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THÈSE DE DOCTORAT

Présentée par

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Discipline: Science de la vie
Spécialité: Pharmacologie et Toxicologie

VALORISATION PHARMACOLOGIQUE DE *ROSMARINUS OFFICINALIS* ET DE *LAVANDULA OFFICINALIS*: TOXICITE AIGUË, POTENTIEL PSYCHOTROPE ET ANTIBACTERIEN

Soutenue le 8 Avril 2014

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Dédicace

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Liste des abréviations

ATCC: American Type Culture Collection

ATP: Adénosine Triphosphate

CMI: Concentration Minimale Inhibitrice

CPG/MS: Chromatographie en Phase Gazeuse couplée à la spectrométrie de masse

CPG: Chromatographie en Phase Gazeuse

DCI: Dénomination Commune Internationale

DL: Dose Léthale

DL0: Dose Léthale Zéro

DL50: Dose Léthale Médiane

DMM: Dose Minimale Mortelle

DMM: Dose Mortelle Minimale

DMSO: DiMethyl SulfOxyde

DO: Densité Optique

DZI: Diamètre de Zone D'inhibition

EA: Extrait Aqueux

EM: Extrait Méthanolique

g: Gramme

GABA: Gamma Amino Butyrate Acid

GHB: Gamma-Hydroxy-Butyrate

IMAO: Inhibition Monoamines Oxydases

IP: Intrapéritonéale

mg: Milligramme

min: Minute

ml: Millilitre

mm: Millimètre

MS: Spectrométrie de masse

MTT: 3-(4.5-dimethyl thiazol-2-y1)-2.5-diphenyl tetrazolium bromide

OMS: Organisation Mondiale de la Santé

PAM: Plantes Aromatiques et Médicinales

PTZ: Pénthyléne TetraZole

SC: Sous Cutanée

SNC: Système Nerveux Central

SVF: Sérum de Veau Fœtal

VO: Voie Orale

µg: Microgramme

µL: Microlitre

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MATERIELS ET METHODES

RESULTATS

Ce travail a fait l'objet de:

1. Publications Internationales

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1. **Rachad Alnamer**, Katim Alaoui, El Houcine Bouidida, Abdelaziz Benjouad, Yahia Cherrah: «*Psychostimulant activity of Rosmarinus officinalis Essential oils* »; **Journal of Natural Products,** 2012; Vol. 5: 83-92.

2. **Rachad Alnamer**, Katim Alaoui, El Houcine Bouidida, Abdelaziz Benjouad, Yahia Cherrah: « Sedative and Hypnotic Activities of the Methanolic and Aqueous Extracts of *Lavandula officinalis* from Morocco »; **Advances in Pharmacological Sciences,** 2012; Vol. 2012: 1-5.

3. **Rachad Alnamer**, Katim Alaoui, El Houcine Bouidida, Abdelaziz Benjouad, Yahia Cherrah: « *Psychostimulant activity of the Methanolic and Aqueous Extracts of Rosmarinus officinalis »;* **Journal of Medicinal Plants Research,** 2012; Vol. 6(10): 1860-1865.

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1. Ahmed Alwashli, Mosa'd Al-sobarry, **Rachad Alnamer,** Yahia Cherrah and Katim Alaoui :«Analgesic and Anti-inflammatory Activities of *Boswellia elongate balf* Methanolic Extracts, as endemic Plants in Yemen»**; Journal of Biologically Active Products from Nature,** 2012; Vol. 2 (2): 90 – 98.

2. Latifa Doudach, Bouchra Meddah, **Rachad Alnamer,** Fatiha Chibani, Yahia Cherrah:«in vitro antibacterial activity of the methanolic and aqueous extracts of

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II. Communications à des congrès Internationaux

1. Rachad Alnamer, Katim Alaoui, El Houcine Bouidida, Abdelaziz Benjouad, Yahia Cherrah:« Psychostimulant activity Rosmarinus officinalis Essential oils». **Premier Congrès International de Pharmacologie.** Organisé par Société Marocaine de Pharmacologie, Les 3 et 4 Mai 2012 à Rabat.

2. Rachad Alnamer, Katim Alaoui, El Houcine Bouidida, Abdelaziz Benjouad, Yahia Cherrah: « Toxicity and Psychotropic Activity of Essentials Oils of Rosmarinus officinalis and Lavandula officinalis from Morocco »; **Premier Congrès International de Pharmacologie.** Organisé par Société Marocaine de Pharmacologie, Les 3 et 4 Mai 2012 à Rabat.

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5. Ahmed Alwashli, Mosa'd Al-sobarry, **Rachad Alnamer,** Yahia Cherrah and Katim Alaoui: «Analgesic and Anti-inflammatory Activities of *Boswellia elongate* Balf Methanolic Extracts, as endemic Plants in Yemen*»;* **Journées Internationales sur les Substances Naturelles et le Développement Durable.** Faculté des Sciences Rabat –Agdal (Maroc); Les 22 – 23 Juin 2012.

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III. Membre du Comité Scientifique et Referee aux Revues Internationaux indexées

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INTRODUCTION GENERALE

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Depuis toujours les plantes ont constitué la source majeure de médicaments grâce à la richesse de ce qu'on appelle le métabolisme secondaire, une exclusivité du monde végétal. Parmi les milliers de molécules produites par ce métabolisme, l'homme sélectionne celles qui lui permettent de se défendre contre les agressions d'autres organismes vivants pathogènes (champignons, bactéries, virus…) et de corriger ses troubles métaboliques. Le 21éme siècle s'ouvre et de nombreuses maladies à fort taux de mortalité restent encore sans traitement. La recherche de nouvelles molécules, plus actives, moins conteuse, avec moins d'effets secondaires, est aujourd'hui une urgence pour l'homme.

Ces produits naturels jouent un rôle significatif dans la découverte des médicaments (Newman et al., 2004). Il est rapporté qu'au minimum 119 composés dérivés de 90 espèces de plantes peuvent être considérés comme médicaments importants (Farnsworth et al., 1985). Bien que les médicaments contre la plupart des maladies aient été découverts, beaucoup de problèmes de santé restent sans traitement encore de nos jours.

Ces problèmes incluent divers types de cancer, les infections par les bactéries et les champignons, les maladies cardiovasculaires, les inflammations; approximativement un tiers de ces maladies peuvent être traitées (Muller et al., 2000). Par contre, la recherche des nouveaux agents thérapeutiques continue, et l'objectif reste toujours à découvrir des armes thérapeutiques efficaces.

Les produits naturels ont une grande opportunité de découvrir une médication effective contre les maladies qui n'ont pas été traitées, par l'effet thérapeutique direct, après une modification semi-synthétique ou bien par une nouvelle synthèse d'un modèle moléculaire à partir des produits naturels (Cragg et al., 1997). La découverte des produits naturels à potentiel thérapeutique est affectée par plusieurs facteurs à savoir l'extraction, l'isolement, l'élucidation des structures d'identification, le dosage et l'évaluation des activités biologiques et pharmacologiques: Malgré les progrès de la thérapeutique moderne, il est nécessaire de rechercher de nouvelles médications:

- soit parce que certaines substances actives sont assez mal tolérées, ou au contraire entraient l'accoutumance,
- soit par suite de l'apparition de nouveaux syndromes ou de souche microbiennes résistantes,
- soit enfin parce que l'on est encore désarmé face à un certain nombre de maladies.

La phytopharmacologie représente l'un des aspects de l'avenir des plantes médicinales. Elle consiste à étudier les plantes et les substances naturelles par l'intermédiaire d'une approche scientifique faisant intervenir différents techniques d'analyse plus ciblées.

Cette notion de phytopharmacologie s'avère d'une importance primordiale pour l'évaluation de certaines indications thérapeutiques revendiquées dans les pharmacopées traditionnelles pour plusieurs préparations à base de plantes médicinales. Ainsi, la réalisation des études pharmacologiques permet de vérifier via l'expérimentation différents points importants qui interviennent pendant le traitement, entre autre, le type de préparation, la posologie et la voie d'administration.

Généralement, les méthodes usuelles de collecte de plantes pour la recherche des molécules bioactives sont, soit systématiques, soit basées sur la connaissance des particularités chimiques de certaines familles de plantes (chimiotaxonomie) ou encore, utilisent des médecines traditionnelles locales comme source primaire d'informations.

Très souvent, une drogue est utilisée pour des affections variées, souvent fort différentes, et il est très rare de ne trouver qu'une seule indication traditionnelle.

Nous avons alors orienté notre travail vers la recherche de propriétés psychotropes, en raison de l'importance en médecine contemporaine, des substances dotées tels effets. Nous avons ainsi souhaité valider les utilisations traditionnelles sédatives de *Lavandula officinalis* et stimulantes de *Rosmarinus officinalis*.

Le but de notre travail est d'apporter une contribution aux connaissances ethno pharmacologiques, cherchant à donner une base scientifique à la médecine traditionnelle marocaine fondée sur l'usage empiriques des plantes médicinales.

Ce travail propose de:

- Extraire les huiles essentielles de *Rosmarinus officinalis* et *Lavandula officinalis* originaires de régions marocaines et de comparer leurs compositions chimiques avec celle décrite dans la littérature.

- Etudier la toxicité aiguë des huiles essentielles, des extraits méthanoliques et des extraits aqueux du *Rosmarinus officinalis* et de la *Lavandula officinalis* chez deux espèces de rongeur (souris et rats); par administration orale.
- Etudier chez ces modèles les effets sédatifs et hypnotiques de la lavande (*Lavandula officinalis* L), et de comparer ces effets à ceux d'un médicament de référence en utilisant la voie orale.

- Etudier l'effet psychostimulant des extraits du Rosmarinus officinalis L sur la vigilance (stimulation du système nerveux central) chez le rat.

- Etudier des effets antibactériens des extraits méthanoliques et aqueux des deux plantes étudiées (*Rosmarinus officinalis* et *Lavandula officinalis*) vis-à-vis des bactéries à Gram négatif (*Escherichia coli* ATCC 54127, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 53153) et à Gram positif (*Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633).

REVUE BIBLIOGRAPHIQUE

Plantes médicinales et Phytothérapie

I. Les produits naturels

Les produits naturels attiraient l'attention des chimistes et des biologistes depuis de longues années.
Dans les dernières décennies, on note un développement remarquable dans la biosynthèse des produits naturels couplée avec les approches d'isolement, de caractérisation et de synthèse; la recherche universitaire or industrielle s'en est largement inspirée (Clardy et Walsh, 2004).
La plupart des scientifiques définissent les produits naturels comme des composés chimiques qui se trouvent dans de nombreuses familles et espèces, animales ou végétales (Firn, 2004).

II. La phytothérapie

II. 1 Introduction

La phytothérapie, qui signifie traitement par les plantes (du grec «Phyton», plantes), désigne une science dont les secrets et recettes constituent l'origine de la pharmacie moderne.
La plupart des médicaments, encore aujourd'hui, sont en effet directement issus des plantes, en général par extraction des racines, des feuilles ou de l'écorce. D'autres sont recomposés chimiquement à partir d'une molécule naturelle qu'ils cherchent à imiter (Lahlou, 2007).
La phytothérapie, qui n'est pas dans son principe opposée à la médecine scientifique moderne, privilégie l'utilisation de la plante entière plutôt que de son extrait chimique. Certes, il existe dans chaque plante une molécule particulièrement active, mais elle est accompagnée d'autres molécules qui facilitent son absorption ou qui la rendent moins agressive. C'est pourquoi la phytothérapie est considérée comme une médecine douce, « parce qu'elle a une toxicité très réduite». Son inconvénient est que l'effet se fait

sentir plus lentement, en raison de la faible concentration du produit actif. Son inocuité par ailleurs n'est pas systématique, d'où le développement aujourd'hui de la phytoresistance.

II. 2 Plantes aromatiques commercialement importantes au Maroc

Le Maroc est chanceux d'avoir un climat aussi varié où n'importe quelle plante médicinale peut pousser et être cultivée économiquement (Tahraoui et al., 2007). Les plantes existantes au Maroc sont soit cultivées soit spontanées. Il reste encore très difficile de cerner avec précision la catégorie de plantes spontanées car elle est composée d'espèces de cueillette exploitées parfois par la population. La collecte se fait entre la fin du printemps et le début de l'automne. Cette production est issue de terres relevant généralement du domaine du Haut Commissariat aux Eaux et Forêts et à la Lutte contre la Désertification. Les Offices Régionaux de Mise en Valeur Agricole relevant du ministère de l'Agriculture (ORMVA) sont responsables de leur vente.

Vu le développement rapide de la population, les besoins en produits aromatiques d'origine végétale ont augmenté d'une façon considérable et les importations ont atteint 500 millions de DH. Le poivre, le cumin, la cannelle, les produits importés font l'objet d'un regain d'intérêt pour l'industrie des plantes aromatiques et médicinales (PAM) au Maroc. En témoigne le volume des exportations en 2009 qui a atteint 900 millions de DH contre 500 millions en 2005. Pour leur part, les importations de PAM se sont établies à 500 millions de DH contre 300 millions en 2005. Il s'agit essentiellement d'épices telles que le poivre, le cumin, la cannelle, la noix de muscade, le girofle, le fenugrec… Le volume d'importations est dû à la faible production locale de ces plantes. Au niveau international, les PAM représentent un marché de 64 milliards de dollars par an, brassés par des unités industrielles utilisant plus de 35.000 plantes. L'UE, les Etats-Unis et l'Asie sont les plus gros importateurs. Cependant et selon les experts, la production de ces plantes reste difficile à cerner au Maroc du fait de l'absence de statistiques nationales ou régionales. Les exportations ne sont pas bien codifiées tant au niveau de l'Office des changes que de l'Etablissement Autonome de Contrôle et de Coordination des exportations (EAACE). La nomenclature utilisée par

les deux organismes n'est pas détaillée et ne correspond pas tout à fait à ce qui est exporté. Par ailleurs, au niveau de la production, il faut distinguer entre cultures spontanées (thym, romarin, armoise, origan, myrte…) et plantes cultivées (menthe, cumin, coriandre, absinthe, safran). Les premières sont réparties à travers les terres collectives relevant du ministère de l'Intérieur et les zones dépendant du département des Eaux et Forêts. La multiplicité des intervenants complique l'organisation de ce secteur. Toujours est-il que les cultures spontanées constituent environ 98% de la production, tandis que les plantes cultivées contribuent pour à peine 2%, une production qui reste traditionnelle et irrégulière, loin de répondre à une demande de plus en plus importante. Les PAM sont utilisées dans les industries de transformation telles que le cosmétique, la parfumerie, la parachimie, la pharmacie, l'agrochimie… Mais au Maroc, ces plantes ne sont pas valorisées et sont exportées sans aucune valeur ajoutée. Ces produits sont commercialisés sous quatre catégories, les plantes poussant naturellement qui sont cueillies par les habitants et utilisées pour aromatiser les nourritures, les plantes poussant à l'état naturel telles que le cèdre, l'armoise, le myrte, le pouliot, utilisées pour leurs huiles essentielles. D'autres plantes peuvent être cultivées pour la consommation, ou pour leurs huiles essentielles comme la menthe, l'estragon, le persil, le safran… La quatrième et dernière catégorie est représentée par les plantes cultivées uniquement pour leur utilisation en huiles essentielles telles que le jasmin, le géranium, la camomille, la bergamote… Que ce soit pour le séchage, le nettoyage ou encore la transformation, les technologies utilisées par les unités industrielles restent artisanales et donnent des produits de qualité imparfaite (L'ECONOMISTE, 2010).

Le monde des médicaments, quant à lui, fait le part à trois grandes catégories selon l'origine:

- D'origine naturelle: Citons entre autres des alcaloïdes comme la morphine à propriétés analgésiques, l'ergotamine efficace contre les migraines, la vinblastine et la vincristine, efficaces contre la leucémie, la vincamine qui améliore la circulation cérébrale, les hétérosides comme la digitaline et la digitoxine qui traitent les

insuffisances cardiaques chroniques. La découverte de ces substances a eu un retentissement particulièrement important à l'échelle mondiale.

- Synthétiques: Les principes actifs des végétaux inspirent la préparation des médicaments de synthèse. Ainsi la structure de l'aspirine (acide acétylsalicylique) est voisine de celle de la salicine des écorces de saule; de même pour les anticoagulants, les anesthésique locaux, les anti-malariques et les curarisants de synthèse. Contrairement aux apparences, la chimie de synthèse n'est pas plus aisée que la chimie extractive car elle a souvent un rendement faible. En effet, la synthèse des médicaments comporte de nombreux stades, au cours desquels il se forme des produits secondaires qu'il faudra éliminer.

- Hémisynthétiques: Les plantes fournissent des précurseurs pour la préparation de médicaments; par exemple on trouve chez des végétaux comme le dioscorea, le fenugrec et certaines agaves, des stéroïdes, inactifs par eux- mêmes, mais dont le chimiste peut modifier la structure pour fabriquer des hormones sexuelles et cortico-surrénaliennes (Lahlou, 2007).

III. Description des deux plantes étudiées

III. 1 Le romarin (*Rosmarinus officinalis* L.)

Figure 1: *Rosmarinus officinalis* (Rhayour, 2002).

Règne:	***Plantae***
Sous-Règne:	***Tracheobionta***
Superdivision:	***Spermatophyta***
Division:	***Magnoliophyta***
Classe:	***Magnoliopsida***
Sous-classe:	***Astéridées***
Ordre:	***Lamiales***
Famille:	***Lamiaceae (labiée)***
Sous-famille:	***Nepetoideae***
Genre:	***Rosmarinus***
Espèce:	***Rosmarinus officinalis***

Rosmarinus est un mot latin qui signifie rosée de la mer (ros: rosée, marinus = mer).

Synonymes: Rosemary (anglais), *klîl: ce mot dérive d'îklîl al-jabal* (litt. : couronne de montage), ***barkella*** (Oriental marocain), ***ašîš Lerneb*** (Tissint) (litt. : herbe à lièvres).
Nom vernaculaire: *âzîr, yazîr*.

III. 1. 1 Description botanique, répartition géographique de romarin

Le romarin est une plante vivace de 1à 2 mètres de hauteur, touffu et très rameux, à feuilles opposées et subsessiles, coriaces, à bords repliés en dessous. Les fleurs, bleu pâle ou lilas claire, sont groupées en grappes denses axillaires ou terminales. Plante commune du bassin méditerranéen dont l'aire de répartition s'étend jusqu' au sud-ouest de l'Asie, le romarin se développe sur les sols calcaires, en particulier en France et en Afrique du Nord (Perry et Perry, 2006; Robins, 1999; Gachkar et al., 2007; Al-Sereitia et al., 1999).
Au Maroc, le romarin se trouve sur les rives de la Moulouya, l'Atlas rifain, le Moyen Atlas, le Grand Atlas et l'Oriental. Les peuplements les plus importants sont rencontrés dans les régions de Taourirt, Jerada et Bouarfa. Comme pour l'armoise, plusieurs chémotypes existent à l'intérieur de l'espèce, *Rosmarinus officinalis* et *Rosmarinus touurnefortii* Denoe. On estime la partie couverte par le romarin dans l'oriental à environ 432 748 ha mais il se trouve rarement comme espèce dominante. La production de phytomasse est estimée 828 298 tonnes. Le Maroc produit en moyenne 60 tonnes d'huiles essentielles de romarin soit l'équivalent d'au moins 15 000 tonnes de matériel végétal, mais la production est très variable selon les régions et la pluviométrie annuelle. Les productions s'échelonnent de 449 750 tonnes (Taourirt) à 207 000 tonnes (Figuig) et 171 548 (Jerrada). La région de Ait Boumerieme (Province de Figuig) reste parmi les plus productives du Maroc oriental (568 500 tonnes en 2000, soit 68 % de la production nationale) (source: CRF-Rabat). La période de récolte du romarin s'étend de fin mars à fin octobre. La fermeture des récoltes correspond à la période allant du 15 novembre au 15 mars. Il s'agit souvent de domaines relevant du Haut Commissariat aux Eaux et Forêts. Les

ventes se font sous forme de marché public et les prix sont variables d'une année à l'autre.

III. 1. 2 Usages traditionnels

Le romarin a été long temps utilisé comme aromate et plante médicinale par plusieurs populations à travers le monde. Il est utilisé comme diurétique, antitussif, emménagogue, carminatifs et tonique. Il est également utilisé dans l'industrie agroalimentaire (Tawaeha et al., 2007; Spencer et al., 2005; Tateo et Fellia, 1998; Cheang et Tai, 2007; Rota et al., 2004; Al-Sereitia et al., 1999). Le romarin est réputé actif pour faciliter les fonctions digestives, en particulier le fonctionnement biliaire (Al-Sereitia et al., 1999).

III. 1. 3 Propriétés biologiques et pharmacologiques

Rosmarinus officinalis a fait l'objet de plusieurs études validant ses effets hépato protecteurs (Sotelo-Félix et al., 2002), antibactériens (Gachkar et al., 2007), anti thrombotique (Yamamoto et al., 2005), antiulcéreux (Dias et al., 2000), diurétique (Haloui et al., 2007), antioxydant (Bakiral et al., 2008), anti nococeptique (Gonzàlez-Trujano et al., 2007) et anti-inflammatoire (Altineir et al., 2007). Les extraits du *Rosmarinus officinalis* L. sont utilisés également pour traiter quelques désordres psychologiques comme la dépression (Heinrich et al., 2006 ; Machado et al., 2009). Il est également insecticide (Pavela, 2006; Traboulci et al., 2002; Rozman et al., 2007), et anti parasitaire (Moon et al., 2000). L'administration de l'huile essentielle de romarin par voie orale et inhalation, stimule l'activité respiratoire et locomotrice du système nerveux central chez la souris, suggérant une action directe d'un ou plusieurs de ses constituants.

III. 2. La lavande (*Lavandula officinalis* L.)

Figure 2: *Lavandula officinalis* (Rhayour, 2002).

Règne:	***Plantae***
Sous-Règne:	***Tracheobionta***
Superdivision:	***Spermatophyta***
Division:	***Magnoliophyta***
Classe:	***Magnoliopsida***
Sous-classe:	***Astéridées***
Ordre:	***Lamiales***
Famille:	***Lamiaceae (labiée)***
Sous-famille:	***Nepetoideae***
Genre:	***Lavandula***
Espèce:	***Lavandula officinalis***

Synonymes: *Lavandula angustifolia*, *Lavandula vera*, *Lavandula vraie*, lavander (anglais).

Nom vernaculaire: *khuzama, hzama, khuzama fassiya* (lavande de Fès), *khuzama zerqa* (Algérie, Maroc, Tunisie).

III. 2. 1 Description botanique et répartition géographique

Lavandula officinalis est un arbrisseau dicotylédone de la famille des Lamiacées (ou labiées) et du genre Lavandula, à fleurs le plus souvent mauves ou violettes disposées en épis, dont la plupart des espèces, très odorantes, sont largement utilisées dans toutes les branches de la parfumerie. Le genre Lavandula est originaire des terres qui bordent la mer Méditerranée et le sud de l'Europe à travers le nord et l'Afrique de l'Est et les pays du Moyen-Orient à l'Asie du sud-ouest et sud-est de l'Inde. Au Maroc, la lavande comme plante aromatique et médicinale est cultivée au sud- est où elle occupe des surfaces étendues (Bellakhaddar, 1997; Tahraoui et al., 2007). Elle comprend plus de 30 espèces, des dizaines de sous-espèces, et des centaines d'hybrides sélectionnés. Les différentes variétés de cette gamme de plante poussent à hauteur de 9 pouces à 3 pieds, bien que certains puissent grandir avec l'âge. Les lavandes sont divisés en quatre catégories principales: *L. angustifolia*, communément connue sous le nom de lavande anglaise, est une espèce résistante au gel (autrefois connue sous le nom *L. vera* ou *L. officinalis*); *L. stoechas* est une grande plante à feuillage gris-vert et à floraison tardive avec une odeur très forte (parfois connue sous le nom de lavande française); *L. latifolia,* typiquement méditerranéenne et *L. intermedia*, croisement entre *L. latifolia* stérile et *L. angustifolia.* Les différentes lavandes ont des propriétés ethnobotaniques et des constituants chimiques principaux similaires (Cavanagh et Wilkinson, 2002; Woronuk et al., 2011; Koulivand et al., 2013).

III. 2. 2 Utilisations traditionnelles

Les fleurs de lavande sont utilisées, partout au Maroc, en infusion, comme emménagogue, stomachique, cholagogue, ainsi que comme anti septique urinaire et pulmonaire. Elle est aussi e mployée par voie vaginale, dans les infections du vagin et de l'utérus. Elle sert aussi comme anti-poux, antimites et comme cosmétique ou eau de toilette. (Tahraoui et al., 2007; Erica et al., 2001; Gachkar et al., 2007; El-Hilaly et al., 2003).

III. 2. 3 Indications thérapeutiques

Lavandula officinalis est la meilleure des lavandes pour la qualité de son huile essentielle (Erica et al., 2001). Elle est utilisée comme antibactérien (Cavanagh et al., 2002; Rota et al., 2004; Evandri et al., 2005), antifongique (Cavanagh et al., 2002), carminative (Cavanagh et al., 2002) analgésique (Hajhashemi et al., 2003), anti inflammatoire (Koulivand et al., 2013; Hajhashemi et al., 2003; Peeana et al., 2002; Sosa et al., 2005), pesticide, insecticide (Pavela, 2006; Traboulci et al., 2002; Rozman et al., 2007; Roger et al., 2004; Ghelardini et al., 1999), parasiticide (Moon et al., 2000), diurétique (James et al., 2006), antihypotensive (Tahraoui et al., 2007), antioxydante (Charles, 2013; Tawaeha et al., 2007), antivirale (Spencer et al., 2005; Hopia et al., 1996) et anti- diabètique (Tahraoui et al., 2007; El-Hilaly et al., 2003).

IV. Généralités sur les médicaments psychotropes

IV. 1 Historique

Tout au long de l'histoire de l'humanité, les civilisations humaines ont utilisé des substances psychotropes afin de soulager des souffrances physiques et psychiques, mieux dormir ou mieux travailler, ou encore observer des rites religieux.

Le terme psychotrope apparait à la fin du XIXème siècle, suite à la démonisation de la morphine en médecine.

Le développement de la chimiothérapie psychiatrique remonte aux années 1950 qui ont vu une véritable explosion pharmacologique, dans laquelle la contribution de la recherche française a été déterminante.

En 1950, Charpentier synthétise le premier des neuroleptiques, la chlorpromazine, qui est ensuite utilisée en ‘’cocktail anesthésique’’ en 1952, associée au dolosal et à la prométhazine par Laborit, puis utilisée seule par Delay et Denicker dans les états psychotiques, ce qui révolutionne l'approche avec les patients psychotiques internés. A cette époque, les scientifiques ne voient en ces avancées biotechnologiques que d'incroyables possibilités pour la santé humaine.

D'autres dates méritent d'être retenues: 1954, emploi du méprobamate, 1957, utilisation de l'imipramine, premier des médicaments antidépresseurs tricycliques ainsi que d'un I.M.A.O., l'iprioniazide; 1960, la première benzodiazépine est commercialisée sous la Dénomination Commune Internationale de chlordiazépoxide, suivie rapidement par d'autre molécules: le diazépam en 1964, l'oxazépam en 1965, jusqu'à la vingtaine de spécialités de benzodiazépines actuellement commercialisées. En 1963, des neuroleptiques retard sont mis au point. En 1970, Schou démontre les propriétés préventives des sels de lithium dans la psychose maniacodépressive. C'est dans les années soixante qu'apparaissent les premiers écrits faisant état d'un culte de la drogue tant par la consommation de drogues que de médicaments psychotropes, on les décrit alors comme un phénomène social d'évasion face aux activités normales quotidiennes.

Depuis 1985, de nouvelles molécules antidépressives, Inhibiteurs sélectifs de recapture de la Sérotonine. IRS et autres, et de nouveaux neuroleptiques « atypiques » ou antipsychotiques se développent. Ainsi ces dernières années, de nouveaux médicaments psychotropes voient le jour, dans un souci permanent d'obtenir des molécules toujours plus efficaces avec le minimum d'effets indésirables.

Aujourd'hui, toutes ces nouvelles générations de médicaments psychotropes pouvant réguler l'humeur ou changer certains traits de personnalité ne sont plus utilisées comme moyens d'évasion, mais bien dans une optique d'insertion sociale pour tous.

IV. 2 Définition

Le terme ″psychotrope″ vient du grec « psukhe » signifiant âme (Psyché, dans la mythologie hellénique, est une jeune déesse qui personnifie l'âme), et du grec « tropos » signifiant orienté vers, tourné, affinité pour.

La définition de ce qu'est un médicament psychotrope varie beaucoup d'une étude à l'autre. En 1957, Jean Delay définit comme psychotrope « une substance chimique d'origine naturelle ou artificielle, qui a un tropisme psychologique, c'est-à-dire qui est susceptible de modifier l'activité mentale, sans préjuger du type de cette modification ». La définition anglo-américaine retient les « substances qui modifient les sensations, l'humeur, la conscience et d'autres fonctions psychologiques et comportementales ». Dans le grand dictionnaire terminologique de l'Office de la langue française, cette définition est accompagnée de la note suivante: « les médicaments psychotropes englobent des agents très divers, y compris ceux qui modifient le comportement par action directe ou indirecte sur le système nerveux central ou par action périphérique ». On parle aussi des médicaments psychotropes comme étant ceux « utilisés principalement pour leurs effets sur la conscience, l'humeur et la perception des environnements interne et externe » selon Kalant.

Les psychotropes sont des substances qui agissent au niveau du système nerveux central pour en modifier le fonctionnement cellulaire, modulant ainsi les fonctions supérieures: motrices, cognitives, sensorielles, psychiques. Inhibition ou désinhibition, modifications de la vigilance et de l'attention, excitation, agressivité paradoxale, modifications de la vision, hallucinations, perte de la notion du temps, exacerbation du moi, désintérêt de l'environnement, sont autant d'effets délétères susceptibles d'être provoqués par les unes ou les autres de ces substances.

Les substances psychotropes englobent des substances licites comme les médicaments psychotropes prescrits, et d'autres illicites:

- ✓ substances psychotropes licites: alcool, tabac, médicaments psychotropes (mais les médicaments peuvent aussi avoir un usage illicite lorsqu'ils sont détournés de leur bon usage).

- ✓ substances psychotropes illicites: cannabis, colles et solvants, cocaïne, hallucinogènes (LSD et champignons hallucinogènes), amphétamines, ecstasy, héroïne…

De manière générale, un médicament psychotrope est un produit qui a la propriété d'affecter la psyché d'un individu en agissant sur le système nerveux central. De tels médicaments ont pour but de soulager soit la douleur, le stress, l'anxiété, les troubles du sommeil, la dépression, certaines conditions neuropathologiques ou encore de moduler certains comportements dans le cadre de traitements spécifiques comme pour la schizophrénie ou la maladie bipolaire.

IV. 3 Classification

Les médicaments psychotropes regroupent plusieurs produits provenant de différentes classes de médicaments.

En 1957, Delay et Deniker proposent une classification des psychotropes fondée sur la notion de « tonus mental », selon Janet, ou interviennent comme éléments constituants et toujours en remaniements, la vigilance et l'humeur, elle permet de distinguer:

- ✓ des substances sédatives (ou psycholeptiques): anxiolytiques, hypnotiques et neuroleptiques, auxquels on peut ajouter les thymorégulateurs pour actualiser cette classification,
- ✓ des composés qui élèvent le tonus mental, des stimulants (ou psychoanaleptiques) parmi lesquels on distingue:
  - les stimulants de la vigilance (noanaleptiques): les amphétamines,
  - les stimulants de l'humeur (thymoanaleptiques): les antidépresseurs,
- ✓ les perturbateurs de l'activité mentale (psychodysleptiques): les hallucinogènes.

Tableau 1: Classification générale des psychotropes (Muster et al., 2005).

Psycholeptiques	Psychoanaleptiques	Psychodysleptiques
1. Hypnotiques 2. Tranquillisants, Sédatifs 3. Neuroleptiques 4. Régulateurs de l'humeur	1. Stimulants de la vigilance 2. Antidépresseurs 3. Autres stimulants	1. Hallucinogènes et onirogènes 2. Stupéfiants 3. Alcool et dérivés

IV. 3 1 Les neuroleptiques

IV. 3. 1. 1 Définition

Ce sont des sédatifs majeurs à action anti psychotique symptomatique, ils provoquent la mise au repos du SNC dans le domaine psychique (Kasambe, 1998).

IV. 3. 1. 2 Mécanisme d'action

Tous les neuroleptiques ont en commun un effet de blocage de récepteurs dopaminergiques, et sélectivement les récepteurs dopaminergiques D2.

Tableau 2: Classification des neuroleptiques (Muster et al., 2005).

Classe	DCI
Neuroleptiques de première génération	
Phénothiazines	Chlorpromazine
	Cyamémazine
	Fluphénazine
	Lévomépromazine
	Pipotiazine
	Propériciazine
	Thioridazine
Butyrophénones	Halopéridol
	Penfluridol
	Pipampérone
Thioxanthènes	Flupentixol
	Zuclopenthixol
Benzamides substitués	Amisulpride
	Sulpride
	Sultopride
	Tiapride
Neuroleptiques de deuxième génération	
Benzisoxazoles	Rispéridone
Dérivés de la quinolinone	Aripiprazole
Dibenzo-oxazépines	Loxapine
Dibenzodiazépines	Clozapine

DCI: dénomination commune internationale.

IV. 3. 2 Les hypnotiques

IV. 3. 2. 1 Définition

Les hypnotiques représentent une classe pharmacologique proche des anxiolytiques, dont les propriétés sédatives sont privilégiées. Il s'agit essentiellement de benzodiazépines (BZD) ou d'apparentés.

IV. 3. 2. 2 Mécanisme d'action

Les études de fixation ont permis de montrer que les BZD se fixent au voisinage immédiat des sites GABA, récepteurs couplés à un ionophore chlorique, intervenant sur ce neurotransmetteur grâce à certaines interactions allostériques.
Le GABA a pour propriété principale d'inhiber les autres systèmes monoaminergiques (noradrénergique, dopaminergique, serotoninérgiques) ce qui permettrait d'expliquer, au moins partiellement, l'action plutôt sédative, anxiolytique et anti convulsivante de ces agonistes (Hirokane, 1999; De Beaurepaire, 2005).

Tableau 3: Classification des hypnotiques (Muster et al., 2005).

Classe	DCI
Cyclopyrrolones	Zopiclone
Imidazopyridines	Zolpidem
Benzodiazépines (utilisées comme hypnotiques)	Estazolam Flunitrazépam Loprazolam Lormétazépam Nitrazépam Témazépam Triazolam
Ethanolamines	Doxylamine
Phénothiazines	Acéprométazine Clorazépate dipotassique Acépromazine Alimémazine Niaprazine

DCI: dénomination commune internationale.

IV. 3. 3 Les anxiolytiques ou tranquillisants

IV. 3. 3. 1 Définition

Ce sont des substances de structure chimique très variable qui ont en commun la propriété de réduire ou supprimer l'angoisse et de provoquer une sédation.

IV. 3. 3. 2 Mécanisme d'action

Le mécanisme d'action des benzodiazépines n'a été globalement élucidé qu'en 1987, quand Schqield et al., en ont cloné le récepteur (De Beaurepaire, 2005).

- ✓ les BDZ renforcent les effets du GABA sur ses récepteurs cérébraux qui sont de type GABA A
- ✓ les récepteurs GABA A se situent dans la membrane cellulaire et contrôlent l'ouverture des canaux chlore des cellules nerveuses; il existe un récepteur au GABA B; mais il est très différent et il n'est pas impliqué dans le traitement de l'anxiété.
- ✓ la fixation d'une molécule de BDZ sur ou à proximité immédiate du récepteur GABA A potentialise l'action du GABA.
- ✓ les benzodiazépines sont donc des inhibiteurs très pressants de l'excitabilité neuronale (De Beaurepaire, 2005; Muster et al., 2005; Moulin, 1998; American Psychiatrie Association, 1997).

Tableau 4: Classification des anxiolytiques ou tranquillisants (Muster et al., 2005).

Classe	DCI
Benzodiazépines (utilisées comme anxiolytiques)	Alprazolam Bromazépam Clobazam Clorazépate dipotassique Clotiazépam Diazépam Lorazépam Nordazépam Oxazépam Prazépam
Autres anxiolytiques	Buspirone Captodiame Etifoxine Hydroxyzine Méprobamate

DCI: dénomination commune internationale.

IV. 3. 4 Les antidépresseurs

IV. 3. 4. 1 Définition

Ce sont, selon l'expression de Deniker, les médicaments de l'humeur douloureux ceux qui permettent lorsque leur action est efficace le retournement de l'humeur du déprimé (action thym réversive); les antidépresseurs vont agir sur les différent aspects de la dépression (Muster et al., 2005)

- ✓ sur l'humeur dépressive.

- ✓ sur l'activité psychomotrice.
- ✓ sur les facteurs non spécifiques.

IV. 3. 4. 2 Mécanisme d'action

De point de vue biologique la dépression s'accompagne d'un déficit en monoamines cérébrales ou neurotransmetteur il s'agit de rétablir le taux de monoamines. Plusieurs possibilités sont alors envisageables.

- ✓ inhibition de recaptage de monoamines (antidépresseur tricycliques).
- ✓ Inhibition de la destruction des monoamines oxydase (IMAO).
- ✓ Apport des précurseurs de monoamines.

Les antidépresseurs vont agir sur les monoamines cérébrales (adrénaline, noradrénaline, dopamine, sérotonine) en augmentant leur taux (Warsh, 1998).

Tableau 5: Classification des Antidépresseurs (Muster et al., 2005).

Classe	DCI
Antidépresseurs imipraminiques et apparentés	
Antidépresseurs intermédiaires ou médians	Clomipramine
	Dosulépine
	Imipramine
Antidépresseurs sédatifs et anxiolytiques	Amitriptyline
	Amoxapine
	Doxépine
	Maprotiline
	Trimipramine
Antidépresseurs serotoninérgiques purs ou IRS	
A forte potentiel d'interactions médicamenteuses	Fluoxétine
	Fluvoxamine
	Paroxétine
A faible potentiel d'interactions médicamenteuses	Citalopram
	Escitalopram
	sertraline
Antidépresseurs divers	
Antidépresseurs psychotoniques	Viloxazine
Antidépresseurs intermédiaires ou médians(IRSNA)	Milnacipran
	Tianeptine
	Venlafaxine
Antidépresseurs sédatifs (NaSSA)	Miansérine
	Mirtazapine
Antidépresseurs IMAO	
IMAO sélectifs de type A	Moclobémide
IMAO non sélectifs	Iproniazide

IMAO: inhibiteur des monoamines oxydases A ; IRS: inhibiteur de la recapture de la sérotonine; IRSNA: inhibiteur de la recapture de la sérotonine et de la

noradrénaline; NaSSA: antidépresseurs spécifique de la sérotonine et de la noradrénaline. DCI: dénomination commune internationale.

IV. 3. 5 Les psychostimulants

IV. 3. 5. 1 Définition

Une substance psychostimulante est caractérisée par sa capacité à élever le niveau de vigilance et à stimuler l'activité mentale et comportementale (Muster et al., 2005).

IV. 3. 5. 2 Mécanisme d'action

Les mécanismes d'action qui sous tendent les effets des molécules amphétaminique sur le SNC sont de plusieurs types et convergent tous vers:

- ✓ la libération des catécholamines (noradrénaline et dopamine) des sites de stockage des terminaisons nerveuses.
- ✓ Ils ont la capacité d'inhiber la recapture des monoamines par les terminaisons pré synaptiques.
- ✓ A forte dose, ils peuvent inhiber l'activité de l'enzyme de dégradation des monoamines oxydases (IMAO)
- ✓ Ils ont un effet agoniste post-synaptique direct sur les récepteurs dopaminergiques et serotoninérgiques (De Beaurepaire, 2005).

Tableau 6: Classification des psychostimulants ou nonanaleptiques (Muster et al., 2005).

Classe	DCI
Psychostimulants non amphétaminiques	Adrafinil Modafinil
Psychostimulants amphétaminiques	Méthylphénidate

DCI: dénomination commune internationale.

IV. 3. 6 Les régulateurs de l'humeur

IV. 3. 6. 1 Définition

Ce sont des médicaments qui régularisent l'activité mentale, équilibrent le psychisme, ils sont appelés encore normo thymiques ou thym régulateurs et sont indiqués dans la psychose maniacodépressive.

IV. 3. 6. 2 Mécanisme d'action

Le prototype des thymostabilisateurs ou thymorégulateurs est le lithium. Le mécanisme d'action des thymostabilisateurs est très complexe et toujours assez mal connu.

Depuis le fait observé en 1971, que le lithium provoque une déplétion en inositol libre dans le cerveau, et jusqu'à l'observation vers le milieu des années 1990 d'effets neuroblastiques du lithium, quatre domaines ont été posé:

l'interaction de l'ion lithium avec les autres ions, les effets du lithium sur les systèmes de neurotransmetteurs, sur les voies des seconds messagers et les effets neurotrophiques et neuroprotecteurs du lithium (De Beaurepaire, 2005; De Beaurepaire, 2003; De Beaurepaire, 2002a; De Beaurepaire, 2002b).

Tableau 7: Classification des régulateurs de l'humeur (Muster et al., 2005).

Classe	DCI
Thymorégulateurs	Sels de lithium Acide valproïque Valproate valpromide

DCI: dénomination commune internationale.

IV. 3. 7 Les psychodysleptiques

A coté des hallucinogènes, capables de produire des psychoses artificielles et des inducteurs d'ivresse (alcool, éther), on peut aussi placer les stupéfiants : soit des substances illicites (héroïne, cocaïne) soit des médicaments comme la morphine, le dolosal, le palfium, le temgésic… (De Beaurepaire, 2005).

IV. 4 Les sédatifs d'origine végétale

Le stress, l'anxiété et la nervosité sont à l'origine de troubles du sommeil chez un nombre toujours croissant de personnes. Les sédatifs à base de plante constituent une réelle alternative. Ce ne sont pas des hypnotiques, ils exercent plutôt un effet calmant et tranquillisant. Dépourvus d'effets secondaires, ils sont tout à fait adaptés pour soulager les troubles mineurs du sommeil d'origine nerveuse et leur prise, même prolongée, ne provoque pas d'accoutumance.

Ainsi, la valériane (*Valeriana officinalis* L, valérianacée) est la plante sédative la mieux étudiée. Sa racine contient des dérivés instables appelés valépotriates. Lorsque la racine est arrachée ou lésée, les valépotriates libèrent de l'acide isovalérique qui est responsable de l'odeur caractéristique et désagréable de la valériane. Il existe plusieurs formes d'utilisation de cette plante et la composition des préparations peut varier fortement.

On trouve sur le marché des spécialités standardisées en valépotriates obtenues à partir d'espèces de valériane comme la valériane mexicaine (*Valeriana mexicana* DC).

L'utilisation d'autres plantes est bien établie sans qu'on sache toujours quels en sont les principes actifs. Il en est ainsi du houblon (*Humulus lupulus* L, cannabacées). On raconte que les gens qui le cueillaient autrefois pour la fabrication de la bière s'endormaient.

Houblon et valériane sont souvent associés à la passiflore (*Passiflora incarnata* L, passifloracées) utilisée à l'origine dans la médecine traditionnelle nord et sud-américaine ou à la mélisse (*Melissa officinalis* L, lamiacées) (Hostettman, 1997). La tradition populaire attribue à plusieurs autres plantes des vertus calmantes sans que l'effet ne soit réellement établi. Des études pharmacologiques confirment aujourd'hui leur potentiel sédatif (Tab. 8).

Tableau 8: Action sédative des plantes médicinales et aromatiques.

Plante (Famille/Espèce)	Partie employée	Extrait/ Principe actif	Animal testé	Référence bibliographique
Annonaceae				
Annona diversifolia	Feuilles	Extrait éthanolique	Souris	Gonzalez et al., 1998
Apiaceae				
Centelle asiatica	Feuilles	Extrait hydroalcoolique	Rat	Lucia et al., 1997
Asteraceae				
Lactuca sativa	Graines	Huile	Souris	Said et al., 1996
Caesalpinioideae				
Cassia fistula	Graines	Extrait méthanolique	Souris	Mazumder et al., 1998
Caprifoliaceae				
Viburnum tinus	Feuilles	Iridoides	Souris	Cometa et al., 1998
Compositae				
Matricaria chamomilla	Fleurs	extrait méthanolique	Souris	Avallone et al., 2000
Sphaeranthus senegalensis	Feuilles	flavonoide: apigenin	Souris	Amos et al., 2001
Convolvulaceae				
Convolvulus subhirsutus	Parties aeériennes	Extrait aqueux Alcaloïdes: Convolvine, atropine		Mirzaev et Aripova, 1998
Crassulaceae				
Bryophyllum pinnatum	Feuilles	Extrait méthanolique	Rat Souris	Diddhartha et Chaudhuri, 1999
Euphorbiaceae				
Euphorbia fischeriana	Racines	Diterpenoides	Souris	qingGao et al., 1997
Hypericaceae				
Hypericum hookerianum	Feuilles	Extrait	Souris	Mukherjee et al., 2001

H. patulum		méthanolique		
Juglandaceae				
Juglans regia	Feuilles	extrait chloroformique quinonc : juglone	Souris	Girzu et al., 1998
Lamiaceae				
				Gilani et coll., 2000
Lavandula stoechas	Fleurs		Souris	Gilani et coll., 2000
		Extrait alcoolique		
Melissa officinalis	Feuilles		Souris	Soulimani et al., 1993
Leguminosae				
Lupinus mutabilis	Graine	Alcaloïdes	Souris	Pothier et al., 1998
Malpighiaceae				
Galphimia glauca	Parties aériennes	Triterpenoide: galphimine B	Souris	Tortoriello et Ortega, 1993
Myrtaceae				
Leptospermum scoparium	Feuilles	Flavonoïdes	Rats	Haberlein et al., 1994
Papaveraceae				
Eschscholzia californica	Parties aériennes	Extrait hydro-alcoolique	Souris	Rolland et al., 2001
Passifloraceae				
Passiflora incarnata	Parties aériennes	Extrait aqueux	Souris	Soulimani et al., 1997
Pinaceae				
Cedrus deodara	Bois	Huile essentielle	Rat Souris	Tandan et al., 1998
Poaceae				
Bambusa bambos	Feuilles	Extrait aqueux	Souris Rat	Singh et al., 1998

			Cobaye	
Polygalaceae				
Securidaca longepedunculata	Racines	Extrait méthanolique	Souris	Olajide et al., 1998
Rhamnaceae				
Zizyphus spina	Racines	Extrait aqueux	Souris	Adzu et al., 2002
Rubiaceae				
Sickingia williamsi	Ecorces	extrait chloroform-méthanilique extrait méthanolique	Souris	Capasso et al., 1996
Rutaceae				
Cirtus bergamia	Feuilles	Huile essentielle	Souris	Occhiuto et al., 1995
Solanaceae				
Solanum nigrum	Fruit	Extrait éthanolique	Rat Souris	Perez et al., 1998
Verbenacea				
Lippia multiflora	Feuilles	infusion huile essentielle		Abena et al., 2001
Zingiberaceae				
Aeollanthus suaveolens	Parties aériennes	huile essentielles linalol	Souris	Elisabetsky et al., 1995

IV. 5 Les hypnotiques d'origine végétale

Les hypnotiques permettent un prompt endormissement et évitent les réveils pendant la nuit. Mais ils présentent des inconvénients en réduisant les phases de sommeil lent et de sommeil paradoxal et en créant un état de dépendance.

La médecine à base de plantes propose donc des tisanes et d'autres préparations pour dormir. Elles ont l'avantage d'être naturelles et de ne présenter aucun risque d'accoutumance. Aussi une recherche constante de nouvelles classes d'hypnotiques est essentielle pour surmonter les problèmes de dépendance (Tab. 9).

Tableau 9: Action hypnotique des plantes médicinales et aromatiques.

Plante (Famille/Espèce)	Partie employée	Extrait/ Principe actif	Animal testé	Propriété exercée	Référence bibliographique
Annonaceae					
Annona squamosa	Graines	Extrait éthanolique	Rats	Potentialisation l'effet hypnotique du pentobrabitone	Saluja et Santani 1994
Apiaceae					
Centella asiatica	Feuilles	Extrait yhdroalcoolique	Rats	Potentialisation l'effet hypnotique du pentobrabitone	Lucia et al., 1997
Notopterygium incisum	Rhizomes et racines	Coumarine (notopterol)	Rats	Potentialisation l'effet hypnotique du pentobrabitone	Okuyama et al., 1993
Asteraceae					
Baccharis serrafolia	Feuilles	Extrait méthanolique	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Tortoriello et al., 1996
Lactuca sativa	Graines	Huile	Souris	Potentialisation l'effet hypnotique du Pentobrabitone	Said et al., 1996

Matricaria chamomilla	Parties aériennes	Huile essentielle	Rats	Effet hypnotique	Yamada et al., 1996
Cannabidaceae					
Humulus lupulus	Parties aériennes	Extrait aqueux	Souris	Effet hypnotique	Lee et al., 1993
Compositae					
Sphaeranthus senegalensis	Feuilles	Extrait aqueux	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Amos et al., 2001
Euphorbiaceae					
Euphorbia hirta	Tiges et feuilles	Extrait aqueux	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Lanhers et al., 1996
Fabaceae					
Lupinus albus	Graines	Extrait méthanolique	Rats	Potentialisation l'effet hypnotique du pentobrabitone	Bobkiewicz et al., 1995
Hypericaceae					
Hypericum perforatum	Parties aériennes	Extrait éthanolique Extrait à l'acetate d'thyl	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Jakovljevic et al., 2000
Lamiaceae					
Ocimum sanctum	Parties aériennes	Huile fixe	Rats	Potentialisation l'effet hypnotique du pentobrabitone	Singh et al., 2001
Salvia guaranitica	Feuilles	Cirsiliol (5,3',4'-trihydroxy- 6,7-dimethoxy flavone)	Souris	Effet hypnotique	Marder et al., 1996
Pinaceae					
Cedrus deodara	Bois	Huile essentielle	Souris	Potentialisation l'effet	Tandan et al., 1998

				hypnotique du pentobrabitone	
Piperceae					
Piper solmsianum	Feuilles	Huile essentielle	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Moreira et al., 2001
Poaceae					
Bambusa bambos	Feuilles	Extrait aqueux	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Singh et al., 1998
Rhamnaceae					
Zizyphus spinachristi	Racines	Extrait aqueux	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Adzu et al., 2002
Rhosaceae					
Rubus brasiliensis	Extrait hexanique	Extrait hexanique	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Nogueira et Vassilief, 2000
Rubiaceae					
Xeromphis spinosa	Pulpe	Extrait éthanolique	Rats	Potentialisation l'effet hypnotique du pentobrabitone	Saluja et Santani 1994
Rutaceae					
Citrus bergamia	Feuilles	Huile essentielle (fraction non volatile)	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Occhiuto et al., 1995
Verbenaceae					
Vitex negundo	Feuilles	Ether de pétrole	Souris	Potentialisation l'effet hypnotique du pentobrabitone	Malaya et al., 1997

V. Généralités sur les huiles essentielles

V. 1 Introduction

V. 1. 1 Aromathérapie

L'aromathérapie est une branche de la phytothérapie qui utilise les HE pour traiter un certain nombre de maladies. Le terme aromathérapie vient du chimiste Français René-Maurice Gattefosse, qui a utilisé l'HE de lavande pendant la première guerre mondiale pour soigner des blessures et des infections. Selon lui, la lavande était plus appropriée pour traiter les infections que plusieurs antiseptiques utilisés à cette époque. Cette spécialité préoccupe de plus en plus des médecins et des pharmaciens qui ont publié un nombre important d'ouvrages d'aromathérapie (Roulier, 1992). Les HE sont largement utilisés pour traiter certaines maladies internes et externes (infections d'origine bactérienne ou virale, troubles humoraux ou nerveux). En médecine dentaire, plusieurs HE ont donné des résultats cliniques très satisfaisants dans la désinfection de la pulpe dentaire, ainsi que dans le traitement et la prévention des caries (Kato et al., 1990; Schwartz et al., 1992; Sourai, 1989). La listerine, solution constituée d'HE de thymol et d'eucalyptol, possède une grande activité bactéricide sur les microorganismes de la salive et de la plaque dentaire (Kato et al., 1990). Les huiles essentielles de thym et de romarin ont été utilisées pour soulager la fatigue, les maux de tête, les douleurs musculaires et quelques problèmes respiratoires.

V. 1. 2 Définition et rôle écologique

Les huiles essentielles (H.E) sont des composés aromatiques volatiles constitués par un mélange complexe des molécules odoriférantes des plantes. Ce sont des produits très volatiles, d'excrétion du métabolisme végétale, généralement odorants , constitués d'un grand nombre de substances terpéniques synthétisées par les plantes et extraites à partir du matériel végétal à l'aide de procédés physiques comme la distillation à l'eau (hydrodistillation) ou à la vapeur d'eau (entrainement à la vapeur d'eau) ou par

expression du péricarpe de certains citrus, suivis de décantation ou centrifugation (Mossaddak, 1995). L'histoire des huiles essentielles débuta dans les pays de l'Orient et particulièrement en Egypte, Perse et Inde (Guenter, 1949). Selon Schow, la séparation des principes odorants par la technique de la distillation remonte à la Renaissance (Schow, 1984). Par ailleurs, la première description détaillée de la distillation date du XIIIème siècle grâce au physicien catalan A. de Villanova (Guenter, 1949).

V. 1. 3 Localisation et rôles des huiles essentielles dans la plante

Dans la plante, les huiles essentielles peuvent être stockées dans divers organes: fleurs (origan), feuilles (citronnelle, eucalyptus), écorce (cannelier), bois (bois de rose, santal), racines (vétiver), rhizomes (acore), fruits (badiane) ou graines (carvi). La synthèse et l'accumulation des huiles essentielles, classées parmi les métabolites secondaires, se font généralement au niveau des structures histologiques spécialisées, souvent localisées sur la surface de la plante (Brunechon, 1987; AFN, 1986). Par exemple, pour la famille des Lamiaceae, elle se situe dans les poils sécréteurs, chez les Myrtaceae au niveau des poches sécrétrices ou encore des canaux sécréteurs pour les Asteraceae.

Parmi les composants majoritaires des huiles essentielles, nous trouvons les terpènoïdes qui possèdent un rôle écologique lors des interactions végétales, comme agents allélopathiques, soit inhibiteur de la germination, mais aussi lors des interactions végétal-animal, comme agent de protection contre les prédateurs tels que les insectes. Ils interviennent également, par leurs odeurs caractéristiques, dans l'attraction de pollinisateurs (Langenheim, 1969).

Par ailleurs, les plantes aromatiques productrices d'huiles essentielles, ont fait l'objet de diverses recherches en particulier dans le domaine de la parfumerie.

Les travaux de J.Q. CU et al., (J.Q. CU., 1990), reprennent ceux de TEDDER et BRUNECHON (Tedder, 1970; J.Q. CU., 1990), où il apparaît clairement comment les molécules très volatiles sont synthétisées à partir d'unités méthyle-2-buta-1,3-diène (isoprène). Les diverses combinaisons de ces unités, par réaction d'addition,

conduisent aux terpènes, sesquiterpènes, diterpènes, mais aussi à leurs produits d'oxydation tels que les alcools, aldéhydes, cétones, éthers, et esters terpéniques.

L'ensemble de ces composés est produit dans les cellules sécrétrices, conférant à la plante une odeur caractéristique. Celle-ci peut alors être libérée de la plante et récupérée par diverses méthodes allant de l'enfleurage, pour les odeurs les plus délicates (fleur), à l'extraction à l'aide de solvants organiques (J.Q.CU., 1990).

Une gamme de produits à odeur plus ou moins prononcée selon la concentration en composés et en composés volatils recueillis, est alors obtenue. Les huiles essentielles produites par hydrodistillation, entraînement à la vapeur ou expression de l'écorce des fruits, sont les produits les plus concentrés en composés olfactifs (Tedder, 1970). Chacun de ces composés de par sa volatilité dégage une odeur propre. Ainsi certaines plantes peuvent avoir une odeur similaire due à une molécule commune présente en quantité notable dans l'huile essentielle. Dans ces conditions, une fois le ou les composés responsables d'une odeur identifiés, si le caractère olfactif de ces composés s'avère intéressant, il serait alors rentable, selon le contexte économique, de produire des espèces végétales susceptibles de fournir une huile essentielle à haute teneur moléculaire en composés recherchés, et donc généralement de meilleure qualité (Tedder, 1970). De manière générale, il est assez aléatoire d'évaluer la qualité olfactive d'une huile essentielle d'après la composition chimique de celle-ci, où des rapprochements heureux aussi bien que des dégradations de composés aromatiques sont induites. Seul, un examen organoleptique permettra d'évaluer ces interactions moléculaires. Les principaux atouts olfactifs d'une huile essentielle ne tiennent pas seulement compte de la composition chimique de l'essence mais aussi de son examen organoleptique, de ses constantes physiques permettant de déceler toute adultération (AFN, 1986).

Les essences naturelles sont considérées comme un ensemble harmonieux de composants trop diversifiés dans leurs propriétés physico-chimiques. Ainsi les H.E sont formées de deux types des produits principaux:

- Les composés non polaires, hydrocarbures (mono terpènes, sesquiterpènes).
- Les composés polaires, esters, acides, aldéhydes, cétones, époxydes, alcools, éthers,…

Les multiples fonctions possibles que peuvent jouer les H.E au sein de la plante sont :

- La protection contre les prédateurs de la plante (insecticide)
- L'attraction des insectes pollinisateurs.
- L'inhibition de la multiplication des bactéries et des champignons.
- L'inhibition de la germination et de la croissance des mauvaises herbes.
- La stimulation de l'humeur.

V. 1. 4 Caractéristiques économiques

Si de nos jours quelques centaines de plantes aromatiques, parmi d'innombrables espèces recensées dans la nature, sont exploitées à l'échelle commerciale, c'est en partie parce que les facteurs agronomiques, climatiques, botaniques et olfactifs sont limitatifs mais d'autres critères sont aussi à prendre en compte.

V. 1. 5 Caractéristiques traditionnelles

Un certain nombre de plantes médicinales sont encore utilisées de nos jours sous forme de décoctions et infusions mais la plupart d'entre elles ont été délaissées au profit de produits pharmaceutiques de synthèse. Cependant, les connaissances actuelles permettent d'analyser ces plantes et souvent de comprendre l'activité préconisée par nos ancêtres.

Une relation entre la structure chimique et l'activité biologique est alors tentante, et la production de molécules naturelles pourrait entrer dans la composition de médicaments moins agressifs vis-à-vis de l'organisme, ou à des fins industrielles précédemment exposées.

Cette dernière perspective permet d'élargir le champ de valorisation des plantes aromatiques, (autrefois restreint du point de vue économique, à l'extraction de molécules olfactives), par l'exploitation des nombreuses et diverses activités biologiques, substantiellement évoquées par la médecine traditionnelle, que nous allons recenser et corréler à certains types de structures chimiques. Ce dernier travail

faire apparaître des molécules « bioactives » dans des espèces référencées par la médecine traditionnelle supposant ainsi des activités biologiques (Bourel, 1993).

V. 1. 6 Activités biologiques des huiles essentielles

Les huiles essentielles possèdent de nombreuses activités biologiques. En phytothérapie, elles sont utilisées pour leurs propriétés antiseptiques contre les maladies infectieuses d'origine bactérienne, par exemple contre les bactéries endocanalaires (Pellecuer et al., 1980) ou au niveau de la microflore vaginale (Viollon et Chaumont, 1994) et d'origine fongique contre les dermatophytes (Chaumont et Legeer, 1989).

Cependant, elles possèdent également, des propriétés cytotoxiques (Sivropoulou et al., 1996) qui les rapprochent donc des antiseptiques et désinfectants en tant qu'agents antimicrobiens à large spectre.

Dans des préparations pharmaceutiques, les terpènes phénoliques, comme le thymol et le carvacrol, sont souvent utilisés comme antiseptiques antibactériens et antifongiques. Le thymol est très irritant, astringent et caustique. La dose de thymol applicable sur la peau et les muqueuses est de 0,5%. Ingéré à la dose de 2 g ou à plus fortes doses, il est responsable de gastralgies avec nausées (Zambonelli et al., 2004).

Dans les domaines phytosanitaire et agro-alimentaire, les huiles essentielles ou leurs composés actifs pourraient également être employés comme agents de protection contre les champignons phytopathogènes (Zambonelli et al., 2004) et les microorganismes envahissant les denrées alimentaires (Mangena et Muyima, 1999).

VI. Généralités sur l'activité antibactérienne

VI. I. Introduction

Au cours de la dernière décennie la phytothérapie est devenue un sujet d'importance mondiale, ayant à la fois un impact sur la santé mondiale et le commerce international. Les plantes médicinales continuent de jouer un rôle central dans le système sanitaire de la grande proportion de la population mondiale. Cela est particulièrement vrai dans

les pays en cours de développement, où l'histoire du recours à la médecine à base de plantes a une histoire longue et ininterrompue. En médecine humaine, les bactéries deviennent de plus en plus résistantes aux antibiotiques habituellement utilisées pur le traitement des maladies infectieuses ce qui exige la recherche de nouveaux produits antibactériens (Alanis, 2005).

Dans les pays du tiers monde, où les ressources budgétaires affectées aux dépenses de santé restent inférieures aux besoins de la communauté, les plantes médicinales continuent de représenter une part importante des moyens thérapeutiques accessibles aux couches défavorisées qui constituent 90% de la population (Bellakhddar, 1997).

En effet, l'**O**rganisation **M**ondiale de la **S**anté (OMS) recommande à tous les pays en développement de recenser, d'évaluer et d'intégrer la médecine traditionnelle dans leur système de santé.

La reconnaissance et le développement des vertus médicinales et économiques de ces plantes sont en augmentation dans les pays industrialisés et ceux en cours de développement (Anowi et al., 2012). L'utilisation de la médecine à base de plantes par une large proportion de la population dans les pays en développement est en grande partie en raison du coût élevé des produits pharmaceutiques occidentaux et des soins, les effets indésirables qui suivent leur utilisation (dans certains cas) et la vision culturelle et spirituelle des populations de ces pays (Anowi et al., 2012). Dans les pays occidentaux développés, après un ralentissement dans le rythme d'utilisation des PAM dans les dernières décennies, le rythme s'accélère de nouveau d'autant que les scientifiques se rendent compte que la durée de vie effective de tout antibiotique est limitée (Satyajii and Lutfun, 2007). Il a été prévu que les dépenses mondiales pour la recherche de nouveaux agents anti-infectieux (y compris les vaccins) allaient augmenter de 60% par rapport aux niveaux de dépenses en 1993. De nouvelles sources, notamment des sources végétales, sont également à l'étude. De plus, le public est de plus en plus conscient des problèmes de la sur-prescription et l'utilisation abusive des antibiotiques traditionnels.

Huiles essentielles et différents extraits de plantes ont suscité un intérêt en tant que sources de produits naturels. Ils ont été sélectionnés pour leurs utilisations potentielles, comme des remèdes alternatifs pour le traitement de nombreuses maladies infectieuses (Agunu et al., 2005; Oluwatuyi et al., 2004 ; Derwich et al., 2011; Jarrar et al., 2010 ; Jiang et al., 2011). En particulier, les activités antimicrobiennes et anti-virus d'huiles végétales et d'extraits ont formé la base des demandes, y compris la conservation des aliments bruts et transformés, les produits pharmaceutiques, la médecine alternative et les thérapies naturelles (Friedman et al., 2002). En raison des résistances multiples possibles et les effets secondaires de l'antimicrobien synthétique, une attention croissante a été dirigé vers antimicrobien naturel (Namiki, 1990). Il existe de nombreuses études sur l'activité antimicrobienne des métabolites secondaires ces dernières années mais à notre connaissance, peu d'études comparatives sur l'activité antimicrobienne de *R. officinalis* L. des extraits méthanoliques et aqueux ont été rapportés (Wong and Kitt, 2006; Fagbohun et al., 2010; Derwich et al., 2011).

VI. 1. 1 L'antibiothérapie et le phénomène de résistance

Les antibiotiques, selon Warshan (1972), sont des substances antiseptiques élaborées par les micro-organismes, en particulier les champignons inférieures.

Actuellement, le terme antibiotique désigne toutes les substances chimiques possédant un pouvoir de toxicité sélective et s'opposant à la multiplication microbienne sans nuire aux cellules, de l'hôte, tout en agissant à des concentrations très faibles de l'ordre de microgramme/ml (Muster, 1988).

Les antibiotiques mettent en jeu des mécanismes d'action d'une grande diversité, en relation avec la variété de leurs structure chimiques et la pluralité de germes contre lesquels ils peu vent être appliqués. Ainsi, les antibiotiques ont été classés (Dupont et Drouhet, 1987; Leminor et Veron, 1989) selon leur mode d'action en:

- Antibiotiques inhibiteurs des synthèses du peptidoglycane.
- Antibiotiques actifs sur les enveloppes membranaires.
- Antibiotiques inhibiteurs des synthèses protéiques.

- Antibiotiques inhibiteurs des acides nucléiques.

Tous ces agents ont, grâce à leur action microbicide ou antimicrobienne sur les germes, pu transformer l'évolution et le pronostic des maladies infectieuses. Seulement, l'utilisation intense et abusive de ces produits a rapidement conduit à l'apparition des deux phénomènes importants: les effets secondaires dus aux médicaments et l'acquisition d'une certaine résistance aux antibiotiques par certaines populations microbiennes.

La résistance est définie comme étant la capacité pour une souche de se multiplier dans une concentration d'antibiotique supérieure à celle qui inhibe la majorité des souches appartenant à la même espèce (Acar et al., 1982). Les bactéries peuvent acquérir la résistance aux antibiotiques de deux façons, par mutation au niveau de l'ADN chromosomique et par transmission d'un plasmide provenant d'une bactérie. Ce dernier mécanisme est le plus important puisque 80 à 90% des souches résistantes isolées en clinique révèlent de la résistance plasmidique.

Quelque soit le mode d'acquisition de la résistance, celle-ci est due en général à quatre mécanismes plus fréquents: modification et inactivation de l'antibiotique par des enzymes du germe, modification du site d'action de l'antibiotique, diminution ou perte de la perméabilité membranaire à l'antibiotique et développement d'une autre voie métabolique suppléant à celle inhibée par l'antibiotique.

Face à ces problèmes, les chercheurs concentrent leurs efforts pour trouver des substances inhibitrices idéales, de large spectre d'activité, ses effets secondaires et surtout d'un prix de revient raisonnable. Le potentiel prometteur des PAM pourrait donc jouer un role notoire dans la lutte contre les infections microbiennes.

VI. 1. 2 Mécanisme d'action antibactérienne

Les mécanismes précis impliqués dans l'action antimicrobienne des extraits sont encore loin d'être totalement élucidés. Etant donné le grand nombre de composants présents dans un extrait, il est évident que l'activité antibactérienne ne peut être due à un seul mécanisme d'action spécifique mais plutôt à divers mécanismes (Burt, 2004).

Ces mécanismes sont la conséquence de l'action d'extrait à différents point de la cellule (Fig. 3). Plusieurs études montrent que la membrane des cellules bactériennes est la cible primaire des composés aromatiques bioactifs. En effet une caractéristique importante des extraits fixes et de leurs composants est leur hydrophobicité, qui leur permet de s'unir aux lipides de la membrane cellulaire tout en changeant sa structure et par conséquent sa fonction. On assiste ainsi à une augmentation de la fluidité membranaire et la fuite des potassiums et des protons (Carson et al., 2002; Hada et al., 2003; Inoue et al., 2004) menant à la diminution du gradient de pH à travers la membrane plasmique et la variation du potentiel membranaire. D'autres constituants cytoplasmiques comme l'adénosine triphosphate passent dans le milieu extracellulaire (Hayouni et al., 2008; Ultee et al., 2002).

La membrane plasmique bactérienne est la barrière de perméabilité élective qui protège le cytoplasme de l'environnement extracellulaire. Elle constitue également le siège de nombreuses fonctions vitales telles que la respiration. L'énergie requise pour les processus cellulaires est générée par la force proton matrice (gradient d'ions hydrogène) qui est établie par un gradient de protons à travers la membrane plasmique. Pendant le métabolisme, les cellules transportent les protons à l'extérieur de la membrane plasmique, ce qui crée un excès d'ions hydrogène et une charge positive à l'extérieur de la membrane. La différence de concentration et de charges électriques entre l'intérieur et l'extérieur de la cellule produit la force protons motrice qui constitue le potentiel de proton (ou gradient de pH) et le potentiel électrique (ou potentiel membranaire). Les protons ne peuvent pas diffuser à travers la membrane plasmique. Leur retour vers l'intérieur de la cellule bactérienne à travers l'ATP synthétase libère de l'énergie requis pour la production de l'ATP à partir de l'AP et du phosphate (Jormakka et al., 2003).

La perte du caractère différentiel de la perméabilité de la membrane cytoplasmique est fréquemment identifiée comme étant la cause de la mort cellulaire. Toutefois, certains auteurs voient que la perte des fonctions de la membrane explique seulement en partie l'activité antimicrobienne des huiles essentielles (Walsh et al; 2003).

Les composants des huiles essentielles peuvent aussi agir sur les protéines membranaires. Ils sont capables de s'accumuler dans la couche lipidique et interférer avec l'interaction lipide-protéine. De plus, une interaction directe entre les composés lipophiles et la partie hydrophane de la protéine peut avoir lieu (Juven et al., 1994). Ces interaction ont pour conséquence l'altération de l'activité des enzymes qui régulent la production de l'énergie (Lambert et al., 2002; Ultee e al., 2002) et la synthèse des composants structuraux (Burt, 2004) (Figure 3).

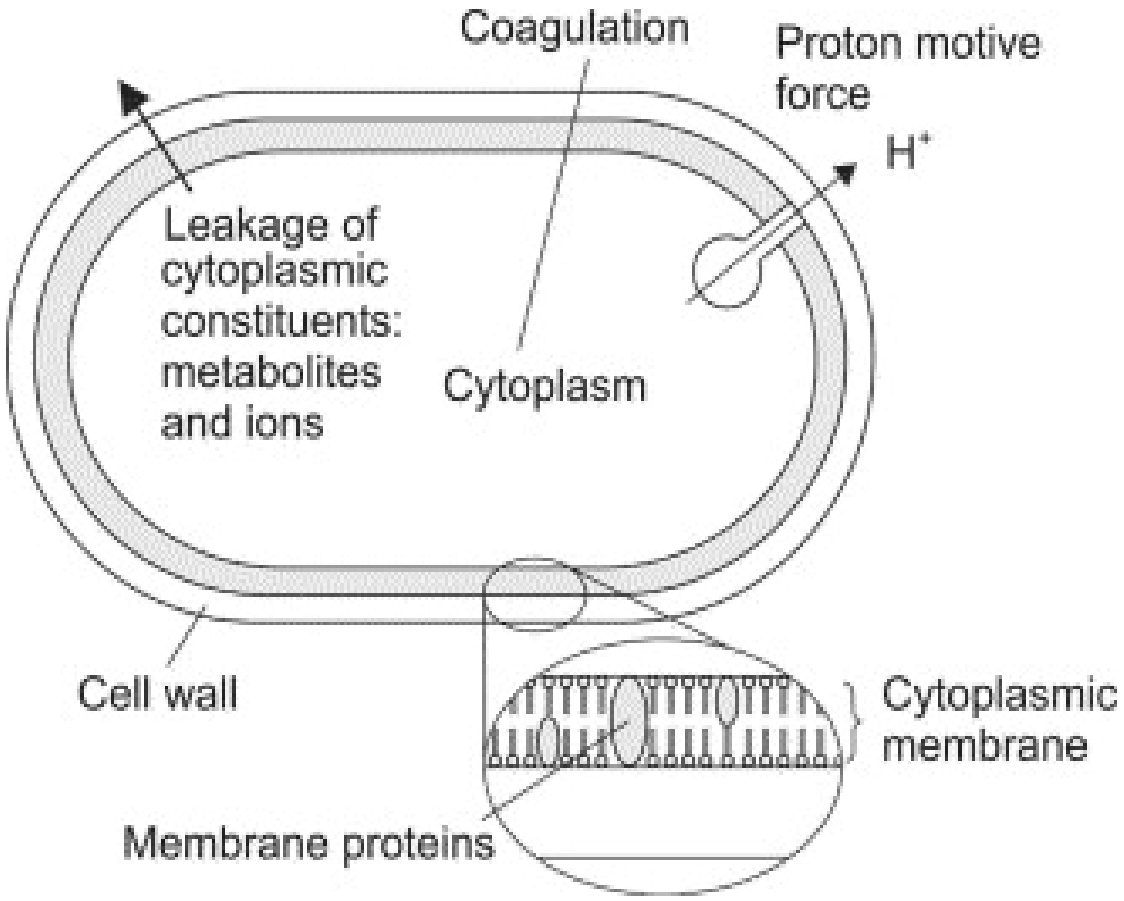


Figure 3: Schéma des principales cibles cellulaires des constituants des extraits méthanoliques et aqueux à effet antibactérien (Burt, 2004). Dégradation de la paroi cellulaire, endommagement de la membrane plasmique, altération des protéines, perte du contenu cellulaire (ions et métabolites), coagulation des constituants du cytoplasme et inhibition de la force proton motrice.

MATERIELS ET METHODES

Etude chimique et toxicologique des huiles essentielles, des extraits méthanoliques, et des extraits aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis*

I. Les formes de transformation des plantes médicinales

Une plante médicinale ne devient utile qu'après avoir subi un certain de transformation qui visent à libérer ses principes actifs et à les rendre assimilables par l'organisme.

Les plantes sont utilisées suivant les cas fraiches ou sèches, stabilisées ou non.

La division a pour but de réduire les corps à l'état de fragments plus ou moins grossiers ou de particules plus ou moins ténues, ou bien à une poudre (tamis).

L'expression est une opération qui a pour effet de séparer d'une substance solide, les liquides qui l'imprègnent. On utilise habituellement une presse qui exercera une pression lente et graduelle sur la substance à exprimer.

L'évaporation consiste à volatiliser des liquides tenant en dissolution des principes médicamenteux que l'on veut isoler.

Plusieurs procédés permettent d'effectuer cette opération:

- évaporation spontanée à l'air libre;
- évaporation par la chaleur à feu nu ou bain-marie;
- évaporation dans le vide pour éviter l'action de la chaleur et de l'oxygène de l'air.

La distillation est une opération qui consiste à chauffer un liquide pour le transformer en vapeur, puis à ramener cette vapeur à l'état liquide, par refroidissement. On peut ainsi séparer les corps volatils des matières fixes ou peu volatiles qui les accompagnent.

La distillation se fait suivant les circonstances à l'alambic, à feu nu, au bain-marie ou à la vapeur.

La nébulisation consiste à obtenir à basse température des extraits secs finement pulvérulents et stables.

On pulvérise le produit sous forme d'infusé ou de solution dans l'air chaud: les particules sont séchées instantanément et la vaporisation enlève la chaleur de sorte que la température reste très faible pendant la durée du séchage.

La dissolution est une opération qui consiste à obtenir un liquide homogène, en mélange un corps solide, liquide ou gazeux avec un autre corps liquide appelé le dissolvant.

On distingue six procédés distincts:

a) La solution simple s'emploie lorsque la substance à dissoudre est soluble dans le dissolvant, sans résidu. Il suffit de mélanger et d'agiter pour obtenir homogène.

b) La macération consiste à laisser séjourner les plantes dans un dissolvant approprié pendant un temps déterminé à la température ordinaire, en agitant fréquemment pour favoriser la dissolution des principes solubles; après décantation le résidu s'appelle le marc et le produit s'appelle macéré.

c) L'infusion se pratique en versant un liquide bouillant sur une partie de plante et en laissant le mélange en contact, le véhicule employé est généralement l'eau et le résultat obtenu s'appelle tisane.

d) La digestion (ou infusion au bain-marie) consiste à maintenir les plantes médicinales pendant un temps déterminer, en contact avec le dissolvant, à une température qui sera toujours inferieure à celle de l'ébullition.

e) La décoction est une opération dans laquelle on soumet les substances à l'action plus ou moins prolongée d'un liquide bouillant.

f) La lixiviation est un procédé qui consiste à épuiser une substance de ses principes solubles, en la faisant traverser lentement par un dissolvant approprié.

II. Procédé classique d'extraction

II. 1 Principe de l'entraînement à la vapeur d'eau des huiles essentielles

Deux méthodes de distillation sont principalement utilisées: l'entraînement à la vapeur d'eau et l'hydrodistillation. Nous nous sommes intéressés plus particulièrement au procédé d'hydrodistillation puisque c'est un équipement que nous possédons au sein de notre laboratoire.

II. 2 L'hydrodistillation

L'hydrodistillation consiste à immerger la matière première dans un bain d'eau.

L'ensemble est porté à ébullition et l'opération est généralement conduite à pression atmosphérique. La distillation peut s'effectuer avec ou sans recyclage communément appelé Cohobage (Figure 4).

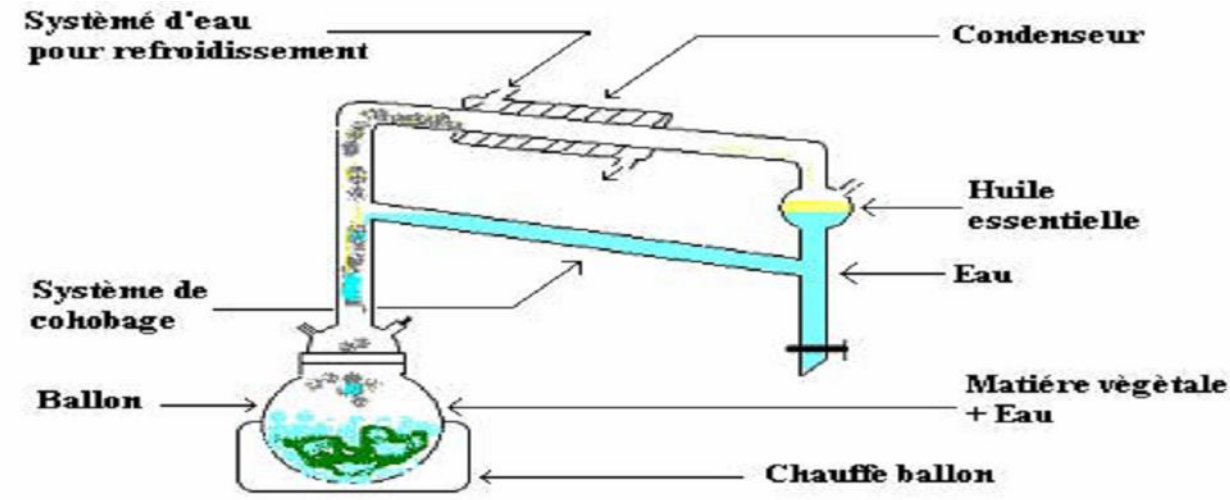


Figure 4: Appareillage utilisé pendant l'hydrodistillation d'huile essentielle (Bourrel, 1993).

Le montage de type SCHILCHER (Bourel, 1993) (Figure 4) est composé de quatre parties principales:

1. le réacteur, un ballon dans lequel on introduit la matière végétale et l'eau;
2. la colonne, un cylindre en verre placé au-dessus du réacteur qui recueille la phase vapeur;
3. le réfrigérant dans lequel se décondensent les vapeurs;
4. le vase florentin où vont se séparer la phase organique (huile essentielle) et la phase aqueuse (eau florale).

Ce système peut être équipé d'un recyclage ou Cohobage: un principe de siphon renvoie l'eau florale du vase florentin vers le réacteur. Un simple robinet au bas du vase permet de recueillir l'huile essentielle à la fin de la réaction.

Le milieu réactionnel constitué par la matière végétale et l'eau est porté à ébullition grâce à un chauffe-ballon. La température est limitée par la température d'ébullition de l'eau 100°C.

La composition chimique des huiles essentielles dépend largement de l'influence des conditions d'hydrodistillation sur l'essence contenue dans la plante.

II. 2. 1 Propriétés physico-chimiques de l'hydrodistillation

Lors de la distillation des huiles essentielles, des phénomènes sont à la base d'échanges de matière entre les phases solide, liquide et vapeur, d'où l'influence d'un grand nombre de paramètres sur la qualité et le rendement de la production.

Les expérimentations (Koedam, 1987; Denny, 1988) conduites jusqu'à épuisement du substrat en essence montrent que la durée de la distillation est plus longue pour les organes de plantes ligneuses que pour les herbacées.

Cette différence est fortement liée à la localisation des structures d'élaboration ou de stockage des essences qui peuvent être superficielles ou internes. De ce fait, elles ont une influence sur le déroulement de l'hydrodistillation, c'est-à-dire sur les mécanismes successifs mis en jeu, et par conséquent sur la durée.

Dans le cas où ces structures sont superficielles, la membrane externe ou la cuticule qui constituent les seules barrières à la libération de l'huile essentielle, est vite rompue à ébullition, les composés volatils sont aussitôt évaporés. Lorsque les essences sont sous-cutanées, elles doivent d'abord diffuser à travers l'épaisseur du tissu végétal avant d'entrer en contact avec l'eau ou sa vapeur. Elles sont alors évaporées comme dans le cas des secrétions superficielles (Koedam, 1987).

En ce qui concerne la localisation des sites producteurs d'essence, les molécules odorantes sont rencontrées dans tous types d'organes : racine, tige, bois, écorce, feuille, fleur, fruit, etc… Elles sont produites par diverses structures spécialement différenciées dont le nombre et les caractéristiques sont très variables (Paupardin et al., 1990).

III. Procédés classique d'extraction des extraits fixes

III. 1 Obtention des extraits méthanoliques

Les espèces végétales ont été séchées séparément et de façon identique sous forme étalée, à l'abri des rayons solaires, à température ambiante. Pour augmenter le rendement en extrait fixe, la partie aérienne de chaque espèce est broyée, puis divisée en deux parties : la première subit une extraction par macération à froid, alors que la deuxième partie est extraite en continu par soxhlet.

III. 1. 1 Extraction par macération à froid

Une quantité de 500g de chaque espèce étudiée a été mise à macérer à l'abri de la lumière, dans deux litres de méthanol de 20h à 48h. L'opération est répétée 3 fois à intervalle régulier. Les extraits méthanoliques sont évaporés et les résidus obtenus sont ensuite placés dans un dessiccateur afin d'éliminer totalement le solvant. A ce stade, le rendement est calculé par rapport au poids obtenu. Le rendement se calcule à partir de l'extrait final par rapport au poids de la plante sèche.

III. 1. 2 Extrait continu par soxhlet

Une quantité (m = 100g) de la poudre de chaque espèce étudiée a été mise dans une cartouche que l'on place dans l'extracteur. Le ballon étant rempli avec deux litres du méthanol, est placé sur le chauffe ballon. En chauffant le solvant s'évapore, se condense dans le réfrigérant, retombe dans l'extracteur, solubilise les principes actifs et retourne dans le ballon de récupération: l'opération est répétée plusieurs fois jusqu'à épuisement total de la poudre, durant 24h.

❖ Obtention des extraits méthanoliques par macération à froid

Les solvants organiques testés, nous avons retenu le méthanol comme solvant d'extraction par macération et ce pour deux raisons:

- ❖ sa faible tension de vapeur
- ❖ son rang élevé en polarité, capable d'entrainer le maximum de métabolites secondaires de diverses natures.

La macération se fait à froid et la durée de l'extraction peut varier de 20h à 48h pour chaque reprise, selon la richesse des espèces en chlorophylle le rendement se calcule à partir de l'extrait final par rapport au poids de la plante sèche.

❖ Obtention des extraits aqueux

Pour l'extrait aqueux, 500g de plante est extraite par infusion dans l'eau bouillant (500ml) pendant 3 jours. Cet extrait aqueux est séparé de ses résidus par une filtration selon la gravité. L'eau est évaporée sous vide à 65°C dans un rotavapeur (Fig. 5). L'extrait qui reste est séché dans un four sous vide à 30°C pendant deux heures, pour la lyophilisation.

Figure 5: Rotavapeur utilisé pendant l'extraction par macération pour évaporer les solvants organiques (Bourel, 1993).

IV. Etude chimique des huiles essentielles des deux plantes étudiées

IV. 1 Protocole d'extraction des huiles essentielles du Rosmarinus officinalis et de la Lavandula officinalis par la technique d'hydrodistillation

Le matériel végétal a été récolté dans la région de Rabat- Salé- Zmour-Zaer. Une masse végétale fraîche sans racines (2kg) a été mise dans un endroit bien aéré et à l'abri de la lumière pendant 45 jours. Après le séchage du matériel végétal, on procède à l'hydrodistillation.

IV. 2 Analyses chimiques des huiles essentielles par chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG-SM)

Nous avons injecté nos huiles essentielles extraites en chromatographie en phase gazeuse couplée à la spectrométrie de mass. Les analyses CPG/SM ont permis d'acquérir les spectres de masse des différents composés constituant les huiles essentielles. Les identifications chimiques ont été réalisées sur la base de la comparaison de nos spectres de masse avec ceux de la banque de données Wiley 275. Les chromatogrammes ont été acquis sur un chromatographe en phase gazeuse Hewlett-Packard 5890. Le gaz vecteur est l'hélium. Les spectres de masse ont été obtenus au moyen d'un spectromètre de masse Hewlett-Packard 5971 A. La colonne choisie est une colonne capillaire SGE de type BPx5 C (nature phase stationnaire: 5% phénylpolysil phénylene siloxane), de dimension 50mm x 0.25mm D.I et 0,25µm d'épaisseur de film. Les injections sont manuelles avec une micro- seringue de 1µl munie d'un répétiteur. Par ailleurs, nous avons également calculé les indices de rétention de chaque composé que nous avons pu ensuite comparer avec les données de la littérature (Kim and Lee, 2002).

V. Détermination de la toxicité aiguë

V. 1 Introduction

Classiquement en présence d'une substance inconnue, la première étape dans la recherche d'une activité pharmacologique débute par l'étude de la toxicité et en particulier l'évaluation de la toxicité aiguë. Les ressources végétales naturelles ne sont certes pas dénuées de toxicité. Ainsi depuis toujours, les toxiques végétaux ont été utilisés comme poison de flèches (curare), ou pour la chasse, ou à des fins criminelles. En effet, le poison donné à Socrate pour sa mise à mort était à base de ciguë (*Conium macculatum* L.) additionné d'extraits de jusquiame (*Hyoscyamus sp*) et plus particulièrement *Hyoscyamus faleslez* (Bisset, 1991).

Ainsi, l'étude des propriétés pharmacologiques des plantes médicinales est insuffisante pour les classer parmi les produits thérapeutiques. Il faut d'abord définir le rapport bénéfice risque dans l'indication thérapeutique de chaque drogue. Ceci ne peut être réalisé que par l'intermédiaire de deux types d'étude, d'une part d'efficacité chez l'animal (pharmacologie expérimentale) et chez l'homme (effets bénéfique), d'autre part une étude de sécurité chez l'animal (toxicologie) et chez l'homme (effets indésirables) (Dupont, 1987; Claude, 1985; Antonious et al., 2006; Buenz, 2006).

Ainsi, l'étude du pouvoir toxique est nécessaire pour définir les limites supérieures de l'utilisation des doses thérapeutiques de ces drogues (Mazué et al., 1983; Cook et Bero, 2006; Gong et al., 2007). La toxicologie doit donc veiller à l'innocuité des plantes médicinales. Mais sa position ne doit pas être seulement négative et réglementaire, bien au contraire elle doit aider aussi à l'innovation, au choix des plantes médicinales à des doses bénéfiques. En définitive, elle doit apporter à la phytothérapie et à la consommation des plantes une certaine garantie et une certaine caution. L'évaluation de la sécurité d'une drogue est une priorité éthique qui a conduit à des réglementations rigoureuses visant la sécurité du consommateur (Laroche et al., 1986; Bounias, 1990).

V. 2 Etude de la toxicité aiguë

V. 2.1 Généralités

C'est une étude qualitative (effet sur le comportement général) et quantitative (détermination de la dose létale 50) du pouvoir toxique résultant d'une administration unique de la substance ou des substances actives contenues dans le médicament (Laroche et al., 1984). L'étude du pouvoir toxique et, notamment, l'évaluation de la morbi-mortalité, doivent être décrites pour chaque espèce animale utilisée et chaque voie d'administration, aux différentes doses étudiées.

La létalité n'est en fait qu'un des nombreux paramètres définissant la toxicité aigué. La pente de la courbe dose- réponse, le moment de la mort, les signes pharmaco-toxiques et les altérations pathologiques sont aussi nécessaire à définir que la DL_{50} (Alaoui et al., 1998, Abbassi, 2001).

V. 2. 2 Protocole d'étude de la toxicité aiguë

La toxicité résultant de l'administration unique d'un xénobiotique dite aussi toxicité aigué correspond habituellement à ce que l'on appelle la dose létale 50 (DL50) qui est la quantité du produits exprimée en mg/kg de poids corporel qui provoque la mort de 50% des animaux d'un lot homogène quant à la race , au sexe, à l'âge et au poids dans un délai conventionnel généralement court (Litchfield et Wilcoxon, 1970)

Généralement, le choix se fait sur deux rongeurs (rat et souris) avec un nombre égal de mâles et de femelles.

Habituellement, les essais se font sur 5à 6 lots de 10 animaux, mais si la toxicité aiguë du produit testé est inconnue, on effectue d'abord une détermination approchée par 5 lots de 3 animaux avec un même nombre de chaque sexe, et il faut que le pourcentage soit compris entre 0 et 100.

Le choix des doses se fait en progression géométrique comme suit:

$$D_N = D_1 \, X \, R^{N-1}$$

Avec;

D_1: première dose

D_N: dose qui suit

R: Facteur arbitraire.

Ces animaux doivent être maintenus dans des conditions rigoureusement constantes (même nourriture, même température).

Les produits à tester ont été administré par voie orale selon le mode d'administration de la plante en médecine traditionnelle.

La solubilité du produits met en œuvre l'eau distillée, le sérum physiologique, le prophylène, glycol dilué, l'huile de coton ou d'arachide. Si le produit est insoluble dans les véhicules ci-dessus, la gomme arabique, la méthylcellulose, ou la carboxyméthylcellulose sont utilisées.

L'observation doit être faite d'une façon très attentive à intervalle réguliers, il faut noter tous les signes de toxicité, l'instant de leur première apparition, leur gravité, le moment d'appréciation de la mort et bien sur la pesée des animaux doit être faite quotidiennement. L'examen microscopique doit être porté sur les morts, sur les moribonds en cours d'expérimentation et sur les survivants. Il comporte un examen des organes, leurs descriptions et le relevé de leurs altérations.

V. 2. 3 Facteurs de variation de la DL50

La valeur numérique de la DL_{50} est affectée par de multiples facteurs, dont la plupart sont liés à l'animal:

*l'espèce: il se peut que la DL_{50} varie brusquement au sein d'une souche de la même espèce.

* l'âge: des fluctuations ont été rapportées.
* le sexe est un facteur moins important.
* le poids doit être pris en considération.
* la pathologie spontanée: symptômes et causes des maladies.

D'autres facteurs qui sont liés aux conditions expérimentales peuvent intervenir:

- la voie d'administration
- la nature du véhicule
- concentration de la substance
- la vitesse de l'injection.
- La température, l'éclairage,…

Cette multitude de causes de fluctuations de DL_{50} explique la mauvaise reproductibilité inter et intra-laboratoire, que l'on reproche souvent aux études de la toxicité aigué, il devient ainsi malaisé de prétendre pouvoir déterminer une DL_{50} « exacte » sans respecter des conditions draconiennes de réalisation.

V. 2. 4 Evaluation de la toxicité aiguë

Nous avons testé la toxicité aiguë des huiles essentielles, des extraits méthanoliques et des extraits aqueux du *Rosmarinus officinalis* et de la *Lavandula officinalis* sur des souris de laboratoire.

a. Matériels

Nous avons utilisé un matériel de routine constitué par une balance pour peser les souris et le matériel pour l'injection des huiles essentielles, des extrait méthanoliques et des extraits aqueux à savoir la sonde et la seringue. Les critères des animaux de l'expérience sont résumés dans le tableau suivant:

Tableau 10: Critères des animaux expérimentés.

Critères	Souris
Souche	Souiss
Sexe	Autant de males que de femelles
Age	2 à 2,5 mois
Etat physiologique	Souris saines non gravides
Poids	20 à 30g

Les animaux doivent être soumis à un jeûne 18h avant l'administration du produit.

b. Méthode

✓ Préparation de l'échantillon

A une quantité de l'huile essentielle, on ajoute un adjuvant qui est dans notre cas l'huile de maïs. Pour l'extrait méthanolique, on ajoute la gamme arabique à 10%. Pour l'extrait aqueux, on utilise l'eau distillée.

✓ Voie d'administration

Nous avons choisi la voie orale (VO) comme mode d'administration qui est souvent utilisée par l'homme en tradition et la voie intrapéritonéale (IP).
Les extraits des deux plantes étudiées à testes ont été administrée par gavage.

- Nous avons d'abord effectué une détermination approchée sur des lots de 3 animaux, ensuite nous avons en choisissant les doses en progression géométrique.

Tableau 11: Protocole d'administration de l'huile essentielle de *Lavandula officinalis*.

Dose mg/kg	Nombre	Sexe		Volume à administration
		F	H	
500	10	5	5	A raison de 0,5 ml pour 20g du poids corporel.
1000	10	5	5	
1500	10	5	5	
2000	10	5	5	
3000	10	5	5	
5000	10	5	5	

Tableau 12: Protocole d'administration de l'huile essentielle de *Rosmarinus officinalis*.

Dose mg/kg; v.o.	Nombre	Sexe		Volume à administration
		F	H	
500	10	5	5	A raison de 0,5 ml pour 20g du poids corporel.
750	10	5	5	
850	10	5	5	
900	10	5	5	
950	10	5	5	
1000	10	5	5	

Tableau 13: Protocole d'administration de l'extrait aqueux de romarin (*Rosmarinus officinalis*).

Dose mg/kg ; VO	Nombre	Sexe		Volume à administration
		F	H	
500	10	5	5	A raison de 0,5 ml pour 20g du poids corporel.
1000	10	5	5	
2000	10	5	5	
3000	10	5	5	
4000	10	5	5	
5000	10	5	5	

Tableau 14: Protocole d'administration de l'extrait méthanolique de la lavande (*Lavandula officinalis*).

Dose en mg/kg, VO	Nombre	Sexe		Volume à administration
		F	H	
500	10	5	5	A raison de 0,5 ml pour 20g du poids corporel.
1000	10	5	5	
1500	10	5	5	
2000	10	5	5	
3000	10	5	5	
5000	10	5	5	

Tableau 15: Protocole d'administration de l'extrait méthanolique de romarin (*Rosmarinus officinalis*).

Dose mg/kg, VO	Nombre	Sexe		Volume à administration
		F	H	
500	10	5	5	A raison de 0,5 ml pour 20g du poids corporel.
1000	10	5	5	
2000	10	5	5	
3000	10	5	5	
4000	10	5	5	
5000	10	5	5	

Tableau 16: Protocole d'administration de l'extrait aqueux de *Lavandula officinalis*.

Dose mg/kg; VO	Nombre	Sexe		Volume à administration
		F	H	
500	10	5	5	A raison de 0,5 ml pour 20g du poids corporel.
1000	10	5	5	
1500	10	5	5	
2000	10	5	5	
3000	10	5	5	
5000	10	5	5	

Les observations ont été étalées sur une durée de deux semaines, au cours de laquelle, tous les signes de toxicité, l'évolution du poids corporel ainsi que le pourcentage de mortalité ont été consignés chaque jour.

Etude pharmacologique des huiles essentielles des extraits méthanoliques et des extraits aqueux de *Rosmarinus officinalis* et *Lavandula officinalis*

I. Evaluation des propriétés psychotropes des huiles essentielles, des extraits méthanoliques et des extraits aqueux du *Rosmarinus officinalis* et *Lavandula officinalis*

1. Introduction

La recherche de propriété sédative est réalisée grâce à l'étude du comportement d'un petit rongeur, la souris, confrontée à diverses situations particulières plus ou moins contraignantes (Loo, 1998).

En effet il faut savoir que cet animal est territorial et que les repères olfactifs jouent un rôle important dans l'expression de son comportement. Ainsi, le fait d'introduire la souris, de force, dans un milieu nouveau, dépourvu de ces repères families, constitue une situation très stressante et a pour effet d'activer le système nerveux central de l'animal, qui ce se traduit entre autre par une activation de l'axe hypothalamus-hypo-physiocoticosurrénalien, phénomène non observé lorsque la continuité topographique entre les milieux nouveau et familier est maintenue (Misslin et al., 1982).

L'administration d'une substance douée de propriétés sédatives induit la réduction de certains paramètres, tels que l'activité générale de l'animal, sa locomotion, son redressement et son activité exploratrice.

II. Evaluation de l'activité sédative

II. 1 Introduction

La détermination du profil psychopharmacologique d'une substance donnée, nécessite l'utilisation d'une batterie de tests pharmacologiques, étant donné qu'il n'existe pas de

méthodes spécifiques pour une classe pharmacologique donnée. En fonction des résultats obtenus sur la base de différents tests, l'on définira le profil psychopharmacologique d'une substance et au terme de ces études, une idée sur son utilisation clinique potentielle sera émise.

Les méthodes d'étude sont classées en 3 groupes:

- Méthodes comportementales ou étude de la réponse de l'animal à l'administration de la molécule à l'aide de différents tests comportementaux.
- Méthodes neurochimiques ou recueil de dialysats et mesure des concentrations en neurotransmetteurs et ses métabolites, étude de la liaison sur les récepteurs et les transporteurs.
- Méthodes électrophysiologiques ou enregistrement de l'activité électrique des neurones l'on a souvent recourt à l'EEG (électro encéphalogramme) et les autres enregistrements polygraphiques, permettent d'apprécier l'activité d'une substance sur le système nerveux (Cohen et al., 2008; Beaulieu, 2006).

Ces trois méthodes sont complémentaires dans la définition du profil psychopharmacologique d'une substance.

Dans le cadre de notre étude, nous avons utilisé les méthodes comportementales pour évaluer l'activité psychotrope des nos extraits.

II. 2 MATERIELS ET METHODES

- **Produits testés:** - Des huiles essentielles de *Rosmarinus officinalis* et de *Lavandula officinalis*.

  - Des extraits méthanoliques et aqueux de *Lavandula officinalis.*

- **Produit de référence:** Diazépam (3mg/kg, IP).

- **Doses:** Les doses utilisées pour déterminer le profil psychopharmacologique de nos extraits ont été fixées sur base des résultats de la toxicité aiguë sur les souris, soit des doses bien inférieures à la DL_{50}.

- **Voies d'administration:** Nous avons utilisé la voie intra péritonéale pour les huiles essentielles et la voie orale pour les extraits méthanoliques et aqueux des deux plantes étudiées. Les extraits sont administrés sous forme de suspension dans la gomme arabique à 10%.
- **Animaux:** Nous avons utilisé les souris Swiss et les rats Wistar issus de l'Animalerie Centrale de la Faculté de Médecine et de Pharmacie de Rabat, en tenant compte: de leur âge, le poids, le sexe et de leur état général.

Dans le cadre de notre étude, nous avons en recourt aux méthodes comportementales suivantes:

II. 2. 1 Test de la traction

Il consiste à suspendre des souris par les pattes antérieures à un fil métallique tendu horizontalement, et un compte le temps mis par la souris pour amener au moins une des pattes postérieure à toucher le fil. Une souris normale effectue un rétablissement en moins de 5 secondes; le cas contraire signifie que la souris est soumise à une action sédative (Cohen et al., 2008; Beaulieu, 2006; Lempérière, 2006; Delorme et al., 2003; Orsini et Pellet, 2005).

III. 2. 2 Test de la planche à trous

Il permet d'explorer la curiosité et le désir de fuite de l'animal. Pour ce faire, on utilise une planche de 40x40 cm et de 1.8cm d'épaisseur, percée de 16 trous de 3cm de diamètre, régulièrement espacés. La souris est déposée au centre de la planche et on compte le nombre de fois que la souris plonge la tête dans un des trous au bout 1, 2, 3 ,4 et 5 minutes. La moyenne de trous explorés est calculée pendant 5 minutes (Cohen et al., 2008; Beaulieu, 2006; Lempérière, 2006; Delorme et al., 2003; Orsini et Pellet, 2005).

III. 2. 3 Test de la cheminée

La souris traitée est mise dans une éprouvette de 2L la tête vers le bas. L'on estimera la durée passée dans l'éprouvette, jusqu'au moment ou la souris cherchera à en sortir. La réponse est positive si la souris remonte le tube en moins de 30 secondes (Cohen et al., 2008; Beaulieu, 2006; Lempérière, 2006; Delorme et al., 2003; Orsini et Pellet, 2005).

III. EVALUATION DE L'ACTION HYPNOTIQUE

III. 1 Introduction

Les hypnotiques constituent une classe très hétérogène (Warot et al., 1979), appartenant à des classes chimiques très variées riches, en alcool, aldéhydes, sulfones carbamate... Le sommeil qu'ils induisent est généralement différent du sommeil physiologique, des modifications pouvant intervenir dans la durée totale du sommeil profond et/ou paradoxal, ainsi qu'au niveau de ces différents stades.

Leur répartition se fait principalement en deux groupes : les barbituriques et les non-barbituriques. Leur caractéristique commune est de provoquer une dépression du système nerveux central, qui se traduit principalement par une sédation, un sommeil et une action de type anesthésique.

Parmi les groupes des non- barbituriques, il est intéressant de mentionner la présence de certaines benzodiazépines utilisées comme hypnotiques sans que l'on sache très bien si ces substances possèdent une action vraie ou si leur seule activité anxiolytique permet l'endormissement. Il existe également d'autres tranquillisants mineurs, certains neuroleptiques sédatifs (chlorpromazine) et antihistaminiques dont on peut se demander s'ils exercent une réelle action hypnotique ou si leur activité n'est pas due uniquement à leur effets sédatifs.

A ces deux groupes viennent s'adjoindre diverses substances, telles que l'hydrate de chloral, le glutéthimide (chimiquement très proche des barbituriques) des dérivés

quinazolones, le L-tryptophane, le 5- hydroxyle – tryptophane (Bossier et Simon, 1966; Passouant et Billard, 1978; Warot et al, 1979).

De nombreuses substances psychotropes, dépourvues de toute activité hypnotique propre, peuvent cependant modifier le sommeil expérimental induit par un barbiturique.

Ainsi, les neuroleptiques, les tranquillisants mineurs et les antidépresseurs peuvent potentialiser cet effet hypnotique, tandis que les noanaleptiques et psychostimulants l'antagonisent (Bossier et Simon, 1966; Simon et al., 1982). De ce fait la recherche d'une influence de l'huile essentielle et de l'extrait fixe de *Lavandula officinalis* sur la narcose barbiturique devrait apporter un certain nombre d'informations supplémentaires nécessaires à son positionnement parmi les substances psychotropes.

III. 2 MATERIEL ET METHODES

III. 2. 1 Animaux

Les caractéristiques et les conditions d'élevage des animaux (souris males Iops OFI) sont détaillées dans la partie I, pages 58 et 64.

IV. 3. 2 Procédure expérimentale

L'huile essentielle de la *Lavandula officinalis* est administrée par voie intrapéritonéale, aux doses non toxiques 1000 et 1500 mg/kg. L'extrait méthanolique de cette plante est administré par voie orale, à 800 et 1000 mg/kg. Les animaux sont maintenus en observation durant 24 heures.

L'action hypnotique est la capacité d'une substance à réduire la vigilance puis induire le sommeil. Les animaux traités sont considérés comme hypnotisés, s'ils perdent leur réflexe de redressement une fois mis sur leurs dos. Deux paramètres sont à mesurer:

- Le temps d'endormissement (TE), ou intervalle de temps entre l'administration du produit et l'apparition du sommeil.

- Le temps de sommeil (TS), ou intervalle de temps entre la perte du réflexe de redressement et sa réapparition.

Le début du sommeil est pris en compte lorsque les animaux acceptent la position en décubitus latéral et le temps de réveil, lorsque la souris quitte cette position (Cohen et al., 2008; Beaulieu, 2006; Lempérière, 2006; Delorme et al., 2003; Orsini et Pellet, 2005).

IV. EVALUATION DE L'ACTIVITE PSYCHOSTIMULANTE DU ROMARIN (*ROSMARINUS OFFICINALIS* L)

IV. 1 Introduction

En vu des résultats préliminaires des tests psychotropes réalisé il a semblé intéressant de faire une étude d'activité psychostimulante des extraits issus de *Rosmarinus officinalis*. La détermination du profil psychopharmacologique d'une substance donnée, nécessite l'utilisation d'une batterie de tests pharmacologiques. Le terme «médicaments psychstimulant» se réfère à une classe diversifiée de composés psychoactifs que ont la capacité l'activer le système nerveux central et le comportement. Physiologiquement, les psychostimulants augmentent généralement les activités fonctionnelles du centre monoaminergique et du système cholinergique (Fibiger and Phillips, 1988; Coury et al., 1992; Marik et al., 1999; Neothby et al., 1997). Considérant de l'utilisation de *R. officinalis* dans la médecine traditionnelle dans le traitement des troubles de l'humeur, le présent travail vise à étudier l'effet des extraits du *R. officinalis* sur les mouvements stéréotypés, l'observation de l'animal en situation libre, et l'interaction avec le pentobarbital.

IV. 2 MATERIEL ET METHODES

✓ **Animaux**

Nous avons utilisé des rats males de la lignée Wistar (CRL), pesant entre 200 et 250 g, provenant d'élevages homogènes.

✓ **Produit testes, extraits de Rosmarinus officinalis:**

- huile essentielle
- extrait méthanolique
- extrait aqueux

✓ **Produit de référence:** Apomorphine (16 mg/kg, SC).

✓ **Doses:** Les doses utilisées pour déterminer le profil psychostimulant ont été fixées sur la base des résultats de la toxicité aiguë sur les souris, soit des doses inférieures à la DL_{50}, et correspondant au maximum 1/10éme de la DL_{50}.

✓ **Voies d'administration:** Nous avons utilisé la voie orale pour les extraits et la voie sous-cutanée pour le produit de référence (Apomorphine). Les substances étudiées ont été mises en suspension dans l'huile végétale pour l'huile essentielle et en suspension dans la gomme arabique à 10% pour l'extrait méthanolique. Les volumes administrés par voie orale sont de 0.5ml/100g de poids corporel chez le rat. Toutes les expériences ont été réalisées à la température ambiante (22±1°C).

- ✓ **Procédure expérimentale**

Pour tester leur effet psychostimulant, les extraits de *Rosmarinus officinalis* sur le rat sont évalués par les tests suivants:

a- Observation des animaux en situation libre

Des observations détaillées du comportement sont menées sur des lots de 3 souris par dose, 15 ; 30 ; 60 ; 120 et 180 min et 24h après l'administration des substances. Les observations du comportement sont réalisées selon une grille inspirée de celle décrite par Irwin (Irwin and Guigues; 1967; Irwin, 1958). En particulier l'effet psychostimulant est apprécié d'après l'hypermobilité et hyperexcitabilité.

b- Interaction avec pentobarbital chez la souris

Les souris (6 par dose) sont placées dans des boites individuelles et reçoivent les substances 30mn avant une dose hypnotique de pentobarbital (60mg/kg, IP). Les temps de suppression et de réapparition du réflexe de retournement sont notés pour chaque animal (Irwin 1958; Chermat et Poncelet, 1983; Stella et al., 1987; Spetit et al., 1990).

c- Recherche de mouvement stéréotypés chez le rat

Les mouvements stéréotypés, caractérisés par des mouvements de la tête et de la sphère buccale, reniflements, léchages ou mâchonnement sont cotés de 0 à 3 selon la méthode décrite par Simon et Chermat (Simon and Chermat, 1972), toutes les 10mn jusqu'à leur disparition chez tous les animaux. Six rats par dose ont été utilisés.
L'huile essentielle est testée aux doses de 50 et 100 mg/kg, et aux doses de 100 et 200 mg/kg pour l'extrait méthanolique et l extrait aqueux. Les témoins reçoivent le véhicule dans les mêmes conditions. Dés l'administration des extraits de *Rosmarinus officinalis* L, chaque animal est isolé dans une cage individuelle, pour être observé toutes les 10 mn, pendant 210mn. L'intensité des stéréotypies est évaluée selon la

cotation de Simon (Simon et al., 1972). Pour chaque lot est calculée la somme des cotations individuelles obtenues par fraction de 10 minutes. Le nombre total des stéréotypies observées durant les 210mn est aussi pris en compte. Ce total général peut alors être exprimé en pourcentage par rapport au lot témoin pris comme 100%.

0 = absence de stéréotypie et tout mouvement normal

1= présence de rares mouvements stéréotypés ou d'un reniflement continu, la tête du rat est dirigée vers le haut.

2= reniflements intenses avec mouvements saccadés et rapides la tête, parfois interrompus par de courtes phases de mâchonnements.

3= mouvements stéréotypés intense et continus (déplacement de la tête, mâchonnements ou léchages), souvent accompagnés de mouvements répétés des pattes antérieures contre les parois de la cage.

V. EVALUATION DE L'ACTIVITE ANTIBACTERIENNE DES EXTRAITS METHANOLIQUES ET AQUEUX DE *ROSMARINUS OFFICINALIS* ET DE *LAVANDULA OFFICINALIS*

V. 1 MATÉRIEL ET MÉTHODES

V. 1. 1 Test de l'activité antibactérienne

V. 1. 2 Les souches bactériennes

Les six micro-organismes suivants (trois bactéries Gram-négatif et trois bactéries Gram-positives, respectivement), ont été utilisés dans cette étude antibactérienne: *Escherichia coli* ATCC 54127, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 53153, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633. Les cultures de bactéries ont été maintenues dans leurs tubes de gélose à +4°C tout au long de l'étude et utilisé en tant que cultures de stock. Les bactéries ont été obtenues auprès du Service de Microbiologie, Laboratoire National de contrôle des Médicaments Vétérinaires, Rabat, Maroc.

V. 1. 3 Préparation des solutions

Une concentration de 200ug/ml de chaque extrait lyophilisé des plantes est ensuite préparée par dilution dans l'eau distillée stérile, utilisée comme solution mère.

V. 1. 4 Détermination de l'activité antibactérienne par la méthode de diffusion sur disque de papier (Aromatogramme)

Il s'agit de la technique la plus utilisée pour déterminer l'activité antimicrobienne des différents extraits. Cette méthode repose sur le pouvoir de diffusion des extraits dans un milieu nutritif solide.

L'activité antibactérienne des extraits méthanoliques et aqueux de *Rosmarinus officinalis et Lavandula officinalis* a été testée par la méthode de diffusion sur disque de papier. L' Agar Muller Hinton stérile (10ml) fondu (45°C) dans un flacon a été inoculé avec un bouillon de culture (1%, contenant 10^6-10^7 ufc/ml) des souches bactériennes respectives, et versé sur des plaques contenant 10ml de l'Agar Muller Hinton dans boîtes de Pétri stériles de 9cm. Cinquante microlitres des dilutions des extraits méthanoliques et aqueux ont été pipetés sur un disque de papier filtre stérile (Whatman N°.1., 5mm de diamètre), qui ont été laissé à sécher dans des boites de Pétri stériles ouvertes dans une hotte biologique à flux laminaire vertical (Nuaire Pétri produits plats à flux laminaire, USA). 200, 100, 50, 25, 12,5, 6,25, 3,125 et 1,56 mg/ml des solutions des extraits méthanoliques et aqueux ont été appliquées sur les disques. Les disques ont été placés sur la surface des plaques inoculées et incubés à 37°C pendant 18 heures. Les zones d'inhibition de croissance microbienne autour du disque de papier contenant les extraits ont été mesurées et enregistrées à la fin d'incubation. La zone d'inhibition a été considérée comme la plus courte distance (mm) à partir de la marge extérieure du disque de papier jusqu'au point de départ de la croissance microbienne. Toutes les analyses ont été effectuées en trois exemplaires (Baydar et al., 2004; Askun et al., 2009). Les disques imprégnés d'eau distillée stérile servent de contrôle négatif. La pénicilline et l'ampicilline (Sigma, St. Louis, USA) ont été utilisées comme contrôle positif (Zampini et al., 2005; Partibha et al., 2012; Geeta and Padma, 2012).

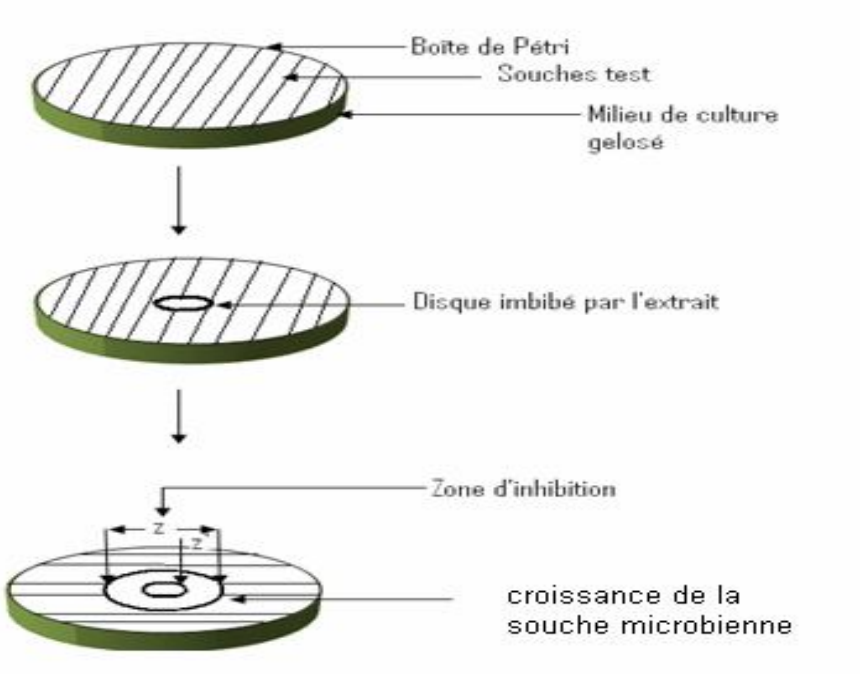


Figure 6: Illustration de la technique d'aromatogramme

La mesure de diamètre d'inhibition reflète la valeur de la CMI de l'extrait. Le résultat de cette activité est exprimé par le diamètre de la zone d'inhibition et peut être symbolisé par des croix. Est considérée respectivement comme souche **résistante (-), sensible (+), très sensible (+ +), extrêmement sensible (+ + +),** celle ayant un diamètre D <8mm, 9mm ≥ D ≤ 14mm, 15mm ≥ D ≤ 19mm, D> 20mm. La lecture des résultats se fait à la lumière du jour et à l'œil nu. La limpidité du milieu implique l'effet antibactérien de l'extrait testé tandis que la présence d'un trouble indique son inefficacité (signe de croissance bactérienne). Les témoins négatifs et positifs sont réalisés respectivement par l'imbibition des disques dans l'eau distillée stérile et l'application d'un disque d'un antibiotique à la place de l'extrait puis ils sont déposés dans les boites et qui sont préalablement ensemencé par les souches à testés (appliquer le même inoculum).

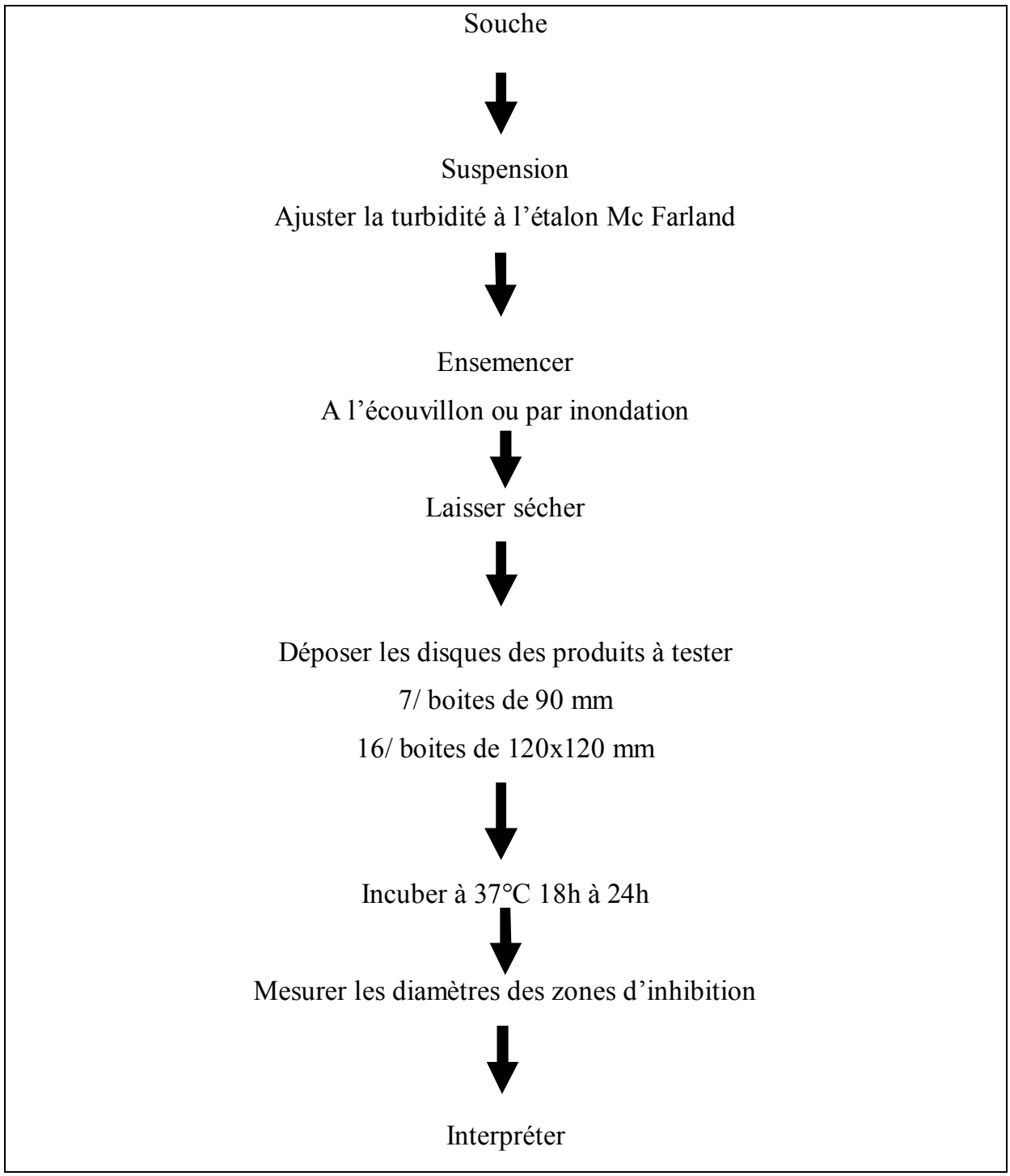


Figure 7: Schéma général de l'antibiogramme.

V. 1. 5 DETERMINATION DE LA CONCENTRATION MINIMALE INHIBITRICE (CMI)

V. 1. 5. 1 Microméthode

La Concentration Minimale Inhibitrice (CMI) de façon générale est la plus faible concentration d'antimicrobien capable d'inhiber toute croissance visible après un temps d'incubation de 18 à 24 heures. Ici sa détermination s'est effectuée à partir de la mesure de la turbidité induite par la croissance des germes étudiés. La CMI correspondra donc à la plus petite concentration pour laquelle il y a absence de turbidité.

Des microplaques à fond en U (plaque à microtitration) sont utilisables pour la détermination de la CMI. Une plaque de 96 puits permet la détermination de la CMI d'un produit vis-à-vis une souche bactérienne. Dans les cupules d'une même ligne, les dilutions d'un produit et la souche sont introduit à l'aide d'une micropipette. S'ensuit une incubation pendant 24h à la température optimale, puis une observation de la plaque et une éventuelle pousse dans chaque cupule (ou un dépôt au fond de la cupule).

La technique consiste à réaliser pour chaque extrait une gamme de dilutions en milieu liquide à partir la solution mère d'un extrait, puis on ajoute ces différentes concentrations à la même quantité de l'inoculum bactérien. La concentration minimale inhibitrice correspond à la plus petite quantité d'extrait capable d'inhiber totalement la croissance de la bactérie.

Les souches test ont été suspendues dans Muller Hinton Agar (MHA). La turbidité de la suspension a été ajustée grâce au spectrophotomètre pour correspondre à une turbidité égale à 0,5 sur l'échelle McFarland (c.à.d. obtenir une densité finale de 10^7cfu/ml). L'indicateur de viabilité MTT (3 - (4, 5 diméthylthiazol-2-yl) -2, 5 bromure de diphényltétrazolium, Sigma, Aldrich) et la méthode rapide de lecture de microplaques ont été utilisés pour la détermination de la concentration inhibitrice minimale (CMI) (Grare et al., 2008; Eloff, 1998; Oumzil et al., 2002; Andrews, 1992). Les dilutions en série des extraits ont été faites dans de l'eau distillée, puis dans une

gamme de concentrations de 1,56 mg/ml à 200 mg/ml dans des tubes à essai stériles. Chaque tube à essai a été inoculé avec 20 µl de chacune des différentes dilutions des extraits méthanoliques et aqueux de R. officinalis et *L. officinalis* ont été ajoutés à 5 ml de l'Agar Muller Hinton, l'ensemble dans des tubes contenant 10^7cfu/ml de cellules bactériennes vivantes. Les tubes sont ensuite incubés dans des conditions optimales à 37°C pendant 18 heures. A la fin de l'incubation bactérienne, 1 mg/ml de MTT a été ajouté à chaque puits comme indicateur de croissance, et après une incubation allant de 3 à 5h à 37°C, un contrôle avec MTT et inoculations bactériennes et sans les extraits de la plante a été utilisé comme contrôle négatif. La suspension bactérienne vire au bleu quand la croissance bactérienne a lieu. La dilution la plus élevée ne montrant aucune croissance visible est considéré comme la CMI. La suspension cellulaire (0,1 ml) des tubes ne présentant pas de croissance est sous cultivée dans des plaques de Muller Hinton en triple afin de déterminer si l'inhibition est réversible ou permanente. Tous les tests ont été réalisés en triple.

V. 1. 5. 2 Macrométhode

Dans une série de tubes stériles 1 ml de chaque dilution de l'extrait est testé. On ajoute dans tous les tubes le même volume d'inoculum et on l'incube 24 heures en présence de MTT à la température optimale de la souche à tester, puis on Observe les tubes après incubation (on observe le changement de coloration à l'œil nu et on mesure la densité optique à 578 nm). La CMI sera la concentration en extrait la plus faible pour laquelle il n'y a pas de croissance visible**,** témoin est préparé sans extrait de plante.
La préparation de la culture en phase exponentielle en milieu liquide de la bactérie à étudier est réalisée comme précédemment (Méthode des disques). Cette technique confirme les résultats obtenus par la méthode de microtitration (*Microméthode*).

L'objectif de cette étude consiste donc à sélectionner, parmi quatre extraits, ceux qui possèdent un fort pouvoir antibactérien, en évaluant l'action de ces extraits vis-à-vis de bactéries Gram-positives et Gram-négatives appartenant aux groupes pathogènes ou responsables de l'altération des produits alimentaires et par conséquent, de déterminer

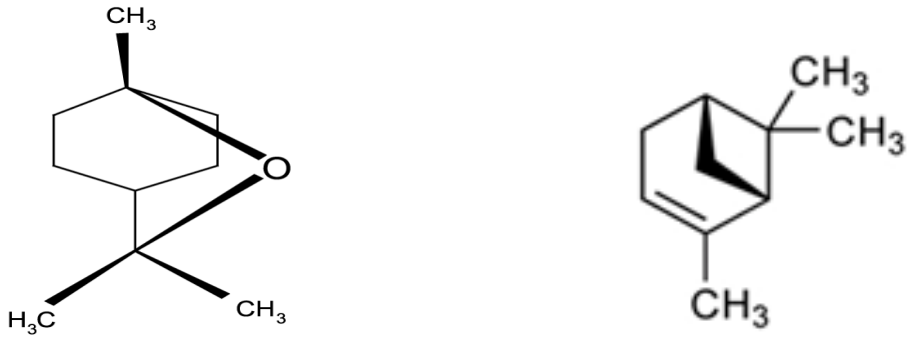
la base scientifique pour ses utilisations dans la médecine traditionnelle pour traitement des maladies infectieuses.

RESULTATS

I. Etude chimique et toxicologique des huiles essentielles, des extraits méthanoliques, et des extraits aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis*

I. 1 Composition chimique de l'huile essentielle du *Rosmarinus officinalis* L

Le rendement obtenu est de **0,256%** par rapport à la matière fraiche. La composition de l' H.E est semblable aux données de la littérature, mais avec le pourcentage différent, ainsi l'interprétation du profil chromatographique a montré qu'à coté de plusieurs constituants mineurs c'est le 1,8-cinèole, le **α**-pinène et le camphre qui représentent les constituants majoritaires (Tab. 17, Fig. 8 et Fig. 9).



α-pinène **1,8-cinèole**

Figure 8: Structure chimique de α-pinène et de 1,8-cinèole.

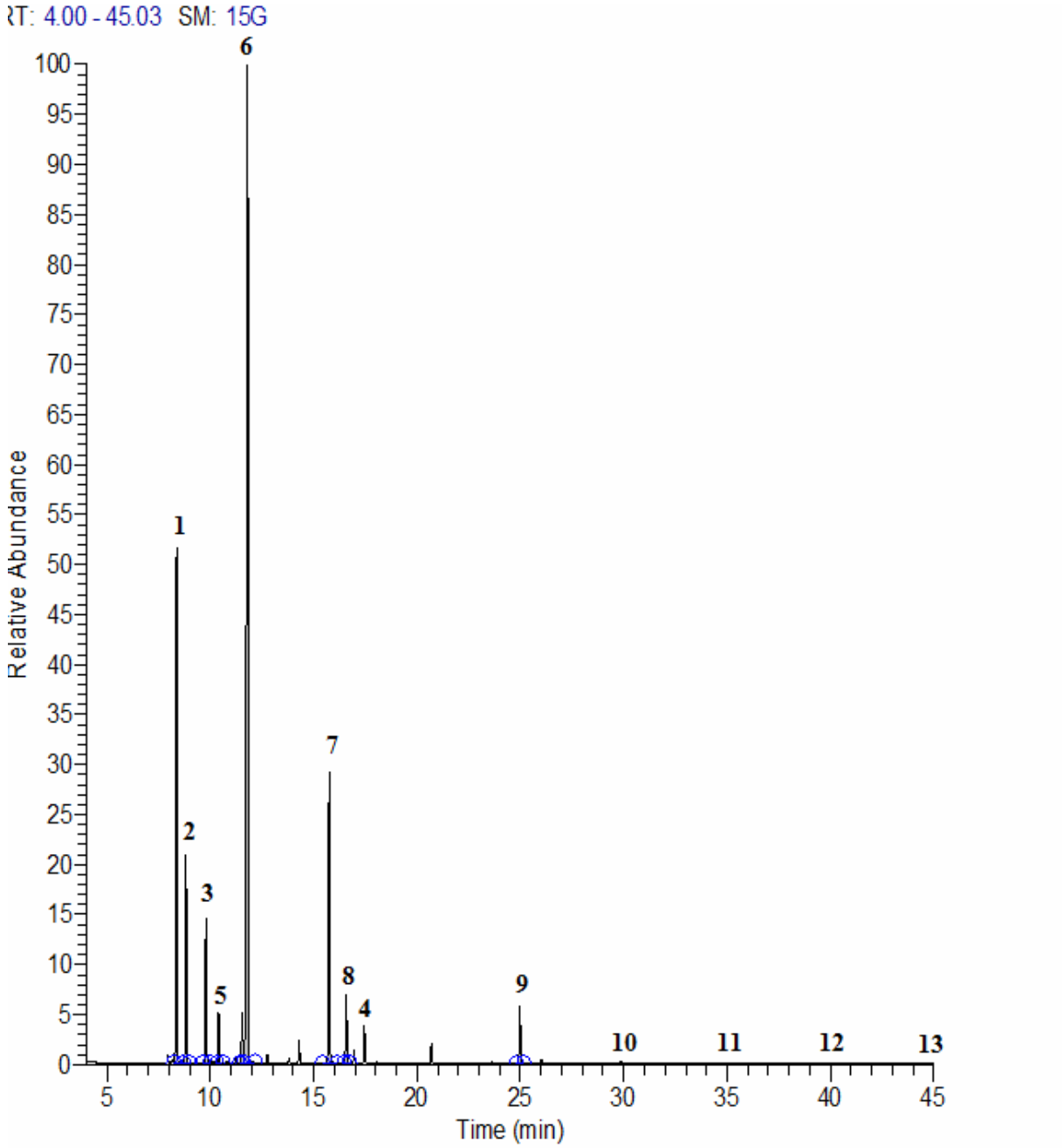


Figure 9: Chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG/SM) de l'huile essentielle du *Rosmarinus officinalis*.

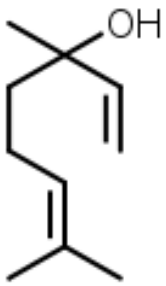
Tableau 17: Composition chimique de l'huile essentielle du *Rosmarinus officinalis*.

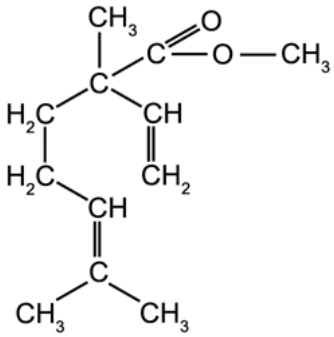
N Pic	Composé identifié	Tr (Temps de rétention)	Pourcentage relative %
1	α – pinène	8,37	15,82
2	Camphène	8,83	6,80
3	β-pinène	9,79	4,75
4	Myrcène	10,40	1,70
5	*p*-cymène	11,54	2,16
6	1,8-cinèole	11,80	50,49
7	Camphre	15,75	11,61
8	Bornèol	16,57	2,58
9	Acétate de bornyle	24,98	2,08
10	Tan*s*-caryophyllene	29,85	Tr
11	NI	35,59	Tr
12	NI	39,13	Tr
13	Bisabolène	43,35	Tr

Tr: trace; NI: non identifie.

I. 2 Composition chimique de l'huile essentielle de la *Lavandula officinalis*

Le rendement obtenu est de **0,24%** par rapport à la matière fraiche. La composition de l'H.E est semblable aux données de la littérature, ainsi l'interprétation du profil chromatographique a montré qu'à coté de plusieurs constituants mineurs, c'est le linalol et son acétate qui représentent les constituants majoritaires (Tab. 18 et Fig. 10 et Fig. 11).





Linalol **Linalyle d'acétate**

Figure 10: Structure chimique de linalol et de linalyle d'acétate.

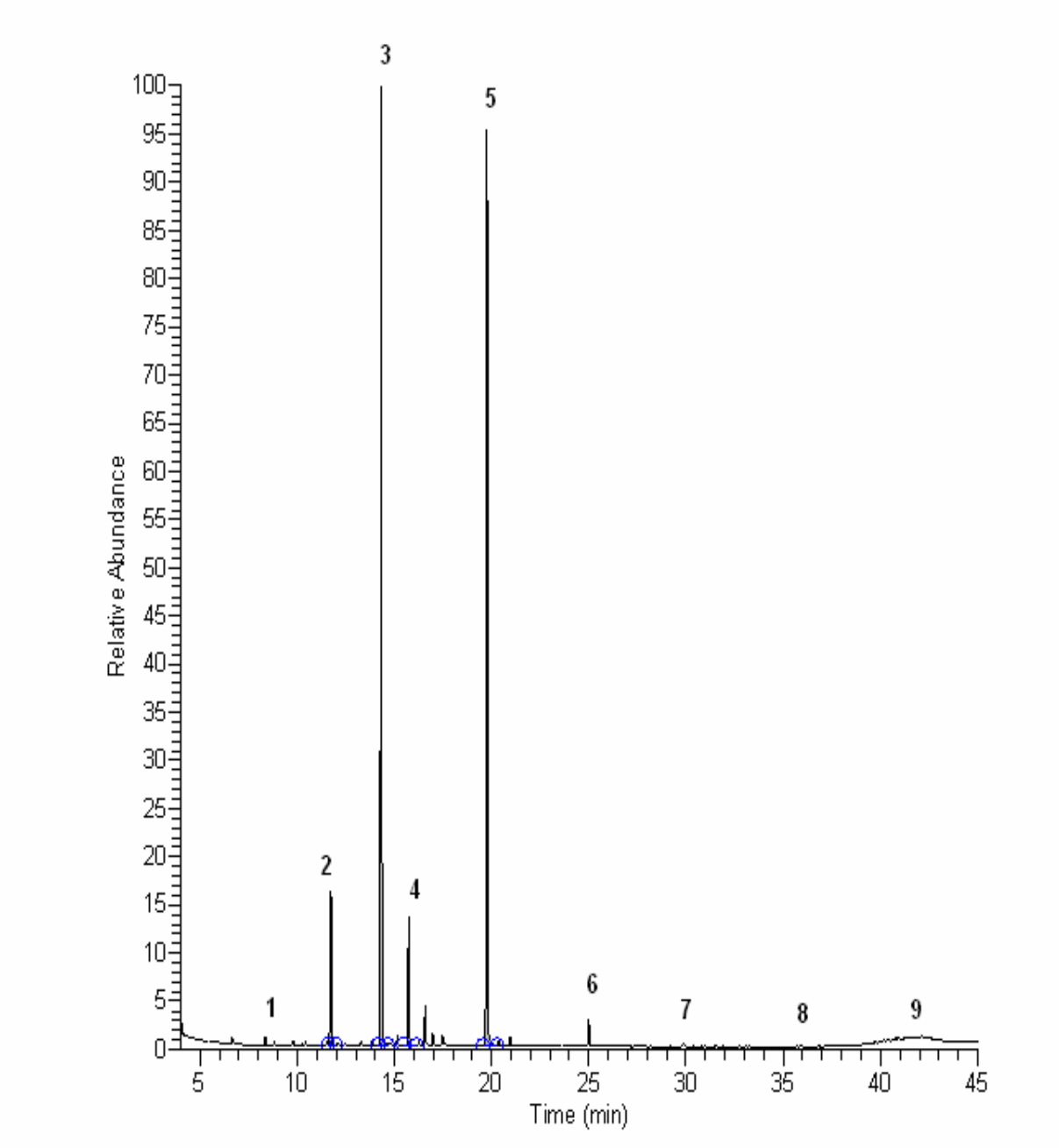


Figure 11: Chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG/SM) de l'huile essentielle de la *Lavandula officinalis*.

Tableau 18: Composition chimique de l'huile essentielle de la *Lavandula officinalis*.

N Pic	Composé identifié	Tr (Temps de rétention)	Pourcentage relative %
1	α-pinène	8,35	T r
2	1,8-cinèole	11,73	5,30
3	Linalool	14,32	44,67
4	Camphre	15,72	6,02
5	Acétate de linalyle	19,75	42,00
6	Acétate de bornyle	24,99	T r
7	Iso-eugénole	29,86	T r
8	Iso-eugénole methyl ether	35,70	T r
9	Farnesol	42,10	T r

Tr: trace; NI: non identifie.

I. 3 Rendement des extraits méthanoliques et aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis*

Le rendement se calcule à partir de l'extrait final par rapport au poids de la plante sèche. L'extrait méthanolique final de *Rosmarinus officinalis* et de *Lavandula officinalis* est une poudre vert foncé avec pourcentage du poids sec de 21,8 et 22,8%, respectivement. Mais, l'extrait aqueux final brut est obtenu sous forme de poudre jaune graisseuse avec un pourcentage du poids sec de 15,7 et 18,7%, respectivement.

I. 4 Signes de toxicité

Nous avons remarqué qu'à partir de la dose 1000 mg/kg tous les animaux prennent une position de repli avec tête penchée, yeux fermés et respiration accélérée.

Cependant un retour à l'état normal (activité et nutrition) a été observé chez certains, tandis que d'autres conservent l'état d'affaiblissement jusqu'à la mort.

I. 5 Evolution pondérale

La variation du poids corporel des animaux a été notée chaque jour à la même heure. Une baisse du poids corporel peut s'expliquer par une consommation alimentaire réduite suite à l'ingestion du toxique.

Le suivi de l'évolution pondérale des souris testées pendant les 14 jours a montré deux cas de figures (Figure 12 et 13):

- Le poids corporel a subit une régression dés les premiers jours d'observation, puis lorsque les signes de toxicité commencent à disparaitre, le poids augmente progressivement et dépasse le poids initial (Figure 12).
- Le poids corporel a subit une progression normale dés le premier jour d'observation témoignant la sensibilité de l'animal à l'extrait (Figure 13 et 14).

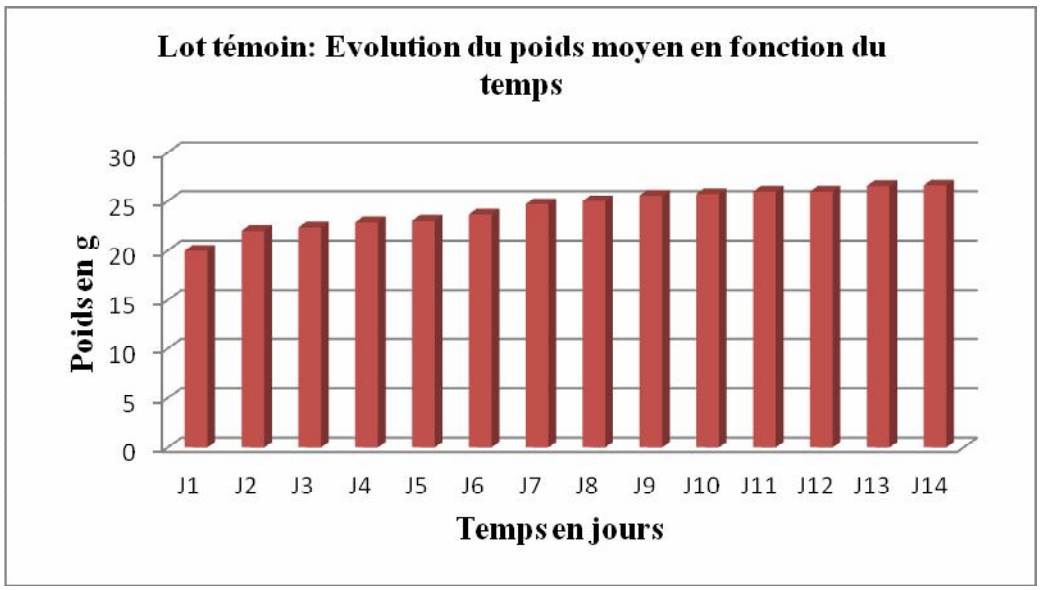


Figure 12: Evolution du poids moyen en fonction du temps chez la souris témoin.

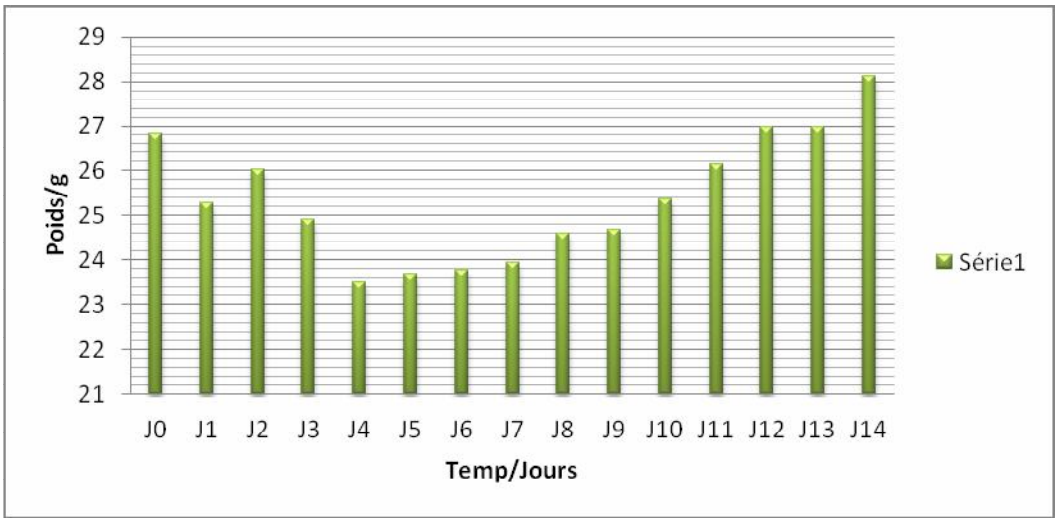


Figure 13: Evolution du poids moyen des souris traitées par l'huile essentielle de *Rosmarinus officinalis* administré par voie intrapéritonéale en fonction du temps en jours.

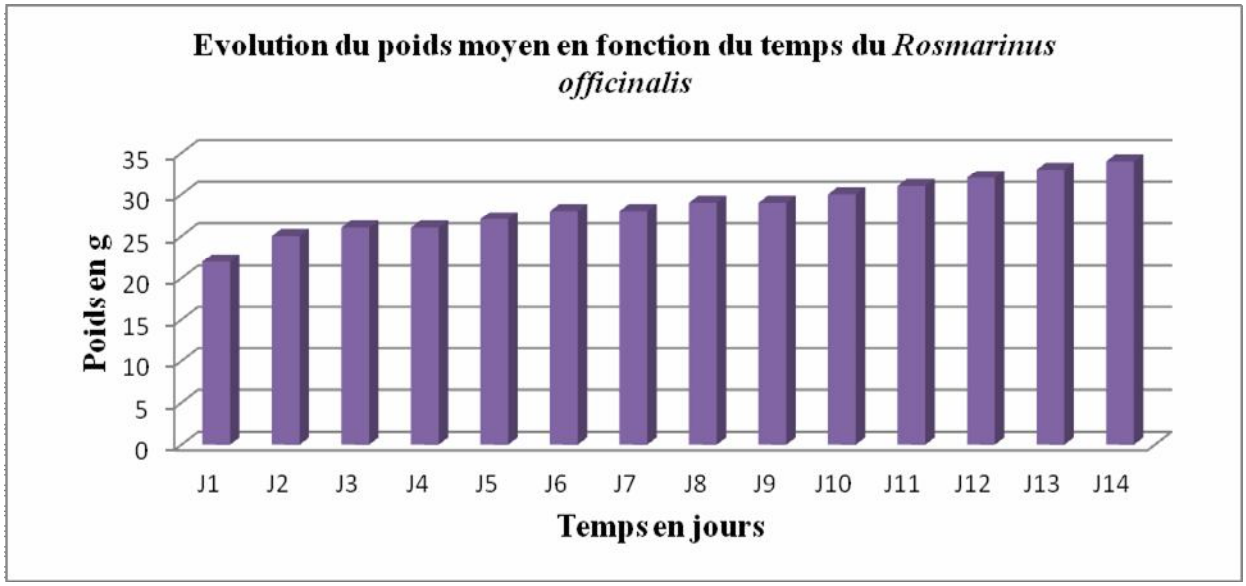


Figure 14: Evolution du poids moyen des souris traitées par l'extrait méthanolique de *Rosmarinus officinalis* administré par voie orale en fonction du temps en jours.

II. Détermination de la DL_{50}

Après avoir établi les pourcentages de mortalité pour chaque dose de l'huile essentielle de *Rosmarinus officinalis*, nous avons reporté ces résultats à un programme, qui permet le calcul de la dose efficace par la méthode du probit (Huet, 1972). La (DL_{50}) calculée par ce programme est de **897,85mg/kg** avec des limites de confiance située entre **885,90 mg/kg** et **909,97 mg/kg (**Figure 16).
La DL_{50} de l'extrait méthanoliques du *Rosmarinus officinalis* est évaluée à **2104,85 mg/kg** avec des limites de confiance entre **1890 mg/kg** et **2343 mg/kg** (Figure 15).

Les tableaux ci- dessus regroupent les pourcentages de mortalité des différentes doses utilisées (Tab. 19 et 20).

Tableau 19: Mortalité en fonction de la dose de l'extrait méthanolique du *Rosmarinus officinalis* administré par voie orale (VO).

Doses (mg/kg; VO)	% Mortalité	DL_{50} (mg/kg)
500	0	
1000	30	DL_{50}= 2104,85 mg/kg
2000	40	1890 mg/kg < DL_{50} <2343 mg/kg, avec
3000	50	limites de confiance à 95%
4000	70	
5000	100	

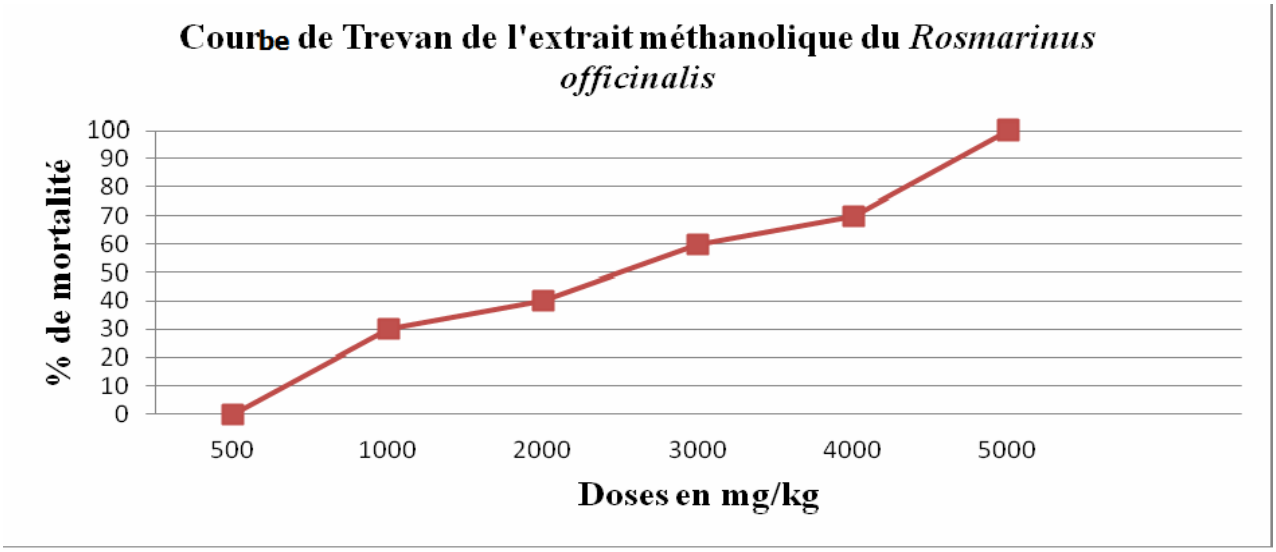


Figure 15: Taux de Mortalité en fonction des doses de l'extrait méthanolique de *Rosmarinus officinalis* administré par voie orale.

Aucun signe de toxicité n'a été enregistré suite à l'administration orale des différents doses d'extrait méthanolique de *Rosmarinus officinalis*.

Tableau 20: Pourcentage de mortalité observé après administration par voie orale des doses graduelles de l'huile essentielle de *Rosmarinus officinalis*.

Doses (mg/kg VO)	% Mortalité	DL_{50} (mg/kg)
500	0	
750	10	DL_{50}= 897,85mg/kg
850	30	885,90 mg/kg < DL_{50} < 909,97 mg/kg, avec
900	40	limites de confiance à 95%
950	60	
1000	100	

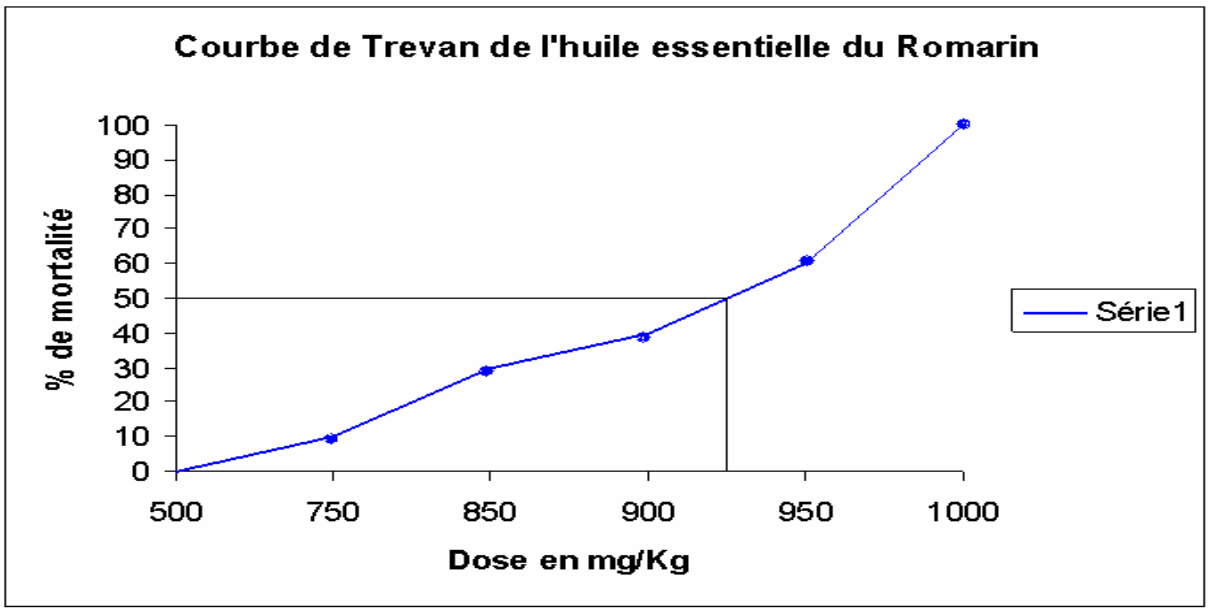


Figure 16: Taux de Mortalité en fonction des doses de l'huile essentielle de *Rosmarinus officinalis* administré par voie orale chez la souris.

Aucun signe de toxicité n'a été enregistré suite à l'administration orale de différentes doses d'huile essentielle de *Rosmarinus officinalis*.

III. Evaluation de l'activité sédative

Les résultats des effets sédatifs des huiles essentielles des deux plantes étudiées, et des extrait méthanoliques et extrait aqueux de la *Lavandula officinalis* sont exprimés en comparaison avec le groupe contrôle. Les tests pharmacologiques sont donc réalisés aux doses thérapeutiques non toxiques *i.e.* 50 et 100mg/kg pour l'huile essentielle de *Rosmarinus officinalis* et 300, 600 mg/kg pour l'huile essentielle de *Lavandula officinalis*, (100, 200, 400, 600) pour l'extrait méthanolique de la *Lavandula officinalis*, (100, 200, 400) pour l'extrait aqueux de la *Lavandula officinalis*.

III. 1 Test de la traction

On observe que l'animal traité par l'huile essentielle du *Rosmarinus officinalis* aux doses 50 et 100 mg/kg réalise un rétablissement normal immédiat (Tab. 21). Ceci

indique que cette huile essentielle n'a pas des effets sédatifs significatifs sur les comportements de la souris. Par contre l'huile essentielle de la *Lavandula officinalis* a un effet sédatif significatif sur le système nerveux central (SNC) comme indique par le temps relativement élevé du rétablissement de la souris (7±0,8) (Tab. 3).

En augmentant la dose à la 600 mg/kg, la moyenne du temps de rétablissement est augmentée.

Pour l'extrait méthanolique de la lavande administré par voie orale à 100mg/kg, induit chez tous les animaux un rétablissement normal immédiat (p> 0,05). Cependant, l'extrait aux doses 200, 400 et 600 mg/kg induit un effet sédatif significatif sur le système nerveux central (SNC) comme l'indique le temps relativement élevé rétablissement de la souris.

A 400 et 600 mg/kg, la moyenne du temps de rétablissement augmente par rapport au groupe contrôle (p<0,001) (Tab. 21 et 22). L'administration par voie orale de l'extrait aqueux à 100 mg/kg tous animaux effectuent un rétablissement normal (il est noté que le temps de rétablissement diminue). Ceci indique que l'extrait aqueux n'a pas d'action sédative à cette dose. Par augmentation des doses à 200 et 400mg/kg, le temps de rétablissement est augmenté, et les souris échouent à se rétablir immédiatement (p<0,001). Ce qui confère à l'extrait aqueux de *Lavandula officinalis* un effet sédatif significatif aux doses 200 et 400 mg/kg par voie orale (VO) (Tab. 24).

III. 2 Test de la cheminée

Les animaux traités à l'huile essentielle du *Rosmarinus officinalis* à 50 et 100 mg/kg, ne montrent pas de perte de l'initiative et de la curiosité. Par contre, l'administration intrapéritonéale de l'huile essentielle de la lavande à la dose 600 mg/kg, induit chez toutes les souris une perte de leur initiative et leur curiosité, les animaux n'essaient pas de monter dans le tube pour s'échapper. Parailleurs, l'extrait méthanolique de la *Lavandula officinalis* à 100mg/kg VO, ne provoque pas de perte de l'initiative et de la curiosité. Contrairement à 200, 400 et 600 mg/kg, aucune souris n'essaye de remonter le tube pour s'échapper (p<0,01).

L'extrait aqueux de la lavande à 100mg/kg VO, ne provoque pas d'effet sédatif mais à des doses de 200 et 400mg/kg VO, par voie orale, tous les animaux perdent l'initiative et la curiosité (p<0,001) (Tab. 24).

III. 3 Test de la planche à trous

Dans ce test l'huile essentielle du *Rosmarinus officinalis* (50 et 100mg/kg, IP) ne réduit pas le nombre cumulatif des trous explorés (en relation avec leur curiosité). Par contre, l'huile essentielle de la lavande (600mg/kg, IP) réduit le nombre cumulatif des trous explorés et le nombre des espaces entre deux trous (Tab. 21 et 22). Ces données mène à conclure que les huiles essentielles de la *Lavandula officinalis* ont un effet sédatif potentiel sur le système nerveux central à la dose 600mg/kg. Alors que l'huile essentielle su *Rosmarinus officinalis*, en regroupant les testes de la traction, de la cheminée et de la planche à trous n'induit aucun action sédative aux doses 50 et 100 mg/kg par voie intrapéritonéale (p<0,001). Ainsi, les animaux traités avec l'extrait méthanolique de la *Lavandula officinalis* administrée par voie orale aux doses de 200, 400, et 600mg/kg, réduisent significativement le nombre d'explorations de trous effet inexistant à 100mg/kg VO. De même, l'animal traité à l'extrait aqueux à 100mg/kg VO, ne voit pas sa curiosité réduite, ne réduit pas le nombre où la souris plonge de la tête à trous (p>0,05). En augmentant les doses à 200 et 400mg/kg, cet extrait réduit le nombre cumulatif des trous explorés et le nombre des espaces entre les trous explorés (p<0,001) (Tab. 23 et 24).

Ces données, mène à conclure que les extraits méthanoliques et aqueux de la *Lavandula officinalis* L ont un effet sédatif potentiel sur le système nerveux central dés 200mg/kg VO.

Tableau 21: Action sédative de l'huile essentielle de *Rosmarinus officinalis*.

Tests		Témoins	Huile essentielle du romarin	
			50mg/kg (IP)	100mg/kg (IP)
Test de la traction	Tm de rétablissement (en secondes)	0,9± 0 n = 6	0,7 ± 0,1 n = 6	1± 0 n = 5
Test de la cheminée	Tm pour remonter le tube	7± 0,5 n = 6	7,2 ± 2 n = 6	7,4 ± 1,7 n = 6
Test de la planche à trous	Trous explorés au cours de 5 minutes	10±1 n =6	12 ± 1 n = 6	11± 1,8 n = 5

Tm: temps moyen de redressement (en secondes); n: nombre des animaux par groupe; IP: voie intrapéritonéale.

Tableau 22: Action sédative de l'huile essentielle de *Lavandula officinalis*.

Tests		Témoins	Huile essentielle de la lavande	
			300mg/kg (IP)	600mg/kg (IP)
Test de la traction	Tm de rétablissement (en secondes)	0,9 ± 0 n = 6	1 ± 0 n = 6	7 ± 0,8 n = 6
Test de la cheminée	Tm pour remonter le tube (en secondes)	7 ± 0,5 n = 6	14,4 ± 1,5 n = 6	45 ± 1 n = 6
Test de la planche à trous	trous explorés au cours de 5minutes	10 ± 1 n = 6	11,7 ± 2 n = 6	0,1± 0 n = 6

Tm: temps moyen en secondes ; n: nombre des animaux par groupe ; IP: voie intrapéritonéale.

Tableau 23: Action sédative de l'extrait méthanolique de *Lavandula officinalis*.

Test		**Témoins**	**Diazépam, IP**	**Extrait méthanolique de *Lavandula officinalis* en mg/Kg, VO**			
			DZP (3mg/Kg)	**EM (100mg/Kg)**	**EM (200mg/Kg)**	**EM (400mg/Kg)**	**EM (600mg/Kg)**
Test de la traction	Temps de rétablissement	0,9 sec±0,0 n=5	10 sec±0,3 n=5	0,8 sec±0,5 n=5	5 sec±0,5* n=5	18 sec±0,5 n=5	27 sec±01* n=5
Test de la cheminée	Temps pour remonter le tube	7 sec± 0,5 n = 5	> 2 min n = 5	2 sec± 0,5 n = 5	30 sec± 1* n=5	58 sec±1* n=4	> 2min* n=4
Test de la planche à trous	Trous explorés au cours de 5minutes	10 ± 1 n = 5	0,0± 0,0 n = 5	8 ± 0,1 n = 5	2 ± 0,0* n =5	1 ± 0,0* n = 5	0,0 ± 0,0* n = 5

VO: voie orale; IP: voie intrapéritonéale; n: nombre de souris par groupe; sec: seconde; ME: extrait méthanolique; DZP: diazépam.

Les données sont exprimées en moyenne ± écart-type; *p<0,001 par rapport aux témoins.

Tableau 24: Action sédative de l'extrait aqueux de *Lavandula officinalis*.

Test		Témoins	Diazépam, IP	Extrait aqueux de *Lavandula officinalis* en mg/Kg, VO		
			DZP (3mg/Kg)	EA (100 mg/Kg)	EA (200 mg/Kg)	EA (400 mg/Kg)
Test de la traction	Temps de rétablissement	0,9 sec± 0,0 n =5	10 sec± 0,3 n = 5	2 sec± 0,3 n = 5	12 sec± 0,5* n = 4	30 sec± 1* n = 5
Test de la cheminée	Temps pour remonter le tube	7 sec± 0,5 n = 5	> 2 min n = 5	12 sec± 0,1 n = 5	45 sec ± 1* n = 5	> 2 min* n = 4
Test de la planche à trous	Trous explorés au cours de 5minutes	10 ± 1 n = 5	0,0±0,0 n =5	6 ± 0,2 n = 5	1 ± 0,0* n = 4	0,0 ± 0,0* n = 4

VO: voie orale; IP: voie intrapéritonéale; n: nombre de souris par groupe; sec: seconde; EA: extrait aqueux; DZP: diazépam. Les données sont exprimées en moyenne ± écart-type; *p<0,001 par rapport aux témoins.

IV. EVALUATION DE L'ACTION HYPNOTIQUE

L'huile essentielle de la *Lavandula officinalis* présente un effet hypnotique significatif à la dose de 1000mg/kg. Cette action hypnotique est augmentée à la dose 1500mg/kg (Tab. 26). Ceci confirme l'action hypnotique d'huile essentielle à doses élevées par voie intra- péritonéale. De même l'extrait méthanolique de la lavande induit un effet hypnotique à 800 mg/kg, qui augmente à 1000 mg/kg (Tab. 25). L'extrait aqueux lui, ne présente aucun effet inducteur du sommeil (Tab. 25). Cependant, si aucun animal ne s'endort, tous restent plus ou moins, blottis les uns contre les autres dans un coin de la cage. Cette immobilisation se manifeste surtout aux doses de 800 et 1000mg/kg. Après une heure, ces phénomènes semblent disparaitre totalement. En comparaison avec l'effet inducteur du thiopental, si la dose de 60mg/kg de ce composé conduit au même temps d'endormissement que la dose 1500mg/kg d'huile essentielle et la dose 1000 mg/kg pour d'extrait méthanolique, les animaux s'endorment mois rapidement et dorment plus longtemps. Le temps d'endormissement de des extraits méthanoliques et de l'huile essentielle à 1000 et 1500 mg/kg, respectivement, est moins rapide que le thiopental, mais le temps sommeil est plus long. A 60mg/kg, le thiopental induit le sommeil chez tous les animaux, le temps d'endormissement moyen est très proche de celui obtenu avec la plus forte dose de l'huile essentielle et de l'extrait méthanolique, soit 1500 et 1000 mg/kg. Toute fois, le sommeil est nettement prolongé avec les extraits de cette plante. La durée moyenne est d'environ 106 et 112 minutes, respectivement, celle du thiopental étant de 36 minutes.

Tableau 25: Effet de l'extrait méthanolique de *Lavandula officinalis* sur le début et la durée du sommeil chez les souris traitées par thiopental.

Groupe	Dose (mg/kg)	Temps d'endormissement TE (min)	Temps de sommeil TS (min)
Normale	60	8±1	36±3
DZP	3	6±1*	75±3*
EM	800	12±0.5*	45±2*
EM	1000	6±1*	112±3*

VO: voie orale; IP: voie intrapéritonéale; (n=5): nombre des souris par groupe; min: minute; EM: extrait méthanolique; DZP: diazépam. Les données sont exprimées en moyenne ± écart-type; *p<0,001 par rapport aux témoins.

Tableau 26: Effet hypnotique de l'huile essentielle de *Lavandula officinalis*.

Temps	Thiopental (60 mg/kg; IP)	HE (1000 mg/kg; IP)	HE (1500 mg/kg; IP)
T.E	8 min ±1 n = 5	16 min ± 1* n = 5	9 min ± 1* n = 5
T.S	33 min ± 6 n = 5	54 min ± 6* n = 5	106 min ± 3* n = 5

TE: temps d'endormissement; TS: temps de sommeil; IP: voie intrapéritonéale; (n=5): nombre des souris par groupe; min: minute; HE: huile essentielle. Les données sont exprimées en moyenne ± écart-type; *p<0,001 par rapport aux témoins.

V. EVALUATION DE L'ACTIVITE PSYCHOSTIMULANTE DU ROMARIN (*ROSMARINUS OFFICINALIS* L)

V. 1 Activité psychostimulant de l'huile essentielle, de l'extrait méthanolique et de l'extrait aqueux sur le système nerveux central

Les résultats des effets psychostimulants de l'huile essentielle, de l'extrait méthanolique et de l'extrait aqueux ont été déterminés par comparaison avec le groupe témoin. Les essais pharmacologiques ont été réalisés à des doses de 50 et 100 mg/kg, VO, pour l'huile essentielle et 100 et 200 mg/kg, VO pour l'extrait méthanolique et l'extrait aqueux. Ces doses n'ont pas provoqué aucun effet neurologique parallèle.

V. 1. 1 Observation des animaux en situation libre

Nous avons observé que l'administration orale de l'huile essentielle de *Rosmarinus officinalis* à la dose de 100 mg/kg et des extraits méthanoliques et aqueux de *Rosmarinus officinalis* à 100 et 200mg/kg, ont provoqué l'apparition de mouvements rapides de la tête et de reniflement. Les souris rongent également la paroi de la cage, suggérant une activité locomotrice croissante. De même sont notées une hypermobilité et une hyperexcitabilité du système nerveux central (mouvements stéréotypés). L'extrait aqueux s'est révélé plus actif que l'huile essentielle et l'extrait méthanolique (Tab. 27, 28 et 29).

V. 1. 2 Interaction avec le thiopental chez la souris

L'administration de 50 et 100 mg/kg, VO, d'huile essentielle et 100 and 200mg/kg, VO de l'extrait méthanolique et aqueux du *Rosmarinus officinalis*, 30 minutes avant l'injection de la dose hypnotique de thiopental (60mg/kg, IP), ont augmenté de façon significative le temps d'endormissement (à 100 mg/kg VO d'huile essentielle et à 100 et 200 mg/kg, VO d'extrait méthanolique et d'extrait aqueux) et ont provoqué une diminution du temps de sommeil (à 50 et 100 mg/kg, VO). L'extrait aqueux est significativement plus actif que l'extrait méthanolique (Tab. 30, 31 et 32).

V. 1. 3 Recherche des mouvements stéréotypés chez le rat

D'après le tableau 33, l'apomorphine (16mg/kg, SC) induit chez le groupe Témoins l'apparition de mouvements stéréotypés après seulement 10 minutes. Les mouvements stéréotypés diminuent progressivement jusqu'à disparaître à 210 min. Les phénomènes stéréotypés sont augmentés en durée et en intensité. Cependant, l'huile essentielle (100mg/kg) a produit une augmentation significative (P<0,001) de potentialisation des effets de l'apomorphine. En effet, les mouvements stéréotypés atteignent un pic à 40 min (total des cotations = 18) pour disparaître à 280 min (Tab. 33).

Les extraits aqueux et méthanoliques (100 et 200mg/kg) ont produit une augmentation significative (P <0,001) de potentialisation des effets de l'apomorphine, mais à la dose de 100 mg/kg, l'agonisme des mouvements stéréotypés est partielle en durée et intensité par rapport au groupe de contrôle. En effet, les mouvements stéréotypés sont apparus à 10 min, pour atteindre un pic à 40 et 80 min, respectivement (total des cotations à 16 et 18, respectivement) pour disparaître à 280 min (Tab. 34 et 35).

Tableau 27: Observation des animaux en situation libre après l'administration orale de l'huile essentielle de *Rosmarinus officinalis* à 100 mg/kg, VO.

Temps (min) / **Souris**	**15**	**30**	**60**	**120**	**180**
Témoins **S1**	- normal -mobilité vers l'aliment et l'eau	Normal	Normal	Normal	Normal
S2	- absence de stéréotypie, tous les mouvements sont normaux	//	//	//	//
S3	//	//	//	//	//
Romarin **S4**	-mouvements normaux - redressement normal -mouvement de grattage	- hypermobilité - rares mouvements stéréotypés -reniflement continu - la tête est dirigée vers le	- reniflement intense -mouvements rapides de la tête -courtes phases de mâchonnement	-déplacement de la tête -mouvements répétés des pattes antérieures contre la paroi de la	-hypermobilité -hyperexcitabilité -mâchonnements -déplacement de la tête -léchage

		le haut de la cage - position spécifique par leurs pattes antérieures	-sauts -suspension - direction de la tête vers le haut de la cage	cage - direction continue de la tête vers le haut de la cage.	-exploration de la sciure de bois
S5	-début d'hypermobilité -absence de stéréotypie -se grattent	-hypermobilité -respiration rapide - sauts	-apparition de mouvement stéréotypie -se grattent -déplacements -mouvement rapide de la tête.	-mouvement rapide de la tête -reniflement de la sphère buccale -morsure des parois de la cage - direction de la tête vers le haut de la cage	-hypermobilité élevée -hyperexcitabilité -rongement -rongent les de parois de la cage
S6	-sédation -gratter	-déplacement -sauter -hypermobilité	-mouvement en position circulaire -suspension -mouvement rapide de la tête.	Mêmes signes //	-hyperexcitabilité -suspension continue - mouvement répétés des pattes antérieures contre les parois de la cage -se grattent

Tableau 28: Observation des animaux en situation libre après l'administration orale de l'extrait méthanolique de *Rosmarinus officinalis* à 200 mg/kg.

Temps / Souris	**15**	**30**	**60**	**120**	**180**
Témoins S1 S2 S3	-endormis aux coins de la cage -vont vers l'eau et l'aliment	//	//	//	//
Romarin S4	-Se redressent normalement -se grattent	-rare mouvement de stéréotypie -la tête est dirigée vers le haut de la cage.	-position spécifique par leurs pattes antérieures	-hypermobilité -mouvements rapides de la tête -phase courte de mâchonnement.	-déplacement de la tête -mouvements répétés des pattes antérieures contre la paroi de la cage.
S5	-début d'hypermobilité. -absence de mouvement stéréotypie -grattent leurs peaux par les pattes antérieures et postérieures	-appariation de mouvement stéréotypie -léchage -exploration de la sciure de bois	-hypermobilité -reniflement intense -courtes phases de mâchonnement -suspension	-mouvement répétés des pattes antérieures contre la paroi de la cage -hochement de la tête	-direction continu de la tête vers le haut de la cage -mouvements rapides de la tête

S6	-phase courte de sédation -absence de mouvements stéréotypie.	-mâchonnement -léchage -direction de la tête vers le haut de la cage. -retranchement contre la paroi de la cage	-hochement de la tête -hypermobilité -rongent la paroi de la cage.	-mouvements répétés des pattes contre la paroi de la cage -position spécifique par leur patte -hyperexcitabilité	-rongent la paroi de la cage -hochement de la tête -hyperexcitabilité

Tableau 29: Observation des animaux en situation libre après l'administration orale de l'extrait aqueux de *Rosmarinus officinalis* à 200 mg/kg.

Temps / Souris	**15**	**30**	**60**	**120**	**180**
Témoins S1 S2 S3	-endormis aux coins de la cage -vont vers l'eau et l'aliment	//	//	//	//
Romarin S4	-rare mouvement de stéréotypie -se grattent leurs	-mouvement stéréotypie -reniflement intenses -courtes phases de mâchonnement	-hypermobilité -hyper excitabilité -hochement de la tête - mâchonnement	-mouvement stéréotypie intense et continus -léchages -mouvement répétés des pattes antérieures	-hyperexcitabilité -rongent de la paroi de la cage -diminution partielle des mouvements de stéréotypie
S5	-hypermobilité. -direction de la tête vers le haut de la cage	//	//	//	-mouvement répétés des pattes antérieures contre la paroi de la cage -hypermobilité diminuée

S6	-hypermobilité -se grattent -hochement de la tête.	//	//	//	-hyperexcitabilité -hochement de la tête - mâchonnement ou léchages -courte phase de mâchonnements

Tableau 30: Influence de l'huile essentielle de *Rosmarinus officinalis* (HE) sur le temps d'endormissement et la durée du sommeil induit chez la souris, par une dose hypnotique de thiopental (60 mg/kg, IP).

Temps	**Référence**	**Huile essentielle de *Rosmarinus officinalis***	
T.E (min)	18 ± 1	**50 mg/kg; VO**	**100 mg/kg; VO**
		20 ± 1	42 ± 1
T.S (min)	34 ± 2	10 ± 0,1	3 ± 0,2

Tableau 31: Influence de l'extrait méthanolique de *Rosmarinus officinalis* (EM) sur le temps d'endormissement (TE) et la durée du sommeil (TS) induit chez la souris, par une dose hypnotique de thiopental (60 mg/kg, IP).

Temps	**Référence**	**Extrait méthanolique de *Rosmarinus officinalis***	
T.E (min)	18 ± 1	**100 mg/kg; VO**	**200 mg/kg; VO**
		34 ± 1	58 ± 0,2
T.S (min)	34 ± 2	8 ± 1	3 ± 0,1

Tableau 32: Influence de l'extrait aqueux de *R. officinalis* (EA) sur le temps d'endormissement (TE) et le temps de sommeil (TS) induit chez la souris, par une dose hypnotique de thiopental (60mg/kg, IP).

Temps	**Référence**	**Extrait aqueux de *Rosmarinus officinalis***	
T.E (min)	18 ± 1	**100 mg/kg; VO**	**200 mg/kg; VO**
		48 ± 1	50 ± 0,2
T.S (min)	34 ± 2	6 ± 1	2 ± 0,1

Tableau 33: Influence de l'huile essentielle de *Rosmarinus officinalis* L sur les stéréotypies induites par l'apomorphine (16mg/kg, SC), chez le rat.

Somme des cotations individuelles stéréotypiques																											
Temps (min)	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**	**130**	**140**	**150**	**160**	**170**	**180**	**190**	**200**	**210**	**220**	**230**	**240**	**250**	**260**	**270**	**280**
Référence	9	11	12	12	15	16	18	18	16	15	14	10	8	8	6	5	5	4	3	2	0	0	0	0	0	0	0	0
HE 50mg/kg	8	10	12	13	15	16	16	14	14	12	11	10	10	8	8	6	5	3	2	1	0	0	0	0	0	0	0	0
HE 100mg/kg	15	15	16	18	18	18	16	16	14	15	15	15	14	13	12	10	10	9	8	8	6	6	5	5	3	2	1	0

(n=6) nombre d'animaux par lots; S.C= sous-cutanée. Cotation individu stéréotypé:

0 = absence de mouvement stéréotypés, tous les animaux étaient normaux; 1 = présence rare de mouvements stéréotypés; 2 = intense reniflant avec des mouvements rapides de la tête; 3 = intense et continu mouvement stéréotypés (déplacement de la tête, lèchements) ($p< 0.01$).

Tableau 34: Influence de l'extrait méthanolique de *Rosmarinus officinalis* L sur les stéréotypies induites par l'apomorphine (16mg/kg, SC), chez le rat.

Somme des cotations individuelles stéréotypiques																												
Temps (min)	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**	**130**	**140**	**150**	**160**	**170**	**180**	**190**	**200**	**210**	**220**	**230**	**240**	**250**	**260**	**270**	**280**
Référence	**9**	**11**	**12**	**12**	**15**	**16**	**18**	**18**	**16**	**15**	**14**	**10**	**8**	**8**	**6**	**5**	**5**	**4**	**3**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
EM 100 mg/kg	**10**	**10**	**12**	**13**	**16**	**16**	**16**	**18**	**18**	**15**	**14**	**12**	**12**	**10**	**10**	**8**	**8**	**6**	**5**	**3**	**2**	**2**	**1**	**0**	**0**	**0**	**0**	**0**
EM 200 mg/kg	**16**	**16**	**18**	**18**	**18**	**18**	**16**	**16**	**16**	**15**	**15**	**15**	**15**	**15**	**14**	**12**	**12**	**11**	**10**	**10**	**8**	**8**	**6**	**6**	**3**	**2**	**1**	**0**

(n=6) nombre d'animaux par lots; S.C= sous-cutanée. Cotation individu stéréotypé:

0 = absence de mouvement stéréotypés, tous les animaux étaient normaux; 1 = présence rare de mouvements stéréotypes; 2 = intense reniflant avec des mouvements rapides de la tête; 3 = intense et continu mouvement stéréotypés (déplacement de la tête, lèchements) ($p< 0.01$).

Tableau 35: Influence de l'extrait aqueux de *Rosmarinus officinalis* L sur les stéréotypies induites par l'apomorphine (16mg/kg, SC), chez le rat.

Somme des cotations individuelles stéréotypiques																												
Temps (min)	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**	**130**	**140**	**150**	**160**	**170**	**180**	**190**	**200**	**210**	**220**	**230**	**240**	**250**	**260**	**270**	**280**
Référence	9	11	12	12	15	16	18	18	16	15	14	10	8	8	6	5	5	4	3	2	0	0	0	0	0	0	0	0
EA 100 mg/kg	10	10	12	13	15	16	16	16	14	14	14	12	12	11	10	10	8	8	8	6	5	3	2	1	0	0	0	0
EA 200 mg/kg	16	16	16	18	18	18	18	16	16	15	15	15	15	14	14	13	13	12	12	10	10	10	8	6	5	3	2	0

(n=6) nombre d'animaux par lots; S.C= sous-cutanée. Cotation individu stéréotypé:

0 = absence de mouvement stéréotypés, tous les animaux étaient normaux; 1 = présence rare de mouvements stéréotypés; 2 = intense reniflant avec des mouvements rapides de la tête; 3 = intense et continu mouvement stéréotypés (déplacement de la tête, lèchement) ($p< 0.01$).

VI. Evaluation de l'activité antibactérienne des extraits méthanoliques et aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis*

Les extraits méthanoliques et aqueux ont été évalués pour l'activité antimicrobienne contre les bactéries Gram positif (*S. aureus*, *M. luteus et B. subtilis*) et Gram négatif (*P. aeruginosa, K. pneumoniae et E. coli*). L'extrait méthanolique de romarin et l'extrait aqueux de lavande se sont avérés les plus actifs contre toutes les souches bactériennes. L'extrait méthanolique de *R. officinalis* a montré de bonnes activités, inhibant la croissance de *E. coli, P. aeruginosa* et *K. pneumoniae* avec des DZI (diamètre de la zone d'inhibition) de 19, 20 et 18 mm respectivement, mais avec plus d'activité contre trois bactéries Gram positif (*S. aureus, M. luteus et B. subtilis*), avec un diamètre de zone d'inhibition de 24, 22 et 20 mm respectivement (P <0,001). Le diamètre de la zone d'inhibition de l'extrait méthanolique du romarin variait de 19 à 50 mm, tandis que l'DZI de l'extrait aqueux variait de 12 à 30 mm (P <0,01) (Tab. 37 et 38). Pour *Lavandula officinalis*, les résultats des concentrations minimales inhibitrices et les zones d'inhibitions des extraits méthanoliques et aqueux contre les Gram négatives et les Gram positives sont résumés dans les tableaux 39, 40 et 41. L'extrait aqueux de *Lavandula officinalis* s'est avéré le plus actif contre toutes les souches bactériennes. L'extrait aqueux de *Lavandula officinalis* a montré de bonnes activités, inhibant la croissance de *E. coli, P. aeruginosa* et *K. pneumoniae* avec des DZI de 20, 22 et 18 mm respectivement, mais avec plus d'activité contre trois bactéries Gram positif (*S. aureus, M. luteus et B. subtilis*), avec un diamètre de zone d'inhibition de 24, 20 et 22 mm respectivement (P <0,001). Le diamètre de la zone d'inhibition de l'extrait aqueux de lavande variait de 17 à 48 mm, tandis que le DZI de l'extrait méthanolique variait de 12 à 36 mm (P <0,01) (Tab. 40 et 41).

Les résultats présentés dans cette étude mettent en évidence le potentiel des extraits du romarin et de la lavande en tant que source de composés modifiants la résistance aux antibiotiques. Les CMI de l'extrait méthanolique du romarin et l'extrait aqueux de la lavande variaient de 1,56 à 6,25 mg/ml pour tous les micro-organismes du test (P<0,001), tandis que les CMI de l'extrait aqueux du romarin et l'extrait méthanolique

de la lavande variaient de 3,13 à 6,25 mg/ml ($p < 0,01$) (Tab. 36 et 39). En général, l'extrait méthanolique de *Rosmarinus officinalis* et l'extrait aqueux de la *Lavandula officinalis* ont montré une plus grande activité antimicrobienne que les autres extraits (Tab. 36 et 39). Prenant les DZI les plus faibles des contrôles (pénicilline et l'ampicilline) comme point de rupture, la plupart des extraits dépassent ce point de rupture (Tab. 37, 38, 40 et 41).

De cette étude analytique d'évaluation de l'activité antibactérienne des extraits méthanoliques et aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis,* il ressort que:

- Toutes les souches bactériennes étudiées sont sensibles à ces extraits.
- Cette sensibilité est différente selon les souches. Les souches sont très sensibles, les souches sont moyennement sensibles et les souches sont moins sensibles.
- L'extrait méthanolique du *Rosmarinus officinalis* et l'extrait aqueux de la *Lavandula officinalis* sont plus actifs que l'extrait méthanolique de *Lavandula officinalis* et l'extrait aqueux de *Rosmarinus officinalis*.

Tableau 36: Concentration Minimale Inhibitrice (CMI) des extraits méthanoliques et aqueux de *Rosmarinus officinalis sur* les souches bactériennes.

	Concentration Minimale Inhibitrice (mg/ml)									
	Concentrations différentes de l'extrait méthanolique								Contrôle positif	
Souches de Référence	**200**	**100**	**50**	**25**	**12,5**	**6,25**	**3,13**	**1,56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	50	40	30	28	26	24	20	19	32	28
P. aeruginosa					3,13± 0,00				6,25± 0,00	
K. pneumoniae	40	34	30	24	22	20	20	18	28	24
M. luteus					1,56± 0,00				6,25± 0,00	
P. aeruginosa	40	26	20	20	19	18	18	17	18	18
S. aureus					3,13± 0,00				6,25± 0,00	
M. luteus	60	52	34	30	28	26	24	24	33	30
B. subtilis					1,56± 0,00				3,13± 0,00	

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341. Les valeurs sont des moyennes ± écart-type de trois déterminations. Les données indiquent une différence significative (P <0,05) par rapport au contrôle positif (pénicilline et l'ampicilline).

Tableau 37: Inhibition par l'extrait méthanolique de *Rosmarinus officinalis* (taille de la zone, mm).

S. aureus	46	34	28	26	24	24	22	20	26	24
B. subtilis	36	34	30	26	24	22	20	20	32	28

Concentrations différentes de l'extrait aqueux (mg/ml)	**Contrôle positif**

Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442, *Kb, Klebsiella pneumoniae* ATCC 53153, *Ec, Escherichia coli* ATCC 54127, *Sa, Staphylococcus aureus* ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341, Am, Ampicillin, Pe, Pénicilline. Les valeurs sont des moyennes ± écart-type de trois déterminations. Les données indiquent une différence significative (P <0,05) par rapport au contrôle positif (pénicilline et l'ampicilline).

Tableau 38: Inhibition par l'extrait aqueux de *Rosmarinus officinalis* (taille de la zone, mm).

Souches de Référence	**200**	**100**	**50**	**25**	**12,5**	**6,25**	**3,13**	**1,56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	26	24	20	18	18	16	16	14	32	28
K. pneumoniae	26	22	20	20	18	17	14	12	28	24
P. aeruginosa	24	22	20	20	19	16	14	12	18	18
M. luteus	24	18	18	18	16	16	14	12	33	30
S. aureus	24	20	20	19	19	18	17	14	26	24

B. subtilis	30	28	26	26	24	22	20	20	32	28

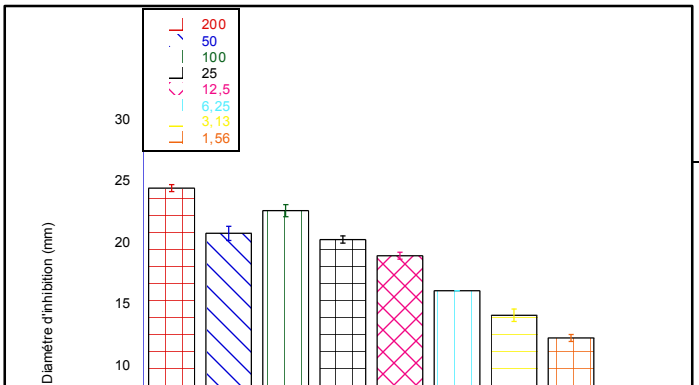
Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442, *Kb,*

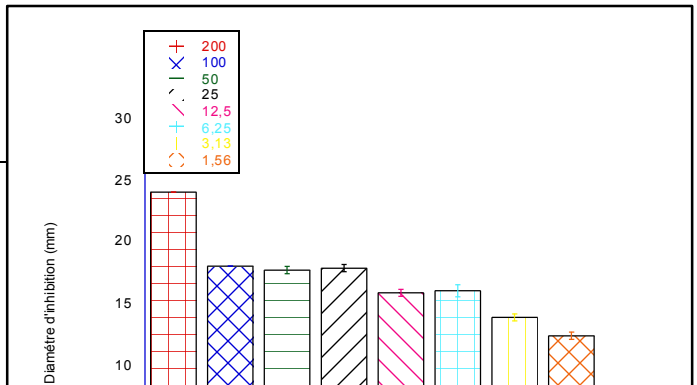
	Concentrations différentes de l'extrait aqueux (mg/ml)

Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, *Sa, Staphylococcus aureus* ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341, Am, Ampicillin, Pe, Penicillin. Les valeurs sont des moyennes ± écart-type de trois déterminations. Les données indiquent une différence significative (P <0,05) par rapport au contrôle positif (pénicilline et l'ampicilline).

Tableau 39: Concentration Minimale Inhibitrice (CMI) des extraits méthanoliques et aqueux de *Lavandula officinalis sur* les souches bactériennes.

Souches de Référence	Extrait méthanolique	Extrait aqueux
E. coli	3,13 ± 0,00	6,25± 0,00
K. pneumoniae	6,25 ± 0,00	6,25± 0,00
P. aeruginosa	6,25± 0,00	6,25± 0,00
M. luteus	1,56± 0,00	6,25± 0,00





Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442, *Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli* ATCC 54127, *Sa, Staphylococcus aureus* ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341, Am, Ampicilline, Pe, Pénicilline. Les valeurs sont des moyennes ± écart-type de trois déterminations. Les données indiquent une différence significative (P <0,05) par rapport au contrôle positif (pénicilline et l'ampicilline).

Tableau 40: Inhibition par l'extrait aqueux de *Lavandula officinalis* (taille de la zone, mm).

Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442, *Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli* ATCC 54127, *Sa,*

Concentrations différentes de l'extrait aqueux (mg/ml)									**Contrôle positif**	
Souches de Référence	**200**	**100**	**50**	**25**	**12,5**	**6,25**	**3,13**	**1,56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	48	40	30	28	26	24	20	19	32	28
K. pneumoniae	40	32	28	26	22	22	20	18	28	24
P. aeruginosa	42	26	22	20	19	18	18	17	18	18
M. luteus	52	52	34	30	28	26	24	24	33	30
S. aureus	46	34	28	26	24	24	22	20	26	24
B. subtilis	36	34	32	28	24	22	20	20	32	28

Staphylococcus aureus ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341, Am, Ampicilline, Pe, Pénicilline. Les valeurs sont des moyennes ± écart-type de trois déterminations. Les données indiquent une différence significative ($P < 0,05$) par rapport au contrôle positif (pénicilline et l'ampicilline).

Table 41: Inhibition par l'extrait méthanolique de *Lavandula officinalis* (taille de la zone, mm).

Concentrations différentes de l'extrait méthanolique (mg/ml)									Contrôle positif	
Souches de Référence	**200**	**100**	**50**	**25**	**12,5**	**6,25**	**3,13**	**1,56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	36	24	22	20	18	18	16	14	32	28
K. pneumoniae	28	22	22	20	18	18	14	12	28	24
P. aeruginosa	26	24	20	20	18	16	14	12	18	18
M. luteus	24	18	18	18	16	16	14	12	33	30
S. aureus	28	20	20	19	19	18	17	14	26	24
B. subtilis	30	28	26	26	24	22	20	20	32	28

Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442, *Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli* ATCC 54127, *Sa, Staphylococcus aureus* ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341, Am, Ampicilline, Pe, Pénicilline. Les valeurs sont des moyennes ± écart-type de trois déterminations. Les données indiquent une différence significative ($P < 0,05$) par rapport au contrôle positif (pénicilline et l'ampicilline).

DISCUSSION ET CONCLUSION GENERALE

Par la présente étude, l'on a pu évaluer la toxicité aiguë des huiles essentielles, des extraits méthanoliques et des extraits aqueux du *Rosmarinus officinalis* et de la *Lavandula officinalis* L.

L'absence de toxicité a pu être confirmée puisque l'étude symptomatologique sur les 14 jours d'observation n'a pas montré de perturbations affectant le comportement, l'aspect extérieur ou certaines fonctions de l'organisme, en dehors d'une réduction attendue de l'activité générale des animaux qui s'est manifestée dans la première heure après l'administration des extraits pour disparaitre dans les heures qui suivent.

L'apathie des animaux, observée durant la première phase du test et se traduisant par la réduction de l'activité générale, pourrait expliquer la légère diminution du poids corporel visible dans les 2 ou 3 jours qui suivant l'injection des extraits, les animaux restent blottis dans un coin de la cage et ne s'alimentent pas. Les (DL_{50}) ainsi déterminées par voie orale et Intrapéritonéale pourront servir de repérer pour des études pharmacologiques ultérieures des huiles essentielles, des extraits méthanolique et aqueux du *Rosmarinus officinalis* et de la *Lavandula officinalis* L., (*Lavandula angustifolia*). Les doses les plus élevées produisent un changement significatif du comportement général des souris. Cet état persiste jusqu'à la mort chez la plupart des animaux testés.

Les plantes étudiées n'entrainent pas de toxicité après administration unique par la voie orale. Horn, 1956, a rapporté qu'une DL50>2-3g/kg d'une substance ou des substances actives contenues dans un médicament peut être considérée comme non toxique.

D'autre part, les souris sont beaucoup plus sensibles que l'homme (Zbenden et Flury, 1981). L'effet adverse observé suite à l'administration intrapéritonéale peut être attribué au fait que les substances toxiques sont métabolisées, hydrolysées ou non absorbées au niveau du tube digestif lors du gavage. Seule sera préconisée la voie orale chez l'Homme.

Les travaux antérieurs ont démontré que l'α –pinène, le 1,8-cinèole, le camphre et le bornèol, représentent 80-90,6% des huiles essentielles de *R. officinalis*. Dans notre étude, ces composés représentent 97,6%. Ces différences dans la composition chimique des huiles essentielles peuvent être dûes à des facteurs développementaux et environnementaux qui influencent le métabolisme des plantes (Kaloustian et al., 2008; Jarrar et al., 2010; Moysaluwa et al., 2004; Jians et al., 2011; Derwich et al., 2011; Kadri et al., 2011; Miresmailli et al., 2006; Moghtader and Afzali, 2009; Takaki et al., 2008 ; Ozcan and Chalchat, 2008; Touafek et al., 2008; Soliman et al., 1993).

L'ensemble de cette étude toxicologique permet de confirmer la faible toxicité de l'huile essentielle du *Rosmarinus officinalis*, et la très faible toxicité de l'extrait méthanolique. Par ailleurs, l'étude de la toxicité aiguë de l'huile essentielle, de l'extrait méthanolique et de l'extrait aqueux de la *Lavandula officinalis,* ainsi que l'extrait aqueux du *Rosmarinus officinalis*, a montré que ces extraits ne provoquent aucune toxicité par voie d'administration. L'estimation de la (DL_{50}) de l'huile essentielle et de l'extrait méthanolique de *Rosmarinus officinalis* chez la souris est évaluée à 897,85mg/kg et 2104mg/kg, respectivement. Cette étude rassure quant à l'utilisation des extraits issus du *Rosmarinus officinalis* et de la *Lavandula officinalis* dans l'industrie ou dans l'aromathérapie.

Les huiles essentielles de romarin et de lavande, grandes plantes d'aromathérapie, ont une odeur attractive et sont incorporées dans de nombreux produits. Leurs huiles essentielles sont connues pour leurs effets anticonvulsif, sédatif, hypnotique, antidépresseur et sont utilisées pour traiter les « crises de nerfs », tension nerveuse et dépression (Trisserand et al., 1993; Umezu et al., 2006, Yamad et al., 1994; Show et al., 2006; Lehner et al., 2009). L'étude a porté ici sur l'effet neuropharmacologique de différentes doses d'huiles essentielles de romarin et de lavande. L'administration par voie intrapéritonéale est choisie pour la meilleure biodisponibilité qu'elle offre par rapport à la voie orale. L'administration par voie intrapéritonéale des huiles essentielles de lavande et romarin induit un effet sédatif et stimulant, respectivement. L'huile essentielle de la lavande a provoqué un effet sédatif à 600mg/kg VO, suggérant que le linalol et l'acétate de linalyle, en tant que composants majeurs de l'huile essentielle de lavande, pourraient en être les principaux actifs

pharmacologiques, ce qui conforte la littérature qui rapporte que le linalol et l'acétate de linalyle réduisent l'activité locomotrice de la souris (Buchabauer et al., 1991; Buchabauer et al., 1993). Les propriétés sédatives de l'extrait méthanolique et aqueux de la *Lavandula officinalis* ont ici été mise en évidence par leur influence sur le temps de rétablissement et la perte de l'initiative et de la curiosité chez la souris.

Parailleurs, les benzodiazépines sont dépresseurs du système nerveux central, elles sont utilisées pour contrôler les désordres du sommeil comme l'insomnie; elles agissent en se liant au récepteur GABA de type A couplé a un ionophore (Huang et al., 2002). Elles réduisent le temps d'apparition du sommeil induit par le thiopental et augmentent sa durée induit, et diminuent également l'activité exploratrice de l'animal par leur potentiel sédatif (File et Wardill, 1975; Roy-Byrne, 2005). L'extrait de lavande augmente le temps de rétablissement de la souris dans le test de la traction, aux doses 200, 400 et 600mg/kg VO. Après administration par voie orale, l'effet sédatif induit par cet extrait à ces doses est similaire à celui de 0.3 mg/kg de benzodiazépine, IP. A cet égard, l'extrait de la lavande a provoqué une réduction dose-dépendante de la curiosité dans le test de la planche à trous similaire ou plus important que le diazépam. Il est admis que l'activité locomotrice résulte de l'activation du cerveau, et se manifeste par une excitation des neurones incluant différents mécanismes neurochimiques et une augmentation du métabolisme cérébral. Ainsi, il serait possible que l'activité sédative de l'extrait méthanolique et aqueux de *Lavandula officinalis* soit médiée par la voie GABA ergique, la modulation de la transmission GABA ergique étant impliquée dans une sédation profonde chez la souris (Gouttes Mann, 2002). L'action inhibitrice du GABA consiste en l'ouverture des canaux chlorure pour permettre l'hyperpolarisation de la membrane (Forte et al., 2010), entrainant une dépression du SNC et induisant une activité sédative. Le glutamate et le GABA sont quantitativement les plus important excitateurs et inhibiteurs neurotransmetteurs au niveau cérébral chez les mammifères (Range et al., 2007). Les récepteurs de ces neurotransmetteurs seraient des cibles importantes des médicaments psychotropiques. Des études phytochimiques ont identifié les composants actifs de *Lavandula officinalis*, comme les coumarines, les chalcones, les flavines, les flavonols, les quercetin et les dérivés de kaempferol, ce qui suggère leur implication dans

l'activité sédative (Zapata- Sudo et al., 2010; Pérez-Ortege et al., 2008; Huang et al., 2007).

L'huile essentielle de la *Lavandula officinalis* quant à elle, présente un effet hypnotique significatif à la dose de 1000mg/kg IP, augmenté à la dose 1500mg/kg IP. De même l'extrait méthanolique de la lavande induit un effet hypnotique à 800 mg/kg VO, qui augmente à 1000 mg/kg VO. L'extrait aqueux lui, ne présente aucun effet inducteur du sommeil. Cependant, si aucun animal ne s'endort, tous restent plus ou moins, blottis les uns contre les autres dans un coin de la cage. Cette immobilisation se manifeste surtout aux doses de 800 et 1000mg/kg VO. Après une heure, ces phénomènes semblent disparaitre totalement. En comparaison avec l'effet inducteur du thiopental, si la dose de 60mg/kg IP de ce composé conduit au même temps d'endormissement que la dose 1500mg/kg IP d'huile essentielle et la dose 1000 mg/kg VO d'extrait méthanolique, les animaux s'endorment moins rapidement et dorment plus longtemps. Le temps d'endormissement de l'extrait méthanolique et de l'huile essentielle à 1500 et 1000 mg/kg VO est moins rapide, respectivement, que le thiopental, mais le temps de sommeil est plus long. A 60mg/kg IP, le thiopental induit le sommeil chez tous les animaux, le temps d'endormissement moyen est très proche de celui obtenu avec la plus forte dose d'huile essentielle et d'extrait méthanolique, soit 1500 et 1000 mg/kg. Toutefois, le sommeil est nettement prolongé sous l'effet de *Lavandula officinalis*, sa durée moyenne est d'environ 106 et 112 minutes, respectivement, celle du thiopental étant de 36 minutes. En conclusion, l'administration par voie orale de l'extrait méthanolique et de l'extrait aqueux et par voie intrapéritonéale de l'huile essentielle de la *Lavandula officinalis* induit des effets sédatifs qui restent similaires, appuyant son utilisation en médecine traditionnelle, ce qui fournit des bases pharmaceutiques pour son efficacité thérapeutique sur l'insomnie.

L'aromathérapie est un traitement efficace pour soigner toute une gamme de troubles psychiatriques (Perry et Perry, 2006; Sarris, 2007; Steflitsch and Steflitsch, 2004; Akhondzadeh, 2007). Les huiles essentielles sont censés posséder des effets antidépresseurs utiles pour traiter la dépression nerveuse (Tisserand, 1993; Fibiger, et al., 1988; Machado, et al., 2009). Nous avons analysé ici l'activité psychostimulante de l'huile essentielle, de l'extrait méthanolique et de l'extrait aqueux des tiges et des feuilles de romarin (*Rosmarinus officinalis*) à différentes doses (50, 100 et 200 mg/kg) administrées par voie orale.

Il convient de noter d'emblée le nombre très faible de publications sur l'action stimulante de *Rosmarinus officinalis.*

Dans cette étude, l'activité psychostimulante a été étudiée par l'enregistrement de l'activité locomotrice spontanée chez les souris et rats. Dans le test de l'observation en situation libre, l'huile essentielle à 100 mg/kg VO et les extraits méthanoliques et aqueux aux doses 100 et 200 mg/kg VO produisent une augmentation de la curiosité et de l'activité motrice, par l'induction d'une agression spontanée, d'une hypermobilité et d'une hyperexcitabilité.

Ces observations suggèrent que l'action psychostimulante de cette plante serait médiée par la voie dopaminergique puisque la transmission dopaminergique peut produire des effets stimulants profonds chez la souris (Bossier et al., 1972; Simon et al., 1982).

La transmission de la dopamine est due à la libération des catécholamines (noradrénaline et dopamine) stockées dans les vésicules neuronales (Costa et al., 1972; Samanin et al., 1977). L'huile essentielle, l'extrait aqueux et l'extrait méthanolique de *Rosmarinus officinales* auraient donc un potentiel neuro-dopaminergique considérable. On a également observé que l'extrait aqueux (200 mg/kg VO) a provoqué une augmentation significative (P <0,001) de l'activité motrice spontanée et de l'induction des mouvements stéréotypés.

Dans le test du sommeil induit par le thiopental, le temps d'endormissement augmente significativement (P <0,001) à l'application d'huile essentielle (100 mg/kg VO) et des extraits méthanoliques et aqueux (200 mg/kg VO). De même, le temps de sommeil

diminue nettement sous l'effet de l'huile essentielle (100 mg/kg VO) et des extraits méthanoliques et aqueux (200 mg/kg VO).

L'induction du comportement explorateur, la prolongation du temps d'endormissement et la diminution du temps de sommeil par les huiles essentielles, les extraits méthanoliques et aqueux de *Rosmarinus officinalis* sont nettement en faveur d'une activité stimulante du système nerveux central.

Dans le test de l'induction des mouvements stéréotypés par l'apomorphine chez le rat, l'extrait méthanolique et aqueux (200mg/kg VO) produisent un effet significatif (P<0,001) potentialisant l'apomorphine; les mouvements stéréotypés ont augmenté en durée et en intensité. De plus, la dose de 100mg/kg VO des deux extraits augmente, même partiellement par rapport aux témoins, les mouvements stéréotypés en durée et en intensité. Un mécanisme potentiellement dopaminergique de l'huile essentielle et de l'extrait méthanolique et aqueux de *Rosmarinus officinalis* est ainsi posé.

Des études phytochimiques ont identifié les composants actifs de l'extrait méthanolique et aqueux de *Rosmarinus officinalis* tels que les flavonoïdes, y compris la diosmétine, la diosmine, la lutéoline, l'apigénine, la quercétine et le kaempherol. Les phénols tels que les acides caféique et rosmarinique et des terpènoïdes comme le carnosol, l'acide carnosique, rosmanol, l'oleonolic et l'acides ursolique sont également contenus dans la plante (Okamura, et al., 1994; Frankel et al., 1996; Al-Sereitia et al., 1999; Barnes et al., 2001; Nolkemper, et al., 2006; Altinier et al., 2007; Bentayeb et al., 2007; Yesil-Celiktas$_{a,b}$ et al., 2007; Erkan et al., 2008; Askun et al., 2009; Machado et al., 2009). Le criblage de l'extrait méthanolique et éthanolique de la partie aérienne de *R. officinalis* a signalé la présence de ces composés mais pas celle d'alcaloïdes détectés dans l'extrait aqueux (Hosseinzadeh and Nourbakhsh, 2003; Maria-José et al., 2003; Machado et al., 2009). Plus d'analyses chimiques de l'extrait sont à mener pour isoler, caractériser et doser les principes actifs de l'activité psychostimulante.

L'activité antimicrobienne des extraits méthanoliques et aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis* a été évaluée contre les bactéries Gram positif (*S. aureus*, *M. luteus et B. subtilis*) et Gram négatif (*P. aeruginosa, K. pneumoniae et E. coli*). L'extrait méthanolique de romarin et l'extrait aqueux de lavande se sont avérés les plus actifs contre toutes les souches bactériennes. L'extrait méthanolique de *R. officinalis* inhibe la croissance de *E. coli, P. aeruginosa* et *K. pneumoniae* avec des diamètres de la zone d'inhibition de 19, 20 et 18 mm respectivement. Il s'est montré plus actif contre trois bactéries Gram positif (*S. aureus, M. luteus et B. subtilis*), avec un diamètre de zone d'inhibition de 24, 22 et 20 mm respectivement (P <0,001). Le diamètre de la zone d'inhibition de l'extrait méthanolique du romarin varie de 19 à 50 mm, tandis que le DZI de l'extrait aqueux varie de 12 à 30 mm (P <0,01). Les résultats de la diffusion sur disque de papier soutiennent et permettent d'étendre la conclusion précédente selon laquelle le romarin contient de nombreux composés biologiquement actifs dont certains ont été fréquemment utilisés dans la médecine traditionnelle pour leurs propriétés antibactériennes. En outre, les valeurs de CMI appuient les résultats de la méthode de diffusion sur disque de papier.

L'extrait aqueux de *Lavandula officinalis* s'est avéré le plus actif contre toutes les souches bactériennes. L'extrait aqueux de *Lavandula officinalis* inhibe la croissance de *E. coli, P. aeruginosa* et *K. pneumoniae* avec des DZI de 20, 22 et 18 mm respectivement, et s'est plus actif contre trois bactéries Gram positif (*S. aureus, M. luteus et B. subtilis*), avec un diamètre de zone d'inhibition de 24, 20 et 22 mm respectivement (P <0,001). Le diamètre de la zone d'inhibition de l'extrait aqueux de lavande varie de 17 à 48 mm, tandis que le DZI de l'extrait méthanolique varie de 12 à 36 mm (P <0,01).

Par ailleurs, les souches qui présentent les plus grandes zones d'inhibition ne sont pas toujours celles qui sont les plus sensibles (valeurs de CMI plus basses).

En effet, le diamètre de la zone d'inhibition ne reflète pas l'efficacité antibactérienne d'un produit, il peut être affecté par la solubilité et la diffusion de l'extrait.

Les bactéries Gram négatives étudiées dans ce travail sont généralement moins sensibles aux extraits que les Gram positives.

L'activité biologique du romarin contre les bactéries testées pourrait être attribuée à la présence de flavonoïdes, acides phénoliques (caféique et rosmarinique), des huiles essentielles (camphre et cinéol) et des diterpènes (carnosol) (Faixova and Faix, 2008; Barnes et al., 1996; Rozman and Jerek, 2009). Des composants biologiquement actifs sont supposés perturber la perméabilité de la membrane cytoplasmique et faciliter ainsi l'afflux des antibiotiques (Zhao et al., 2000). Les résultats présentés dans cette étude mettent en évidence le potentiel des extraits du romarin et de la lavande en tant que source de composés modifiants la résistance aux antibiotiques. Les CMI de l'extrait méthanolique du romarin et l'extrait aqueux de la lavande varient de 1,56 à 6,25 mg/ml pour tous les micro-organismes du test ($P<0,001$), tandis que les CMI de l'extrait aqueux du romarin et l'extrait méthanolique de la lavande varient de 3,13 à 6,25 mg/ml ($p <0,01$). Ces différences dans la sensibilité des micro-organismes testés aux échantillons d'essai pourraient être dues à la variation du taux de pénétration des échantillons à travers la paroi et la membrane cellulaire des structures (Cox et al., 2000; Cox et al., 2001). En général, l'extrait méthanolique de *Rosmarinus officinalis* et l'extrait aqueux de la *Lavandula officinalis* ont montré une plus grande activité antimicrobienne que les autres extraits. Prenant les DZI les plus faibles des contrôles (pénicilline et l'ampicilline) comme point de rupture, la plupart des extraits dépassent ce point de rupture.

Il est assez difficile d'attribuer l'effet antimicrobien d'un extrait d'un ou de quelques principes actifs, les extraits contenant toujours un mélange de différents composés chimiques (Caillet et al., 2005; Caillet et al., 2006). En plus des principaux composants, des éléments mineurs peuvent apporter une contribution significative à l'activité antimicrobienne des extraits. Il serait légitime de déduire, d'après nos résultats, que l'activité antimicrobienne des extraits méthanoliques et aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis* résulte de la synergie de leurs composants. Ces extraits aqueux et méthanoliques pourraient devenir un agent antimicrobien naturel potentiel dans le domaine des industries alimentaires et pharmaceutiques.

Afin de poser scientifiquement ces différents potentiels pharmacologiques, il est nécessaire de compléter l'évaluation de ces différents extraits par l'étude de leur mécanisme d'action psychotrope et anti infectieux et de leur pharmacocinétique. Au préalable, une étude phytochimique analytique complète permettrait de caractériser et doser les différents principes actifs contenus dans les extraits et faciliterait une étude Relation-Activité indispensable à toute validation scientifique des utilisations traditionnelles recherchée.

PERSPECTIVES

PERSPECTIVES

Il serait donc intéressant de procéder à:

- ✓ L'analyse chimique analytique des extraits pour isoler, caractériser et doser les principes actifs responsables de l'activité psychotrope potentielle des huiles essentielles, des extraits méthanoliques et des extraits aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis*.

- ✓ L'étude du mécanisme de l'action psychotrope des huiles essentielles, des extraits méthanoliques et des extraits aqueux du *Rosmarinus officinalis* et de la *Lavandula officinalis* sur le système nerveux central(SNC).

- ✓ L'étude du mécanisme de l'action anti infectieuse des huiles essentielles, des extraits méthanoliques et aqueux de *Rosmarinus officinalis* et de *Lavandula officinalis* via à vis des bactéries à Gram négatif et à Gram positif. Il serait important de compléter ce travail en abordant l'effet des extraits sur d'autres fonctions et structures cellulaires, et étudier l'action des extraits fixes sur le profile lipidique et protéique des membranes bactériennes ainsi que sur l'activité enzymatique et l'expression génétique.

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RESUME

Résumé

Les feuilles de *Rosmarinus officinalis* et les fleurs de *Lavandula officinalis* ont été utilisés comme médicaments pour le traitement des troubles du système nerveux, en médecine traditionnelle marocaine. Nous avons évalué les effets psychotropes des huiles essentielles, des extraits méthanoliques et aqueux des feuilles de *R. officinalis* et des fleurs de *L. officinalis* sur le système nerveux central en utilisant une batterie de tests psychopharmacologiques comportementaux. Les huiles essentielles extraites par hydrodistillation ont été caractérisés par GC-MS. L'administration par voie intrapéritonéale chez la souris de l'huile essentielle de *L. officinalis* à 300 et 600 mg/kg induit de forts effets sédatifs par rapport à la substance de référence (Diazépam) et un effet hypnotique à 1000 et 1500 mg/kg. Cependant, l'huile essentielle extraite de *R. officinalis* aux doses de 50 et 100 mg/kg IP, ne produit aucun effet sédatif significatif sur le système nerveux central. L'extrait méthanolique a induit de façon significative un effet sédatif aux doses de 200, 400 et 600 mg/kg VO, par rapport à la substance de référence (Diazépam), et un effet hypnotique à des doses de 800 et 1000 mg/kg VO, tandis que le traitement des souris avec l'extrait aqueux à des doses de 200 et 400 mg/kg VO réduit de manière significative tous les deux, le temps rétablissement d'équilibre et la curiosité de l'animal.

Cette étude a aussi apporté de manière innovante les preuves scientifiques de l'activité psychostimulante de *Rosmarinus officinalis* L. (Lamiaceae) sur le système nerveux central. Les effets des extraits méthanoliques et aqueux de romarin ont été en effet étudiés en utilisant trois modèles de comportement, soit l'observation des animaux en situation libre, la recherche des mouvements stéréotypés chez le rat et l'interaction avec le thiopental chez la souris. Les extraits de *R. officinalis* augmentent l'activité locomotrice et induisent une hypermobilité et hyperexcitabilité par rapport au groupe témoin. Le traitement par les extraits aqueux et méthanoliques de *R. officinalis* à 200 mg/kg VO, diminue significativement de la durée du temps de sommeil induite par le thiopental à partir de 34 min ± 0,2 à 3 min ± 0,1 et 2 min ± 0,1, respectivement ($P < 0,001$). L'huile essentielle de *Rosmarinus officinalis* à 100 mg/kg VO augmente de manière significative l'activité locomotrice et induit une hyperexcitabilité et une hypermobilité, par rapport au groupe témoin; ainsi qu'une diminution significative de la durée du temps de sommeil induit par le thiopental de 34 min ± 0,2 à 2 min ± 0,2 ($P < 0,001$).

Par ailleurs, a été déterminée l'activité antibactérienne des fleurs de *Lavandula officinalis* et des feuilles de *Rosmarinus officinalis* contre les microorganismes pathogènes par la

détermination de la concentration minimale inhibitrice. Les concentrations minimales inhibitrices contre trois bactéries à Gram-positif (*Micrococcus luteus, Staphylococcus aureus* et *Bacillus subtilis*), et trois bactéries à Gram négatif (*Klebsiella pneumoniae, Pseudomonas aeruginosa* et *Escherichia coli*) ont été déterminées pour les extraits méthanoliques et aqueux de *Lavandula officinalis* et *Rosmarinus officinalis*. L'extrait aqueux de *Lavandula officinalis* et l'extrait méthanolique de *Rosmarinus officinalis* ont montré antibactérienne plus prononcée que l'extrait méthanolique et l'extrait aqueux, vis-à-vis de tous les microorganismes testés. La concentration minimale inhibitrice de l'extrait aqueux est de 1,56, 1,56, 3,13, 3,13, 6,25 et 6,25 mg/ml, respectivement, contre *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, et *Pseudomonas aeruginosa*, alors que l'extrait méthanolique inhibe avec une CMI de 6,25 mg/ml toutes les souches bactériennes testées Gram-positives et Gram-négatives. En conclusion, ces résultats suggèrent que les extraits méthanoliques et aqueux de *Lavandula officinalis* possèdent des activités sédatives et hypnotiques puissants, qui soutiennent son utilisation traditionnelle pour traiter l'insomnie. En outre, ces résultats suggèrent que l'huile essentielle, l'extrait méthanolique et l'extrait aqueux des tiges et feuilles de *Rosmarinus officinalis* possèdent une activité potentielle psychostimulante, qui pourrait justifier son utilisation en médecine traditionnelle comme stimulant général. De plus, les extraits méthanoliques et aqueux de *Lavandula officinalis* et *Rosmarinus officinalis ont* démontré un potentiel antibactérien qui pourrait constituer une base pharmacologique pour son efficacité thérapeutique contre les maladies infectieuses.

Mots-clés: *Rosmarinus officinalis*; *Lavandula officinalis*; Huiles essentielles; Extrait aqueux; Extrait Méthanolique; GC-MS; Toxicité aiguë; Criblage antibactérien; Concentration minimale inhibitrice; Système nerveux central; Effets sédatifs; Action hypnotique; Activité psychostimulante.

Summary

Leaves of *Rosmarinus officinalis* and the flowers of *Lavandula officinalis* have been used as medicine for treatment of nervous disorders, in traditional Moroccan medicine. We evaluate the central nervous system psychotropic effects of the essential oil, methanolic and aqueous extracts from the leaves of *R. officinalis* and the flowers of *L. officinalis* using a battery of comportamental psychopharmacology tests. The essential oil extracted by hydrodistillation was characterized by means of GC-MS. The intraperitonial administration in mice of essential oil from *L. officinalis* at 300 and 600 mg/kg IP induces strong sedative effects compared to reference substance diazepam in mice, and an hypnotic effects at doses 1000 and 1500 mg/kg. However, the essential oil extracted from *R. officinalis* at the doses 50 and 100 mg/kg, produced no sedative activity significant on the central nervous system. The methanolic extract produced significant sedative effect at the doses of 200, 400, and 600 mg/kg PO, compared to reference substance diazepam (DZP), and an hypnotic effect at the doses of 800 and 1000 mg/kg while the treatment of mice with the aqueous extract at the doses of 200 and 400 mg/kg via oral pathway significantly reduced in both the re-establishment time and number of head dips during the traction and hole board tests.

This study is also aimed at determining the psychostimulants activity of *Rosmarinus officinalis* L. (*lamiaceae*) on central nervous system. The effects of the methanolic and aqueous extracts of the stems and leaves of this plant were investigated in three behavioral models including, observation of animals in free situation, search of stereotypes movement in rats and interaction with thiopental in mouse. We observed that extracts of *R. officinalis* increase the locomotors activity and induced hypermobility and hyperexcitability, as compared to the control group. Treatment with *R. officinalis* methanolic or aqueous extracts at the dose of 200 mg/kg PO, significantly decreased the duration of thiopental-induced sleeping time from 34 min ±0.2 to 3 min ± 0.1 and 2 min ±0.1, respectively ($P<0.001$). We observed that essential oil of *Rosmarinus officinalis at*100mg/kg PO, significantly increased the locomotors activity and induced hypermobility and hyperexcitability, as compared to a control group. Treatment with *Rosmarinus officinalis* essential oil at the dose of 100 mg/kg PO, significantly decreased the duration of thiopental-induced sleeping time from 34 min ± 0.2 to 2 min ± 0.2 ($P<0.001$).

In addition, this study is aimed at determining the antibacterial activity of *Lavandula officinalis* flowers and *Rosmarinus officinalis* leaves against pathogenic microorganisms by determination the minimal inhibitory concentration. Minimum inhibitory concentrations (MICs) against three Gram-positive bacteria (*Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus subtilis*), three Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) were determined for the methanolic and aqueous extracts of *Lavandula officinalis* and *Rosmarinus officinalis*. The aqueous extract of *Lavandula officinalis* and methanolic extract of *Rosmarinus officinalis* showed pronounced antibacterial than the Methanolic extract and aqueous extract, respectively against all of the tested microorganisms. Aqueous extract inhibited with minimal inhibitory concentration of 1.56, 1.56, 3.13, 3.13, 6.25 and 6.25 mg/ml against *Micrococcus luteus*, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*, *and Pseudomonas aeruginosa,* respectively, while the methanolic extract inhibited with minimal inhibitory concentration of 6.25 mg/ml against all tested bacterial strains both Gram-positive and Gram-negative bacteria. In conclusion, these results suggest that the essential oils, the methanolic and the aqueous extracts of *Lavandula officinalis* possess potent sedative and hypnotic activities, which supported its therapeutic use for insomnia. Moreover, these results suggest that, the essential oil, the methanolic and aqueous extracts from stems and leaves of *Rosmarinus officinalis* possess potential psychostimulants activity, this may justifies its use in traditional medicine as general stimulant. In addition, the methanolic and aqueous extracts of *Lavandula officinalis* and *Rosmarinus officinalis* demonstrated activities against certain bacteria, these data provide pharmacological basis for its therapeutic efficacy against infectious diseases.

Keywords: *Rosmarinus officinalis; Lavandula officinalis*; Essential oils; Aqueous extract; Méthanolique extract; GC-MS; Acute toxicity; Antibacterial screening; Minimal inhibitory concentration; Central nervous system; Sedative effects; Hypnotic action; Psychostimulant activity.

ANNEXE

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Research Article

In Vivo Potential Anti-Inflammatory Activity of *Melissa officinalis* L. Essential Oil

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Melissa officinalis L. (Lamiaceae) had been reported in traditional Moroccan medicine to exhibit calming, antispasmodic, and strengthening heart effects. Therefore, this study is aimed at determining the anti-inflammatory activities of *M. officinalis* L. leaves. The effect of the essential oil of the leaves of this plant was investigated for anti-inflammatory properties by using carrageenan and experimental trauma-induced hind paw edema in rats. The essential oil extracted from leaves by hydrodistillation was characterized by means of gas chromatography-mass spectrometry (GC-MS). *M. officinalis* contained Nerol (30.44%), Citral (27.03%), Isopulegol (22.02%), Caryophyllene (2.29%), Caryophyllene oxide (1.24%), and Citronella (1.06%). Anti-inflammatory properties of oral administration of essential oil at the doses of 200, 400 mg/kg p.o., respectively, showed significant reduction and inhibition of edema with 61.76% and 70.58%, respectively, ($P < 0.001$) induced by carrageenan at 6 h when compared with control and standard drug (Indomethacin). On experimental trauma, *M. officinalis* L. essential oil showed pronounced reduction and inhibition of edema induced by carrageenan at 6 h at 200 and 400 mg/kg with 91.66% and 94.44%, respectively ($P < 0.001$). We can conclude that the essential oil of *M. officinalis* L. possesses potential anti-inflammatory activities, supporting the traditional application of this plant in treating various diseases associated with inflammation and pain.

1. Introduction

The varied climate and heterogeneous ecologic conditions in Morocco have favoured the proliferation of more than 42,000 species of plants, divided into 150 families and 940 genuses [1–4]. Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population [3]. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are increasing in both developing and industrialized nations. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some cases), and the cultural, spiritual point of view of people [5–7]. In western developed countries, however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited [8–10]. Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the overprescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over

their medical care. All these make the knowledge of chemical, biological, and therapeutic activities of medicinal plants used as folklore medicine become necessary [11–14].

Generally, the inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischemia, antigen-antibody interaction, and thermal or physical injury. Inflammation is usually associated with pain as a secondary process resulting from the release of analgesic mediators: nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immunosuppressant drugs, which have been used usually in the relief of inflammatory diseases by people around the world for a long time [9].

However, these drugs were often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcers [9]. Recently, many natural medicines derived from medicinal plants were considered as effective and safer for the treatment of various diseases including inflammation and pain [15].

There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration, and granuloma formation represent such components of inflammation [16]. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [17, 18].

M. officinalis L. (Lamiaceae) is a herbal medicine native to the Eastern Mediterranean region and Western Asia. *M. officinalis* has been traditionally used for different medical purposes such as tonic, antispasmodic medicine drug, carminative, diaphoretic medicine drug, surgical dressing for wounds, and sedative/hypnotic, and it is used for strengthening the memory and relief of stress-induced headache [19–21]. In our previous study, we have proven the efficacy of the extract of the essential oil of this plant on central nervous activity [22]. To the best of our knowledge, this is the first study to provide data that the essential oil of the leaves of *M. officinalis* L. evaluated against inflammations. Thus, the aim of this study is to evaluate the anti-inflammatory effect of the essential oil of the leaves of *M. officinalis* L. and, therefore, to determine the scientific basis for its use in traditional medicine in the treatment of inflammation.

2. Materials and Methods

2.1. Plant Material. Fresh leaves of *Melissa officinalis* L. (Lamiaceae) were collected based on ethnopharmacological information from villages around the region Eljadida, middle Morocco in January 2013, with the agreement from the authorities with respect to the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of the Department of Medicinal and Aromatic Plants, National Institute for Agricultural Research, Morocco. A voucher specimen (no. RAB76712) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat.

2.2. Preparation of the Essential Oil. Fresh leaves of *Melissa officinalis* L. were hydrodistilled in Clevenger apparatus for 4 hours to obtain the essential oil with (v/w) yield. The extract was stored in a refrigerator at 4°C [23] and protected against light and heat until use. The essential oil was produced from leaves of *M. officinalis* by hydrodistillation method. Plant materials (100 g) cut into small pieces were placed in distillation apparatus and hydrodistilled for 4 h after the oils were dried over hydrous K_2CO3; they were stored at +4°C until used for GC-MS analysis. The yield of extraction (ratio weight of EO/weight of dry plant) was 0.5% [1–3].

2.3. Phytochemical Analysis of Melissa officinalis L. Essential Oil by Combined Gas Chromatography-Mass Spectrometry (GC-MS). The essential oil was submitted to quantitative analysis in a Hewlett-Packard 575, GC condition: carrier gas N2 (0.5 bar) at flow rate of 1.0 m/min, sample size: 0.2 μL injected, and capillary column (30 m siloxane 5% HP EM). The temperature of the injector and detector was set at 250°C. The oven temperature was programmed from 50°C to 250°C (5 min). The MS was taken at 70 eV. The components of the essential oil were identified by comparison of their mass spectra with those in the Wiley-NIST 7th edition library of mass spectral data. The percentage composition on the oil sample was calculated from GC-MS peak areas [1, 2].

2.4. Animals. Male Wistar rats weighing 180–220 g were used in this study. The animals were obtained from the animal centre of Mohammed V-Souissi University, Medicine and Pharmacy Faculty, Rabat, Morocco. All animals were kept in a room maintained under environmentally controlled conditions of 23 ± 1°C and 12 h light-12 h dark cycle. All animals had free access to water and standard diet. They were acclimatized at least one week before the experiments started. The animals submitted to oral administration of the extracts or drugs were fasted for 18 h before the experiment (water was available). All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509 [1–3, 24, 25].

2.5. In Vivo Anti-Inflammatory Activity. The evaluation of the anti-inflammatory activity of *M. officinalis* L. essential oil was carried out by using two different methods that used chemical stimuli (winter test) [26] and mechanical stimuli (Riesterer and Jacques test) [27] induced paw edema in rats. In both methods, all animals were fasted 18 h before testing and received 5 mL of distilled water by gavages to minimize individual variations in response to the swelling of the paws. The right hind paw (RP) is not treated, and it is taken as control.

2.6. Carrageenan-Induced Rat Paw Edema. The carrageenan-induced paw edema model [9, 26–28] was used to evaluate the anti-inflammatory effect of *M. officinalis* essential oil. The initial paw volume was recorded using an Ugo Basile model LE750 plethysmometer.

Rats groups were orally administered essential oil of *M. officinalis* L. (200 and 400 mg/kg); Indomethacin (10 mg/kg)

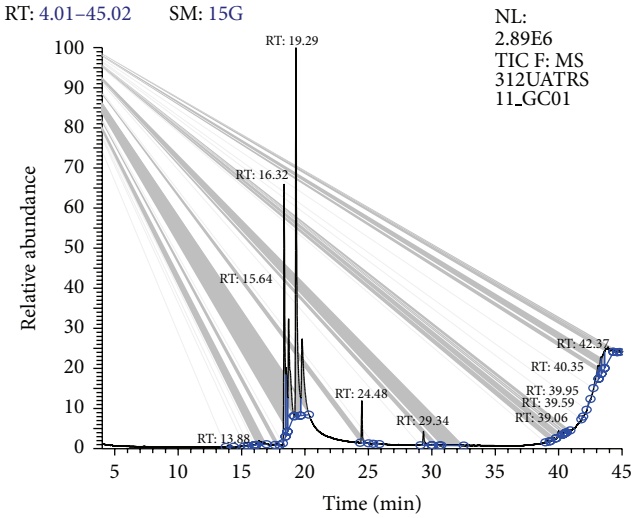

Figure 1: Gas chromatography-mass spectrometry (GC-MS) of *Melissa officinalis* L. essential oil.

was used as reference drug while distilled water (5 mL/kg) was used as negative control. After 60 min had passed, carrageenan (0.05 mL of a 1% w/v solution, prepared in sterile saline) was injected subcutaneously into subplantar region of the left hind paw of each rat. The right hind paw is not treated; it is taken as a witness. One hour 30 minutes, 3 hour and 6 hours after the injection of carrageenan, the paw volumes of each rat were measured. Mean differences of treated groups were compared with the mean differences of the control group. The percentages of inhibition of inflammation were calculated according to the following formula:

$$\%\ \text{of inhibition} = \frac{\text{mean}\left[V_{\text{left}} - V_{\text{right}}\right]_{\text{control}} - \left[V_{\text{left}} - V_{\text{right}}\right]_{\text{treated}}}{\left[V_{\text{left}} - V_{\text{right}}\right]_{\text{control}}} * 100, \quad (1)$$

where V_{left} is the mean volume of edema on the left hind paw and V_{right} is the mean volume of edema on the right hind paw.

2.7. Experimental Trauma-Induced Rat Paw Edema. This assay was determined as described by Riesterer and Jacques test [27]. The test groups of rats were given orally 200 and 400 mg/kg of *M. officinalis* essential oil, the control group received 5 mL/kg of distilled water, and the standard group received the reference drug (Indomethacin 10 mg/kg). One hour after oral administration of different substances dropping a weight of 50 g onto the dorsum of the left hind paw of all animals. The right hind paw is not treated; it is taken as a witness.

The difference volume of two paws was measured and taken as the edema value by using digital plethysmometer LE750 at 1 h 30 min, 3 h and 6 h after induction of inflammation [29]. Mean differences of treated groups were compared with the mean differences of the control group. The percentages of inhibition of inflammation were calculated according to the following formula:

$$\%\ \text{of inhibition} = \frac{\text{mean}\left[V_{\text{left}} - V_{\text{right}}\right]_{\text{control}} - \left[V_{\text{left}} - V_{\text{right}}\right]_{\text{treated}}}{\left[V_{\text{left}} - V_{\text{right}}\right]_{\text{control}}} * 100. \quad (2)$$

2.8. Statistical Analysis. The results are expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) followed by Student's t-test. A value of $P < 0.001$ was considered significant.

3. Results

3.1. Chemical Composition of the Essential Oil. The results obtained by GC-MS analyses of the essential oils of *Melissa officinalis* L. are presented in Figure 1. Thirteen compounds were identified in this essential oil by GC-MS analyses (Figure 1); *M. officinalis* L. contained six major compounds, that is, Nerol (30.44%), Isopulegol (22.02%), Citral (27.03%), Caryophyllene (2.29%), Caryophyllene oxide (1.24%), and Citronella (1.06%) as the main constituents of essential oil of *M. officinalis* L. Nerol and Citral as have been previously reported major chemical components of *M. officinalis* L. [18–22], but Isopulegol has never been reported as the main component of *M. officinalis* L. (Figure 1).

3.2. Carrageenan-Induced Rat Paw Edema. The results of the effect of the *M. officinalis* essential oil on carrageenan-induced edema are shown in Tables 1 and 2. At doses of 200 and 400 mg/kg via oral pathway, *M. officinalis* essential oil exhibited significant ($P < 0.001$) anti-inflammatory activity as compared to the control and standard group (Table 1). At 1 h 30 min, the extract of the essential oil showed similar inhibition of edema by 70.58% and 76.47% at 200 and 400 mg/kg, respectively, as compared to the standard drug Indomethacin (10 mg/kg) by 76.47%. However, at the sixth hour the *M. officinalis* essential oil showed greater inhibition with 61.76% and 70.58% at 200 and 400 mg/kg, p.o., respectively, as compared to reference drug Indomethacin (10 mg/kg) by 52.94% (Table 2).

3.3. Experimental Trauma-Induced Rat Paw Edema. The effect of two doses of the *M. officinalis* essential oil on experimental trauma-induced inflammation is shown in Tables 3 and 4, and the results are comparable to that of the control and standard drug Indomethacin (10 mg/kg, p.o.). The *M. officinalis* essential oil at all DOE levels significantly decreased inflammation induced by experimental trauma (Table 3).

At 200 and 400 mg/kg, p.o., *M. officinalis* essential oil exhibited maximum anti-inflammatory activity of 91.66% and 94.44%, respectively, at the sixth hour (Table 4). This inhibition of edema was significantly similar to that obtained with Indomethacin (10 mg/kg, p.o.) by 91.66% during the same time.

Table 1: Effect of essential oil of *Melissa officinalis* L. on carrageenan-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Mean volume of edema (left paw-right paw) induced by carrageenan (mL)		
		1 h 30 min	3 h	6 h
Control		0.17 ± 0.013	0.26 ± 0.01	0.34 ± 0.023
Indomethacin	10	0.04 ± 0.004*	0.07 ± 0.005*	0.16 ± 0.008*
EOMO	200	0.05 ± 0.009*	0.1 ± 0.011*	0.13 ± 0.01*
EOMO	400	0.04 ± 0.01*	0.09 ± 0.008*	0.1 ± 0.013*

Values are expressed as mean ± S.E.M. ($n = 6$), p.o.: oral route, n: number of animals per group, EOMO: essential oil of *Melissa officinalis* L., $^*P < 0.001$ statistically significant relative to the control and reference drug (Indomethacin).

Table 2: Percentage of inhibition of inflammation of essential oil of *Melissa officinalis* L. using carrageenan-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Percentage of inhibition of inflammation induced by carrageenan (%)		
		1 h 30 min	3 h	6 h
Indomethacin	10	76.47	73.07	52.94
EOMO	200	70.58	61.53	61.76
EOMO	400	76.74	65.38	70.58

$N = 6$; these results compared with standard drug (Indomethacin, 10 mg/kg, p.o.) were administered by the oral route.

4. Discussion

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilizations. Driven by their intuition and their sense of observation, they were able to find answers to their health problems in the plant environment [4–6]. Recently, the search for novel pharmacotherapy from medicinal plants for inflammation diseases has progressed significantly owing to their less side effects and better tolerability. Aromatherapy is currently used worldwide in the management of chronic pain [1–5]. Oil compositions of *M. officinalis* L. have already been reported [18–22]. Thus, it has been shown that Nerol, Isopulegol, Citral, Caryophyllene, Caryophyllene oxide, and Citronella account for 80% of *M. officinalis* L. essential oils, but in our study, these compounds represent 84.08%. These differences in chemical composition of essential oil may be due to both developmental and environmental factors that influence plant metabolism. We analyzed the effects of different doses of essential oil from leaves of *M. officinalis* L. for their anti-inflammatory activity.

Following oral administration of *M. officinalis* L. extract at the doses of 300 and 2000 mg/kg, p.o., no toxicity and no significant changes in the body weight between the control and treated groups were demonstrated at these doses. This result indicates that the LD_{50} was higher than 2000 mg/kg. These results were previously reported by Bounihi et al. [22].

In the present study, anti-inflammatory effect of the essential oil of *M. officinalis* L. was investigated after subplantar injection of carrageenan and experimental trauma in rat paw.

The method of carrageenan induced paw edema is the most widely used to evaluate the anti-inflammatory effect of natural products. Edema formation due to carrageenan in the rat paw is the biphasic event [30]. The initial phase, which occurs between 0 and 2.5 h after the injection of the phlogistic agent, has been attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability [30–32]. It has been reported that histamine and serotonin are mainly released during the first 1.5 h while bradykinin is released within 2.5 h after carrageenan injection [32, 33]. While the late phase is associated with the release of prostaglandins and may be occurs from 2.5 h to 6 h post-carrageenan injection [33, 34].

In the inflammatory response there is an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines (interleukins and tumors necrosis factor-α (TNF-α)). At the same time, there is also an induction of the activity of several enzymes (oxygenases, nitric oxide synthases, and peroxidases) as well as the arachidonic acid metabolism. In the inflammatory process there is also the expression of cellular adhesion molecules, such as intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) [34].

The carrageenan-induced hind paw edema in rat is known to be sensitive to cyclooxygenase inhibitors, but not to lipooxygenase inhibitors, and has been used to evaluate the effect of nonsteroidal anti-inflammatory drugs which primarily inhibit the cyclooxygenase involved in prostaglandins synthesis. It has been demonstrated that the suppression of carrageenan-induced inflammation after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents [33].

M. officinalis essential oil at doses of 200 and 400 mg/kg, p.o., reduced and inhibited significantly ($P < 0.001$) the edema in the early and late phases of inflammation induced by carrageenan. In the experimental trauma-induced edema in rats, the extract was also reduced and inhibited significantly ($P < 0.001$) the edema in the different phases of inflammatory response. Based on the results obtained, *M. officinalis* essential oil was able to effectively inhibit the increase in paw volume during the phases of inflammation. This indicates that the extract of the *M. officinalis* essential oil, has a significant anti-inflammatory activity perhaps by inhibiting the release of the inflammatory mediators;

Table 3: Effect of essential oil of *Melissa officinalis* L. on experimental trauma-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Mean volume of edema (left paw-right paw) induced by experimental trauma (mL)		
		1 h 30 min	3 h	6 h
Control		0.153 ± 0.005	0.22 ± 0.021	0.36 ± 0.015
Indomethacin	10	0.04 ± 0.009*	0.03 ± 0.01*	0.03 ± 0.01*
EOMO	200	0.085 ± 0.005*	0.05 ± 0.007*	0.03 ± 0.005*
EOMO	400	0.068 ± 0.004*	0.04 ± 0.004*	0.02 ± 0.005*

Values are expressed as mean ± S.E.M. ($n = 6$), EOMO: essential oil of *Melissa officinalis* L., * $P < 0.001$ statistically significant compared to the control and reference drug (Indomethacin).

Table 4: Percentage of inhibition of inflammation of essential oil of *Melissa officinalis* L. using experimental trauma-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Percentage of inhibition of inflammation induced by experimental trauma (%)		
		1 h 30 min	3 h	6 h
Indomethacin	10	65.81	86.36	91.66
EOMO	200	44.44	77.27	91.66
EOMO	400	55.55	81.81	94.44

$N = 6$; these results compared with standard drug (Indomethacin, 10 mg/kg, p.o.) were administered by the oral route.

serotonin and histamine also suppressed prostaglandin and cytokine.

Carrageenan and experimental trauma-induced paw edema in rats are a suitable experimental animal model to evaluate the antiedematous effect of diverse bioactive compounds such as plant extracts and essential oils [35–38]. If this method allows screening the anti-inflammatory of samples, very little information is given about its mechanism.

The exact mechanism of the anti-inflammatory activity of the essential oil used in the present study is unclear. However, other investigators have reported that the *M. officinalis* essential oil contains Nerol, Citral, Caryophyllene and Citronella as the main components. The same phytochemicals also investigated in this extract by us [22]. Citral constitutes the main components of *Cymbopogon citrates* stapf essential oil. This EO is revealed to be capable to suppress IL-1β and IL-6 in LPS-stimulated peritoneal macrophages of normal mice [37, 38]. Whether some essential oils are able to inhibit the production of proinflammatory cytokines such as TNF-α, some of them, their main components (Citral, geraniol, citronellol, and carvone), can also suppress TNF-α-induced neutrophil adherence responses [38]. Another work [39] revealed that Citral inhibited TNF-α in RAW 264.7 cells stimulated by lipopolysaccharide. According to these authors, the *M. officinalis* essential oil we used can be associated with anti-inflammatory activity at least due to the presence of the Citral as the main component.

Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the anti-inflammatory activity. We can conclude that the essential oil of *M. officinalis* L. possesses potential anti-inflammatory activities, supporting the traditional application of this plant in treating various diseases associated with inflammation and pain.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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IN VITRO ANTIBACTERIAL ACTIVITY OF THE METHANOLIC AND ETHANOLIC EXTRACT OF *INULA VISCOSA* USED IN MOROCCAN TRADITIONAL MEDICINE

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ABSTRACT

Inula viscosa (*Dittrichia viscosa* L.) had been reported in traditional medicine, to exhibit antimicrobial properties. Therefore, this study is aimed at determining the antibacterial activity of *I. viscosa* leaves and flowers against pathogenic microorganisms by determination the minimal inhibitory concentration and to serve as criteria to recommend the ethno pharmacological uses of the plant. Plant leaves and flowers were dried, powdered and extracted by cold maceration with methanol and ethanol for 48h. The extracts were screened against 24h broth culture of bacteria seeded in Muller Hinton Agar at concentration 5000 to 19µg/ml in sterile distilled water and incubated at 37°C, for 18h and measuring the inhibition zone diameter (IZD). Minimum inhibitory concentrations (MICs) against Gram-positive bacteria and Gram-negative bacteria were determined for the methanolic and ethanolic extracts of *Inula viscosa*. The ethanolic extract showed pronounced antibacterial than the aqueous extract against all of the tested microorganisms. Methanolic extract inhibited with minimal inhibitory concentration of 78, 78, 156, 156, 156, 312, 312, 312, 312, 312, 312, 625,

625, 625, 1250 and 2500µg/ml against *Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli1, Escherichia coli17, Klebsiella oxytoca, Escherichia coli12, Escherichia coli15, Escherichia coli16, Providencia stuartii*, *Salmonella paratyphi A*, *Serratia marcescens*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Salmonella paratyphi B*, *Citrobacter freundii*, *Escherichia coli14, Escherichia coli13,* respectively, while the methanolic extract inhibited with minimal inhibitory concentration of 19, 39, 39, 39, 39, 78, 78, 78, 78, 78, 78, 78, 156, 156, 156, 156, 156, 156, 312 and 625µg/ml against *Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli1, Escherichia coli17, Salmonella paratyphi A*, *Escherichia coli12, Escherichia coli15, Acinetobacter baumannii*, *Salmonella paratyphi B*, *Citrobacter freundii*, *Providencia stuartii*, *Escherichia coli14, Escherichia coli13, Klebsiella oxytoca, Pseudomonas aeruginosa, Edwardsiella tarda, Streptococcus feacalis*, respectively. In conclusion, the extract of *Inula viscosa* contains some major bioactive compounds that inhibit the growth of microorganisms thereby proving very effective as alternative source of antibiotics. Our results support the ethno-pharmacological uses of this plant in folk medicine and could provide useful data for the utilization of this extracts in pharmaceutical, cosmetic and food industries.

Keywords: *Inula viscosa*, minimal inhibitory concentration, antibacterial screening, bacterial strains, medicinal plant.

INTRODUCTION

The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants; divided into 150 families and 940 geniuses [1-4]. Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population [3]. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations[8]. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some cases) and the cultural, spiritual point of view of the people of the countries [5-7]. In western developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that

the effective life span of any antibiotic is limited [8-10]. Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these make the knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine become necessary [11-14]. Some studies have reported that the extract of *Inula viscosa* have a number of pharmacological activities, such as anti-inflammatory, antidiabetic, antipyretics, antiseptic, for treating gastro duodenal disorders [27-35].

Inula viscosa is a common dense, evergreen, aromatic shrub grown in many parts of the world. The fresh and dried flowers are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods. They are extensively used in cooking, and a distinct mustard smell gives off while they are burned, therefore, they often are used to flavor foods while barbecuing. Essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [15-17]. Particularly, the antimicrobial and antivirus activities of plant oils and extracts have formed the basis of applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [8]. Because of the possible multiple resistances and side effects of the synthetic antimicrobial, increasing attention has been directed towards natural antimicrobial [9-10]. There are many studies on the antimicrobial activity of secondary metabolites in recent years, but to our knowledge, fewer comparative studies on antimicrobial activity of *I. viscosa* methanolic and aqueous extracts have been reported [25-32].

To the best of our knowledge, this is the first study to provide data that the methanolic and ethanolic extracts of *Inula viscosa* evaluated against a wide range of bacteria. Thus, the aim of this study is to evaluate the toxicity and antimicrobial activity of *I. viscosa* (*Dittrichia viscosa* L.) methanolic and ethanolic extracts, and to, therefore, determine the scientific basis for its use in traditional medicine in the treatment of infection diseases.

MATERIALS AND METHODS

Plant material

Inula viscosa was collected based on ethnopharmacological information, from villages around the region Ain Taoujtat, middle Morocco on April and August 2010, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB11577) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco.

Preparation of methanolic and ethanolic extracts

Leaves and flowers of *I. viscosa* (*Dittrichia viscosa* L.) were successively extracted with methanol and methanol by maceration at room temperature (25°C) over period of 48h. 100 g of plant material and one liter of each extracts of methanol and ethanol were used in the extraction. Methanol and ethanol containing the extract were then filtered through Whatman paper and the solvent was vacuum-distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for 2h to ensure the removal of any residual solvent. Final extracts were in percentage dry weight 44.95% and 29.9, respectively; these methanolic and ethanolic extracts were kept in deep freeze at -20°C until use [1-4].

Antibacterial Activity Test

Bacterial strains: This study was made on seven strains of *Escherichia coli,* bacteria often found in the diarrhea and urine of children from 0 to 5 years old. Among these seven strains, thirteen are referenced strains namely *Klebsiella pneumoniae, Staphylococcus aureus, Edwardsiella tarda, Providencia stuartii, Salmonella paratyphi B, Citrobacter freundii, Salmonella paratyphi A, Serratia marcescens, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella oxytoca, Streptococcus feacalis* and *Proteus vulgaris*: A strain was retrieved from well and another from hospital. The cultures of bacteria were maintained in their appropriate agar slants at +4°C throughout the study and used as stock cultures. The strains used in this work are widely encountered in various diseases in humans and multiple antibiotic-resistant bacteria. The bacteria were obtained from the **L**aboratory of **M**icrobiology, Makoudi Private Medical Laboratory, Meknes, Morocco (Table 1).

Table 1: Origin of Sampling of *Escherichia coli.*

Strains	Age of Man (years)	Age of woman (years)	Origin of Sampling
E.coli$_1$	35	_	Urine
E.coli$_2$	3month	_	Urine
E.col$_3$	_	30	Vaginal
E.coli$_4$	_	23	Urine
E.coli$_5$	_	60	Urine
E.coli$_6$	60	_	Urine
E.coli$_7$	23	_	Urine

Determination of antibacterial activity by the paper disc diffusion method

The methanolic and ethanolic extracts of *Inula viscosa* were tested for antibacterial activity by the paper disc diffusion method. Molten (45°C) sterile Muller Hinton Agar (10ml) in a flask was inoculated with a broth culture (1%, containing 10^6-10^7 cfu/ml) of the respective bacterial strains and poured over plates containing 10ml Muller Hinton Agar in sterile 9cm Petri dishes. Fifty microliters of dilutions of the methanolic and ethanolic extracts were pipette on sterile filter paper disc (Whatman No.1. 5mm in diameter), which were allowed to dry in an open sterile Petri dishes in a biological safety cabinet with vertical laminar (Nuaire Petri dishes Laminar Flow Products, USA). 5000 to 19µg/ml of the methanolic and ethanolic extracts solutions were applied to the discs. Discs were placed on the surface of the inoculated plates and incubated at 37°C for 18h. Zones of inhibition of microbial growth around the paper disc containing the extracts were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (mm) from the outside margin of the paper disc to the initial point of the microbial growth. All analyses were applied in triplicate [15-22]. Discs impregnated with sterile distilled water served as negative control. Penicillin and Ampicillin which purchased from Sigma (St. Louis, USA) was used as a positive control [23-24].

Determination of Minimum Inhibitory Concentration (MIC) and Bactericidal Minimal Concentration (BMC)

Test strains were suspended in Muller Hinton Agar (MHA). The suspension was adjusted spectrophoto-metreically to match the turbidity of a McFarland 0.5 scale (*i.e.* to give a final density of 10^7cfu/ml). The viability indicator MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5

diphenyltetrazolium bromide, Sigma, Aldrich) and the quick microplates method were used for the determination of the Minimum Inhibitory Concentration (MIC) and Bactericidal Minimal Concentration (BMC) [20-28]. Serial dilutions of extracts were dissolved in distilled water and made in a concentration range from 19µl/ml to 5000µl/ml in sterile test tubes. Each test tube was inoculated with 50µl from each various dilutions of the methanolic and aqueous extracts of *I. viscosa* were added to 5ml of Muller Hinton Agar both in tubes containing 107cfu/ml of live bacterial cells. The tubes were then incubated under optimal conditions at 37°C for 18h. After incubation, as an indicator of bacterial growth 1mg/ml of MTT was added to each well, after incubation periods ranging from 3 to 5h at 37°C, control without plant extracts with MTT and bacterial inoculums were used as negative control. The bacterial suspension changed to blue when bacterial growth occurred. The highest dilution (lowest concentration), showing no visible growth was regarded as the MIC. Cell suspension (0.1ml) from the tubes showing no growth was sub cultured on Muller Hinton Agar plates in triplicate to determine if the inhibition was reversible or permanent. All tests were performed in triplicate.

Statistical analysis

Results of the research were tested for statistical significance by one way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The methanolic and ethanolic extracts of *Inula viscosa* were evaluated for antimicrobial activity against Gram positive and Gram negative bacteria. *Inula viscosa* methanolic extract was found to be the most active against all of the bacterial strains. The inhibition diameters vary from 14 to 60 mm according to this diffusion method, an extract is regarded as active when it induces an inhibition zone greater than or equal to 10 mm. The methanolic extract of leaf has revealed to be the most active by inducing the Diameters (60, 57, 56 and 55 mm) on *Staphylococcus aureus, Klebsiella pneumoniae, E.coli6 and Salmonella Paratyphi A,* respectively. The strains *Klebsiella pneumoniae and Staphylococcus aureus* are shown the greatest sensitivity to various extracts ($P<0.001$) (Figure 1). The ethanolic extract of leaf has revealed active by inducing the Diameters (43, 49, 35 and 40 mm) on *Klebsiella pneumoniae, Staphylococcus aureus and Providencia stuartii, E.coli1* respectively, representing the most sensitive strains. But ethanolic extract of flowers has revealed active by inducing the Diameters (50, 47, 40 and 32 mm) on *Klebsiella pneumoniae, Staphylococcus aureus,*

Serratia marcescens and E.coli$_{6}$, respectively, represents the most sensitive strains (*P<0.01*) (Fig. 1).

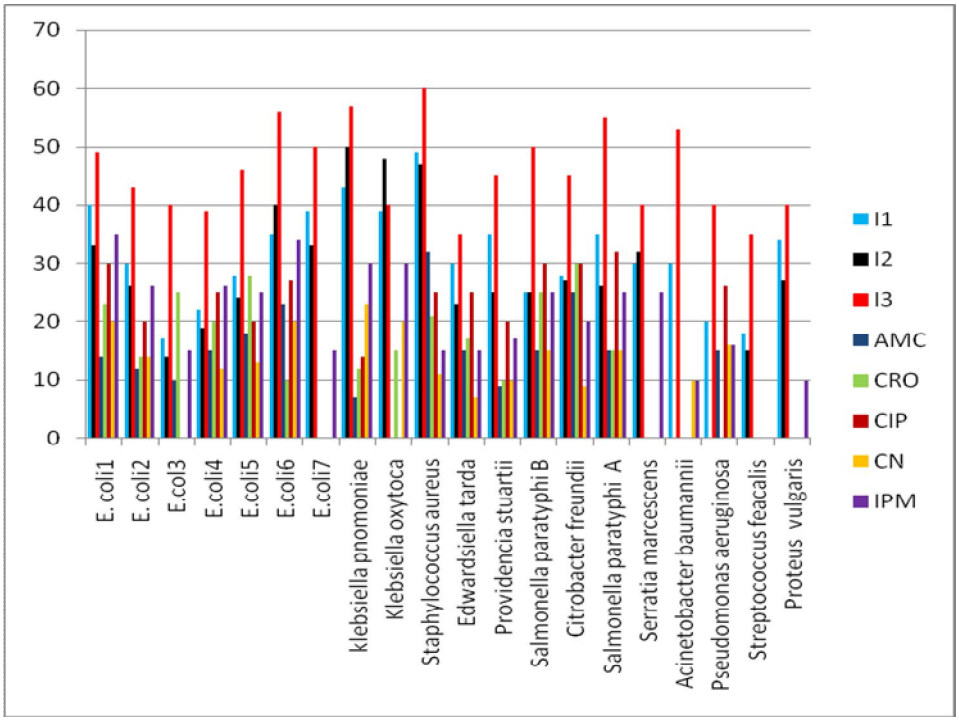

Figure 1: The average inhibition zones for all tested germs under the different concentrations of extracts (IZD in mm).

The results of the paper disc diffusion support and extend previous finding that *I. viscosa* extracts contains numerous biologically active compounds and some of these have been frequently used in folk medicine for their antibacterial properties. In addition, the MIC values support the finding of the paper disc diffusion method (Table 2 and 3). The biological activity of *I. viscosa* against the tested bacteria could be attributed to the presence of flavonoids; phenolic acids [32-37]. Biologically active components are believed to disturb permeability of the cytoplasm membrane and thereby facilitate the influx of antibiotic [23-26] .The results presented in this report highlight the potential of *Inula viscosa* extracts as a source of antibiotic resistance modifying compounds. The MICs for the *I. viscosa* methanolic extract ranged from 19 to 312 µg/ml for all test microorganisms (*P>0.001*), while MICs for ethanolic extract was ranged from 39 to 625 µg/ml (*P<0.01*) (Table 2). These differences in the susceptibility of the test microorganisms to the test samples could be attributed to variation in the rate of samples' penetration through the cell wall and cell membrane structures [1-7]. In general, the methanolic extract showed greater antimicrobial activity than ethanolic extract (Table 2).

Table 2: Determination of Minimum Inhibitory Concentration (MIC, µg/ml), Minimum Bactericidal Concentration (MBC, µg/ml) and report MBC/MIC of the methanolic and ethanolic extracts.

Germes	MIC				MBC				MBC / MIC			
	I1	I2	I_3	CIP	I_1	I_2	I_3	CIP	I_1	I_2	I_3	Interpretation
E. coli$_1$	156±1.2	625±0.1	39±0.0	240±0.0	156±0.1	625±0.0	78±0.0	480±0.0	1	1	2	Bactericidal
E. coli$_2$	312±0.0	1250±0.0	78±0.2	1950±0.0	312±0.0	1250±0.1	78±0.0	1950±0.0	1	1	1	Bactericidal
E. coli$_3$	2500±0.1	2500±0.3	156±0.0	_	2500±0.0	2500±0.0	156±0.0	_	1	1	1	Bactericidal
E. coli$_4$	1250±0.0	1250±0.2	156±0.2	970±0.1	1250±0.2	1250±0.1	156±0.1	970±0.2	1	1	1	Bactericidal
E. coli$_5$	312±0.1	1250±0.0	78±0.2	1950±0.0	312±0.0	1250±0.0	78±0.0	1950±0.0	1	1	1	Bactericidal
E. coli$_6$	312±0.0	156±0.0	19±0.1	61±0.3	312±0.0	312±0.1	39±0.0	61±0.0	1	2	2	Bactericidal
E. coli$_7$	156±0.2	625±0.0	39±0.1	_	312±0.1	625±0.0	39±0.0	_	2	1	1	Bactericidal
Klebsiella pneumoniae	78±0.0	78±0.0	39±0.0	3900±0.0	78±0.0	78±0.2	39±0.0	3900±0.0	1	1	1	Bactericidal
Klebsiella oxytoca	156±0.2	78±0.1	156±0.1	_	156±0.2	78±0.1	156±0.0		1	1	1	Bactericidal
Staphylococcus aureus	78±0.0	78±0.0	19±0.0	970±0.0	78±0.0	78±0.0	19±0.0	970±0.1	1	1	1	Bactericidal

Edwardsiella tarda	312±0.0	1250±0.0	312±0.0	970±0.2	625±0.0	1250±0.3	625±0.2	970±0.0	2	1	1	Bactericidal
Providencia stuartii	312±0.1	1250±0.1	78±0.2	1950±0.0	312±0.0	1250±0.2	78±0.0	1950±0.1	1	1	1	Bactericidal
Salmonella paratyphi B	625±0.0	1250±0.0	78±0.0	240±0.0	625±0.0	1250±0.0	78±0.1	480±0.2	1	1	1	Bactericidal
Citrobacter freundii	625±0.0	1250±0.0	78±0.0	240±0.0	625±0.0	1250±0.0	156±0.0	480±0.0	1	1	2	Bactericidal
Salmonella paratyphi A	312±0.0	1250±0.0	39±0.2	240±0.0	312±0.0	1250±0.0	39±0.3	240±0.1	1	1	1	Bactericidal
Serratia marcescens	312±0.2	625±0.0	156±0.0	_	625±0.0	625±0.0	156±0.0	_	2	1	1	Bactericidal
Acinetobacter baumannii	312±0.1	_	78±0.0	_	625±0.0	_	78±0.2	_	2	_	1	Bactericidal
Pseudomonas aeruginosa	625±0.1	_	156±0.2	61±0.0	1250±0.2	_	312±0.2	61	2	_	2	Bactericidal
Streptococcus feacalis	2500±0.0	2500±0.0	625±0.0	_	2500±0.1	2500±0.0	625±0.1	_	1	1	1	Bactericidal
Proteus vulgaris	312±0.0	1250±0.0	156±0.1	_	312±0.0	1250±0.0	156±0.0	_	1	1	1	Bactericidal

Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive Control (CIP, Ciprofloxacine).

Taking the least IZD of the standard (Ciprofloxacin) as the breaking point, most of the extracts passed the breaking point (Table 2 and 3).

Table 3: Classification of extracts according Aligiannis *et al* .[34]

MIC	I_1	I_2	I_3
Strong inhibition: MIC lower than 500µg/ml	*E1, E2, E5, E6, E7* *k. pneumoniae* *K. oxytoca* *S. aureus* *S. paratyphi A* *A .baumannii* *C. freundii* *P. stuartii* *S. marcescens* *P. vulgaris* *E. tarda*	*S. aureus* *k. pneumoniae* *E. coli*$_{6,}$ *K. oxytoca*	*E1, E2, E3, E4, E5, E6 E7* *k. pneumoniae* *K. oxytoca* *S. aureus* *S. paratyphi A* *S. paratyphi B* *A .baumannii* *C. freundii* *P. stuartii* *S. marcescens* *P. aeruginosa* *P. vulgaris* *E. tarda*
Moderate inhibition: MIC varies from 600µg/ml to 1500 µg/ml	*P. aeruginosa* *S. paratyphi B* *E. coli*$_4$	*E1, E7, E2, E*$_{4,}$ *E5* *S. marcescens* *P.vulgaris* *E. tarda* *P. stuartii* *S. paratyphi B* *C. freundii* *S. paratyphi A*	*S. feacalis*
weak inhibition: MIC greater than 1600µg/ml	*E. coli*$_3$ *S. feacalis*	*E. coli*$_3$ *S. feacalis*	

E: E. coli

It is quite difficult to attribute the antimicrobial effect of an extract to one or a few active principles, because extracts always contain a mixture of different chemical compounds [5-7]. In addition to the major components, also minor components may make a significant contribution to the antimicrobial activity of extracts. Monoterpenes and terpinenes have also shown antimicrobial properties that appear to have strong to moderate antibacterial activity against Gram positive bacteria [35-39]. In most of the cases, the bactericidal action is considered the MBC/MIC ratio. The action of the whole extract is bactericidal against all strains. Aligiannis and colleagues [34] have proposed a classification of extracts of plant material on the basis of MIC results, as shown in table 3. Thus, according to this classification, there is a very strong inhibition with methanolic extract of *Inula viscosa* on all strains (Table 3).

Following the results above, we could infer that the antimicrobial activity of *I. viscosa* methanolic and ethanolic extracts is the synergistic effect of their compositions. It provided evidence that *I. viscosa* methanolic and ethanolic extracts may become the potential natural antimicrobial in the field of food and pharmaceutical industries.

CONCLUSION

The data indicate that, the methanolic and ethanolic extracts of the *Inula viscosa* inhibit efficaciously some resistant bacterial strains which make serious sanitary problems worldwide and confirm that there is a good correlation between the reported traditional uses of the plant for infectious diseases. This indicates that this plant may be useful for developing alternative compounds to treat infections caused by these antibiotic resistant pathogens. According to these results, it is possible to conclude that *Inula viscosa* had a strong and a broad spectrum of antibacterial activity.

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Research Article

Sedative and Hypnotic Activities of the Methanolic and Aqueous Extracts of *Lavandula officinalis* from Morocco

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We evaluate the sedative and hypnotic activities of the methanolic and aqueous extract of *Lavandula officinalis* L. on central nervous system (CNS). In this study, the effect of the methanolic and aqueous extracts of this plant was investigated in a battery of behavioural models in mice. Stems and flowers of *Lavandula officinalis* L. have several therapeutic applications in folk medicine in curing or managing a wide range of diseases, including insomnia. The methanolic extract produced significant sedative effect at the doses of 200, 400, and 600 mg/kg (by oral route), compared to reference substance diazepam (DZP), and an hypnotic effect at the doses of 800 and 1000 mg/kg while the treatment of mice with the aqueous extract at the doses of 200 and 400 mg/kg via oral pathway significantly reduced in both the reestablishment time and number of head dips during the traction and hole-board tests. In conclusion, these results suggest that the methanolic and aqueous extracts of *Lavandula officinalis* possess potent sedative and hypnotic activities, which supported its therapeutic use for insomnia.

1. Introduction

Morocco is fortunate to have such varied climate that almost any medicinal plant can grow. The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants, divided into 150 families and 940 genuses [1–3]. Insomnia defined as persistent difficulty in falling or staying a sleep that affects function can induce significant psychological and physical disorder. Sedatives are drugs that decrease activity and have a calming, relaxing effect. At higher doses, sedatives usually cause sleep. Drugs used mainly to cause sleep are called hypnotics. The difference between sedatives and hypnotics, then, is usually the amount of the dose; lower doses have a calming effect and higher doses cause sleep [4]. Recent studies have shown that herbal drugs exert good sedative and hypnotic effect on the central nervous system [4–6]. In recent years, *Lavandula officinalis* flowers exhibit such various biological and pharmacological activities as anti-tumour, anti-inflammatory, antihistaminic, antidiabetic, and antimicrobial activity and modulating the central nervous system [2, 7–11]. The aim of this experiment is to evaluate the sedative and hypnotic activities of *Lavandula officinalis* methanolic and aqueous extract, and to, therefore, determine the scientific basis for its use in traditional medicine in the management of central nervous system disorders.

2. Materials and Methods

2.1. Plant Material. Stems and flowers of *Lavandula officinalis* L. were collected based on ethnopharmacological information from the villages around the region Rabat-Sale-Zemour-Zaers, with the agreement from the authorities and

respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (N°256) was deposited in the Herbarium of Botany Department of the Scientific Institute of Rabat.

2.2. Preparation of Extract. Stems and flowers of *Lavandula officinalis* were successively extracted with methanol by maceration at room temperature (25°C) over period of 48 hours. 500 g of plant material and one litre of methanol were used in the extraction. Methanol containing the extract was then filtered through Whatman paper and the solvent was vacuum distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for two hours to ensure the removal of any residual solvent (lyophilisation). Final extract was a dark green powder in percentage dry weight 21.8%. For the aqueous extract, 500 g of plant material was extracted by infusion boiled water (500 mL) for three days. The respective aqueous extracts were separated from its residues by gravity filtration. The final crude extract was obtained as yellow greasy powder in percentage from dry weight (15.7% d.w). These extracts were kept in deep freeze at −20°C until use.

2.3. Animals. Male Swiss mice (20–25 g) (Iffa-credo, France) were used in pharmacological tests and females of the same strain in the LD_{50} calculation. The animals were fed *ad libitum* with standard food and water except when fasting was required in the course of the study. The animals were acquired from the animal experimental centre of Mohammed V Souissi University, Medicine and Pharmacy Faculty, Rabat.

2.4. Acute Toxicity. Median lethal dose (LD_{50}) values were determined as described by Litchfield and Wilcoxon [12]. Seven groups of mice of both sexes ($n = 10$, 5 males and 5 females) received or not single oral doses at different concentrations (500, 1000, 1500, 2000, 3000, and 5000 mg/kg, p.o.). The control group received only the water or saline solution. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 6 h and at 24 h time interval to detect any eventual side effects. The number of animals, which died during this period, was expressed as percentile. The LD_{50} of the extract were estimated by the p.o. route using the procedure reported by Litchfield and Wilcoxon; the method estimated the dose of the extract that would kill 50% of a reduced sample of animals by a given route. In a first phase, the extract was given to ten mice per group at doses of 500 and 1000 mg/kg; when no mortality was observed, the doses were increased to 1500, 2000, 3000, and 5000 mg/kg. Mice were kept under observation for 14 days to register possible mortality, their weights were registered, and at the end of the study they were sacrificed for macroscopic tissue examination [13], and the LD50 was determined by probit test using death percent versus doses log [14–16]. Of note, drugs used as control were given to mice in similar conditions.

2.5. Drugs. All drugs and extracts were freshly prepared on the day of the experiments. A control group received distilled water (10 mL/kg, p.o.) as vehicle. Diazepam (3 mg/kg, i.p., a conventional sedative) and thiopental (60 mg/kg, i.p., a conventional hypnosis) were used as positive control.

2.6. Pharmacological Evaluations. The activity of methanolic and aqueous extract from *Lavandula officinalis* on the central nervous system was then studied, using a battery of behavioral tests used in psychopharmacology. We analyzed the effect of different doses of the methanolic extracts (100, 200, 400, and 600 mg/kg, p.o.) and aqueous extracts (100, 200, and 400 mg/kg, p.o.) from Lavandula officinalis for their sedative and hypnotic activities. For testing sedative effect, the effect of extract on mice was qualified in one of the following tests.

2.7. Traction Test. Mice were individually suspended by anterior limbs to a wire stretched horizontally. Abnormal mice that fail to make a reestablishment at least one of its posterior limbs to reach the wire are considered as subject under a sedative action. When the animals perform normal reestablishment immediately, the reaction is known as positive; other wise, the reaction is called negative; also, the behaviours of animals were recorded during the period of the experiment [17, 18].

2.8. Fireplace Test. The apparatus used for this test consist of a vertical glass tube 30 cm in length. Mice were individually placed vertically in the glass test tube, a normal mouse typically attempts to escape in thirty seconds, and the mice considered as subject to the sedative effect when performing the rise of cylinder greater than 30 sec [19].

2.9. Hole-Board Test. Mice were individually placed in the centre of a perforated board, and the number of head dips was registered during a 5 min. The perforated board test was made by using a wood floor board, 40 cm × 40 cm × 25 cm, in which evenly spaced holes were made. The number of explored holes provide a measure of the number of head dips [20, 21].

2.10. Thiopental-Induced Sleep in Mice. Thiopental (a sub-hypnotic dose) 60 mg/kg was injected i.p. 30 min after administration of methanolic and aqueous extracts. The mice were treated with different doses of methanolic and aqueous extracts (800, 1000 mg/kg, p.o., $n = 5$), the control group ($n = 5$) was treated with distilled water (10 mL/kg, p.o.), and positive control group ($n = 5$) was administrated with diazepam (3 mg/kg, i.p.), respectively. The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex [5, 22].

2.11. Statistical Analysis. The statistical analysis was done using ANOVA. The results with $P < 0.05$ were considered significant. The data are expressed as mean ± SD.

3. Results

3.1. Acute Toxicity of Lavandula officinalis Methanolic and Aqueous Extracts. Following oral administration of *Lavandula officinalis* extract at the doses of 500, 1000, 1500, 2000, 3000, and 5000 mg/kg, p.o., no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at these doses. This result indicates that, the LD_{50} was higher than 5000 mg/kg.

3.2. Sedative Activity of the Methanolic and Aqueous Extract on the Central Nervous System (CNS). The results of psychotropic effects of methanolic and aqueous extracts were expressed by comparison with control groups. Pharmacological tests were then performed at nontoxic doses (i.e., 100, 200, 400, and 600 mg/kg, p.o.), for the methanolic extract and (100, 200, and 400 mg/kg, p.o.), for the aqueous extract.

3.3. Traction Test. The methanolic extract of *Lavandula officinalis* given by oral route at 100 mg/kg did not significantly alter the reestablishment time; all animals performed normal reestablishment immediately ($P > 0.05$). However, the extract at the dose of 200 mg/kg produced significant sedative effect on the central nervous system (CNS) as indicated by the relatively high time for the reestablishment of the mice (Table 1). By increasing the doses to 400 and 600 mg/kg, the average reestablishment time was increased. The reestablishment time was notably higher than control group ($P < 0.001$) (Table 1). For the aqueous extract, after oral administration at the dose of 100 mg/kg, all animals performed normal reestablishment time (notably decreased the reestablishment time). This indicates that this extract produced no significant sedative effect on mice behavior at this dose ($P > 0.05$) (Table 2). By increasing the doses to 200 and 400 mg/kg, the reestablishment time was increased; the mice fail to make a reestablishment immediately ($P < 0.001$). So, the aqueous extract of *Lavandula officinalis* produced significant sedative effect at the doses of 200 and 400 mg/kg p.o. (Table 2). In addition, the dose of 100 mg/kg of both extracts did not decreased the reestablishment time.

3.4. Fireplace Test. Animal treated with the methanolic extract of *Lavandula officinalis* at the dose of 100 mg/kg via oral route do not show loss of initiative and curiosity. By increasing the doses to 200, 400, and 600 mg/kg, all mice lose initiative and curiosity; that is, animal did not attempt to mount the tube for escape ($P < 0.01$) (Table 1). While, the aqueous extract, at the doses of 100 mg/kg, produced no sedative effect, at the doses of 200 and 400 mg/kg p.o., all animals treated showed loss of initiative and curiosity ($P < 0.001$) (Table 2).

3.5. Hole-Board Test. In the hole-board test, a significant reduction in the number of head dips at the doses of 200, 400, and 600 mg/kg by oral route administration; with the exception at the dose of 100 mg/kg, the methanolic extract did not reduce the number of head dips. However, animal treated with the aqueous extract at the doses of 100 mg/kg did not reduce the number of head dips ($P > 0.05$). By increasing the doses to 200 and 400 mg/kg, this extract reduced the cumulative number of holes explored and the number of spaces between two holes explored (en relation with motor activity) ($P < 0.001$) (Table 1). The data lead to conclude that the methanolic and aqueous extract of *Lavandula officinalis* possess potential sedative effects on the central nervous system at the doses of 200, 400, and 600 mg/kg via oral route administration (Tables 1 and 2).

3.6. Thiopental-Induced Sleep in Mice. We observed that the lavender methanolic extract (ME) had induced hypnotic effect significant ($P < 0.001$). Hypnosis induced by methanolic extract (800 and 1000 mg/kg, p.o.) was evaluated by observation of the duration of thiopental-induced sleeping time. The extract showed a reduction in the time of onset of sleep induced by thiopental. The effects of the extract on onset of sleep at 800 and 1000 mg/kg were comparable to that of diazepam at 3 mg/kg. The highest prolongation of sleep produced by the methanolic extract was comparable to that of diazepam (3 mg/kg). Only the highest dose tested for ME significantly increased from 45 ± 2 to 112 ± 3 min; the duration of hypnos is induced by thiopental (Table 3). However, the aqueous extract of *Lavandula officinalis* at the doses of 800 and 1000 mg/kg, p.o., produced no hypnotic activity significant on the central nervous system confirming, thus, the hypnotic action of lavender methanolic extract at high doses by oral pathway (Table 3).

4. Discussion

In aromatherapy, the methanolic and aqueous extracts of *Lavandula officinalis* are believed to possess anticonvulsive, sedative, hypnosis, and antidepressive effects and to be useful for treating nervous breakdown, nervous tension, depression, and insomnia [23–25]. In this paper, we observed the sedative and hypnotic properties of methanolic and aqueous extract from *Lavandula officinalis* L. in mice. Therefore, in order to study the comprehensive effect of our drugs, the following targets were observed: reestablishment time, number of head dips, and loss of initiative and curiosity in mice.

Diazepam is central nervous system depressant used in the management of sleep disorders such as insomnia; these compounds have a binding site on GABA receptor type-A ionophore complex ($GABA_A$) [4, 5]. It decreases activity, moderates excitement, and calms the recipient. Substances like diazepam (which has been chosen as the standard reference drug in this study) reduce onset of and increase duration of barbiturate-induced sleep and reduce exploratory activity possessing potentials as sedative [21, 26]. Lavender extract increased the time of reestablishment by mice in the traction test (Table 1), after oral administration of 200, 400, and 600 mg/kg dosages, producing sedative effect similar to that observed with 3 mg/kg diazepam. Diazepam is a very well-known anxiolytic benzodiazepine (BDS) which produces not only anxiolytic-like effect but also important sedative action. In this respect, lavender extract produced

Table 1: Sedative action of *Lavandula officinalis* methanolic extract. p.o. means oral route; i.p. means intraperitoneal route; *n* means number of mice per group; sec means seconds; ME: mean methanolic extract; DZP means diazepam. Data are expressed as mean ± SD; $P < 0.001$ versus the control group.

Test		Control	Diazepam i.p. DZP (3 mg/kg)	Methanolic extract of *Lavandula officinalis* in mg/kg p.o. ME (100 mg/kg)	ME (200 mg/kg)	ME (400 mg/kg)	ME (600 mg/kg)
Traction test	Re-establishment time	0.09 sec ± 0.0	10 sec ± 0.3	0.08 sec ± 0.5	5 sec ± 0.5*	18 sec ± 0.5*	27 sec ± 1*
		$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$
Fireplace test	Time to go back the tube in seconds	7 sec ± 0.5	>2 min	sec ± 0.5	30 sec ± 1*	0.58 sec ± 1*	>2 min*
		$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$
Hole-board test	Explored holes during 5 minutes	10 ± 1	0.0 ± 0.0	8 ± 0.1	2 ± 0.0*	1 ± 0.0*	0.0 ± 0.0*
		$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$

Table 2: Sedative effect of aqueous extract of *Lavandula officinalis.* p.o. means oral route; i.p. means intraperitoneal route; *n* means number of mice per group; sec means seconds; AE means aqueous extract; DZP means diazepam. Data are expressed as mean ± SD; $P < 0.001$ versus the control group.

Test		Control	Diazepam i.p. DZP (3 mg/kg)	Aqueous extract of *Lavandula officinalis* p.o. AE (100 mg/kg)	AE (200 mg/kg)	AE (400 mg/kg)
Traction test	Re-establishment time	0.09 sec ± 0.0	10 sec ± 0.3	2 sec ± 0.3	12 sec ± 0.5*	30 sec ± 1*
		$n = 5$	$n = 5$	$n = 5$	$n = 4$	$n = 5$
Fireplace test	Time to go back the tube in seconds	7 sec ± 0.5	>2 min	12 sec ± 0.1	45 sec ± 1*	>2 min*
		$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 4$
Hole-board test	Explored holes during 5 minutes	10 ± 1	0.0 ± 0.0	6 ± 0.2	1 ± 0.0*	0.0 ± 0.0*
		$n = 5$	$n = 5$	$n = 5$	$n = 4$	$n = 4$

Table 3: Effect of the methanolic extract of *Lavandula officinalis* on the onset and duration of sleep in thiopental-treated mice. Mice received thiopental (60 mg/kg, i.p.) 30 min after the pretreatment of methanolic extract (800 and 1000 mg/kg, p.o.) and diazepam (3 mg/kg, i.p.). i.p. means intraperitoneal route; p.o. means oral route; ($n = 5$) means number of mice per group; ME means methanolic extract; D ZP means diazepam. Data are expressed as mean ± SD; $P < 0.001$ versus the control group.

Group	Dose (mg/kg)	Sleep latency (min)	Sleeping time (min)
Normal	60	8 ± 1	36 ± 3
DZP	3	6 ± 1*	75 ± 3*
ME	800	12 ± 0.5*	45 ± 2*
ME	1000	6 ± 1*	112 ± 3*

a dose-dependent reduction in the number of head dips in the hole-board test similar and/or greater than diazepam (Tables 1 and 2). It is generally believed that locomotor activity results from brain activation, which is manifested as an excitation of central neurons involving different neurochemical mechanism and an increase in cerebral metabolism. It is possible that the sedative activity of methanolic and aqueous extract of Lavandula officinalis is mediated by GABAergic pathway, since GABAergic transmission can produce profound sedation in mice [27]. The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnosis activity. Glutamate and GABA are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain [28]. Thus, receptors for these two neurotransmitters are regarded as important targets for psychotropic drugs. In the test of thiopental-induced sleep in mice, the potentiated effect of lavender extract in mice was represented. It not only prolonged the sleeping time but also decreased the latency of falling asleep and increases the rat of sleep onset. The lavender extract has produced hypnosis at high doses' that is, 800 and 1000 mg/kg. Since the effect of thiopental on the CNS involves the activation of the inhibition GABAergic system [29, 30], this finding suggests that some constituents in lavender extract produce facilitation of this inhibitory system (Table 3). Phytochemical studies have identified active components in this plant such as coumarin, chalcones, flavanones, flavones, flavonols, quercetin, and kaempferol derivatives, suggesting that they are the main responsible for sedative and hypnotic activities [4, 6, 31]. Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the sedative and hypnotic effect. In conclusion, p.o. administration of methanolic and aqueous extract of Lavandula officinalis induces similar sedative effects, supporting its use in folk medicine. Given that the LD_{50} value for these extracts was beyond 5000 mg/kg for oral administration, as determined

by Litchfield and Wilcoxon [12]; our results suggest a remote risk of acute toxicity and good tolerance of these extracts in traditional medicine. To sum up, this work represents that the methanolic and aqueous extracts of *Lavandula officinalis* have obvious sedative and hypnotic activity; these data provide pharmacological basis for its therapeutic efficacy on insomnia.

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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF THE METHANOLIC AND AQUEOUS EXTRACTS OF *ANACYCLUS PYRETHRUM* USED IN MOROCCAN TRADITIONAL MEDICINE

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ABSTRACT

The antibacterial activity against six standard bacterial strains evaluated with the disc diffusion assay and the MTT assay to determinate the Minimum Inhibitory Concentration (MIC). The result of the antimicrobial activity indicated by zone of inhibition of growth ranged from 13.0 to 22.2mm**.** The antibacterial activity demonstrated that all extracts have an inhibitory activity against bacteria with the lowest MIC at 3.125mg/ml. The extract contains some major bioactive compounds that inhibit the growth of microorganisms thereby proving very effective as alternative source of antibiotics. Our results support the ethno-pharmacological uses of this plant in folk medicine and could provide useful data for the utilization of this extracts in pharmaceutical, cosmetic and food industries.

Keywords: *Anacyclus perythrum*, Root extract, Medicinal plants, Antibacterial activity, Minimum inhibitory concentration

INTRODUCTION

All over the world, infectious disease is the number one cause of death accounting for approximately one-half of all the deaths in tropical countries. This perhaps may be attributed to the increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases[1]. Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value[2-4]. Very few antimicrobial studies have focused on in vivo models[5, 6]. Recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. However, there have been some efforts to mimic the traditional use of plant material as noted by the addition of aqueous extracts in screening assays[7-12]. Several methods are currently available to detect the antibacterial activity of plant extracts using different principles to assess bacterial growth or its inhibition. Some reports have focused on the antimicrobial screening together with other pharmacological investigations including toxicity[13, 14]. In Morocco, there is more than 42,000 species of plants; divided into 150 families and 940 genus used in traditional medicine[2, 15]. *Anacyclus Perythrum* (AP) locally known as ''Aud el-attas'', 'Akkar Karha' and 'Agargarha' is a species commonly used in Moroccan traditional medicine. The powder of the root is well known as sternutatory, diaphoretic and used for many ailments. Showing the root is considered to be sialagogue, and to relieve toothache. In liver diseases is recommended, mixed with olive oil it is used in rheumatism, sciatic, colds, neuralgic, and paralysis[16]. In addition, the AP is also used as cardiotonic to treat typhus fever[17]. The chemical analysis of the roots shows that they contain three fatty acids, one sterol and ten unsaturated amides, more specifically: pellitorine, anacycline, phenylethylamide, enetriyne alcohol, inulin, polyacetylenic amides I-IV, and sesamin. The plant contains also tannins, gum and essential volatile oil. Pyrethrine, an alkaloid, yielding pyrethric acid, is stated to be one of the active principles[32]. However the antibacterial activity has not yet been studied. Thus, the aim of this study is to evaluate the acute toxicity and the antibacterial activity of the methanolic and aqueous extracts of *Anacyclus perythrum* against different bacterial strains which may be involved in such diseases, using a prelimary bioassay screening and to quantify the minimum inhibition concentration (MIC) using colorimetric method[18].

MATERIALS AND METHODS

Plant material

Anacyclus perythrum, from *Asteraceae* family, was collected in 28 March 2009, 5km east of Morocco with collection number 77765. The plant was identified by a botanist M. Fennane from the Scientific Institute of Rabat (Morocco), where a voucher specimen has been deposited with number 3949.

Extraction procedure

The roots were air dried for 20 days, and then washed free from soil, powdered and stored in airtight containers at room temperature until extraction. 1kg of the powdered roots was extracted with methanol through a maceration process within a period of 48h. The dark residues were obtained after the evaporation of the solvent under reduced pressure at 40°C; the percentage of extracts yield was 12% (w/w). Simultaneously, water extract was prepared by adding 1.5L of distilled water to 1Kg of powdered plants roots and macerated within a period of 48h. It was then filtered and concentrated the extract under reduced pressure and percentage of extracts yield was 10% (w/w) for plant material.

Acute toxicity

The toxicological study was conducted according to the method of OECD Guideline 423. Swiss mice (20-30g) were kept at 23°C of temperature, 50% humidity, on a 12h light/dark cycle and were fed *at libitum* with standard feed and water in the course of the study. The animals were obtained from the elevage of animal centre of Faculty of Medicine and Pharmacy of Mohammed-V Souissi University, Rabat. The extracts of plant were administered by oral route in a single dose. The mice of both sexes received the initial dose of methanolic and aqueous extracts of AP that was chosen 2000mg/kg of body weight. The control group received only the water. The animals must be individually observed, the symptoms and weight variation was recorded during the first 30min and regularly during the first 24h after treatment and daily for 14 days. Care and treatment of the mice were in compliance with the guidelines of the guide for the care and use of laboratory animals (commission on life science, national research council, 1996).

Antibacterial Activity Test

Bacterial strains

Six bacterial strains, namely *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 53153, *Escherichia coli* ATCC 54127, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, were used for antibacterial testing; The cultures of bacteria were maintained in their appropriate agar slants at +4°C throughout the study and used as stock cultures.

Disk diffusion method and Minimum inhibitory concentration

Antibacterial activity of aqueous and methanolic extracts diluted in sterile distilled water, was evaluated by paper disc diffusion method.

Disks impregnated with sterile distilled water served as negative controls, Cephaxitin and Amikacin are used as positive control[19, 30, 31]. The viability indicator MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide, Sigma, Aldrich) and the quick microplate metho[20] were used for the determination of the minimum inhibitory concentration (MIC) [21]. For the inoculum, turbidity was adjusted to 1.5 x 108 CFU/ml with the reference to Mc Farland turbidometer[22]. Serial dilutions of extracts were dissolved in distilled water and made in a concentration range from 25mg/ml to 200mg/ml in sterile test tubes. Each well of micro-titer plate was inoculated with 20µL of the bacterial suspension (15µl of Mueller Hinton broth and 5µl of the bacterial Inoculum) and 20µl of sample of various concentrations of the extracts. The plate was incubated under optimal conditions at 37°C for 24h. After incubation, as an indicator of bacterial growth 1 mg/ml of MTT was added to each well, after incubation periods ranging from 3 to 5h at 37°C, control without plant extracts with MTT and bacterial inoculums were used as negative control. Optical densities were measured at 570nm by using a microplate reader. The bacterial suspension changed to blue when bacterial growth occurred. The MIC is defined as the lowest concentration of antibiotic or extract at which there is no visible growth. All tests were performed in triplicate.

Statistical analysis

The mean values and standard deviations of all replicates described in the above tests were calculated using Delta Graph (USA). The data of the extracts were statistically analysed by ANOVA. A student's t-test was computed for the statistical significance of the results. Differences were considered significant with $p < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity of *Anacyclus pyrethrum*

During the 14 days, the evolution of the weight was established. Abdominal contraction was observed by 20-25min after the oral administration of aqueous extract of *Anacyclus pyrethrum* and no mortality was recorded. Generally, no toxicity was demonstrated at the dose of 2000mg/kg and the extracts of the plant does not lead to mortality neither by oral route. Under the system of global harmonization of chemicals (GHS), this product is classified category 5, which the LD50 was higher than 2000mg/kg.

Antibacterial potential of plant extracts

The antibacterial activity of methanol and aqueous extracts of AP were assayed in vitro by a disk diffusion method (Table1) using Cephaxitin and Amikacin as positif control against Gram positive and Gram negative pathogenic bacteria. All the assayed extracts significantly inhibited the growth of the bacterial strains, the methanol extract showed the highest antibacterial activity ($P<0.05$), the diameter of the zone inhibition ranging from 16 - 40mm (Fig.1).

Table 1: Diameters of the inhibition zones of the growth of the bacterial strains in mm induced by Antibiotics of third generation.

	Standard reference bacterial strains					
	Gram-negative organisms			Gram-positive organisms		
The antibiotics	EC	KB	BS	SA	PA	ML
Cephaxitin 30 mcg/disc	28	32	33	26	18	35
Amikacin 30 ng/disc	24	26	26	23	14	25

BS: *Bacillus subtilis* ATCC 6633, PA: *Pseudomonas aeruginosa* ATCC 15442, KB: *Klebsiella pneumoniae* CIP 53153, EC: *Escherichia coli* ATCC 54127, SA: *Staphylococcus aureus* ATCC 6538 and ML: *Micrococcus luteus* ATCC 9341.

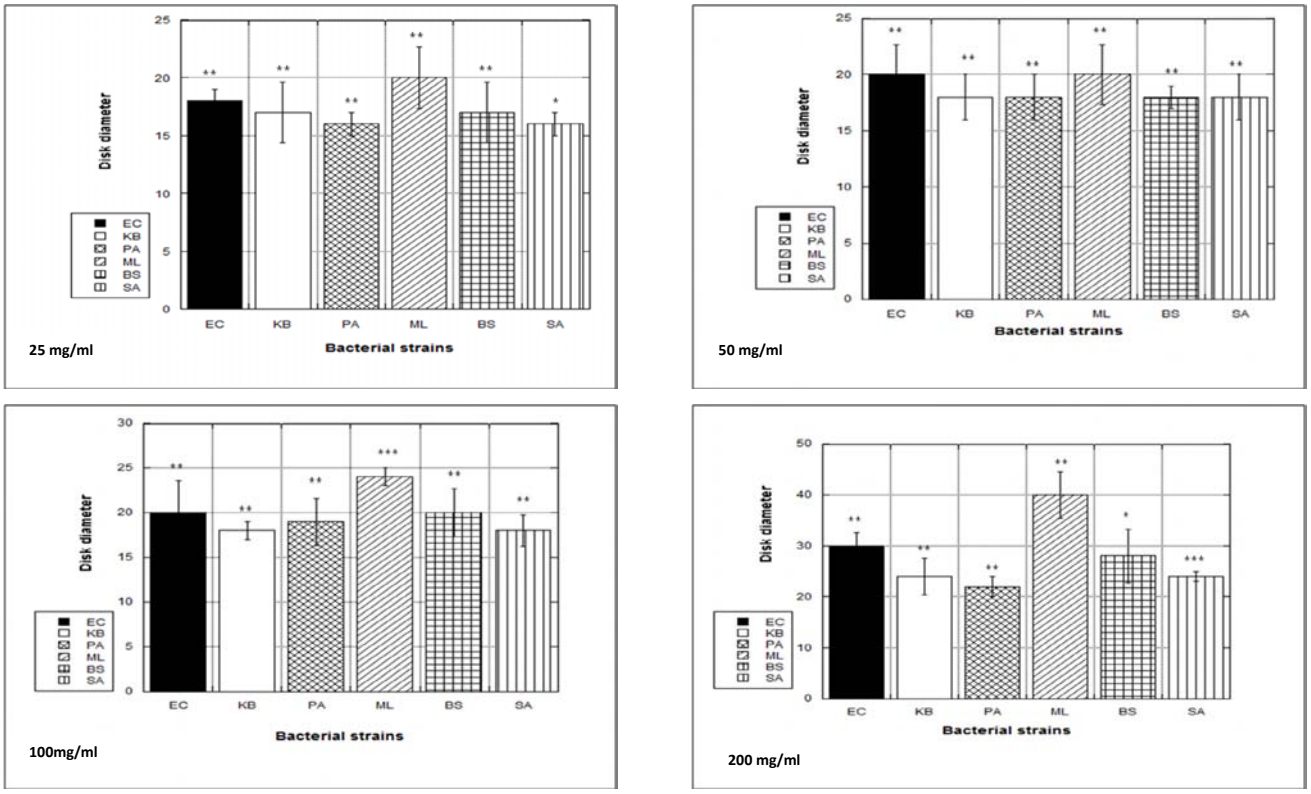

Fig. 1: Graph of the inhibition diameters in mm of methanolic extract tested on bacterial strains.

The significance was evaluated by means values ± S.E. of Student test in triplicate experiments: *$p < 0.05$ **$p < 0.01$ ***$p<0.001$).

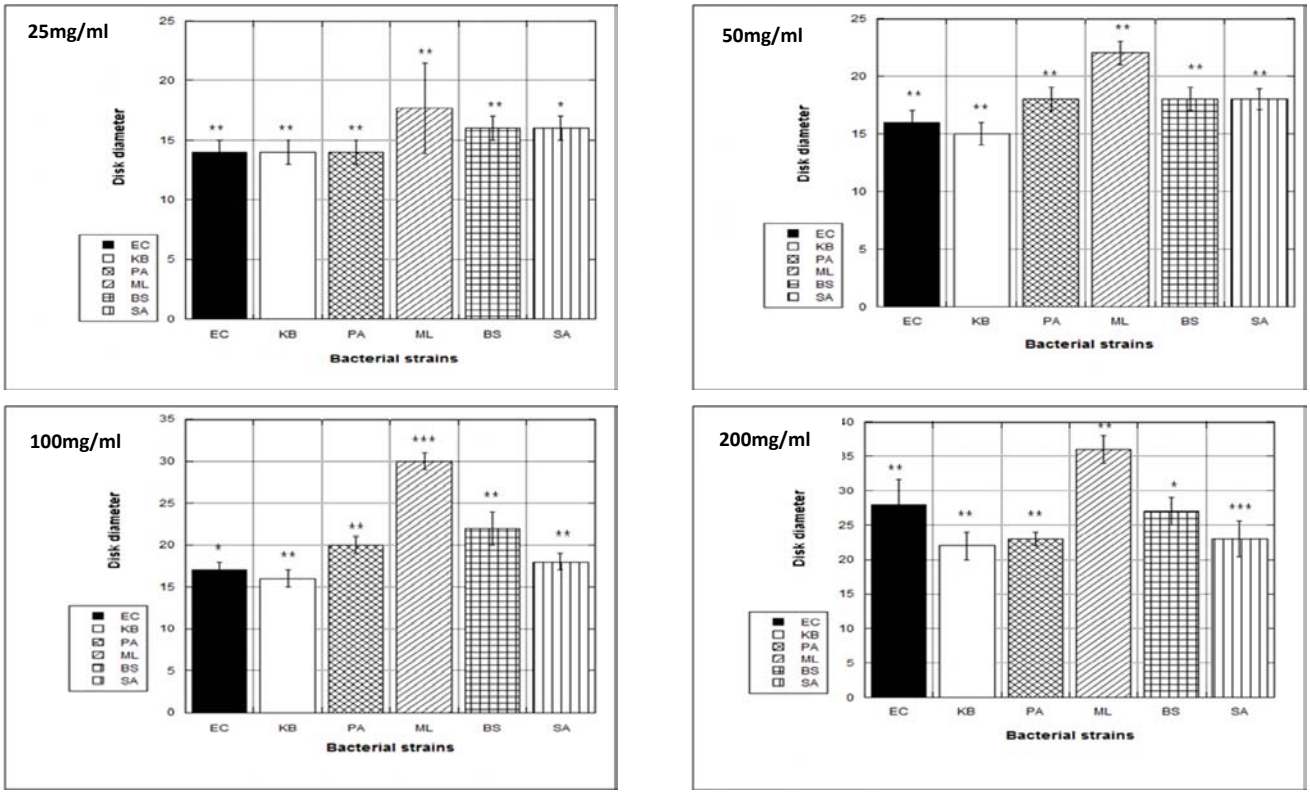

Fig. 2: Graph of the inhibition diameters in mm of aqueous extract tested on bacterial strains.

The significance was evaluated by means values ± S.E. of Student test in triplicate experiments: *p < 0.05 **p <0.01 ***p<0.001).

According to the results, methanolic and aqueous extracts (Figure 2) were found to be active against all pathogenic bacteria and the best results were observed with methanolic extract. The strongest antibacterial activity was seen against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Micrococcus luteus* with a MIC value of 3.125mg/ml followed by MIC 6.25mg/ml while *Klebsiella pneumoniae* methanolic extracts and *Pseudomonas aeruginosa* aqueous extracts of AP. Overall, the reduction in bacterial viability was dose dependent and it was higher (p<0.05) (Table 2).

Most of the researchers have evaluated first the antibacterial spectra of plant extracts; secondly they have determined the chemical composition of the extracts, and thirdly they have retroactively attributed such activity to such molecule[23-27]. In this study the extracts of AP showed higher antibacterial potential against different bacterial strains, this antibacterial activity is may be related to their secondary metabolites; alkaloids, phenolics and terpenoids reported in this plant (Guide to Medicinal Plants in North Africa).

Table 2: The minimum inhibitory concentration (MIC) of the extracts of the plant tested against different bacteria. Data represent the MICs (mg/mL).

	Standard reference bacterial strains					
	Gram-negative organisms			Gram-positive organisms		
Extracts	EC	KB	PA	SA	ML	BS
AE. AP	3.125	3.125	6.25	3.125	3.125	3.125
ME. A P	3.125	6.25	3.125	3.125	3.125	3.125

BS: *Bacillus subtilis* ATCC 6633, PA: *Pseudomonas aeruginosa* ATCC 15442, KB: *Klebsiella pneumoniae* CIP 53153, EC: *Escherichia coli* ATCC 54127, SA: *Staphylococcus aureus* ATCC 6538 and ML: *Micrococcus luteus* ATCC 9341, AE: aqeous extract; ME: methanolic extract; AP: *Anacyclus perythrum.*

Gram-negative bacteria which are responsible for a large number of infectious diseases have a unique outer membrane that contains lipopolysaccharides which render them impermeable to certain antibacterial compounds[28]. All extracts (methanolic and aqueous extracts of AP) showed antibacterial activity against both Gram-positive and Gram-negative bacteria. This gives an indication of the presence of promising antibacterial compounds. The selection of bacterial strains was based on their pathogenicity lead to infectious diseases, poor sanitary conditions, unclean unsafe drinking water and inappropriate food storage conditions are common in poor families that frequently suffer from diarrhea and vomiting. In most cases, they treat themselves by using local herbs[29]. Our results against these bacterial strains suggest the efficacy of plant claimed by traditional healers. This plant may be a source which could yield drug that could improve the treatment of infections caused by this organism.

CONCLUSION

The data indicate that the extracts of the *Anacyclus pyrethrum* species inhibit efficaciously some resistant bacterial strains which make serious sanitary problems worldwide and confirm that there is a good correlation between the reported traditional uses of the plant for infectious diseases. This indicates that this plant may be useful for developing alternative compounds to treat infections caused by these antibiotic resistant pathogens. According to these results, it is possible to conclude that *Anacyclus pyrethrum* had a strong and a broad spectrum of antibacterial activity. To the best of our knowledge, this is the first study to provide data that the methanolic

and aqueous extracts of *Anacyclus pyrethrum* evaluated against a wide range of bacteria.

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In vitro Antibacterial Potential Activity of the Methanolic and Aqueous Extracts of Corrigiola telephiifolia Pourr. and Mesembryanthemum nodiflorum

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In vitro Antibacterial Potential Activity of the Methanolic and Aqueous Extracts of *Corrigiola telephiifolia* Pourr. and *Mesembryanthemum nodiflorum*

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Abstract: The present study was conducted to investigate antibacterial activity of methanolic and aqueous extracts obtained from aerial parts of *Mesembryanthemum nodiflorum* and *Corrigiola telephiifolia* Pourr., root, using the disc-diffusion and microdilution assays. The extracts were tested against Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The methanol extracts of plants screened revealed promising antibacterial activities against most pathogens bacteria. Maximum activity of *corrigiola telephiifolia* Pourr., and *Mesembryanthemum nodiflorum* methanol extracts were observed against *Pseudomonas aeriginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Micrococcus luteus* with minimal inhibitory concentration (MIC) values ranging from 3.12 to 6.25 mg/ml. The antibacterial activity of aqueous extract was less than this of methanolic extract (MIC 3.12 and 12.5 mg/ml) were observed against all tested bacteria. These species might provide promising therapeutic agents against infections with multi-resistant and pathogens bacteria.

Key words: Medicinal plants; Minimal inhibitory concentration; Antibacterial activity; Bacterial strains; Methanolic extract; Aqueous extract.

Introduction

Knowledge of medicinal plants is now essential and is even crucial in health sector and of large proportion of the world's population; there has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants [1]. The improvement of medicinal plants value can be achieved also by searching newer, more effective and less toxic therapeutic molecules, which will add great values to the resources that can be later, integrated into the therapeutic arsenal already in use. Herbal aqueous extracts, either in the form of infusions or as decoctions, have been used since ancient times as remedies form many human and animal diseases [2]. Natural products and pure compounds

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from plant extracts provide opportunities for screening new antimicrobials. The development of microbial resistance to the classical antibiotics led researchers to investigate the antimicrobial activity of several medicinal plants [3]. Several studies have documented the use of plants in the ethnotherapy and the treatment of several diseases; many studies have been carried out to extract various purs products for screening antibacterial activity [4-8]. The knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine becomes necessary [9]. In Africa there is a large variety of medicinal plants [10]. Morocco is one of the Mediterranean countries have a long history and knowledge of traditional therapy [11]. Moreover the ethnomedicinal tradition is still alive in all regions in this country, showed the wealth of ethnomedicinal knowledge accumulated during centuries, actually in Morocco there is about 42,000 species of plants; divided into 150 families and 940 genuses are traditionally used for their medicinal values [13-15].

Corrigiola telephiifolia Pourr., is a perennial species, woody distributed throughout the north of Africa, this plant used in traditional Mediterranean preparations, one of its medicinal uses is the application of a mixture of *Corrigiola telephiifolia* powder with honey for the treatment of stomach aches, chills, lung diseases and rheumatic diseases, it is considered as fortifying and appetizer, the root is the most effective part, it is used to treat flu, dermatological diseases, inflammation, ulcer, cough, andjaundice; it is also used as an anesthetic and a diuretic [16]. Various species are commonly used in Moroccan traditional medicine like *Mesembryanthemum nodiflorum* (Aizoaceae) locally known as ¨ Ghassul Legsi¨ has been used as a medicinal agent to treat gastric disease, skin and infectious pathology used as an emetic in acute poisoning (such as arsenic) [17].

In this study we report for the first time the antibacterial activity of *Mesembryanthemum nodiflorum* and *Corrigiola telephiifolia* plants extracts, the selection of plants for this study was based on ethnobotanical data and on their traditional use in the treatment of infectious diseases, this study may justifies its use in traditional medicine.

Materials and methods

Plant material

Root of *Corrigiola telephiifolia* Pour. and aerial part of *Mesembryanthemum nodiflorum* were collected based on ethnopharmacological information, from Ben Slimane and Tiznit in Morocco, with the agreement from the authorities and with assistance of traditional medical practitioner. The plants were identified by Pr. M. Fennane a botanist of Scientific Institute, University Mohammed- V, Rabat, Morocco. The voucher specimen of *C. telephiifolia* Pour., (RAB77766) and *M. nodiflotum* (RAB77765) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco.

Extraction procedure

The powdered sample of the plant material was subjected to phytochemical screening to test for the presence or otherwise of alkaloids, tannins, flavonoids, saponins, and terpenes using standard procedures [18, 19].

Methanolic extracts

The extracts were produced by maceration method. Plant materials (100 g), cut into small pieces were extracted with methanol at room temperature (25°C) over period of 48 h.The preparations were filtered through Whatman No. 1 filter paper. They were concentrated under reduced pressure at 65°C; the remaining extracts were finally dried in the oven at 30°C for two hours to ensure the removal of any residual solvent [20, 21]. The yield of extraction was 4.56 % for *Corrigiola telephiifolia* and 3.21 % for *Mesembryanthemum nodiflorum*. Final extracts were maintained at 4°C until use.

Aqueous extracts

Water extracts were prepared by adding (175 ml) of distilled water to 100 g of powdered plant's root and macerated within a period of 48 h. It's were then filtered by gravity filtration, concentrated the extract under reduced pressure

and then lyophilized (Free Zone® Dry 4.5, USA). The percentage of *Corrigiola telephiifolia* and *Mesembryanthemum nodiflorum* extracts yield was 5.27 and 4.74 % (w/w) respectively. For assuring stability, the lyophilized material was stored at -20°C.

Antibacterial activity test

Disc-diffusion assay

Antibacterial test systems should ideally be simple, rapid, reproducible, inexpensive and maximize sample throughput in order to cope with a varied number of extracts [21], the disc-diffusion assay [22], was used to determine anti-bacterial activity of the plant extracts using three Gram-positive bacteria: *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633 and three Gram-negative bacteria: *Klebsiella pneumoniae* CIP 53153, *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 54127. A suspension of the tested microorganism (106-108 cfu/ml) was added on the surface of each plate containing 10 ml of Mueller Hinton. Sterile filter discs (5 mm in diameter, Whatman No. 1) were impregnated with ten microliters of dilutions of known extracts concentrations (25-200 mg/ml), placed on the inoculated plates, and after staying at 4°C for 1h. Plates were incubated for 24 h under appropriate cultivation conditions. Discs impregnated with sterile distilled water served as negative control. Cephaxitin (30 mcg/disc1) and Amikacin (30 ng/disc) were used as a positive control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate [23-25].

Micro-dilution antibacterial assay

This test was performed in sterile 96-well microplates [26, 27] into each well were dispensing 5 µl of the bacterial inoculum and 15 µl of Mueller Hinton broth. The inoculum, turbidity was adjusted to 1.5 x 10^7cfu/ml with the reference to Mc Farland turbidometer. A number of wells were reserved in each plate for sterility control (no inocula added), inocula viability (no extract added), and solvent effect (Methanol or destilled water). Serial dilutions of extracts were dissolved in distilled water and made in a concentration range from 25 mg/ml to 200 mg/ml were added. Plates were incubated under optimal conditions at 37°C for 24 h. Each experiment was tested in triplicate. As an indicator of bacterial growth, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Sigma, Aldrich (MTT), colorimetric methods could represent an alternative approach, using tetrazolium salts as indicators, since bacteria convert them to coloured formazan derivatives that can be quantified [28, 29]. The MTT dissolved in water (1 mg/ml) was added to the wells and incubated at 37°C for 3 to 5 h; bacterial growth was assayed by absorbance measurement at 550 nm. The bacterial suspension changed to blue when bacterial growth occurred. The lowest concentration of each extract showing no growth was taken as its minimal inhibitory concentration (MIC).

Statistical analysis

Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level.

Results and discussion

The initial screening of antibacterial activities of *Corrigiola telephiifolia* Pourr., and *Mesembryanthemum nodiflorum* extracts was assayed *in vitro* by the agar diffusion method. Table 1 and 2 summarizes the bacterial growth inhibition by *Corrigiola telephiifolia* Pourr., plant extracts. According to the results, Corrigiola methanolic extract was found to be the most active against pathogenic bacteria. The strongest antibacterial activity was seen against *Pseudomonas aeruginosa* and *Micrococcus luteus* with IZD value of 32 and 36 mm respectively ($P < 0.001$), followed by *E. coli*, giving a zone size of 27 mm, this compared well with the Amikacin control (14 mm) (Fig. 1 and 2). The diameters of the inhibition zones of the aqueous extract were similar to, or less than the IZD of methanolic extract against corresponding bacteria, showed the greatest activity, zone size 24 mm (range especially against *Pseudomonas*

Table 1. Inhibition by Aqueous extract of *Corrigiola telephiifolia* Pour., (Zone size, mm)

Bacterial strains	Zone of Inhibition (mm)					
	E. coli	***K. pneumoniae***	***P. aeruginosa***	***M. luteus***	***S. aureus***	***B. subtilis***
200	22	22	24	20	20	19
100	20	20	24	17	18	18
50	20	17	22	17	16	17
25	18	16	18	16	16	17
Positive control						
Ce (30 mcg/disc)	28	32	18	36	26	33
Am (30 ng/disc)	24	26	26	14	23	26

Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442,
Kb, Klebsiella pneumoniae ATCC 53153, *Ec, Escherichia coli* ATCC 54127,
Sa, Staphylococcus aureus ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341,
Am, Amikacin, *Ce*, Cephaxitin.
Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive control (Amikacin and Cephaxitin).

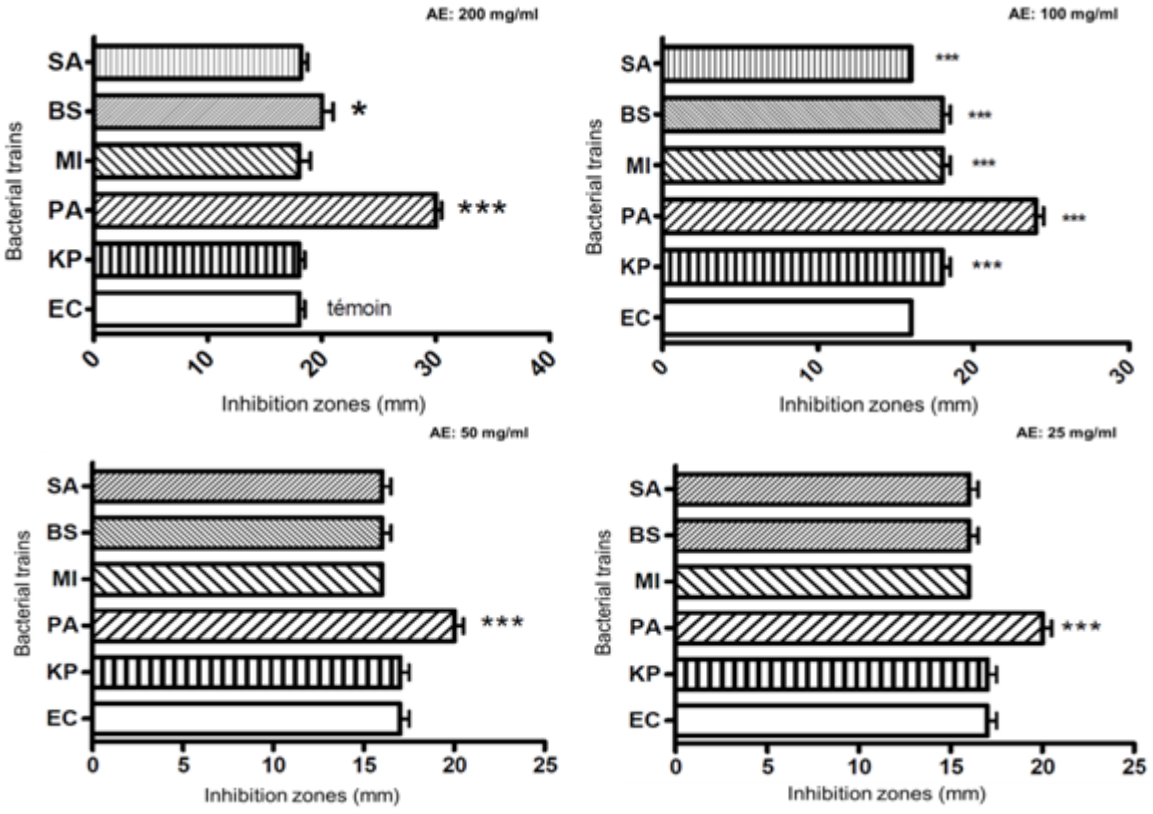

Figure 1, Graph of the inhibition by aqueous extract of *Mesembryanthemum nodiflorum* (zone size, mm). AE, aqueous extract, , BS, *Bacillus subtilis* ATCC 6633, PA, *Pseudomonas aeruginosa* ATCC 15442, KP, *Klebsiella pneumoniae* ATCC 53153, EC, *Escherichia coli* ATCC 54127, SA, *Staphylococcus aureus* ATCC 6538, Ml, *Micrococcus luteus* ATCC 9341. Values are means±SD of three determinations. Data indicate significant difference (*$p < 0.05$ **$p < 0.01$ ***$p<0.001$) (with respect to positive control (Amikacin and Cephaxitin).

Table 2. Inhibition by Methanolic extract of *Corrigiola telephiifolia* Pour., (zone size, mm).

Bacterial strains	Zone of Inhibition (mm)					
	E. coli	***K. pneumoniae***	***P. aeruginosa***	***M. luteus***	***S. aureus***	***B. subtilis***
200	27	24	32	36	20	20
100	19	20	24	24	17	18
50	17	18	20	18	17	17
25	16	17	18	17	17	16
Positive control						
Ce (30mcg/disc)	28	32	18	36	26	33
Am (30 ng/disc)	24	26	26	14	23	26

Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442,
Kb, Klebsiella pneumoniae ATCC 53153, *Ec, Escherichia coli* ATCC 54127,
Sa, Staphylococcus aureus ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341,
Am, Amikacin, *Ce*, Cephaxitin.
Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive control (Amikacin and Cephaxitin).

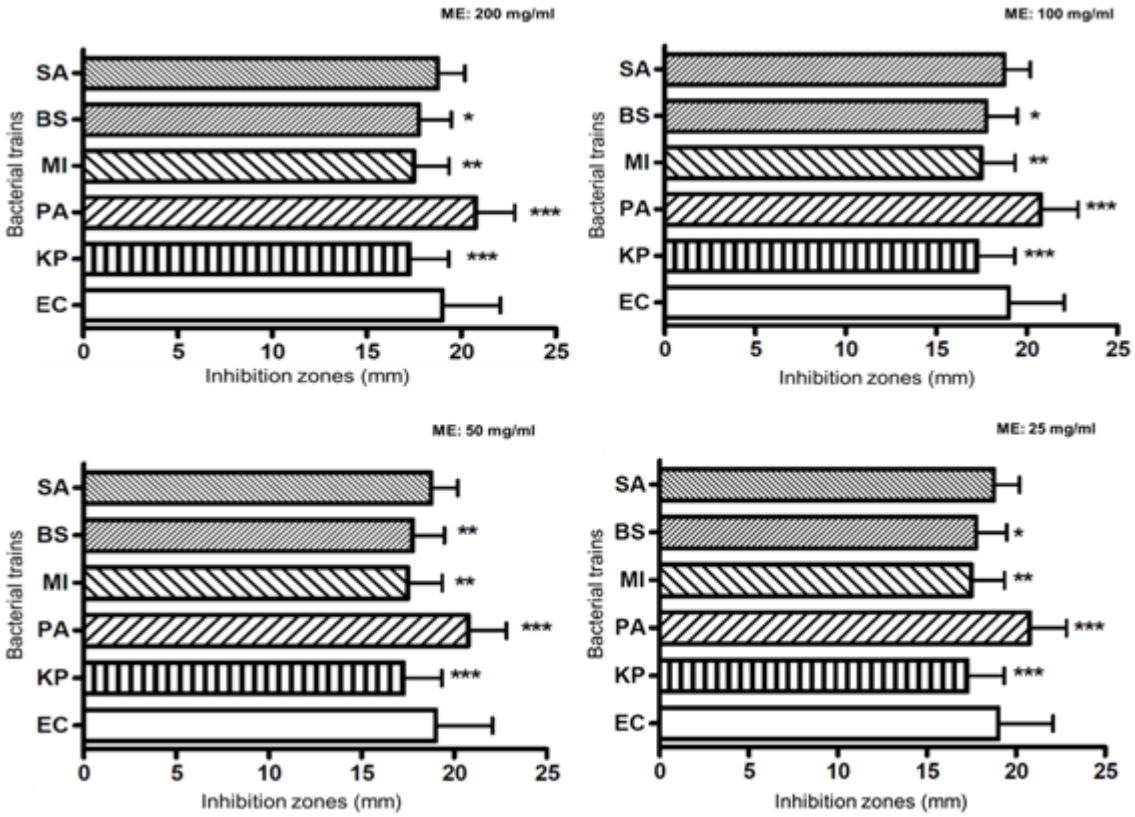

Figure 2. Graph of the inhibition by methanolic extract of *Mesembryanthemum nodiflorum* (zone size, mm). ME, methanolic extract, BS, *Bacillus subtilis* ATCC 6633, PA, *Pseudomonas aeruginosa* ATCC 15442, KP, *Klebsiella pneumoniae* ATCC 53153, EC, *Escherichia coli* ATCC 54127, SA, *Staphylococcus aureus* ATCC 6538, Ml, *Micrococcus luteus* ATCC 9341. Values are means±SD of three determinations. Data indicate significant difference (*$p < 0.05$ **$p < 0.01$ ***$p<0.001$) with respect to positive control (Amikacin and Cephaxitin).

aeruginosa ($P < 0.001$). (Fig. 1 and 2). In view of the results obtained by the disc diffusion method, the MIC values of the *Corrigiola telephiifolia* extract were determined by agar microdilution assays. The MIC values obtained confirm the existence of a significant activity of methanolic extract against the bacterial strains tested in our study, with MIC values ranging from 3.12 to 6.25 mg/ml for all test microorganisms ($P < 0.01$) (Table 3). Plant extracts have been used for thousands of years [30], especially in, pharmaceuticals preparations, alternative medicine and natural therapies [31]. It is necessary to investigate plants scientifically, which have been used, in traditional medicine to improve the quality of healthcare. Plants are potential sources of novel antimicrobial com-pounds especially against bacterial pathogens [32].

In this paper, the aqueous and methanol extracts of *Corrigiola telephiifolia* Pour., and *Mesembryanthemum nodiflorum* inhibited bacterial growth but their effectiveness varied. *M. nodiflorum* methanolic extract displayed good activities, inhibiting the growth of *M. luteus*, *P. aeruginosa* and *K. pneumoniae* with IZD of 24, 20 ($P < 0.001$) and 20 mm ($P < 0.01$), while IZD for aqueous extract against *P. aeruginosa, B. subtilis* and *M. luteus* ranged from 18 to 30 mm ($P < 0.001$), 16 to 20 mm and 16 to 18 mm (Fig. 1 and 2). MIC values showed by the methanol and aqueous extracts were in the range of 3.12-6.25 mg/ml and 6.25-12.5 mg/ml regarding the standard drug. The results indicated that the tested crude extracts showed antibacterial activity towards the "Gram-positive" bacteria. These results are consistent with previous reports on related plants regarding "Gram-positive" bacteria. Usually most of the "Gram-negative" bacteria are more resistant than "Gram-positive" bacteria [33]. The main reason for the differences in bacterial susceptibility could be the outer membrane surrounding the cell wall in gram-negative bacteria, which restricts diffusion of compounds through its lipopoly-saccharide covering, as previously reported [34]. In addition, the periplasmatic space contains enzymes which are capable of breaking down foreign molecules introduced from the outside [34]. The antibacterial activity in this study is related to the different secondary metabolites; alkaloids, phenolics and terpenoids reported or identified in these plant species need further investigations [34, 35]. The

Table 3. Determination of Minimal Inhibitory Concentration (MIC) of *plants screening* (mg/ml).

Plant extracts	**Minimal Inhibitory Concentration (mg/ml)**					
	E. coli	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***M. luteus***	***B. subtilis***
Corrigiola telephiifolia						
AE	3.12±0.00	3.12±0.00	3.12±0.00	3.12±0.00	6.25±0.00	6.25±0.00
ME	3.12±0.00	3.12±0.00	3.12±0.00	6.25±0.00	3.12±0.00	3.12±0.00
Mesembryanthemum nodiflorum						
AE	6.25±0.00	12.5±0.00	6.25±0.00	12.5±0.00	12.5±0.00	6.25±0.00
ME	3.12±0.00	6.25±0.00	6.25±0.00	6.25±0.00	6.25±0.00	6.25±0.00

AE, Aqueous extract, *ME, Methanolic extract,*
Bs, Bacillus subtilis ATCC 6633, *Pa, Pseudomonas aeruginosa* ATCC 15442,
Kb, Klebsiella pneumoniae ATCC 53153, *Ec, Escherichia coli* ATCC 54127,
Sa, Staphylococcus aureus ATCC 6538, *Ml, Micrococcus luteus* ATCC 9341,
Am, Amikacin, *Ce, Cephaxitin.*
Values are means±SD of three determinations.
Data indicate significant difference ($P < 0.05$) with respect to positive control (Amikacin and Cephaxitin).

active molecules could be attributed by the interactions with the membrane constituents and their arrangement [31, 35]. The antibacterial activity in plants aqueous and methanolic extracts against the resistant bacterial strains leads us to investigate these species in order to isolate the active principles.

Conclusion

Multiple drug resistance in bacterial pathogens is a continuing problem throughout the world. There is an established need to develop new antibacterial agents to combat these pathogens. The antibacterial property of *Corrigiola telephiifolia* Pourr., and *Mesembryanthemum nodiflorum* assessed by the present work, authenticates its use in traditional medicine and may be a source of drugs that could improve the treatment of infections caused by bacteria.

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ANALGESIC ACTIVITY OF ETHANOLIC AND ALKALOIDIC EXTRACTS OF *DELPHINIUM STAPHISAGRIA* SEED

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KEYWORDS:

Analgesic Activity, Diterpenoids alkaloids, *Delphinium staphisagria*, Ethanolic extract, Medicinal plants.

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ABSTRACT

We evaluate the analgesic activity of the ethanolic and Alkaloidic extracts obtained from *Delphinium staphisagria* seeds, using chemical and the Tail flick models which will induce acute pain in mice and rats. This study was carried out by using sex Swiss mice (20-30g) and Wistar male rats (180-200g). The ethanolic extract was prepared by using maceration at room temperature (25°C) over the period of 24 hours. The effect of ethanolic and alkaloidic extracts of *Delphinium staphisagria* seeds was investigated for analgesic activity using acetic acid-induced abdominal writhing (Koster test) and Tail immersion method (Tail flick test). The analgesic activity of ethanolic and alkaloidic extracts of *Delphinium staphisagria* seeds at the dose of (40 and 20 mg/kg, p.o.) respectively showed significant ($p<0.001$) reducing in abdominal writhing when compared with control and standard drug (Aspirin, 200 mg/kg, p.o.). However, ethanolic extract at the dose 40mg/kg; p.o., and Alkaloidic extract at the dose 20 mg/kg, p.o., showed significant ($p<0.001$) central analgesic action when compared with control and morphine (5 mg/kg, s.c.) as reference drug. We conclude that, the alkaloidic extract of *Delphinium staphisagria* is a notable, central, and peripheral analgesic activity, these data provide pharmacological basis for its therapeutic efficacy on pains.

INTRODUCTION:

Morocco is fortunate to have such varied climate that almost any medicinal plant can grow. The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants, divided into 150 families and 940 genuses[1–3]. Diterpenoids types have been the targets of considerable interest of medicinal chemists for a broad range of demonstrated pharmacological properties[1]. *Delphinium staphisagria* owned Renonculaceae family is a species characterized by its richness in diterpenoids alkaloids. Analgesia is to reduce the perception of pain without altering the level of consciousness, vigilance or memory of the patient, a large number of products have been used as analgesic, most of these agents produced several side effects including dyspepsia and gastrointestinal complications ranging from unspecified symptoms (nausea, vomiting and diarrhoea) to severe complications (ulcer, bleeding and perforation) and represents major limitations in their clinical use.

Recently, many natural medicines derived from medicinal plants, were considered as the effective and safer for the treatment of various diseases including inflammation and pain[5]. *Aconitum* and *Delphinium* plants have been medicinally used for centuries[6]. The pharmacological effects of *Aconitum* plants are attributed to their characteristic diterpenoides alkaloids, a group of complex natural products displaying a lot of interesting chemistry[7, 8] and biological activities[5]. The analgesic activities of C18- and C19-diterpenoid alkaloids have been extensively investigated since 1981, among which 3-acetylaconitine[9], lappaconitine[10, 11] and crassicauline A[8], have been reported to exhibit marked analgesic activities and have been developed to be analgesic drugs clinically used for the treatment of various pains in China[13-15]. As compared with the known analgesics, such as morphine, methadone, etc., all these three alkaloids induced neither morphine-like tolerance nor physical dependence.

Delphinium plants are a large species within the family Renonculaceae, largely distributed throughout the northern hemisphere region, such as Asia, Europe, and North America, while a few occur in equatorial Africa[9].

The chemical constituents of plants belonging to genus Delphinium have been extensively studies and are best known for their diverse C19 and C20 Diterpene alkaloids[7, 8, 16, 20].

Over 40 alkaloids with differing structures and toxicity have been identified, the amount and types of alkaloids varies greatly between the different larkspur species, within different larkspur populations, and different phenological grown stages. Individual plants often contain 15 or more alkaloids[14, 21-23].

In 1976, Pelletier isolated[14], by chromatography and crystallization, a methoxyl- containing bis-diterpene alkaloids which they designated as Staphisine $C_{43}H_{60}N_2O_2$ and they find that Jacobs

Staphisine is a mixture of Staphisine and a companion non methoxyl staphidine$C_{42}H_{58}N_2O$. In addition, they have isolated two new amine-containing bis-diterpene alkaloids named staphinine $C_{42}H_{56}N_2O_2$ and Staphimine $C_{41}H_{54}N_2O$. The mother liquors which had been accumulated during the isolation of delphinine from the seeds of *Delphinium staphisagria* were found to contain a relatively large amorphous fraction of alkaloids. By a combination of gradient pH extractions and chromatographic technique Pelletier and al.[14, 16], isolated Delphidine $C_{26}H_{41}NO_7$. Three new diterpenoids alkaloids and eight known alkaloids were isolated from the aerial parts of *Delphinium staphisagria* gathered in Morocco[24]. Diterpenoid alkaloids, found in plants of the genera Aconitum and Delphinium, are of the C_{18}, C_{19} and C_{20} diterpenoides types[25] have been the targets of considerable interest of medicinal chemists for a broad range of demonstrated pharmacological properties: arrhythmogenic (neurocardiotoxic), local anesthetic, antiarrhythmic, curariform, analgesic, hypotensive, anti-inflammatory, spasmolytic, neurotropic and psychotropic[22-25].

In this study we report for the first time the analgesic activity of *Delphinium staphisagria* seeds extracts, the selection of plants for this study was based on ethnobotanical data and on their traditional use in the treatment of inflammations, this study may justifies its use in traditional medicine.

MATERIALS AND METHODS:

Plant materials: Seeds of *Delphinium staphisagria* were collected based on ethnopharmacological information, from villages around the region Chefchaoun, northern Morocco on September 2010, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (N° RAB 65077) was deposited in the herbarium of botany department of scientific institute of Rabat.

Preparation of extracts

Ethanolic extract

Seeds of *Delphinium Staphisagria* were extracted with 80% Ethanol by maceration at room temperature (25°C) over the period of 24 hours. 250 g of seeds material and one liter of 80% Ethanol were used in the extraction. Ethanol, containing the extract, was then filtered through Whatman paper and the solvent was vacuum distilled at 50°C in rotary evaporator. Final extract was a dark green semi-solid in percentage dray weight of 5.58%.

Alkaloidic extract

The ethanolic extract was treated with 0.5 M H_2SO_4 and filtered. The acid solution was extracted with $CHCl_3$ to give a crude material (6.44g). Acid aqueous phase was neutralized to pH 7 and

extracted with $CHCl_3$ to give a crude materiel (5.36g). Neutral aqueous phase was basified with 20% NaOH to pH 12 and extracted with $CHCl_3$ to give a crude alkaloidic material (1.45g). Ethanolic and Alkaloid extracts were kept in deep freezer at –20°C until use[20].

Animals

Female Swiss mice (20-30g) (Offa-credo, France) were used in the acute toxicity from estimation of the LD_{50}; The Analgesic activity was done using male Wistar rats (180-200g) and Swiss mice. The animals were acquired from the animal centre of Mohammed V-Souissi University, Medicine and Pharmacy Faculty-Rabat. All animals had free access to food and water; they were housed under standard environmental conditions on a 12/12h light/dark cycle. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509.

Acute toxicity

LD_{50} values were determined as described by OECD 423[22, 23, 24]. The test consists on a stepwise procedure with the use of three female mice per step. The method permits estimation of an LD_{50} with a confidence interval and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) [25]. After a single dose administration, mice were placed in individual clear plastic cages and all animals were observed for possible mortality cases (24h) and behavioural changes followed by daily weight monitoring for 14 days.

Analgesic activity

The evaluation of the analgesic activity of the ethanolic and Alkaloidic extracts obtained from *Delphinium staphisagria* seeds was carried out by using two different methods that used thermal stimuli (Tail Flick test), and chemical stimuli (Koster test)[26,27].

Acetic acid-induced writhing response in mice

A chemical visceral pain model used in this test has been described by Koster. Swiss Offa-credo mice were selected one day prior to each test and were divided into groups of six mice each. The total number of cramps following intraperitonial administration of acetic acid solution (3% with 300 mg /kg i.p) was recorded over a period of 20 min, starting 5 min after acetic acid injection, the mice were treated with ethanolic extract of seeds of Delphinium staphisagria (20 and 40 mg/kg, p.o); alkaloidic extract (10 and 20mg/kg, p.o) or standard drug (aspirin, 200 mg/kg, p.o), 30 min before administration of acetic acid, the number of cramps and stretching was recorded and permitted to express the percentage of protection using the following ratio (control mean-treated mean) x 100/control mean.

Tail flick test

The nociception was assessed by using a meter LE 7106 Tail-flick PANLAB (part of the Harvard Bioscience Family Spain), the tail flick test response was done by measuring the time taken to withdraw the tail from the heat source. Each rat was encased in a small aluminum chamber with the middle portion of the tail placed over the light Bern of the tail flick apparatus. A maximum cut-off time of 12 seconds was observed to minimize undue tissue damage as a result of over exposure of the tail to heat[39, 40]. Morphine (5mg/kg s.c), was used as positive control and *Delphinium staphisagria ethanolic* extract was administrated (20, 40 mg/kg; p.o.), and alkaloid extract (10, 20 mg/kg; p.o.) The reading was taken 15, 30, 60 and 120 min after administration of ethanolic extract (20, 40mg/kg; p.o.) and alkaloid extract (10, 20mg/kg; p.o.) of *Delphinium staphisagria* seeds to different groups of six animals.

Statistical analysis

The results were reported as mean±S.E.M., and analyzed by one-way ANOVA followed by student's t-test used for statistical evaluation. A value of $p< 0.05$ was considered significant.

RESULTS

Acute toxicity

During the 14 days, the evolution of the weight was established. Abdominal contraction was observed by 20-25min after the oral administration of *Delphinium staphisagria* seeds ethanolic and alkaloidic extracts. All the animals treated with this extract presented feebleness, hypothermia and insufficiency respiratory. The LD_{50} value was ranged from 200 to 300mg/kg. These results showed that, the ethanolic extract was evaluated as less toxic extract for seeds of *Delphinium staphisagria* (LD_{50}=300 mg/kg). However, the alkaloid extract to be the most toxic extract with LD50 equate to 200 mg/kg. Under the system of global harmonization of chemicals (GHS), these products are classified category 3 and 4, respectively, due to the LD_{50} was lower than 2000mg/kg (Table 1).

Table. 1 LD_{50} (mg/kg) and extraction yields (%) of *Delphinium staphisagria* seeds ethanolic and alkaloidic extracts

Extracts	Ethanolic extract	Acid extract	Alkaloidic extract	Alkaloidic extract
Phytochemical analysis	Alkaloides, Flavonoides, Glycosides Anthraniliques	Delphinine	Delphinine 14-acetylneoline Chasmanine 14-acetylchasmanine Neoline	19-oxodihydroatisine, Isoazitine Azitine
LD_{50} (mg/kg; p.o.)	300	300	200	200
Extraction yields (%)	5.58	2.57	2.14	0.58

Mice of each group (*n*=3) were received single dose and they were examined for 14 days to determine LD_{50} and any behavioral changes. p.o.: mean oral route.

Acetic acid-induced writhing in mice

The inhibition percentages of writhing for extracts are shown in Table 2. The reference drug Aspirin inhibited 51.81% of the number of writhing elicited by acetic acid. The ethanolic extract (40 mg/kg; p.o.) of *Delphinium staphisagria* seeds restrained the writhing reflex induced by acetic acid with an inhibition percentage of 50.17% .The alkaloidic extract (20mg/kg; p.o.) possess the highest analgesic properties with an inhibition percentage of 69.3%. The lowest activity was observed for ethanolic extract (20mg/kg; p.o.) (Table 2).

Table.2 The effect of *Delphinium staphisagria* ethanolic extract (EE) and Alkaloidic extracts (AE) on acetic acid induced writhing in mice

Treatment response	Dose (mg/kg, p.o.)	Number of writhing (per 20 min)	Inhibition of writhing (%)
Control		50.50 ± 2.81	
Aspirine	200mg/kg	24.33 ± 2.87	51.82%
Ethanolic extract	20mg/kg	31.50 ± 3.08	37.62%
Ethanolic extract	40mg/kg	25.16 ± 3.06	50.17%
Alkaloidic extract	10mg/kg	21.66 ± 2.42	57.10%
Alkaloidic extract	20mg/kg	15.50 ±1.51	69.3%

Data are expressed as mean ± S.E.M.; *n*= 6 per group; *P*< 0.001 versus the control group; n: mean number of animals; p.o.: mean oral, EE: ethanolic extract, AE: Alkaloidic extract.

Effect of ethanolic and alkaloidic extracts on Tail Flick test

As shown in figure 1, it can be clearly observed that morphine produced a significant increase (p<0.001) in the response time in the Tail flick experiment. The ethanolic (40 mg/kg, p.o.) and the alkaloidic extract (20 mg/kg, p.o.) significant (p<0.001) increased the reaction time respectively (6.46 ± 0.17); (7.50 sec ±0.22 sec) at 45 min in the Tail flick experiment which comparable with morphine (5 mg/kg, s.c.), the reaction time was (7.59 ±0.10sec) (p<0.001) (Fig.1).

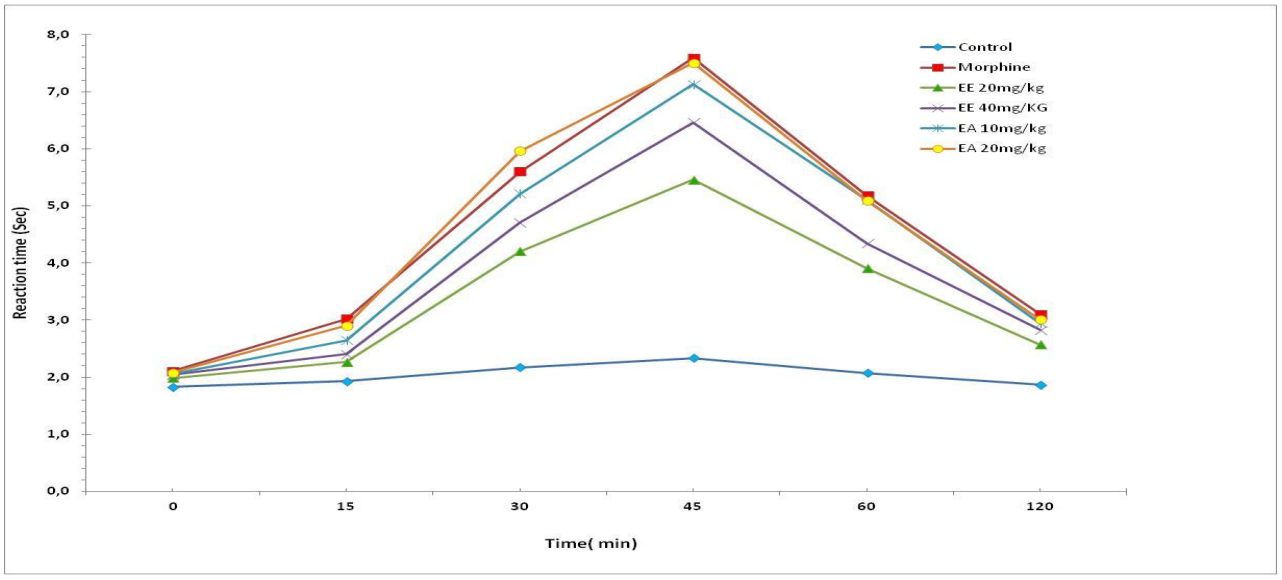

Fig.1 Central Analgesic activity of ethanolic and alkaloidic extracts of *Delphinium staphisagria* by Tail Flick test. Data are expressed as mean ± S.E.M., (n = 6) *P*<0.001, statistically significant relative to control at 45 min.

DISCUSSION

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilizations. Driven by their intuition and their sense of observation, they were able to find answer to their health problems in the plant environment[1, 2, 40]. Recently, the search for novel pharmacotherapy from medicinal plants for inflammation diseases has progressed significantly owing to their less side effects and better tolerability. Aromatherapy is currently used worldwide in the management of chronic pain[3, 4]. We analyzed the effect of different doses of ethanolic extract and alkaloidic from *Delphinium staphisagria* seeds for their acute toxicity and analgesic activities. The LD_{50} value was ranged from 200 to 300mg/kg. These results showed that, the ethanolic extract was evaluated as less toxic extract for seeds of *Delphinium staphisagria* (LD_{50}=300 mg/kg). However, the alkaloid extract to be the most toxic extract with LD50 equate to 200 mg/kg. Preliminary phytochemical analysis of the ethanolic extract revealed the presence of flavonoides, alkaloids and dianthramides glycosides[20, 28, 29]. The alkaloidic is the most toxic, so seeds toxicity is in relation with the presence of alkaloids[26].

The analgesic activities were evaluated using two laboratory models. The Tail Flick test was selected to investigate the central analgesic activity. Acetic acid-induced writhing response was selected to evaluate the peripheral analgesic effects.

In the acetic acid induced writhing test, all tested doses reduced significantly the number of writhing, as ethanolic as alkaloidic extracts demonstrated a significant analgesic effects, with an inhibition percentage of 37.62 %, 50.17 % at the doses 20 mg/kg and 40 mg/kg, respectively, for ethanolic extract, and with an inhibition percentage of 57.10%, 69.3% at the doses 10mg/kg and 20mg/kg respectively, for alkaloidic extract, as compared to the control group. Related studies have demonstrated that acetic acid indirectly induces the release of endogenous mediators of pain (such as prostaglandin, kinin, histamine….etc), that stimulate the nociceptive neurons, which are sensitive to non-steroidal anti-inflammatory drugs and opioïds[31]. The results obtained in this current study suggest that, the extracts for *Delphinium staphisagria* seeds possess peripheral analgesic properties; this particular activity is probably linked to their anti-inflammatory effects[30].

In the Tail Flick test, the central analgesic effects were observed (Fig. 1), indicating that the ethanolic extracts of *Delphinium staphisagria* at the dose 20 mg/kg, don't exhibit significant analgesic activity when compared with control and morphine treated animal. By increasing the doses to (40 mg/kg, 20mg/kg; p.o.) the ethanolic and alkaloidic extracts of *Delphinium staphisagria* produces significant ($p<0.001$) central analgesic action, because this extract increase the reflex time of removal the tail of rats by inhibition the pain respectively (6.46 sec±0.17) (7.50 sec ±0.18) at 45 min, these results were compared with morphine, (7.65 sec ±0.26) at the same time.

These observations suggest that, the ethanolic and alkaloidic extracts of *Delphinium staphisagria* have a significant inhibitory activity in inflammation pain, and this activity may be related with the suppression of synthesis and/or release of endogenous pro-inflammatory substances.

Alkaloids are commonly found to have analgesic activities[30-33]are detected in the ethanolic seeds extract are known to have analgesic activity. The result obtained in this work demonstrated a high activity at low alkaloids extracts doses (10 and 20 mg/kg). However, alkaloids detected in the seeds extracts can be solely responsible for the analgesic activity. With those analgesic property alkaloids of *D. staphisagria* seeds can be considered an effective agent to treat diseases. As analgesics, this could be beneficial in searching for the potential analgesics that is equal or more active, but with lower toxicity, than currently clinical used C18- and C19-alkaloid- type analgesics[30, 32]. These chemical compositions were also identified in others plants extracts such as, *Stylosanthes fruticosa*[33], *Pistacia integerrim*[38,39], *Hedyotis puberula*[35-37]and *Argania spinosa*[38], that have been provided to possess analgesic activity.

Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the analgesic effect.

CONCLUSION

We conclude that, the alkaloidic extract of *Delphinium staphisagria* is a notable, central, and peripheral analgesic activity, these data provide pharmacological basis for its therapeutic efficacy on pains.

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Bio Sciences **RESEARCH ARTICLE……!!!**

IN VITRO POTENTIAL ANTIBACTERIAL ACTIVITY OF THE DICHLOROMETHANE AND HEXANE EXTRACT OF *INULA VISCOSA* From MOROCCO

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KEYWORDS:

Inula viscosa, Minimal inhibitory concentration, Bactericidal minimal concentration, Antibacterial screening, Hexane extract, Dichloromethane extract.

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ABSTRACT

The present study was conducted to investigate the antibacterial activity against twenty standard bacterial strains evaluated with the disc diffusion assay and the MTT assay to determinate the Minimum Inhibitory Concentration and Bactericidal Minimal Concentration. The extracts of plants screened revealed promising antibacterial activities against most pathogens bacteria. The result of the antimicrobial activity indicated by zone of inhibition of growth ranged from 15 to 48 mm**.** The antibacterial activity demonstrated that all extracts have an inhibitory activity against bacteria with the lowest MIC at 156 µg/ml. The extract contains some major bioactive compounds that inhibit the growth of microorganisms thereby proving very effective as alternative source of antibiotics. The antibacterial activity of dichloromethane extract was less than this of hexane extract (MIC 312 and 625 µg/ml) was observed against all tested bacteria. These species might provide promising therapeutic agents against infections with multi-resistant and pathogens bacteria. To sum up, this work represents that the dichloromethane and hexane extracts from leaves of *Inula viscosa* have obvious antibacterial activity; these data provide pharmacological basis for its therapeutic efficacy on infectious diseases.

INTRODUCTION:

All over the world, infectious disease is the number one cause of death accounting for approximately one-half of all the deaths in tropical countries. This perhaps may be attributed to the increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases[1]. Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value[2-4]. Very few antimicrobial studies have focused on in vivo models[5, 6]. Recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. However, there have been some efforts to mimic the traditional use of plant material as noted by the addition of aqueous extracts in screening assays[7-12]. Several methods are currently available to detect the antibacterial activity of plant extracts using different principles to assess bacterial growth or its inhibition. Some reports have focused on the antimicrobial screening together with other pharmacological investigations including toxicity[13, 14]. In Morocco, there is more than 42,000 species of plants; divided into 150 families and 940 genus used in traditional medicine[2, 15]. Some studies have reported that the extract of *Inula viscosa* have a number of pharmacological activities, such as antibacterial, anti-inflammatory, antidiabetic, antipyretics, antiseptic, for treating gastro duodenal disorders[16-25]. *Inula viscosa* is a common dense, evergreen, aromatic shrub grown in many parts of the world. The fresh and dried flowers are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods. They are extensively used in cooking, and a distinct mustard smell gives off while they are burned, therefore, they often are used to flavor foods while barbecuing. Essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. Thus, the aim of this study is to evaluate the antibacterial activity of the dichloromethane and hexane extracts of *Inula viscosa* against different bacterial strains which may be involved in such diseases, using a prelimary bioassay screening and to quantify the minimum inhibition concentration (MIC) using colorimetric method[26-28].

MATERIALS AND METHODS

Plant material

Inula viscosa was collected based on ethnopharmacological information, from villages around the region Ain Taoujtat, middle Morocco on April and August 2010, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB11577) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco.

Preparation of dichloromethane and hexane extracts

Leaves of *I. viscosa* (*Dittrichia viscosa* L.) were successively extracted with dichloromethane and hexane by cold maceration and soxhlet, respectively, at room temperature (25°C) over period of 48h. 100 g of plant material and one liter of each extracts of dichloromethane and hexane were used in the extraction. Dichloromethane and hexane containing the extract were then filtered through Whatman paper and the solvent was vacuum-distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for 2h to ensure the removal of any residual solvent. Final extracts were in percentage dry weight 15.39 and 25.34%, respectively; these dichloromethane and hexane extracts were kept in deep freeze at -20°C until use[29-32].

Antibacterial Activity Test

Bacterial strains

This study was made on seven strains of *Escherichia coli,* bacteria often found in the diarrhea and urine of children from 0 to 5 years old. Among these seven strains, thirteen are referenced strains namely *Klebsiella pneumoniae, Staphylococcus aureus, Edwardsiella tarda, Providencia stuartii, Salmonella paratyphi B, Citrobacter freundii, Salmonella paratyphi A, Serratia marcescens, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella oxytoca, Streptococcus feacalis* and *Proteus vulgaris*: A strain was retrieved from well and another from hospital. The cultures of bacteria were maintained in their appropriate agar slants at +4°C throughout the study and used as stock cultures. The strains used in this work are widely encountered in various diseases in humans and multiple antibiotic-resistant bacteria. The bacteria were obtained from the **L**aboratory of **M**icrobiology, Makoudi Private Medical Laboratory, Meknes, Morocco (Table 1).

Table 1: Origin of Sampling of *Escherichia coli.*

Strains	Age of Man (years)	Age of woman (years)	Origin of Sampling
E.coli$_1$	35	_	Urine
E.coli$_2$	3month	_	Urine
E.col$_3$	_	30	Vaginal
E.coli$_4$	_	23	Urine
E.coli$_5$	_	60	Urine
E.coli$_6$	60	_	Urine
E.coli$_7$	23	_	Urine

Determination of antibacterial activity by the paper disc diffusion method

The Dichloromethane and hexane extracts of *Inula viscosa* were tested for antibacterial activity by the paper disc diffusion method. Molten (45°C) sterile Muller Hinton Agar (10ml) in a flask was inoculated with a broth culture (1%, containing 10^6-10^7 cfu/ml) of the respective bacterial strains and poured over plates containing 10ml Muller Hinton Agar in sterile 9cm Petri dishes. Fifty microliters of dilutions of the dichloromethane and hexane extracts were pipette on sterile filter paper disc (Whatman No.1. 5mm in diameter), which were allowed to dry in an open sterile Petri dishes in a biological safety cabinet with vertical laminar (Nuaire Petri dishes Laminar Flow Products, USA). 5000 to 61µg/ml of the dichloromethane and hexane extracts solutions were applied to the discs. Discs were placed on the surface of the inoculated plates and incubated at 37°C for 18h. Zones of inhibition of microbial growth around the paper disc containing the extracts were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (mm) from the outside margin of the paper disc to the initial point of the microbial growth. All analyses were applied in triplicate[35-44]. Discs impregnated with sterile distilled water served as negative control. Ciprofloxacin, Ceftriaxone, Cefaexin and Ampicillin which purchased from Sigma (St. Louis, USA) were used as a positive control[45-46].

Determination of Minimum Inhibitory Concentration (MIC) and Bactericidal Minimal Concentration (BMC)

Test strains were suspended in Muller Hinton Agar (MHA). The suspension was adjusted spectrophoto-metreically to match the turbidity of a McFarland 0.5 scale (*i.e.* to give a final density of 10^7cfu/ml). The viability indicator MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide, Sigma, Aldrich) and the quick microplates method were used for the determination of the Minimum Inhibitory Concentration (MIC) and Bactericidal Minimal Concentration (BMC)[18, 42-48]. Serial dilutions of dichloromethane and hexane extracts were dissolved in distilled water and made in a concentration range from 61µl/ml to

5000µl/ml in sterile test tubes. Each test tube was inoculated with 50µl from each various dilutions of the dichloromethane and hexane extracts of *I. viscosa* were added to 5ml of Muller Hinton Agar both in tubes containing 10_7cfu/ml of live bacterial cells. The tubes were then incubated under optimal conditions at 37°C for 18h. After incubation, as an indicator of bacterial growth 1mg/ml of MTT was added to each well, after incubation periods ranging from 3 to 5h at 37°C, control without plant extracts with MTT and bacterial inoculums were used as negative control. The bacterial suspension changed to blue when bacterial growth occurred. The highest dilution (lowest concentration), showing no visible growth was regarded as the MIC. Cell suspension (0.1ml) from the tubes showing no growth was sub cultured on Muller Hinton Agar plates in triplicate to determine if the inhibition was reversible or permanent. All tests were performed in triplicate.

Statistical analysis

Results of the research were tested for statistical significance by one way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Most of the researchers have evaluated first the antibacterial spectra of plant extracts; secondly they have determined the chemical composition of the extracts, and thirdly they have retroactively attributed such activity to such molecule. In this study the dichloromethane and hexane extracts of *Inula viscosa* were evaluated for antimicrobial activity against Gram positive and Gram negative bacteria. *Inula viscosa* hexane extract was found to be the most active against all of the bacterial strains. Our results show that the antibacterial activities are related to the origin of the method of extraction, the nature of the solvent and the strain tested. The diameter of the zone of inhibition differs from bacteria to another and from an extract to another. The inhibition diameters vary from 15 to 48 mm according to this diffusion method, an extract is regarded as active when it induces an inhibition zone greater than or equal to 10 mm. The hexane extract of leaf has revealed active over the whole strain tested except over *Serratia marcescens, Acinetobacter baumannii, Pseudomonas aeruginosa, Streptococcus feacalis, E. coli*$_2$, *E. coli*$_3$, and *E. coli*$_5$. While the dichloromethane extract of leaf has revealed active except over *Klebsiella pnomoniae, Staphylococcus aureus, Salmonella paratyphi B, Providencia stuartii, E. coli*$_1$, *E. coli*$_4$, *E. coli*$_6$, and *E. coli*$_7$ (Figure 1).

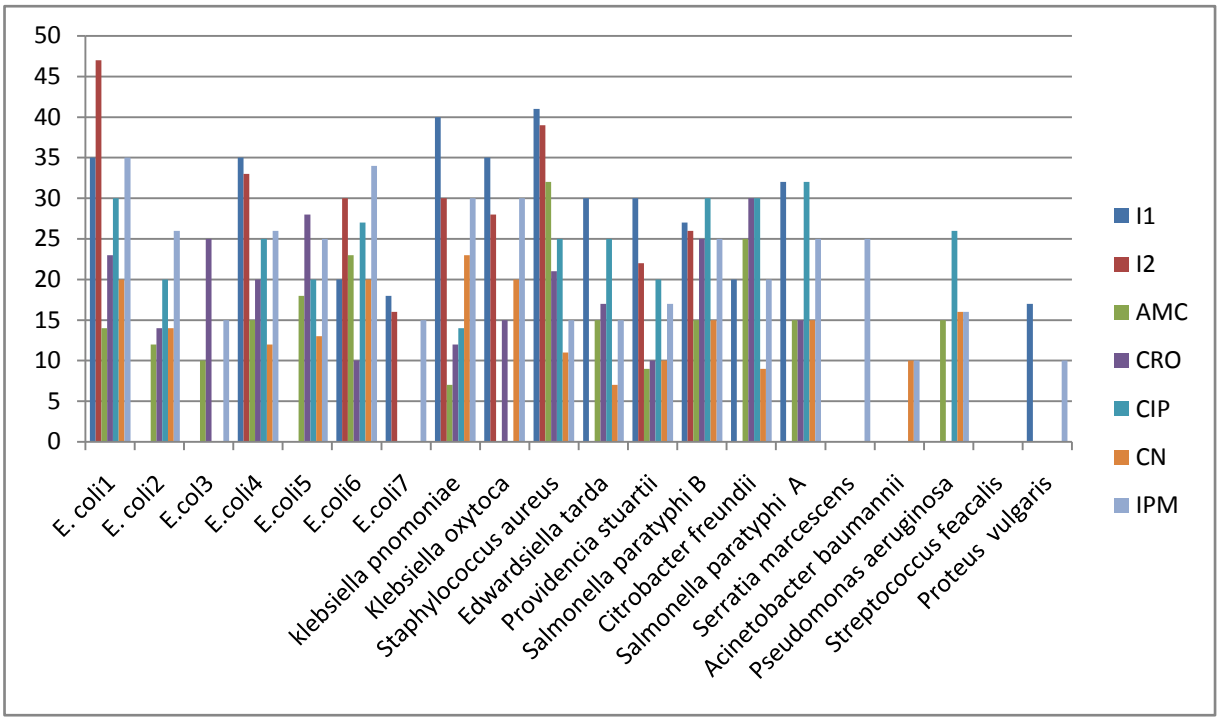

Figure 1: Diameter of inhibition of growth of tested germs and the antibiotics by disc diffusion method (IZD in mm).

The results of the paper disc diffusion support and extend previous finding that *I. viscosa* extracts contains numerous biologically active compounds and some of these have been frequently used in folk medicine for their antibacterial properties. In addition, the MIC values support the finding of the paper disc diffusion method (Table 2 and 3). Gram-negative bacteria which are responsible for a large number of infectious diseases have a unique outer membrane that contains lipopolysaccharides which render them impermeable to certain antibacterial compounds[36]. The biological activity of *I. viscosa* against the tested bacteria could be attributed to the presence of flavonoids, terpenoids and phenolic acids[22-25, 49-51]. Biologically active components are believed to disturb permeability of the cytoplasm membrane and thereby facilitate the influx of antibiotic[45-48]. The results presented in this report highlight the potential of *Inula viscosa* extracts as a source of antibiotic resistance modifying compounds. The MICs for the *I. viscosa* hexane extract ranged from 156 to 625 µg/ml for all test microorganisms ($P>0.001$), while MICs for dichloromethane extract was ranged from 312 to 2500 µg/ml ($P<0.01$) (Table 2). These differences in the susceptibility of the test microorganisms to the test samples could be attributed to variation in the rate of samples penetration through the cell wall and cell membrane structures[20-35]. In general, the hexane extract showed greater antimicrobial activity than dichloromethane extracts (Table 2).

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Table 2: Determination of Minimum Inhibitory Concentration (MIC, µg/ml), Bactericidal Minimum Concentration (BMC, µg/ml) and report MBC/MIC of the hexane and dichloromethane extracts.

Germes	MIC			BMC			BMC / MIC		
	I_1	I_2	CIP	I_1	I_2	CIP	I_1	I_2	Interpretation
***E. coli*$_1$**	312±0.2	312±0.1	240±0.0	312±0.2	312±0.2	480±0.0	1	1	Bactericidal
***E. coli*$_2$**	_	_	1950±0.0	_	_	1950±0.0	_	_	Bactericidal
***E. coli*$_3$**	_	_	_	_	_	_	_	_	Bactericidal
***E. coli*$_4$**	312±0.1	625±0.2	970±0.0	312±0.2	625±0.1	970±0.0	1	1	Bactericidal
***E. coli*$_5$**	_	_	1950±0.0	_	_	1950±0.0	_	_	Bactericidal
***E. coli*$_6$**	2500±0.0	625±0.1	61±0.1	2500±0.0	625±0.1	61±0.2	1	1	Bactericidal
***E. coli*$_7$**	2500±0.0	2500±0.0	_	2500±0.0	5000±0.0	_	1	2	Bactericidal
Klebsiella pnomoniae	156±0.1	625±0.0	3900±0.0	312±0.2	625±0.0	3900±0.0	2	1	Bactericidal
Klebsiella oxytoca	312±0.1	625±0.0	_	312±0.1	625±0.1		1	1	Bactericidal
Staphylococcus aureus	156±0.2	156±0.2	970±0.0	156±0.2	312±0.1	970±0.0	1	2	Bactericidal
Edwardsiella tarda	625±0.1	_	970±0.0	625±0.0	_	970±0.0	1	_	Bactericidal
Providencia stuartii	625±0.1	5000±0.0	1950±0.0	625±0.1	5000±0.0	1950±0.0	1	1	Bactericidal
Salmonella paratyphi B	1250±0.0	2500±0.0	240±0.0	1250±0.0	5000±0.0	480±0.0	1	2	Bactericidal
Citrobacter freundii	1250±0.0	_	240±0.0	1250±0.0	_	480±0.0	1	_	Bactericidal
Salmonella paratyphi A	625±0.2	_	240±0.0	625±0.1	_	240±0.0	1	_	Bactericidal
Serratia marcescens	_	_	_	_	_	_	_	_	Bactericidal
Acinetobacter baumannii	_	_	_	_	_	_	_	_	Bactericidal
Pseudomonas aeruginosa	_	_	61±0.2	_	_	61±0.2	_	_	Bactericidal
Streptococcus feacalis	_	_	_	_	_	_	_	_	Bactericidal
Proteus vulgaris	2500±0.0	_	_	2500±0.0	_	_	1	_	Bactericidal

Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive Control (CIP, Ciprofloxacine).

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It is quite difficult to attribute the antimicrobial effect of an extract to one or a few active principles, because extracts always contain a mixture of different chemical compounds[33-35]. In addition to the major components, also minor components may make a significant contribution to the antimicrobial activity of extracts. Monoterpenes and terpinenes have also shown antimicrobial properties that appear to have strong to moderate antibacterial activity against Gram positive bacteria[25, 49-52]. In most of the cases, the bactericidal action is considered the BMC/MIC ratio. The action of the whole extract is bactericidal against all strains. Aligiannis and colleague [24] have proposed a classification of extracts of plant material on the basis of MIC results, as shown in table 3. Thus, according to this classification, there is a very strong inhibition with hexane extract of *Inula viscosa* on all strains (Table 3).

Table 3: Classification of extracts according Aligiannis *et al*.[24]

MIC	I_1	I_2
Strong inhibition: MIC lower than 500µg/ml	*K. pnomoniae* *S. aureus* *E_1 E_4* *K. oxytoca*	*S. aureus* *E_1*
Moderate inhibition: MIC varies from 600µg/ml to 1500 µg/ml	*E. tarda* *P. stuartii* *S. paratyphi A* *S. paratyphi B, C. freundii*	*E4E6* *k. pnomoniae* *K. oxytoca*
Weak inhibition: MIC greater than 1600µg/ml	*P. vulgaris* *E6 E7*	*E7* *S. paratyphi B, P. stuartii*

I_1: Hexane extract; I_2: Dichloromethane extract; MIC: Minimum Inhibitory Concentration.

By taking into account both MIC and MBC, the sensitivity decreases in the following order: for the hexane extract: *Staphylococcus aureus= Klebsiella pnomoniae > E. $coli_1$ = E. $coli_4$ = Klebsiella oxytoca > Edwardsiella tarda = Providencia stuartii =Salmonella paratyphi* A *> Salmonella paratyphi* B*= Citrobacter freundii > Proteus vulgaris = E. $coli_6$ = E. $coli_7$.*

While the dichloromethane extract: *Staphylococcus aureus > E. $coli_1$ > E. $coli_4$ = E. $coli_6$ = Klebsiella pnomoniae = Klebsiella oxytoca > E. $coli_7$= Salmonella paratyphi* B *> Providencia stuartii.*

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Our results against these bacterial strains suggest the efficacy of plant claimed by traditional healers. This plant may be a source which could yield drug that could improve the treatment of infections caused by this organism.

CONCLUSION

Multiple drug resistance in bacterial pathogens is a continuing problem throughout the world.

There is an established need to develop new antibacterial agents to combat these pathogens.

To sum up, this work represents that the dichloromethane and hexane extracts from leaves of *Inula viscosa* have obvious antibacterial activity; these data provide pharmacological basis for its therapeutic efficacy on infectious diseases.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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IN VITRO ANTIBACTERIAL ACTIVITY OF *ROSMARINUS OFFICINALIS* METHANOLIC AND AQUEOUS EXTRACTS

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ABSTRACT

Rosemary (Rosmarinus officinalis L., family *Lamiaceae*) had been reported in traditional medicine, to exhibit antimicrobial properties. Therefore, this study is aimed at determining the antibacterial activity of *R. officinalis* leaves against pathogenic microorganisms by determination the minimal inhibitory concentration and to serve as criteria to recommend the ethno pharmacological uses of the plant. Plant leaves were dried, powdered and extracted by cold maceration with methanol for 48h. The extracts were screened against 24h broth culture of bacteria seeded in Muller Hinton Agar at concentration 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56mg/ml in sterile distilled water and incubated at 37°C, for 18h and measuring the inhibition zone diameter (IZD). Minimum inhibitory concentrations (MICs) against three Gram-positive bacteria (*Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus subtilis*), three Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) were determined for the methanolic and aqueous extracts of *Rosmarinus officinalis*. The methanolic extract showed pronounced antibacterial than the aqueous extract against all of the tested microorganisms. Methanolic extract inhibited with minimal inhibitory concentration of 1.56, 1.56, 3.13, 1.56, 3.13 and 3.13mg/ml against *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*, respectively, while the aqueous extract inhibited with minimal inhibitory concentration of 6.25 mg/ml against all tested bacterial strains both Gram-positive and Gram-negative bacteria. The methanolic and aqueous extracts of *R. officinalis* demonstrated activities against certain bacteria confirming the use of the plant in ethno pharmacology.

Keywords: *Rosmarinus officinalis*, minimal inhibitory concentration, antibacterial screening, bacterial strains, medicinal plant.

INTRODUCTION

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations. [1] Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that

follow their use (in some cases) and the cultural, spiritual point of view of the people of the countries. [1] In western developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. [2] Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine become necessary. [3] In Morocco, there is more than 42,000 species of plants; divided into 150 families and 940 genus used in traditional medicine. [4, 5]

Rosmarinus officinalis L., commonly referred to as rosemary, belongs to the mint family. It is a common dense, evergreen, aromatic shrub grown in many parts of the world. [6] The fresh and dried leaves are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods. A tisane can also be made from them. They are extensively used in cooking, and a distinct mustard smell gives off while they are burned, therefore, they often are used to flavor foods while barbecuing.

Historically, rosemary has been used as a medicinal agent to treat renal colic and dysmenorrheal. It has also been used to relieve symptoms caused by respiratory disorders and to stimulate the growth of hair. Extracts of rosemary are used in aromatherapy to treat anxiety-related conditions and to increase alertness. [7-9] Essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. [7, 10] Particularly, the antimicrobial and antivirus activities of plant oils and extracts have formed the basis of applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. [11] Because of the possible multiple resistances and side effects of the synthetic antimicrobial, increasing attention has been directed towards natural antimicrobial. [12] There are many studies on the antimicrobial activity of secondary metabolites in recent years, but to our knowledge, fewer comparative studies on antimicrobial activity of *R. officinalis* L. methanolic and aqueous extracts have been reported. [13]

To the best of our knowledge, this is the first study to provide data that the methanolic and aqueous extracts of *Rosmarinus officinalis* evaluated against a wide range of bacteria. Thus, the aim of this study is to evaluate the antimicrobial activity of rosemary (*Rosmarinus officinalis*) methanolic and aqueous extract, and to, therefore, determine the scientific basis for its use in traditional medicine in the treatment of infection diseases.

MATERIALS AND METHODS

Plant material: *Rosmarinus officinalis* was collected based on ethnopharmacological information, from villages around the region Rabat-Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco.

Preparation of extract

Methanolic extract: Stems and leaves of *R. officinalis* were successively extracted with methanol by maceration at room temperature (25°C) over period of 48h. 500 g of plant material and one liter of methanol were used in the extraction. Methanol containing the extract was then filtered through Whatman paper and the solvent was vacuum-distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for 2h to ensure the removal of any residual solvent. Final extract was a yellow powder in percentage dray weight 22.8%; this methanolic extract was kept in deep freeze at -20°C until use.

Aqueous extract: 500 g of plant material was extracted by infusion in boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration and then lyophilized (Free Zone® Dry 4.5, USA). For each study, the lyophilized aqueous extract was carefully prepared under the same condition used throughout the studies (time, temperature and the amount of plant material and water used for extraction under reflux and lyophilization) and each time the quality of extraction was checked by the yield of the lyophilization material. The final crude extract was obtained as dark brown powder in

percentage from dry weight (18.7%). For assuring stability, the lyophilized material was stored at -20°C.

Antibacterial Activity Test

Bacterial strains: The following 6 microorganisms (three Gram-negative and three Gram-positive bacteria), were used in this study: *Escherichia coli* ATCC 54127, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 53153, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633 were used for antibacterial testing. The cultures of bacteria were maintained in their appropriate agar slants at +4°C throughout the study and used as stock cultures. The bacteria were obtained from the **L**aboratory of **M**icrobiology, **N**ational **L**aboratory of **V**eterinary **D**rugs **C**ontrolled, Rabat, Morocco.

Determination of antibacterial activity by the paper disc diffusion method: The methanolic and aqueous extracts of *R. officinalis* were tested for antibacterial activity by the paper disc diffusion method. Molten (4 5°C) sterile Muller Hinton Agar (10ml) in a flask was inoculated with a broth culture (1%, containing 10^6-10^7 cfu/ml) of the respective bacterial strains and poured over plates containing 10ml Muller Hinton Agar in sterile 9cm Petri dishes.

Fifty microliters of dilutions of the methanolic and aqueous extracts were pipette on sterile filter paper disc (Whatman No.1. 5mm in diameter), which were allowed to dry in an open sterile Petri dishes in a biological safety cabinet with vertical laminar (Nuaire Petri dishes Laminar Flow Products, USA). 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56mg/ml of the methanolic and aqueous extracts solutions were applied to the discs. Discs were placed on the surface of the inoculated plates and incubated at 37°C for 18h.

Zones of inhibition of microbial growth around the paper disc containing the extracts were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (mm) from the outside margin of the paper disc to the initial point of the microbial growth. All analyses were applied in triplicate. [14, 15] Discs impregnated with sterile distilled water served as negative control. Penicillin and Ampicillin which purchased from Sigma (St. Louis, USA) was used as a positive control. [17-19]

Determination of Minimum Inhibitory Concentration (MIC): Test strains were suspended in Muller Hinton Agar (MHA). The suspension was adjusted spectrophoto-metreically to match the turbidity of a McFarland 0.5 scale (*i.e.* to give a final density of 10^7cfu/ml).

The viability indicator MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide, Sigma, Aldrich) and the quick microplates method were used for the determination of the minimum inhibitory concentration (MIC)**.** [19-22] Serial dilutions of extracts were dissolved in distilled water and made in a concentration range from 1.56mg/ml to 200mg/ml in sterile test tubes. Each test tube was inoculated with 20µl from each various dilutions of the methanolic and aqueous extracts of *R. officinalis* were added to 5ml of Muller Hinton Agar both in tubes containing 10^7cfu/ml of live bacterial cells. The tubes were then incubated under optimal conditions at 37°C for 18h. After incubation, as an indicator of bacterial growth 1 mg/ml of MTT was added to each well, after incubation periods ranging from 3 to 5h at 37°C, control without plant extracts with MTT and bacterial inoculums were used as negative control. The bacterial suspension changed to blue when bacterial growth occurred. The highest dilution (lowest concentration), showing no visible growth was regarded as the MIC. Cell suspension (0.1ml) from the tubes showing no growth was sub cultured on Muller Hinton Agar plates in triplicate to determine if the inhibition was reversible or permanent. All tests were performed in triplicate.

Statistical analysis: Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The methanolic and aqueous extracts were evaluated for antimicrobial activity against Gram-positive (*S. aureus, M. luteus* and *B. subtilis*), Gram-negative (*P. aeruginosa, K. pneumoniae* and *E. coli*) bacteria. Rosemary methanolic was found to be the most active against all of the bacterial strains. *R. officinalis* methanolic extract displayed good activities, inhibiting the growth of *E. coli*, *P. aeruginosa* and *K. pneumoniae* with IZD of 28, 24 and 20 mm respectively but with less activity against three Gram positive bacteria (*S. aureus, M. luteus* and *B. subtilis*), with inhibition zone diameter of 20 mm ($P < 0.001$). The inhibition zone diameter for the rosemary methanolic extract ranged from 20 to 50 mm, while IZD for aqueous extract ranged from 18 to 30 mm ($P < 0.01$) (Table 2 and 3). The results of the paper disc diffusion support and extend previous finding that rosemary contains numerous biologically

active compounds and some of these have been frequently used in folk medicine for their antibacterial properties. In addition, the MIC values support the finding of the paper disc diffusion method (Table 1, 2 and 3). Phytochemical studies have identified active components in the methanolic and aqueous extract of this plant, such as flavonoids including diosmetin, diosmin, luteolin, apigenin, quercetin and kaempherol, phenols such as caffeic and rosmarinic acids, and terpenoids like, carnosol, carnosic acid, rosmanol, and oleonolic and ursolic acids. [23-33] The biological activity of rosemary against the tested bacteria could be attributed to the presence of flavonoids, phenolic acids (caffeic, chorogenic and rosmarinic) and essential oils (camphor and cineole) and diterpenes (carnosol). [8, 9, 31-34] Biologically active components are believed to disturb permeability of the cytoplasm membrane and thereby facilitate the influx of antibiotic. [35] The results presented in this report highlight the potential of rosemary extract as a source of antibiotic resistance modifying compounds. The MICs for the rosemary methanolic extract ranged from 1.56 to 6.25 mg/ml for all test microorganisms ($P < 0.001$), while MICs for aqueous extract ranged from 3.13 to 6.25 mg/ml ($P < 0.01$) (Table 1). These differences in the susceptibility of the test microorganisms to the test samples could be attributed to variation in the rate of samples' penetration through the cell wall and cell membrane structures. [36] In general, the methanolic extract showed greater antimicrobial activity than aqueous extract (Table 1). Taking the least IZD of the standard (Penicillin and Ampicillin) as the breaking point, most of the extracts passed the breaking point (Table 2 and 3).

It is quite difficult to attribute the antimicrobial effect of an extract to one or a few active principles, because extracts always contain a mixture of different chemical compounds. In addition to the major components, also minor components may make a significant contribution to the antimicrobial activity of extracts. Following the results above, we could infer that the antimicrobial activity of rosemary methanolic and aqueous extract is the synergistic effect of their compositions. It provided evidence that rosemary methanolic and aqueous extracts may become the potential natural antimicrobial in the field of food and pharmaceutical industries.

CONCLUSION

To sum up, this work represents that the methanolic and aqueous extracts from stems and leaves of *Rosmarinus officinalis* have obvious antibacterial activity; these data provide pharmacological basis for its therapeutic efficacy on infectious diseases.

ACKNOWLEDGEMENTS

The authors wish to thank all the individuals and institutions who made this survey possible.

Table 1: Determination of Minimal Inhibitory Concentration (MIC) of *Rosmarinus officinalis* methanolic and aqueous extracts (mg/ml).

	Minimal Inhibitory Concentration (mg/ml)	
Bacterial strains	**Methanolic extract**	**Aqueous extract**
E. coli	1.56 ± 0.00	6.25± 0.00
K. pneumonia	3.13 ± 0.00	6.25± 0.00
P. aeruginosa	3.13± 0.00	6.25± 0.00
M. luteus	1.56± 0.00	6.25± 0.00
S. aureus	3.13± 0.00	6.25± 0.00
B. subtilis	1.56± 0.00	3.13± 0.00

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341. Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive control (Penicillin and Ampicillin).

Table 2: Inhibition by methanolic extract of *Rosmarinus officinalis* (zone size, mm)

	Different concentration of the methanolic extract (mg/ml)								Positive control	
Bacterial strains	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	50	40	30	28	26	24	20	19	32	28
K. pneumonia	40	34	30	24	22	20	20	18	28	24
P. aeruginosa	40	26	20	20	19	18	18	17	18	18
M. luteus	60	52	34	30	28	26	24	24	33	30
S. aureus	46	34	28	26	24	24	22	20	26	24
B. subtilis	36	34	30	26	24	22	20	20	32	28

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341, Am, Ampicillin, Pe, Penicillin. Values are means±SD of three determinations. Data indicate significant difference (P < 0.05) with respect to positive control (Penicillin and Ampicillin).

Table 3: Inhibition by aqueous extract of *Rosmarinus officinalis* (zone size, mm)

	Different concentration of the aqueous extract (mg/ml)								Positive control	
Bacterial strains	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	26	24	20	18	18	16	16	14	32	28
K. pneumonia	26	22	20	20	18	17	14	12	28	24
P. aeruginosa	24	22	20	20	19	16	14	12	18	18
M. luteus	24	18	18	18	16	16	14	12	33	30
S. aureus	24	20	20	19	19	18	17	14	26	24
B. subtilis	30	28	26	26	24	22	20	20	32	28

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341, Am, Ampicillin, Pe, Penicillin. Values are means±SD of three determinations. Data indicate significant difference (P < 0.05) with respect to positive control (Penicillin and Ampicillin).

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ANTISPASMODIC EFFECT OF *LAVANDULA OFFICINALIS* AQUEOUS EXTRACT

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KEYWORDS:

Antispasmodic, Aqueous extract, *Lavandula officinalis*, Rat jejunum

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ABSTRACT

The fresh flowers of *Lavandula officinalis* are frequently used in traditional Moroccan medicine. This study is aimed at determining the effect of *Lavandula officinalis* flowers aqueous extract on rat isolated jejunum and its possible mechanism. 500 g of plant material was extracted by infusion in boiled water (500 ml) for three days in order to withdrawal the largest number of the secondary metabolites of aqueous extracts. The respective aqueous extract was separated from its residues by gravity filtration and then lyophilized (Free Zone® Dry 4.5, USA). Distal segment of jejunum rat was mounted in an organ bath containing Tyrode's solution. Tissue contractility was isometrically recorded. At the concentration of 1 µg/mL of *L. officinalis* aqueous extract, the rate of spontaneous contraction was decreased at 5.3±0.57 mm/sec. By increasing the concentration to 500µg/mL, the rate of spontaneous contraction was notably decreased with 0.5 mm/sec.The *L. officinalis* aqueous extract decrease this frequency to 23±1 contractions per seconds with 1 µg/ml of lavender aqueous extract and 18.6± 3 contractions per seconds with a maximum inhibition of about 40 % at 500µg/mL of lavender extract. In conclusion, the extract appears to be more potent than trimebutine to produce relaxation and to reduce frequency and velocity of spontaneous contraction. The result showed that *Lavandula officinalis* aqueous extract produce a significant antispasmodic effect supporting its use in folk medicine.

INTRODUCTION:

Morocco is fortunate to have such varied climate that almost any medicinal plant can grow. The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants, divided into 150 families and 940 genuses[1-3]. Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population[4]. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care. Many plants are used in folk medicine to treat gastrointestinal disorders, such as spasms or indigestion and also many people nowadays turn to the use of natural product medicine for treatment of intestinal disorders. Natural products have served as a source of medicines for centuries, and about half of the pharmaceuticals in use today are derived from natural products[5]. Lavander (*Lavandula officinalis* L., family Lamiaceae) had been reported in traditional medicine, to exhibit antispasmodic properties. However the antispasmodic effect has not yet been studied. Thus, the aim of this present study was to investigate the antispasmodic effect of *Lavandula officinalis* aqueous extract on isolated rat jejunum and its possible mechanism.

MATERIAL AND METHODS

Chemicals

All reagents were purchased and used for research use only. Acetylcholine, trimebutine (Sigma-Aldrich, St-Quentin Fallavier, France) and crude extract were dissolved in distilled water prior to use. We analyzed the effect of different doses of the aqueous extract of *Lavandula officinalis* (1 to 500µg/ml).

Plant material and preparation of crude extract

Lavandula officinalis was collected based on ethnopharmacological information, from villages around the region Rabat-Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco. 500 g of fresh flowers of *L. officinalis* was extracted by infusion in boiled water (500 ml) for three days, in order to withdrawal the largest number of

the secondary metabolites of aqueous extracts[3]. The respective aqueous extract was separated from its residues by gravity filtration and then lyophilized (Free Zone® Dry 4.5, USA). For each study, the lyophilized aqueous extract was carefully prepared under the same condition used throughout the studies and each time the quality of extraction was checked by the yield of the lyophilization material. The final crude extract was obtained as yellow greasy powder in percentage from dry weight (15.7% d.w). For assuring stability, the lyophilized material was stored at -20°C.

Animals

Male adult Wistar rats weighing 200-250g were kept in cages under standard laboratory conditions with tap water and standard rat chow ad libitum, in a 12-h/12-h light/dark cycle at a temperature of 21–23°C. The study was conducted in accordance with accepted principles outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health and efforts were made to minimize animal suffering and number of animals used. Ethics approval was obtained from Université Paris Diderot- Paris 7.

Preparation of isolated rat jejunum

Male adult rats were fasted 16h with water *ad* libitum. Animals were sacrificed by cervical dislocation and exsanguinations. The jejunum portions were isolated out and cleaned of mesenteries. The mesenteric border was carefully stripped off using forceps. Segments of the jejunum contents, about 2 cm long were removed by flushing with Tyrode's solution of the following composition in mmol/L: NaCl (136.9), KCl (2.7), CaCl2 (1.8), NaHCO3 (11.09), MgCl2 (1.05), NaH2PO4 (0.42), glucose (5.5) at pH 7.4. The tissue was mounted in a 7 mL organ bath containing Tyrode's solution maintained at 37°C ± 0.5 and gassed with 95% O2 and 5% CO2[6]. An initial tension of 1g was applied and the spontaneous muscular contractility was recorded isometrically using Ugo Basile Unirecorder 7050[7]. Each tissue was allowed to equilibrate for at least 30 min before the addition of any product. Drugs and extract were added directly to the organ chamber in volumes not exceeding 1% of the total bath volume. At the end of the 45-min equilibration period, the effect of different drugs and/or crude extract of *Lavandula officinalis* were investigated cumulatively with a contact time for each concentration of 2 min. Acethylcholine (Ach) were used to induce contraction prior to the additions of cumulative doses of plant.

Statistical analysis

Data are expressed as percentage of relaxation of the contraction induced by Ach 1×10^{-7} (100%) and are the mean ± standard error of mean (S.E.M.). The statistical analyses were obtained by the one way analysis of variance (ANOVA), followed by the Dunnett's test where necessary. $P<0.05$ was considered significant. The concentration–response curves were analyzed by non-linear regression (Graphpad program for Windows version 5.01.Graphpad, San Diego, CA, USA).

RESULTS

Firstly, we measured the rate of spontaneous contraction, frequency of spontaneous contraction of tissue before and after addition of the plant extract cumulatively. Further addition of 10-7 M of Ach induced contraction of the tissue pre-treated with crude extract of *Lavandula officinalis.* In control condition, the frequency of spontaneous contraction of jejunum was near to 28±2 contractions per seconds. The *L. officinalis* aqueous extract decrease this frequency to 23±1 contractions per seconds with 1 µg/ml of lavender aqueous extract and 18.6± 3 contractions per seconds with a maximum inhibition of about 40 % at 500µg/mL of lavender extract (Fig.1).

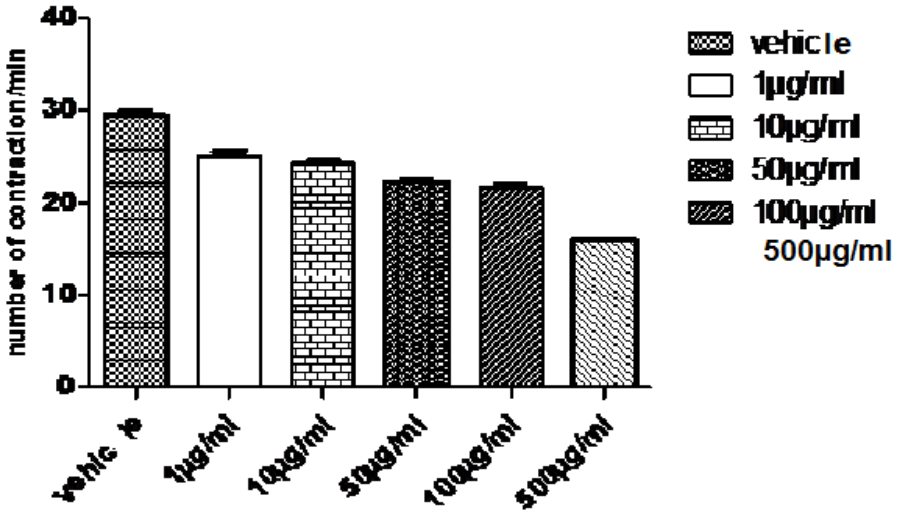

Figure 1: Effect of aqueous extract of *Lavandula officinalis* (1-500µg/ml) on frequency of spontaneous contractions of the isolated rat jejunum. Values expressed as mean ±S.E.M. n= 7 tissues studied

The rate of spontaneous contraction (in mm/sec) of tissue without any addition of product was evaluated at 6mm/sec. At the concentration of 1 µg/mL of *L. officinalis* aqueous extract, the rate of spontaneous contraction was decreased at 5.3±0.57 mm/sec. By increasing the concentration to 500µg/mL, the rate of spontaneous contraction was notably decreased with 0.5 mm/sec. The lavender extract produced a significant antispasmodic effect at 500µg/mL (Fig. 2).

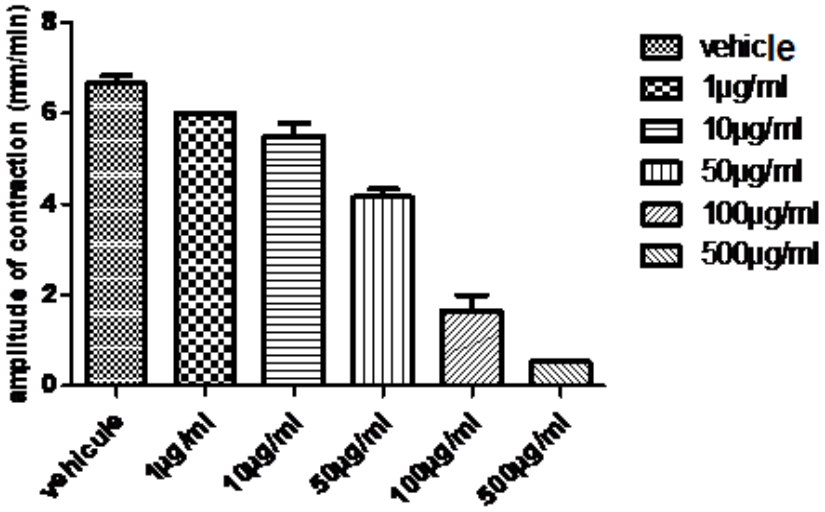

Figure 2: Effect of aqueous extract of *Lavandula officinalis* (1-500µ/ml) the rate of spontaneous contraction of the isolated rat jejunum. Values expressed as mean ±S.E.M. n= 7 tissues studied.

The *L. officinalis* aqueous extract produce a significant relaxation and reduce frequency and velocity of spontaneous contraction from 11.7% at 1 µg/mL up to maximal response with 91.70% at 500µ/ml (Figure 3).

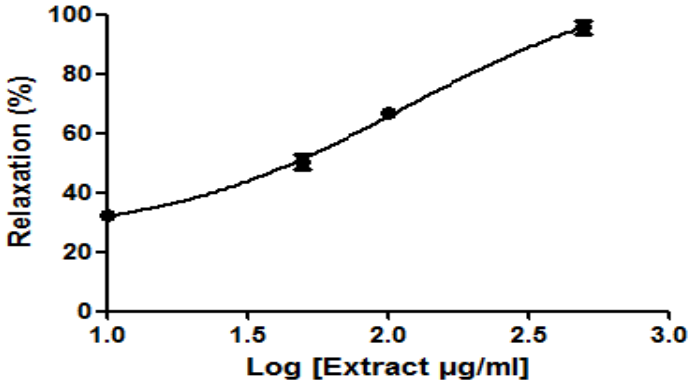

Figure 3: The effect of cumulative concentrations of aqueous extract of *Lavandula officinalis* on relaxation of rat intestine. The extract appears to be more potent (IC50= 88.3±0.1 µg/mL) than trimebutine (IC50 = 91.8±0.1 µg/mL) to produce relaxation and to reduce frequency and velocity of spontaneous contraction. Values of IC50 are expressed as geometric mean with 95 % confidence intervals.

The result that obtained in this study with lavender extract in rat small intestine was described that this plant has antispasmodic and relaxant effect on smooth muscle with IC50 = 88.3 µg/mL, IC 95% confidence intervals [31.2-104.6 µg/mL], IC 50 = 120 ±1 µg/mL of frequency and IC 50 = 78.4 ± 1 µg/mL of the rate of spontaneous contraction. We compared this result with an antispasmodic medicine as trimebutine with IC 50 = 91.8±0.1 µg/mL of relaxation, IC 50 = 246±1 µg/mL of frequency and IC 50 = 106.5±1.4µg/mL of the rate of spontaneous contraction (Table 1).

Table.1 Comparison of *Lavandula officinalis* aqueous extract with antispasmodic medicine

Products	Relaxation	Frequency	Amplitude
Trimebutine	91.8± 0.1*	246 ± 1*	106.5 ±1.4*
LAE	88.3± 0.1**	120 ± 1*	78.4 ± 1**

Values of IC50 are expressed as geometric mean with 95 % confidence intervals. LAE: *Lavandula officinalis* aqueous extract. The statistical analyses were obtained by the one way analysis of variance (ANOVA), followed by the Dunnett's test. *P<0.01, ***p<0.001, n = 7 tissues studied.

DISCUSSION

The present study demonstrates an antispasmodic effect of *Lavandula officinalis* aqueous. The results of the present study clearly demonstrate that in vitro pre-treatment of rat intestine with the crude extract induced dose-dependent relaxation. In addition, this relaxation was accompanied by a reduction of frequency and amplitude of spontaneous contractions.

Acetylcholine, the major excitatory neurotransmitter in visceral smooth muscles, exhibits its action in the gut through stimulation of muscarinic M3 receptors and voltage-dependant Ca2+ channels[8]. Incubation with cumulative doses from 1µg/ml to 500µg/mL of *L. officinalis* aqueous extract reduced the effect spasmogenic of Ach 10-7 M. The present antispasmodic effect compare favourably with commercially available products including trimebutine. The result that obtained in this study with lavander extract in rat small intestine described that this plant has antispasmodic and relaxant effect on smooth muscle with IC50 = 88.3 µg/mL, IC 95% confidence intervals [32.2-105.6 µg/mL], IC 50 = 120±1 µg/mL of frequency and IC 50 = 78.4±0.1 µg/mL of amplitude. We compared this result with an antispasmodic medicine as trimebutine with IC 50 = 91.8±0.1 µg/mL of relaxation, IC 50 = 246±0.1 µg/mL of frequency

and IC 50 = 106.5±1.4 µg/mL of amplitude (Table 1). The actions of trimebutine on the gastrointestinal tract are mediated via an agonist effect on peripheral µ, κ and δ opiate receptors and release of gastrointestinal peptides such as motilin and modulation of the release of other peptides, including vasoactive intestinal peptide, gastrin and glucagon. Recently, trimebutine has also been shown to decrease reflexes induced by distension of the gut lumen in animals and it may therefore modulate visceral sensitivity[9].

The contraction of smooth muscle preparations depends on an increase in the cytoplasmic free Ca2+. This increase can be primarily induced by the influx of Ca2+ via voltage dependent, ligand dependent or Ca2+ channels, which open during the extension since the Ca2 deposits in sarcoplasmic reticulum are small[10]. Tested crude extract inhibited spontaneous contractions in a dose-dependent manner and we could assume that it blocks voltage Ca2+ channels or possibly opens K+ channels which elicit hyper polarisation and lower the excitability of smooth muscles. The antispasmodic effect of plants extract may be justified by their analgesic and anti-inflammatory properties. Several studies confirmed anti-inflammatory and analgesic properties of *Lavandula angustifolia* Mill[3].

Phytochemical studies have identified active components in this plant such as coumarin, chalcones, flavanones, flavones, flavonols, quercetin, and kaempferol derivatives, suggesting that they are the main responsible for antispasmodic[11-13]. The presence of flavonoides, terpenoids and phenolic compound that reported in others plant may be the key of anti spasmodic action[14]. Several studies in vitro and in vivo showed that those compounds can regulate disturbances of gastrointestinal tract such as inhibition of guinea pig intestinal peristalsis by the flavonoids quercetin, naringenin, apigenin and genistein[15]. Their effects depend on the mechanism involved and the tissue. The flavonoid vitexin was considered as spasmolytic in rat duodenum whereas isovitexin was devoid of activity[16]. Flavonoid from licorice isoliquiritigenin plays a dual role in regulating gastrointestinal motility, both spasmogenic and spasmolytic. The spasmogenic effect may involve the activating of muscarinic receptors, while the spasmolytic effect is predominantly due to blockade of the calcium channels[17]. The present antispasmodic effect compare favorably with commercially available drug as trimebutine. Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the antispasmodic effect.

CONCLUSION

In conclusion, the extract appears to be more potent than trimebutine to produce relaxation and to reduce frequency and velocity of spontaneous contraction. The result showed that *Lavandula officinalis* aqueous extract produce a significant antispasmodic effect supporting its use in folk medicine.

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Toxicity and Psychotropic Activity of Essential Oils of *Rosmarinus officinalis* and *Lavandula officinalis* from Morocco

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Abstract: Leaves of *Rosmarinus officinalis* and the flowers of *Lavandula officinalis* have been used as medicine for treatment of nervous disorders, in traditional Moroccan medicine. We evaluate the central nervous system psychotropic effects of the essential oil from the leaves of *R. officinalis* and the flowers of *L. officinalis* using a battery of comportamental psychopharmacology tests. The essential oil extracted by hydrodistillation were characterized by means of GC-MS. *R. officinalis* contained α-pinene (15.82 %), camphene (6.80 %), β-pinene (4.75 %), myrcene (1.70 %), p-cymene (2.16 %), 1,8-cineole (50.49 %), camphor (11.61 %), borneol (2.58 %), and borneol acetate (2.08 %). However, lavender oil contains nine constituents, among which 1,8-cineole (5.30 %), linalool (44.67 %), camphor (6.02 %) and linalyl acetate (42.00 %) were identified. The intraperitonial administration in mice of essential oil from *L. officinalis* at 300 and 600 mg/kg i.p. induces strong sedative effects compared to reference substance diazepam in mice, and an hypnotic effects at doses 1000 and 1500 mg/kg. However, the essential oil extracted from *R. officinalis* at the doses 50 and 100 mg/kg, produced no sedative activity significant on the central nervous system.

Key words: Essential oils; *Rosmarinus officinalis*; *Lavandula officinalis*; Sedative effects; Hypnotic action; Medicinal plants.

Introduction

The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants; divided into 150 families and 940 genuses [13,33]. Medicinal plant can grow naturally or be cultivated due their economic and medicinal value. Among these plants, *Rosmarinus officinalis* and *Lavandula officinalis* essential oils are used in traditional medicine of many countries, especially in Morocco, Middle East and India [4]. Monoterpenoides, the main components in these oils have a large pharmacological effect: against bronchitis, headache, rheumatism, and they present antitumor, antihistaminic, antidiabetic, antiinflammatory and antimicrobial activity [5,16,17,18,27,29,32,34]. The essential oil of lavender (*Lavandula officinalis*) is widely used in the

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treatment of epileptic seizures and nervous disorders. This essential oil significantly reduces spontaneous motor activity and protected mice against penthylen tetrazol-induced convulsion and increased the hypnotic effect induced by pentobarbital via inhalation pathway [7,8,37]. The aim of this experiment is to evaluate the sedative and hypnotic activities of *L. officinalis* and *R. officinalis* essential oils, and to therefore determine the scientific basis for its use in traditional medicine in the management of central nervous system disorders.

Materials and methods

Plant material

Leaves of *Rosmarinus officinalis* and flowering aerial part of *Lavandula officinalis* were collected based on ethnopharmacological information, from the villages around the region Rabat Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plants were identified with botanist of Scientific Institute (Pr. M. Ibn Tatou). A voucher specimen (N° 256) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat- Morocco.

Isolation of essential oils

The essential oils were produced by hydro-distillation method. Plant materials (100 g), cut into small pieces , were placed in a distillation apparatus and hydrodistilled for 3 h after the oils were dried over an hydrous K_2CO_3, they were stored at +4°C until used for GC-MS analyses.

Phytochemical analysis by combined gas chromatography mass spectrometry (GC-MS)

The essential oils were submitted to quantitative analysis in a Hewlett-Packard 575,GC conditions : carrier gas N_2 (0.5 bar) at flow rat of 1.0 ml/min $^{-1}$, sample size, 0.2 µl injected, capillary column (30 m in siloxane 5 % Hp EM). The temperature of the injector and detector was set at 250°C. The oven temperature was programmed from 50°C to 250°C (5min). The MS was taken at 70 eV [11,23].

The components of the oil were identified by comparison of their mass spectra with those in the Wiley-NIST 7th edition library of mass spectral data [22]. The percentage composition of the oil sample was calculated from GC-MS peak areas.

Animals

Male Swiss mice (20-25 g) (Iffa-credo, France) were used in pharmacological test and females of the same strain in the median lethal dose (LD_{50}) calculation, the animals were kept at constant room temperature and were fed *ad libitum* with standard feed and water in the course of the study. The animals were obtained from the animal experimental centre of Mohammed V- Souissi University, Medicine and Pharmacy Faculty-Rabat.

Acute toxicity

LD_{50} (median lethal dose) values were determined as described by Litchfield and Wilcoxon [26]. Seven groups of mice of both sexes (n = 10; 5 males and 5 females) received or not intraperitoneal single doses at different concentrations (500; 750; 850; 900; 950; and 1000 mg /kg, i.p.), for essential oil from *Rosmarinus officinalis***,** and (500; 1000; 1500; 2000; 3000; and 5000 mg/kg; i.p.), for essential oil from *Lavandule officinalis*. The control group received only the water. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 24 h at 6 h time interval to detect any eventual side effects. Boniface *et al.*, have established a program that calculate the percentage of mortality according to the administration dose [6]. The number of animals, which died during this period, was expressed as percentile, and the LD_{50} was determined by probit test using death percent versus doses log [2,12,31,35].

Reference drugs

All drugs and extracts were freshly prepared on the day of the experiments. A control group received distilled water (10 ml/kg, p.o.) as vehicle. Diazepam (3 mg/kg, i.p. a conventional sedative) and thiopental (60 mg/kg, i.p. a conventional

hypnosis) were used as positive control.

Pharmacological evaluations

Pharmacological test were then performed at non toxic doses *i.e.* 50 and 100 mg/kg, for the essential oil of *Rrosmarinus officinalis* and 300 and 600 mg/kg, from the essential oil of *Lavandula officinalis*. These doses did not induce severe neurological side effects *i.e.* reduction of spontaneous motor activity, lack of exploratory motor activity and exploratory behaviour. The activity of essential oils from *Rosmarinus officinalis* and *Lavandula officinalis* on the central nervous system was then studied; using a battery of comportamental testes used in psychopharmacology. For testing sedative effects, the effects of essential oils on mice were qualified in one of the following tests:

Traction test

Mice were individually suspended by anterior limbs to a wire stretched horizontally. Abnormal mice that fails to make a re-establishment at least one of its posterior limbs to reach the wire, is considered as subject under a sedative action. When the animals performs normal re-establishment immediately; the reaction is known as positive, in otherwise, the reaction is called negative. Also, the behaviour and comportamental of animals were recorded during the period of the experiment [10,24].

Fireplace test

The apparatus used for this test consists of a vertical glass tube 30 cm in length. Mice were individually placed vertically in the glass test tube, a normal mouse typically attempt to escape in thirty seconds, and the mice are considered as subject to the sedative effect when performing the rise of cylinder greater than 30 seconds [20].

Hole-board test

Mice were individually placed in the centre of a perforated board and the number of head-dips was registered during a 5 min. The perforated board test was made by using a wood floorboard; 40 cm x 40 cm x 25 cm, in which 16 evenly spaced holes were made .The number of explored holes provide a measure of the number of head-dips [9,15].

Study of the hypnotic action (recovery test)

The mouse is considered as in state of hypnosis, when placed on his back; it loses the righting reflex. Each mouse was observed for the onset of uncoordinated movements (sedative phase) and the loss of righting reflex (hypnosis), but also for duration of sleep (the criterion for sleep is defined as the loss of righting reflex). The time between loss and recovery of the righting reflex was recorded as sleeping time [19,38].

Statistical analysis

Results are expressed as mean ± S.D. Statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by student's *t*-test. A value of $p < 0.05$ or less was considered significant.

Results

Chemical composition of the essential oils

The yield of extraction was 0.256% for *Rosmarinus officinalis* and 0.24 % for *Lavandula officinalis.* The results obtained by GC-MS analyses of the essential oils of *Rosmarinus officinalis* and *Lavandula officinalis* are presented in tables 1 and 2. Thirteen compounds were identified in these essential oils from *Rosmarinus officinalis* and nine from *Lavandula officinalis* by GC-MS analyses (Figure 1 and 2), *Rosmarinus officinalis* contained three major compounds *i.e.*1,8-cineole (50. 49 %), α-pinene (15. 82 %) and camphor (11.61 %), while, the major compounds of *Lavandula officinalis* volatile oil were linalool (44.67 %) and linalyl acetate (42 %). In addition camphene (6.80 %) and β-pinene (4.75 %) were present in rosemary oil (*Rosmarinus officinalis*), while, 1.8-cineole (5.30 %) and camphor (6.02 %) were additional compounds in lavender oil (*Lavandila officinalis*) (Table 1 and 2). Oil composition of *Rosmarinus officinalis* and *Lavandula officinalis* have already been reported [16]. Thus, it has been shown that α-pinene, 1,8-cineole, camphor, verbenone and broneol account for 80 % of total *R. officinalis* essential oil, but in our study, these compounds represent 97.6 %. Data from Literature indicated that essential oil from *Lavandula officinalis* contained 26.12 % linalool

and 26.32 % linalyl acetate. These compounds were also identified in this study but in a high level 46.00 %; for linalool and 42.67 % for linalyl acetate representing thus about 86.67 % of total *Lavandula officinalis*. These differences in chemical composition of essential oils may be due both to developmental and environmental factors that influences plant metabolism.

Table 1. Chemical composition of *Lavandula officinalis* essential oil

No.	Identified compounds	Rt (retention time)	Relative percentage %
1	α-pinene	8.35	tr
2	1,8-cineole	11.73	5.30
3	linalool	14.32	44.67
4	camphor	15.72	6.02
5	linalyl acetate	19.75	42.00
6	bornyl acetate	24.99	tr
7	iso eugenol	29.86	tr
8	methyl eugenol	35.70	tr
9	farnesene	42.10	tr

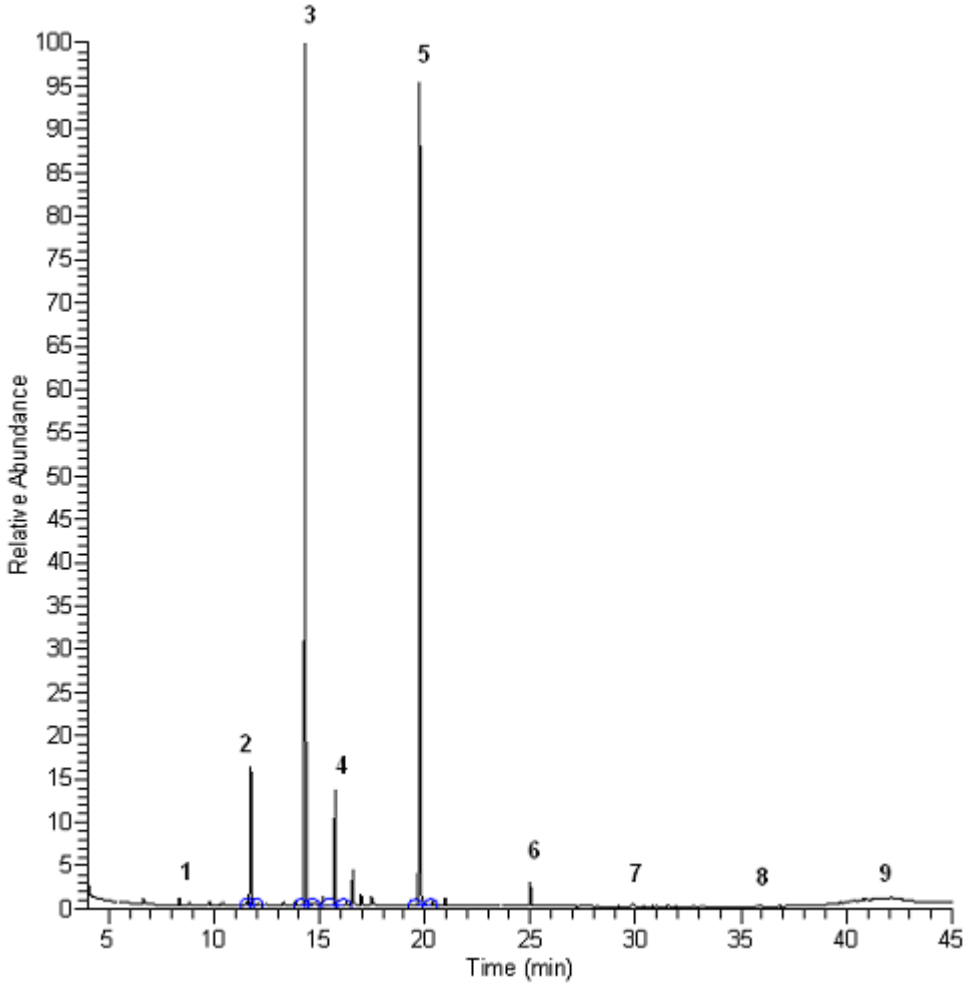

Fig. 1. Gas chromatography-mass spectrometry (GC-MS) of *Lavandula officinalis* essential oil

Table 2. Chemical composition of *Rosmarinus officinalis* essential oil

No.	Identified compounds	Rt (retention time)	Relative percentage %
1	α-pinene	8.37	15.82
2	camphene	8.83	6.80
3	β-pinene	9.79	4.75
4	myrcene	10.40	1.70
5	*p*-cymene	11.54	2.16
6	1,8-cineole	11.80	50.49
7	camphor	15.75	11.61
8	borneol	16 57	2.58
9	bornyl acetate	24.98	2.08
10	*trans*-caryophyllene	29.85	tr
11	γ-cadinene	35.59	tr
12	unknown	39.13	tr
13	bisabolol	43.35	tr

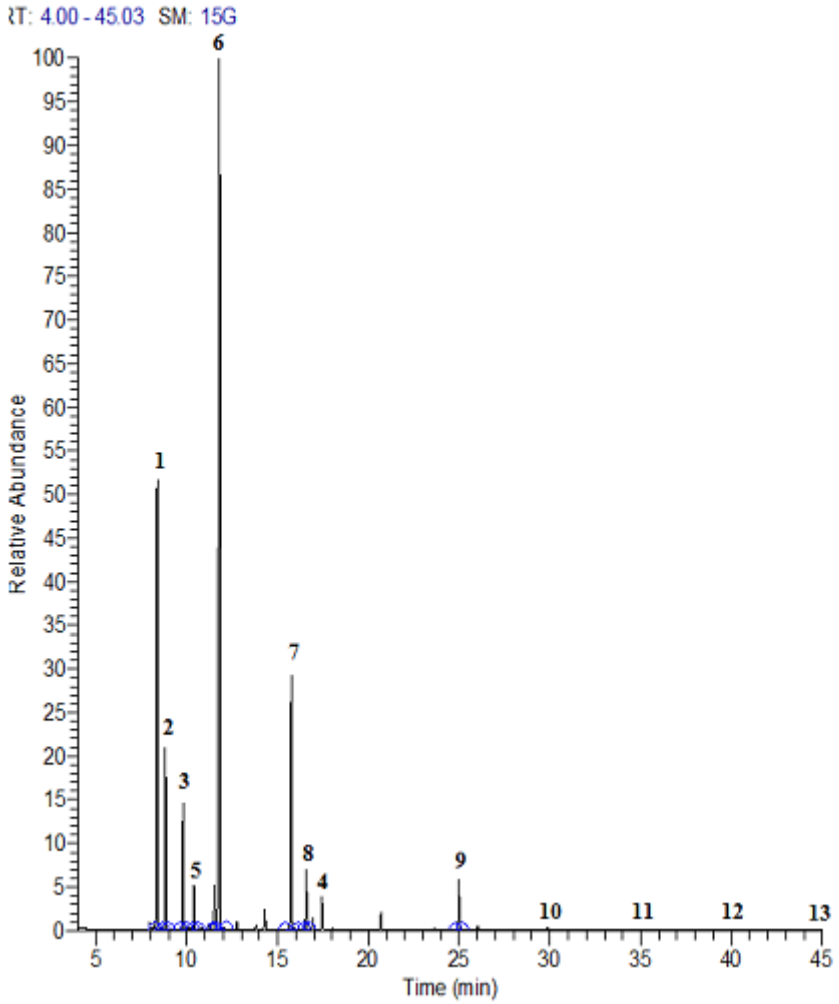

Fig. 2. Gas chromatography-mass spectrometry (GC-MS) of *Rosmarinus officinalis* essential oil

Acute toxicity of *Rosmarinus officinalis* and *Lavandula officinalis* essential oils

Following intraperitoneal administration of *R. officinalis* essential oil at the doses 500 mg/kg, neurological deficit and abdominal contraction were observed by 20-25 min after the injection in 60 % of mice. These effects were reversed in 60 min and might be due to the route of administration of the *Rrosmarinus officinalis* essential oil. In addition to the abdominal contraction, the essential oil reduced spontaneous motor activity and exploratory behaviour. The mortality was recorded 24 h after administration at the doses 850; 900; 950 and 1000 mg/kg. The severity of these effects was increased within 6 h; and mortality was 100 %. The calculation of LD_{50} gives the following results:

LD_{50} = 897.85 mg/kg, 885.90 < LD_{50} < 909.97, with confidence limits at 95 %.

This result indicates that the essential oil had low toxicity. However, after intraperitoneal administration of lavender oil at the doses 500, 1000, 1500, 2000, 3000, and 5000 mg/kg, no toxicity was demonstrated at these doses. This result indicates that this essential oil is not toxic.

Psychotropic activity of the essential oil on central nervous system

The results of psychotropic effects of essential oils were expressed by comparison with control groups.

Traction test

We observed that, animals treated with the Rosemary essential oil at 50 and 100 mg/kg, were performed normal re-establishment immediately (Table 3) ($p>0.05$) in the traction test. This indicates that this essential oil produced no significant effects on mice comportamental. On contrast, the essential oil of *Lavandula officinalis* produced significant sedative effects on the central nervous system (CNS) as indicated by the relatively high time, 7 sec ± 0.8 for the re-establishment of the mice (Table 4). By increasing the dose to 600 mg/kg, the average re-establishment time was increased; re-establishment time was notably higher than control group, 10 min after injection [$F(4, 25) = 6.80$, $p < 0.001$].

Fireplace test

Animals treated with the essential oil of *Rosmarinus officinalis* at 50 and 100 mg/ kg do not show loss of initiative and curiosity. Following intaperitoneal administration of lavender oil at the doses of 600 mg/kg, all mice loss of initiative and curiosity, *i.e*. animals did not attempt to mount of the tube for escape (Table 3 and 4) [$F(4,25) = 8.01$, $p < 0.001$].

Hole-board test

In this test, the essential oil of *Rosmarinus officinalis* does not reduce the cumulative number of holes explored (in relation with curious) and the number covered space between two holes (relation with motor activity) explored by animals exposed to the essential oil. While the essential oil of lavender reduced the cumulative number of holes explored and the number of spaces between two holes (Table 3 and 4). These data lead to conclude that the essential oil of lavender possess potential sedative effects on the central nervous system at the dose of 600 mg / kg. While; the essential oil of *Rosmorinus officinalis*, by joining the traction and fireplace test, no sedative action was recorded with the hole-board test at the doses of 50 and 100 mg/kg (i.p.) ($P > 0.05$).

Hypnotic effects of essential oil of *Lavandula officinalis*

We observed that, the lavender essential oil had induced hypnotic effect significant [$F(4,25) = 6.80$, $p < 0.01$]. Hypnosis induced by essential oil (1000 and 1500 mg/kg, i.p.) was evaluated by observation of the duration of thiopental-induced sleeping time. The essential oil showed a reduction in the time of onset of sleep induced by thiopental. This hypnotic action was increased at the dose of 1500 mg/kg (i.p.). Confirming thus the hypnotic action of lavender essential oil at a high dose via intraperitoneal pathway (Table 5).

Table 3. Sedative effects of *Rosmarinus officinalis* essential oil

Test		Control	Diazepam	*Rosmarinus officinalis* 50 mg/kg; i.p.	100 mg/kg; i.p.
Traction test	Tm1 : falls(in seconds)	0	5	0	0
	Tm2 : re-establishment	0.9 ± 0	7.3 ± 0.1	0.7 ± 0.1	01 ± 0
		n = 5	n = 5	n = 5	n = 5
Fireplace test	Positive response	5	0	0	0
	Tm : go out the tube	7 ± 0.5	t > 2 min	7.2 ± 0.3	7.4 ± 0.2
		n = 5	n = 5	n = 5	n = 5
Hole-board test	Explored holes during 5 min	10 ± 0	0.0 ± 0.0	12 ± 1	11 ± 0.8
		n = 5	n = 5	n = 5	n = 5

Tm 1: mean medium time of falls
Tm 2: mean re-establishment time
n: mean number of animal per group
Data are expressed as mean ± S.D
($p > 0.05$) vs the control group

Table 4. Sedative action of *Lavandula officinalis* essential oil

Test		Control	Diazepam	*Lavandula officinalis* 300 mg/kg; i.p.	600 mg/kg; i.p.
Traction test	Tm1 : falls(in seconds)	0	5	3	0
	Tm2 : re-establishment	0.9 ± 0	9 ±1	1 ± 0	7 ± 0.8*
		n = 5	n = 5	n = 5	n = 5
Fireplace test	Positive response	5	0	3	0
	Tm : For go back the tube	7 ± 0.5	t > 2 min	14.4 ± 0.2	45±1*
		n = 5	n = 5	n = 5	n = 5
Hole-board test	Explored holes during 5 min	10 ± 1	0.0 ± 0.0	10 ± 0.0	0.1 ± 0.0*
		n = 5	n = 5	n = 5	n = 5

Tm 1: mean medium time of falls
Tm 2: mean re-establishment time
i.p.: mean intraperitoneal route
n: mean number of mice per group
Data are expressed as mean ± S.D.
[$F(4,25) = 8.01$, $^*p < 0.001$] vs the control group

Table 5. Hypnotic effects of *Lavandula officinalis* essential oil

	Thiopental (60 mg/kg; i.p.)	EO (1000 mg/kg; i.p.)	EO (1500 mg /kg; i.p.)
TE (min)	8 ± 1	16 ± 1*	9 ± 1*
	n = 5	n = 5	n = 5
TS (min)	33 ± 6	54 ± 6*	106 ± 3*
	n = 5	n = 5	n = 5

Mice received thiopental (60 mg/kg, i.p.) 30 min after the pre-treatment of essential oil (1000 and 1500 mg/kg, i.p.) and thiopental (60 mg/kg, i.p.).
TE: mean hypnotizing time;
TS: mean sleeping time;
i.p.: mean intraperitonial route;
n: mean number of mice per group;
EO: mean essential oil.
Data are expressed as mean ± S.D.; [F (4,25) = 6.80, *p< 0.01] vs the control group.

Discussion

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilization which, driven by their intuition and their sense of observation, were able to find answer to their health problems in the plant environment [1,39]. Aromatherapy is currently used worldwide in the management of chronic pain, depression, anxiety, some cognitive disorders, insomnia and stress-related disorders [21,28].

Lavender and rosemary essential oils are a popular aromatherapy plant that has an appealing scent that has been incorporated in to numerous products. In aromatherapy, the essential oil is believed to possess anticonvulsive, sedative, hypnosis and antidepressive effects and to be useful for treating nervous breakdown, nervous tension and depression [25,36,40]. We analysed the effects of different doses of essential oils from rosemary and lavender for their acute toxicity and neuropharmacological activities. We decided to use intraperitoneal administration of drugs because this pathway allows faster viability of the essential oil than oral pathway. It was found that the i.p. administration of the plant oil induced sedative effects and stimulant; respectively. The essential oil of lavender oil was produced sedative effects at the dose of 600 mg/kg (Table 4), also the essential oil of lavender was able to produced hypnotic action at a high doses (1000 and 1500 mg/kg, i.p.). Since linalool and linalyl acetate are the major compounds of lavender oil this may suggest that these compounds may be the main pharmacologically compounds. This is in line with previous report indicating that, linalool and linalyl acetate reduces the locomotors activity of mice, possessing therefore a sedative effect [7,8]. Also it has been shown that these compounds induce an analgesic effect in mice via inhalation pathway [29,37]. In this respect diazepam is central nervous system depressant used in the management of sleep disorders such as insomnia, these compounds have a binding site on GABA receptor type A-ionophor complex (GABAA). It decrease activity, moderates excitement and calms the recipient. Substances like diazepam (which has chosen as the standard reference drug in this study) reduce onset of and increases duration of barbiturate induced sleep, and reduce exploratory activity possess potentials as sedative [19,30].

Diazepam is a very well known anxiolytic benzodiazepine (BDS) which produces not only anxiolytic-like effect but also important sedative action. The mechanism underlying the effects of linalool and linalyl acetate on brain function remains unclear. To this respect, lavender essential oil produced a dose-dependent reduction in the number of head-dips in the hole-board test similar or/and greater than diazepam. It is generally

believed that locomotors activity results from brain activation, which is manifested as an excitation of central neurons involving different neurochemical mechanism and an increase in cerebral metabolism. It is possible that the sedative activity of essential oil of *Lavandula officinalis* is mediated by GABAergic pathway, since GABAergic transmission can produce profound sedation in mice. The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnotic activity. Glutamate and GABA are quantitavely the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain. Thus, receptors for these two neurotransmitters are regarded as important targets for psychotropic drugs [14]. In the test of Thiopental-induced sleep in mice, the potentiated effect of lavender essential oil in mice was represented. It not only prolonged the sleeping time, but also decreased the latency of falling sleep and increases the rat of sleep onset.

The present study revealed that linalool and linalyl acetate may be the key active constituent of lavender oil, and therefor would be a viable alternative to lavender oil for the treating mental disorders. These results for essential oil of *Lavandula officinalis* was established the advice of traditional practitioners; whereas the essential oil of *Rosmarinus officinalis* by interesting for a study focusing on the opposite nervous stimulation. Aromatherapy confirms this result by advocating the use of rosemary essential oils as stimulant general [3]. Thus, we concluded that, the *Lavandula officinalis* essential oil possess potential sedative and hypnotic activities on the central nervous system via intraperitoneal route, these data provide pharmacological basis for its therapeutic efficacy on insomnia.

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Toxicity and Psychotropic Activity of Essential Oils of *Rosmarinus officinalis* and *Lavandula officinalis* from Morocco

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Abstract: Leaves of *Rosmarinus officinalis* and the flowers of *Lavandula officinalis* have been used as medicine for treatment of nervous disorders, in traditional Moroccan medicine. We evaluate the central nervous system psychotropic effects of the essential oil from the leaves of *R. officinalis* and the flowers of *L. officinalis* using a battery of comportamental psychopharmacology tests. The essential oil extracted by hydrodistillation were characterized by means of GC-MS. *R. officinalis* contained α-pinene (15.82 %), camphene (6.80 %), β-pinene (4.75 %), myrcene (1.70 %), p-cymene (2.16 %), 1,8-cineole (50.49 %), camphor (11.61 %), borneol (2.58 %), and borneol acetate (2.08 %). However, lavender oil contains nine constituents, among which 1,8-cineole (5.30 %), linalool (44.67 %), camphor (6.02 %) and linalyl acetate (42.00 %) were identified. The intraperitonial administration in mice of essential oil from *L. officinalis* at 300 and 600 mg/kg i.p. induces strong sedative effects compared to reference substance diazepam in mice, and an hypnotic effects at doses 1000 and 1500 mg/kg. However, the essential oil extracted from *R. officinalis* at the doses 50 and 100 mg/kg, produced no sedative activity significant on the central nervous system.

Key words: Essential oils; *Rosmarinus officinalis*; *Lavandula officinalis*; Sedative effects; Hypnotic action; Medicinal plants.

Introduction

The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants; divided into 150 families and 940 genuses [13,33]. Medicinal plant can grow naturally or be cultivated due their economic and medicinal value. Among these plants, *Rosmarinus officinalis* and *Lavandula officinalis* essential oils are used in traditional medicine of many countries, especially in Morocco, Middle East and India [4]. Monoterpenoides, the main components in these oils have a large pharmacological effect: against bronchitis, headache, rheumatism, and they present antitumor, antihistaminic, antidiabetic, antiinflammatory and antimicrobial activity [5,16,17,18,27,29,32,34]. The essential oil of lavender (*Lavandula officinalis*) is widely used in the

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treatment of epileptic seizures and nervous disorders. This essential oil significantly reduces spontaneous motor activity and protected mice against penthylen tetrazol-induced convulsion and increased the hypnotic effect induced by pentobarbital via inhalation pathway [7,8,37]. The aim of this experiment is to evaluate the sedative and hypnotic activities of *L. officinalis* and *R. officinalis* essential oils, and to therefore determine the scientific basis for its use in traditional medicine in the management of central nervous system disorders.

Materials and methods

Plant material

Leaves of *Rosmarinus officinalis* and flowering aerial part of *Lavandula officinalis* were collected based on ethnopharmacological information, from the villages around the region Rabat Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plants were identified with botanist of Scientific Institute (Pr. M. Ibn Tatou). A voucher specimen (N° 256) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat- Morocco.

Isolation of essential oils

The essential oils were produced by hydro-distillation method. Plant materials (100 g), cut into small pieces , were placed in a distillation apparatus and hydrodistilled for 3 h after the oils were dried over an hydrous K_2CO_3, they were stored at +4°C until used for GC-MS analyses.

Phytochemical analysis by combined gas chromatography mass spectrometry (GC-MS)

The essential oils were submitted to quantitative analysis in a Hewlett-Packard 575,GC conditions : carrier gas N_2 (0.5 bar) at flow rat of 1.0 ml/min $^{-1}$, sample size, 0.2 µl injected, capillary column (30 m in siloxane 5 % Hp EM). The temperature of the injector and detector was set at 250°C. The oven temperature was programmed from 50°C to 250°C (5min). The MS was taken at 70 eV [11,23].

The components of the oil were identified by comparison of their mass spectra with those in the Wiley-NIST 7th edition library of mass spectral data [22]. The percentage composition of the oil sample was calculated from GC-MS peak areas.

Animals

Male Swiss mice (20-25 g) (Iffa-credo, France) were used in pharmacological test and females of the same strain in the median lethal dose (LD_{50}) calculation, the animals were kept at constant room temperature and were fed *ad libitum* with standard feed and water in the course of the study. The animals were obtained from the animal experimental centre of Mohammed V- Souissi University, Medicine and Pharmacy Faculty-Rabat.

Acute toxicity

LD_{50} (median lethal dose) values were determined as described by Litchfield and Wilcoxon [26]. Seven groups of mice of both sexes (n = 10; 5 males and 5 females) received or not intraperitoneal single doses at different concentrations (500; 750; 850; 900; 950; and 1000 mg/kg, i.p.), for essential oil from *Rosmarinus officinalis*, and (500; 1000; 1500; 2000; 3000; and 5000 mg/kg; i.p.), for essential oil from *Lavandule officinalis*. The control group received only the water. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 24 h at 6 h time interval to detect any eventual side effects. Boniface *et al.*, have established a program that calculate the percentage of mortality according to the administration dose [6]. The number of animals, which died during this period, was expressed as percentile, and the LD_{50} was determined by probit test using death percent versus doses log [2,12,31,35].

Reference drugs

All drugs and extracts were freshly prepared on the day of the experiments. A control group received distilled water (10 ml/kg, p.o.) as vehicle. Diazepam (3 mg/kg, i.p. a conventional sedative) and thiopental (60 mg/kg, i.p. a conventional

hypnosis) were used as positive control.

Pharmacological evaluations

Pharmacological test were then performed at non toxic doses *i.e.* 50 and 100 mg/kg, for the essential oil of *Rrosmarinus officinalis* and 300 and 600 mg/kg, from the essential oil of *Lavandula officinalis*. These doses did not induce severe neurological side effects *i.e.* reduction of spontaneous motor activity, lack of exploratory motor activity and exploratory behaviour. The activity of essential oils from *Rosmarinus officinalis* and *Lavandula officinalis* on the central nervous system was then studied; using a battery of comportamental testes used in psychopharmacology. For testing sedative effects, the effects of essential oils on mice were qualified in one of the following tests:

Traction test

Mice were individually suspended by anterior limbs to a wire stretched horizontally. Abnormal mice that fails to make a re-establishment at least one of its posterior limbs to reach the wire, is considered as subject under a sedative action. When the animals performs normal re-establishment immediately; the reaction is known as positive, in otherwise, the reaction is called negative. Also, the behaviour and comportamental of animals were recorded during the period of the experiment [10,24].

Fireplace test

The apparatus used for this test consists of a vertical glass tube 30 cm in length. Mice were individually placed vertically in the glass test tube, a normal mouse typically attempt to escape in thirty seconds, and the mice are considered as subject to the sedative effect when performing the rise of cylinder greater than 30 seconds [20].

Hole-board test

Mice were individually placed in the centre of a perforated board and the number of head-dips was registered during a 5 min. The perforated board test was made by using a wood floorboard; 40 cm x 40 cm x 25 cm, in which 16 evenly spaced holes were made .The number of explored holes provide a measure of the number of head-dips [9,15].

Study of the hypnotic action (recovery test)

The mouse is considered as in state of hypnosis, when placed on his back; it loses the righting reflex. Each mouse was observed for the onset of uncoordinated movements (sedative phase) and the loss of righting reflex (hypnosis), but also for duration of sleep (the criterion for sleep is defined as the loss of righting reflex). The time between loss and recovery of the righting reflex was recorded as sleeping time [19,38].

Statistical analysis

Results are expressed as mean ± S.D. Statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by student's *t*-test. A value of $p < 0.05$ or less was considered significant.

Results

Chemical composition of the essential oils

The yield of extraction was 0.256% for *Rosmarinus officinalis* and 0.24 % for *Lavandula officinalis.* The results obtained by GC-MS analyses of the essential oils of *Rosmarinus officinalis* and *Lavandula officinalis* are presented in tables 1 and 2. Thirteen compounds were identified in these essential oils from *Rosmarinus officinalis* and nine from *Lavandula officinalis* by GC-MS analyse*s* (Figure 1 and 2), *Rosmarinus officinalis* contained three major compounds *i.e.*1,8-cineole (50. 49 %), α-pinene (15. 82 %) and camphor (11.61 %), while, the major compounds of *Lavandula officinalis* volatile oil were linalool (44.67 %) and linalyl acetate (42 %). In addition camphene (6.80 %) and β-pinene (4.75 %) were present in rosemary oil (*Rosmarinus officinalis*), while, 1.8-cineole (5.30 %) and camphor (6.02 %) were additional compounds in lavender oil (*Lavandila officinalis*) (Table 1 and 2). Oil composition of *Rosmarinus officinalis* and *Lavandula officinalis* have already been reported [16]. Thus, it has been shown that α-pinene, 1,8-cineole, camphor, verbenone and broneol account for 80 % of total *R. officinalis* essential oil, but in our study, these compounds represent 97.6 %. Data from Literature indicated that essential oil from *Lavandula officinalis* contained 26.12 % linalool

and 26.32 % linalyl acetate. These compounds were also identified in this study but in a high level 46.00 %; for linalool and 42.67 % for linalyl acetate representing thus about 86.67 % of total *Lavandula officinalis*. These differences in chemical composition of essential oils may be due both to developmental and environmental factors that influences plant metabolism.

Table 1. Chemical composition of *Lavandula officinalis* essential oil

No.	Identified compounds	Rt (retention time)	Relative percentage %
1	α-pinene	8.35	tr
2	1,8-cineole	11.73	5.30
3	linalool	14.32	44.67
4	camphor	15.72	6.02
5	linalyl acetate	19.75	42.00
6	bornyl acetate	24.99	tr
7	iso eugenol	29.86	tr
8	methyl eugenol	35.70	tr
9	farnesene	42.10	tr

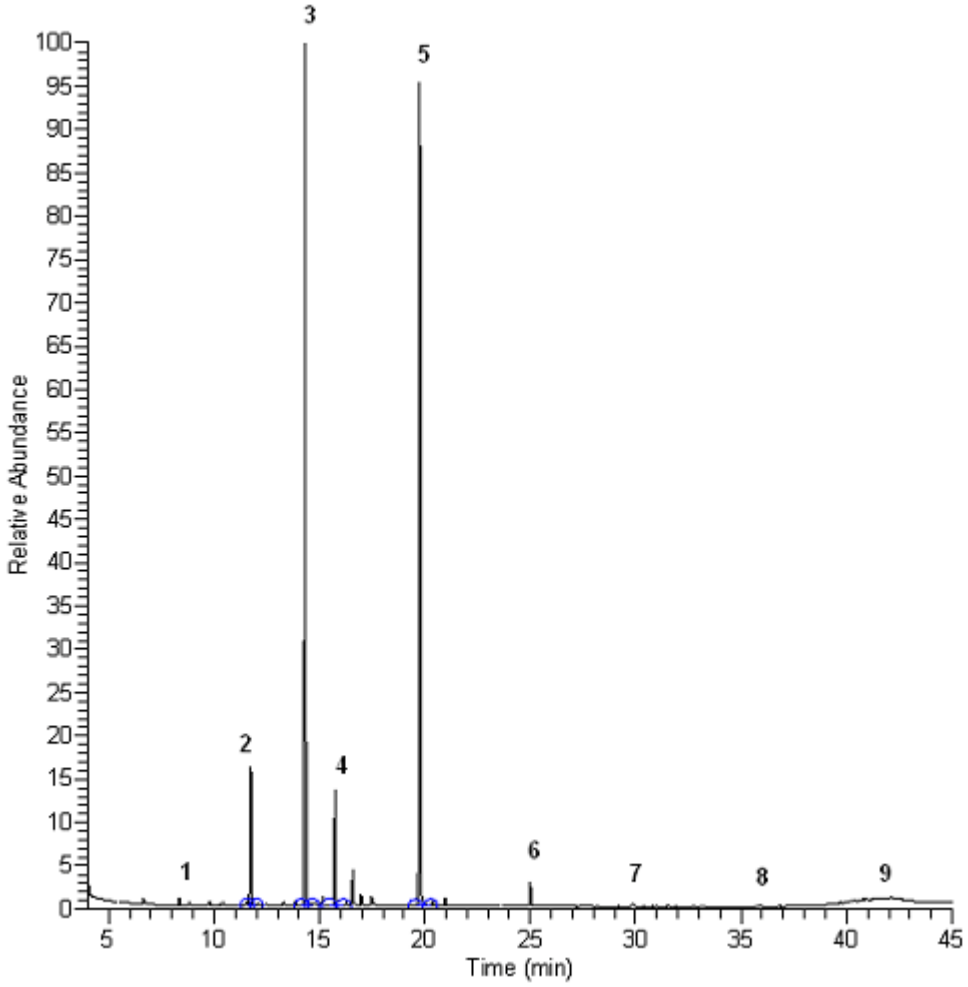

Fig. 1. Gas chromatography-mass spectrometry (GC-MS) of *Lavandula officinalis* essential oil

Table 2. Chemical composition of *Rosmarinus officinalis* essential oil

No.	Identified compounds	Rt (retention time)	Relative percentage %
1	α-pinene	8.37	15.82
2	camphene	8.83	6.80
3	β-pinene	9.79	4.75
4	myrcene	10.40	1.70
5	*p*-cymene	11.54	2.16
6	1,8-cineole	11.80	50.49
7	camphor	15.75	11.61
8	borneol	16 57	2.58
9	bornyl acetate	24.98	2.08
10	*trans*-caryophyllene	29.85	tr
11	γ-cadinene	35.59	tr
12	unknown	39.13	tr
13	bisabolol	43.35	tr

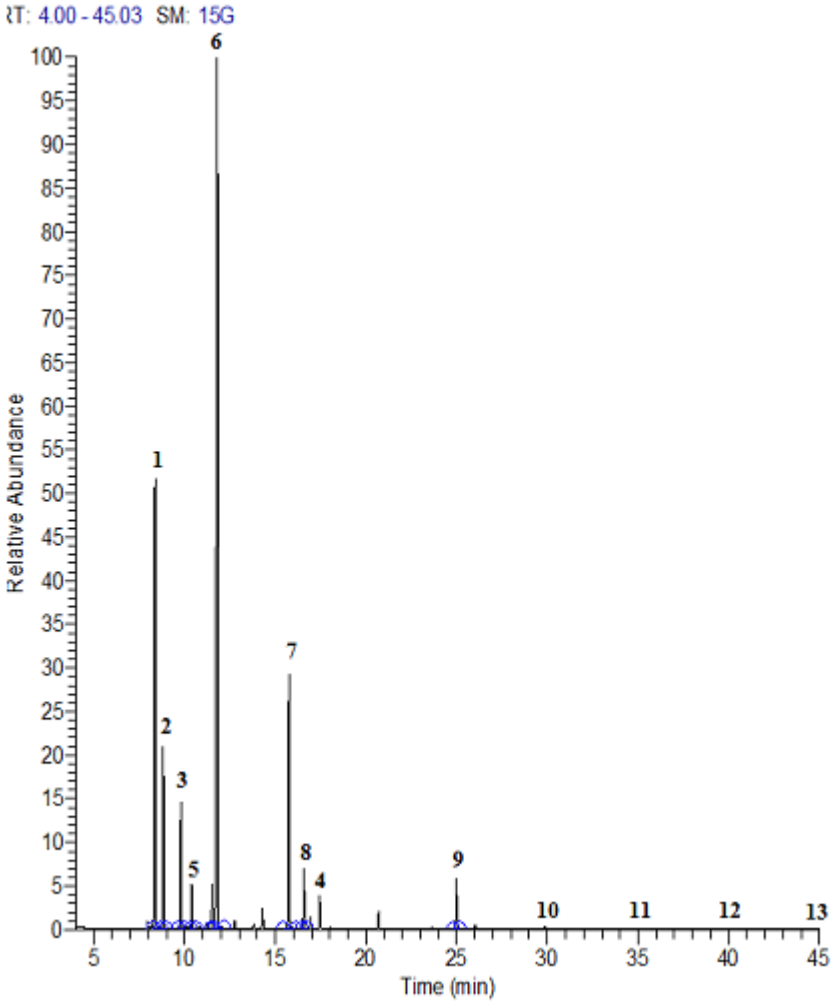

Fig. 2. Gas chromatography-mass spectrometry (GC-MS) of *Rosmarinus officinalis* essential oil

Acute toxicity of *Rosmarinus officinalis* and *Lavandula officinalis* essential oils

Following intraperitoneal administration of *R. officinalis* essential oil at the doses 500 mg/kg, neurological deficit and abdominal contraction were observed by 20-25 min after the injection in 60 % of mice. These effects were reversed in 60 min and might be due to the route of administration of the *Rrosmarinus officinalis* essential oil. In addition to the abdominal contraction, the essential oil reduced spontaneous motor activity and exploratory behaviour. The mortality was recorded 24 h after administration at the doses 850; 900; 950 and 1000 mg/kg. The severity of these effects was increased within 6 h; and mortality was 100 %. The calculation of LD_{50} gives the following results:

LD_{50} = 897.85 mg//kg, 885.90 < LD_{50} < 909.97, with confidence limits at 95 %.

This result indicates that the essential oil had low toxicity. However, after intraperitoneal administration of lavender oil at the doses 500, 1000, 1500, 2000, 3000, and 5000 mg/kg, no toxicity was demonstrated at these doses. This result indicates that this essential oil is not toxic.

Psychotropic activity of the essential oil on central nervous system

The results of psychotropic effects of essential oils were expressed by comparison with control groups.

Traction test

We observed that, animals treated with the Rosemary essential oil at 50 and 100 mg/kg, were performed normal re-establishment immediately (Table 3) ($p>0.05$) in the traction test. This indicates that this essential oil produced no significant effects on mice comportamental. On contrast, the essential oil of *Lavandula officinalis* produced significant sedative effects on the central nervous system (CNS) as indicated by the relatively high time, 7 sec ± 0.8 for the re-establishment of the mice (Table 4). By increasing the dose to 600 mg/kg, the average re-establishment time was increased; re-establishment time was notably higher than control group, 10 min after injection [$F (4, 25) = 6.80$, $p <0.001$].

Fireplace test

Animals treated with the essential oil of *Rosmarinus officinalis* at 50 and 100 mg/ kg do not show loss of initiative and curiosity. Following intaperitoneal administration of lavender oil at the doses of 600 mg/kg, all mice loss of initiative and curiosity, *i.e*. animals did not attempt to mount of the tube for escape (Table 3 and 4) [$F(4,25) = 8.01$, $p <0.001$].

Hole-board test

In this test, the essential oil of *Rosmarinus officinalis* does not reduce the cumulative number of holes explored (in relation with curious) and the number covered space between two holes (relation with motor activity) explored by animals exposed to the essential oil. While the essential oil of lavender reduced the cumulative number of holes explored and the number of spaces between two holes (Table 3 and 4). These data lead to conclude that the essential oil of lavender possess potential sedative effects on the central nervous system at the dose of 600 mg / kg. While; the essential oil of *Rosmorinus officinalis*, by joining the traction and fireplace test, no sedative action was recorded with the hole-board test at the doses of 50 and 100 mg/kg (i.p.) ($P > 0.05$).

Hypnotic effects of essential oil of *Lavandula officinalis*

We observed that, the lavender essential oil had induced hypnotic effect significant [$F (4,25) = 6.80$, $p <0.01$]. Hypnosis induced by essential oil (1000 and 1500 mg/kg, i.p.) was evaluated by observation of the duration of thiopental-induced sleeping time. The essential oil showed a reduction in the time of onset of sleep induced by thiopental. This hypnotic action was increased at the dose of 1500 mg/kg (i.p.). Confirming thus the hypnotic action of lavender essential oil at a high dose via intraperitoneal pathway (Table 5).

Table 3. Sedative effects of *Rosmarinus officinalis* essential oil

Test		Control	Diazepam	*Rosmarinus officinalis* 50 mg/kg; i.p.	*Rosmarinus officinalis* 100 mg/kg; i.p.
Traction test	Tm1 : falls(in seconds)	0	5	0	0
	Tm2 : re-establishment	0.9 ± 0	7.3 ± 0.1	0.7 ± 0.1	01 ± 0
		n = 5	n = 5	n = 5	n = 5
Fireplace Test	Positive response	5	0	0	0
	Tm : go out the tube	7 ± 0.5	t > 2 min	7.2 ± 0.3	7.4 ± 0.2
		n = 5	n = 5	n = 5	n = 5
Hole-board Test	Explored holes during 5 min	10 ± 0	0.0 ± 0.0	12 ± 1	11 ± 0.8
		n = 5	n = 5	n = 5	n = 5

Tm 1: mean medium time of falls
Tm 2: mean re-establishment time
n: mean number of animal per group
Data are expressed as mean ± S.D
($p > 0.05$) vs the control group

Table 4. Sedative action of *Lavandula officinalis* essential oil

Test		Control	Diazepam	*Lavandula officinalis* 300 mg/kg; i.p.	*Lavandula officinalis* 600 mg/kg; i.p.
Traction test	Tm1 : falls(in seconds)	0	5	3	0
	Tm2 : re-establishment	0.9 ± 0	9 ±1	1 ± 0	7 ± 0.8*
		n = 5	n = 5	n = 5	n = 5
Fireplace Test	Positive response	5	0	3	0
	Tm : For go back the tube	7 ± 0.5	t > 2 min	14.4 ± 0.2	45±1*
		n = 5	n = 5	n = 5	n = 5
Hole-board Test	Explored holes during 5 min	10 ± 1	0.0 ± 0.0	10 ± 0.0	0.1 ± 0.0*
		n = 5	n = 5	n = 5	n = 5

Tm 1: mean medium time of falls
Tm 2: mean re-establishment time
i.p.: mean intraperitoneal route
n: mean number of mice per group
Data are expressed as mean ± S.D.
[$F(4,25) = 8.01$, *$p < 0.001$] vs the control group

Table 5. Hypnotic effects of *Lavandula officinalis* essential oil

	Thiopental (60 mg/kg; i.p.)	EO (1000 mg/kg; i.p.)	EO (1500 mg /kg; i.p.)
TE (min)	8 ± 1	16 ± 1*	9 ± 1*
	n = 5	n = 5	n = 5
TS (min)	33 ± 6	54 ± 6*	106 ± 3*
	n = 5	n = 5	n = 5

Mice received thiopental (60 mg/kg, i.p.) 30 min after the pre-treatment of essential oil (1000 and 1500 mg/kg, i.p.) and thiopental (60 mg/kg, i.p.).
TE: mean hypnotizing time;
TS: mean sleeping time;
i.p.: mean intraperitonial route;
n: mean number of mice per group;
EO: mean essential oil.
Data are expressed as mean ± S.D.; [F (4,25) = 6.80, *p< 0.01] vs the control group.

Discussion

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilization which, driven by their intuition and their sense of observation, were able to find answer to their health problems in the plant environment [1,39]. Aromatherapy is currently used worldwide in the management of chronic pain, depression, anxiety, some cognitive disorders, insomnia and stress-related disorders [21,28].

Lavender and rosemary essential oils are a popular aromatherapy plant that has an appealing scent that has been incorporated in to numerous products. In aromatherapy, the essential oil is believed to possess anticonvulsive, sedative, hypnosis and antidepressive effects and to be useful for treating nervous breakdown, nervous tension and depression [25,36,40]. We analysed the effects of different doses of essential oils from rosemary and lavender for their acute toxicity and neuropharmacological activities. We decided to use intraperitoneal administration of drugs because this pathway allows faster viability of the essential oil than oral pathway. It was found that the i.p. administration of the plant oil induced sedative effects and stimulant; respectively. The essential oil of lavender oil was produced sedative effects at the dose of 600 mg/kg (Table 4), also the essential oil of lavender was able to produced hypnotic action at a high doses (1000 and 1500 mg/kg, i.p.). Since linalool and linalyl acetate are the major compounds of lavender oil this may suggest that these compounds may be the main pharmacologically compounds. This is in line with previous report indicating that, linalool and linalyl acetate reduces the locomotors activity of mice, possessing therefore a sedative effect [7,8]. Also it has been shown that these compounds induce an analgesic effect in mice via inhalation pathway [29,37]. In this respect diazepam is central nervous system depressant used in the management of sleep disorders such as insomnia, these compounds have a binding site on GABA receptor type A-ionophor complex (GABAA). It decrease activity, moderates excitement and calms the recipient. Substances like diazepam (which has chosen as the standard reference drug in this study) reduce onset of and increases duration of barbiturate induced sleep, and reduce exploratory activity possess potentials as sedative [19,30].

Diazepam is a very well known anxiolytic benzodiazepine (BDS) which produces not only anxiolytic-like effect but also important sedative action. The mechanism underlying the effects of linalool and linalyl acetate on brain function remains unclear. To this respect, lavender essential oil produced a dose-dependent reduction in the number of head-dips in the hole-board test similar or/and greater than diazepam. It is generally

believed that locomotors activity results from brain activation, which is manifested as an excitation of central neurons involving different neurochemical mechanism and an increase in cerebral metabolism. It is possible that the sedative activity of essential oil of *Lavandula officinalis* is mediated by GABAergic pathway, since GABAergic transmission can produce profound sedation in mice. The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnotic activity. Glutamate and GABA are quantitavely the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain. Thus, receptors for these two neurotransmitters are regarded as important targets for psychotropic drugs [14]. In the test of Thiopental-induced sleep in mice, the potentiated effect of lavender essential oil in mice was represented. It not only prolonged the sleeping time, but also decreased the latency of falling sleep and increases the rat of sleep onset.

The present study revealed that linalool and linalyl acetate may be the key active constituent of lavender oil, and therefor would be a viable alternative to lavender oil for the treating mental disorders. These results for essential oil of *Lavandula officinalis* was established the advice of traditional practitioners; whereas the essential oil of *Rosmarinus officinalis* by interesting for a study focusing on the opposite nervous stimulation. Aromatherapy confirms this result by advocating the use of rosemary essential oils as stimulant general [3]. Thus, we concluded that, the *Lavandula officinalis* essential oil possess potential sedative and hypnotic activities on the central nervous system via intraperitoneal route, these data provide pharmacological basis for its therapeutic efficacy on insomnia.

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Full Length Research Paper

Psychostimulants activity of *Rosmarinus officinalis* L Methanolic and Aqueous Extracts

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We evaluate the psychostimulants activity of *Rosmarinus officinalis* L. (*lamiaceae*) on central nervous system. The effects of the methanolic and aqueous extracts of the stems and leaves of this plant were investigated in three behavioral models including, observation of animals in free situation, search of stereotypes movement in rats and interaction with thiopental in mouse. We observed that extracts of *R. officinalis* increase the locomotors activity and induced hypermobility and hyperexcitability, as compared to the control group. Treatment with *R. officinalis* methanolic or aqueous extracts at the dose of 200 mg/kg, significantly decreased the duration of thiopental-induced sleeping time from 34 min ±0.2 to 3 min ± 0.1 and 2 min ±0.1, respectively (*P<0.001*). Thus, we can conclude that the methanolic and aqueous extracts of *R. officinalis* possess potential psychostimulants activity.

Key words: *Rosmarinus officinalis* L., psychostimulants activity, central nervous system, stereotypes movements, psychiatric disorders.

INTRODUCTION

The term “psychostimulant drugs” refer to a diverse class of psychoactive compound that shares in common the capacity to activate the central nervous system and subsequently behavior. Physiologically psychostimulants typically increase the functional activities of central monoaminergic and cholinergic systems (Coury et al., 1992; Marik et al., 1999; Nesthby et al., 1997). Recently, the search for novel pharmacotherapy from medicinal plants for neurological and psychiatric diseases has progressed significantly owing to their less side effects and better tolerability (Zhang, 2004). Plant extract have been used for the treatment of some psychiatric disorders. Some studies have reported that the extract of *Rosmarinus officinalis* L. have a number of pharmacological activities, such as hepatoprodective (Sotelo-Félix et al., 2002), antibacterial (Gachkar et al., 2007), antithrombotic (Yamamoto et al., 2005), antiulcereogenic (Dias et al., 2000), diuretic (Haloui et al., 2007; Bakirel et al., 2008), antioxidant (Gonzalez–Trujano et al., 2007), antinociceptive (Altinier et al., 2007) and anti-inflammatory (Heinrich et al., 2006). An ethnopharmacological use of *R. officinalis* in the treatment of depression, among other uses, was reported (Fibiger and Phillips, 1988; Machado et al., 2009). However the psychostimulant properties have not yet been studied. Thus, the aim of this study is to evaluate the psychostimulant activity of *R. officinalis* methanolic and aqueous extract in animal models.

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Abbreviations: CNS, Central nervous system; **S.D,** standard deviation.

MATERIALS AND METHODS

Plant material

R. officinalis was collected based on ethnopharmacological information, from villages around the region Rabat-Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with Pr. M. Ibn Tatou of Scientific Institute of Rabat. A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V–Rabat–Morocco.

Preparation of extract

Methanolic extract

Stems and leaves of *R. officinalis* were successively extracted with methanol by maceration at room temperature (25°C) over p eriod of 48 h. 500 g of plant material and one liter of methanol were used in the extraction. Methanol containing the extract was then filtered through Whatman paper and the solvent was vacuum–distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for 2 h to ensure the removal of any residual solvent. Final extract was a yellow powder in percentage dray weight 22.8%; this methanolic extract was kept in deep freeze at -20°C until use.

Aqueous extract

500 g of plant material was extracted by infusion in boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration and then lyophilised (Free Zone® Dry 4.5, USA). For each study, the lyophilized aqueous extract was carefully prepared under the same condition used throughout the studies (time, temperature and the amount of plant material and water used for extraction under reflux and lyophilization) and each time the quality of extraction was checked by the yield of the lyophilization material. The final crude extract was obtained as dark brown powder in percentage from dry weight (18.7%). For assuring stability, the lyophilized material was stored at -20°C.

Animals

Male Swiss mice (20 to 25 g) (IOPS Offa) were used in pharmacological test and females of the same strain in the LD_{50} calculation. Animals were obtained from the animal experimental centre of Mohammed V- Souissi University, Medicine and Pharmacy Faculty Rabat. They were housed three per plastic cage, photoperiod (one light from 6:00 to 18:00 h); air changes and room temperature (22 ± 1°C) were controlled. All animals ha d free access to tap water and at *ad libitum* feeding; except for short fasting period before the treatment with the single dose of the lyophilized methanolic or aqueous extract. The general behavior of mice was observed continuously for 1h after treatment, intermittently for 4 h and over period of 24 h (Twaij et al., 1983). The mice were observed for 14 days following treatment and all signs of toxicity and deaths and their latencies were recorded.

Acute toxicity

LD_{50} (median lethal dose) values were determined as described by Leitchfield and Wilcoxon (1949). Seven groups of mice of both sexes (n=10; 5 males and 5 females) received or not oral single doses at different concentration (500, 1000, 2000, 3000, 4000 and 5000 mg/kg, p.o., for methanolic and aqueous extracts). The control groups received only the water or saline solution. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 6 h and at 24 h time interval to detect any eventual side effect. The number of animals, which died during this period, was expressed as percentile, and the LD_{50} was determined by probit test using death percent versus doses log (Alaoui et al., 1998; Thompson et al., 1952). Of note, drugs used as control were given to mice in similar conditions.

Reference drugs

Thiopental (60 mg/kg, i.p.) and Apomorphine (16 mg/kg, s.c.) were used as the reference drugs (positive control). It was dissolved in distilled water prior to administration.

Pharmacological evaluations

The psychostimulants activity of the methanolic and aqueous extracts from *R. officinalis* on the central nervous system (CNS) was then studied, using a battery of comportamental tests used in psychopharmacology. For testing psychostimulants effect, the effect of *R. officinalis* extract on mice was qualified in one of the following tests.

Observation of animals in free situation

Detailed behavioral observations were made on lots of 3 mice per dose, for 15, 30, 60, 120, 180 min and 24 h after administration of substances, based on the method that described by Irwin (Petit et al., 1990). Especially, the effect of psychostimulant is evaluated after hypermobility and hyperexcitability.

Interaction with thiopental in mice

Mice (6 per dose) were placed in individual boxes and given the substance (methanolic or aqueous extracts) 30 min before administration of Thiopental (a sub-hypnotic dose, 60 mg/kg, i.p.). The mice were treated with different dose of methanolic or aqueous extracts (100, 200 mg/kg, p.o., n=6), the control group (n=6) was treated with distilled water (10 ml/kg, p.o.). The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex (Petit et al., 1990; Simon, 1985).

Search for stereotypes movements in rats

The stereotypes movements, characterized by movements of the head and buckle, sniffing, licking or chewing are scored from 0 to 3 according to the method described by Simon and Chermat (1977) every 10 min until their disappearance in all animals. Six rats per doses were used. Individual quotation stereotypic was calculated as follows:

0 = absence of stereotypes movement and all animals were normal; 1 = rare presence of stereotypes movements; 2 = intense sniffing with rapid movements of the head; 3 = intense and continuous stereotypes movement (displacement of the head, licking).

The methanolic and aqueous extracts were tested at doses of 100 and 200 mg/kg. The control groups have received the solvent under the same conditions. After administration of extracts of *R. officinalis* L., each animal was isolated in individual cages to be observed

Table 1. Mortality according to the administration doses of *R. officinalis* methanolic and aqueous extracts.

Extract	Dose (mg/kg, p.o.)	Mortality (%)
EM	500	0
	1000	30
	2000	40
	3000	50
	4000	70
	5000	100
EA	500	0
	1000	0
	2000	0
	3000	0
	4000	0
	5000	0

Mice of each group (n= 10) were received single dose and they were carefully examined during 14 days to determine LD_{50} and any signs of toxicity and behavioral changes. The lyophilized aqueous and methanolic extracts dissolved in distilled water were administered by oral route (p.o.); EM: mean methanoilc extract; AE: mean aqueous extract.

every 10 min during 210 min. The intensity of stereotypes was assessed according to the quotation of Simon (Simon and Chermat, 1977). For each lot the sum of individual quotations obtained by fraction of 10 min is calculated. The total number of stereotypes observed during 210 min was also taken into account (Simon et al., 1982).

Statistical analysis

Results are expressed as mean ± S.D (standard deviation). Statistical analysis of data was done using one way analysis of variance (ANOVA) followed by student's *t*- test. A level of significance (*P< 0.05 or 0.01*) was considered for each test.

RESULTS

Acute toxicity of *R. officinalis* methanolic extract

After oral administration of *R. officinalis* methanolic extract at the doses of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg some signs of toxicity were produced. The mortality was recorded 48 h after administration at the dose 1000, 2000, 3000, 4000 and 5000 mg/kg, p.o. The severity of these effects was increased within 8 h and mortality was 100% (Table 1). The calculation of LD_{50} by a program of Boniface et al. (1972) gives the following results: LD_{50} **=** 2104.85 mg/kg., 1890< LD_{50}< 2343, with confidence limits at 95%. This result indicates that the methanolic extract had very low toxicity (Table 1).

Acute toxicity of *R. officinalis* aqueous extract

Following oral administration of *R. officinalis* aqueous extract at the doses 500, 1000, 1500, 2000; 3000 and 5000 mg/kg, p.o., no toxicity was observed at these doses. This result indicates that this aqueous extract is not toxic (Table 1).

Psychostimulants activity of the methanolic and aqueous extracts on central nervous system

The results of psychostimulants effect were determined by comparison with control groups. Pharmacological tests were then performed at non toxic doses that is, 100 and 200 mg/kg for methanolic and aqueous extract. These doses did not induce severe neurological side effects.

Observation of animals in free situation

It was observed that oral administration of the methanolic and aqueous extract of *R. officinalis* L. at the therapeutic dose (100 and 200 mg/kg), causes the appearance of rapid movements of the head, sniffing the buckle (gnawing, biting the cage walls) suggesting an increasing locomotors activity and curiosity, induction of spontaneous aggression, hypermobility and hyperexcitability of the central nervous system (stereotypes movements). This psychostimulant effect is appearing significant at the dose of 200 mg/kg. However, the aqueous extract was found to be more active than methanolic extract.

Interaction with thiopental in mice

The oral administration of the therapeutic dose (100 and 200 mg/kg, p.o.) of the methanolic and aqueous extracts

Table 2. Influence of the essential oil, methanolic and aqueous extracts of *Rosmarinus officinlais* L on the onset of sleep (T.E) and sleeping time (T.S) induced in mice by a hypnotic dose of thiopental.

Time	Thiopental (mg/kg, i.p.)	ME (mg/kg, p.o.)		AE (mg/kg, p.o.)	
	60	100	200	100	200
T.E (min)	18 ±1	34 ± 1	58 ± 0.2	48 ± 1	50 ± 0.2
T.S (min)	34 ± 2	8 ± 1	3 ± 0.1	6 ±1	2 ± 0.1

Mice of each group (n= 5); *p< 0.001* vs the control group; p.o.: mean oral route; i.p.: mean intrapeeritonial route; T.E: mean sleep latency; T.S: mean sleeping time. Data are expressed as mean ± SD.

of *R. officinalis* significantly increased sleep latency and caused a decrease in sleep time. The effect was significantly higher in the dose 200 mg/kg than at the dose of 100 mg/kg, but the sleep time reduction by the aqueous extract is longer than the sleep time reduction by the methanolic extract. The reduction of sleep time is significantly longer than the methanolic extract (Table 2).

Search for stereotypes movements in rats

From Table 3, Apomorphine (16 mg/kg, s.c.) induced on the control groups the appearance of stereotypes movements after just 10 min, after the end of 80 min, the total of quotation being 18. The stereotypes movements decreased gradually until disappear at 210 min. The stereotypes Phenomena are increased in duration and intensity. However, the methanolic and aqueous extract (200 mg/kg) produced a significant (*P< 0.001*) potentiating of the effect of Apomorphine, but at the dose 100 mg/kg the agonist of stereotypes movements was partial in duration and intensity compared with controls. For the methanolic extract, stereotypic movements reached a maximum at 80 min (quotation total= 16 and 18, for 100 and 200 mg/kg, respectively) and persist longer time for not disappearing after 210 min (some rats continued to exhibit stereotypic movements up to 270 min with the dose 200 mg/kg) (Table 3). For the aqueous extract of *R. officinalis* L., after the administration of the therapeutic dose (100 and 200 mg/kg, p.o), the stereotypes movements appeared at 10 min, reaching a peak at 40 min (total quotation = 18), remain at 270 min for disappearance at 280 min (Table 3).

DISCUSSION

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilizations. Driven by their intuition and their sense of observation, they were able to find answer to their health problems in the plant environment (Akhondzadeh, 2007; Steflitsch and Steflitsch, 2004). Aromatherapy provides a potentially effective treatment for a range of psychiatric disorders (Sarris, 2007; Perry and Perry, 2006; Steflitsch and Steflitsch, 2004). In aromatherapy, the essential oils are believed to possess antidepressive effect and to be useful for treating nervous breakdown and nervous depression (Fibiger and Phillips, 1988; Machado et al., 2009; Tisserand, 1985). We analyzed the effect of different doses of the methanolic and aqueous extracts from stems and leaves of rosemary (*R. officinalis* L.) for their acute toxicity and psychostimulants activities. In the current study, the psychostimulant activity was investigated by recording the spontaneous locomotors activity in mice. In the test of observation in free situation, the methanolic extract (200 mg/kg) produced an increase in motor activity and curiosity, by the induction of spontaneous aggression, hypermobility and hyperexcitability. It was also observed that, the aqueous extract (200 mg/kg) produced a significant (*P< 0.001*) increase in the spontaneous motor activity and induction of stereotypes movements. These observations suggest that, the psychostimulant action of this plant is mediated by dopaminergic pathway, since dopaminergic transmission can produce profound stimulant effect in mice (Simon et al., 1982). It is possible that, the dopaminergic transmission is due to the releasing of catecholamine (nor epinephrine and dopamine) neural storage vesicles (Costa et al., 1972; Samanin et al., 1977). The methanolic and aqueous extract of *R. officinalis* has considerable dopaminergic potentiating mechanisms. In the test of thiopental-induced sleep, the methanolic (200 mg/kg) extract produced a significant (*P< 0.001*) highest increase in the time of onset of sleep as well as highest reduction of sleep induced by thiopental (Table 2). It was also observed that, the aqueous extract (200 mg/kg) not only induces highest decrease the sleeping time but also induces prolongation the latency of falling asleep and highest decrease in the rate of sleep onset (Table 2). The prolongation in the time of onset of sleep and the highest reduction in the sleep time and the induction of exploratory behavior indicate a central nervous system stimulant activity of *R. officinalis* extract. In the test of apomorphine- induced stereotypes mpvement in rats, the methanolic and aqueous extract (200 mg/kg, p.o.) produced a significant (*P< 0.001*) potentiating of the effect of Apomorphine, the stereotypes movements was increased in duration and intensity. In addition, the dose of 100 mg/kg of both extracts was partial increased the

Table 3. Influence of the methanolic and aqueous extracts of *Rosmarinus officinalis* L on stereotypes movement induced in rats.

Dose (mg/kg)		Time (min)																											
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280
100	ME	10	10	12	13	16	16	16	18	18	15	14	12	12	10	10	8	8	6	5	3	2	2	1	0	0	0	0	0
	AE	10	10	12	13	15	16	16	16	14	14	14	12	12	11	10	10	8	8	8	6	5	3	2	1	0	0	0	0
200	ME	16	16	18	18	18	18	18	16	16	16	15	15	15	15	15	14	12	12	11	10	10	8	8	6	6	3	2	0
	AE	16	16	16	18	18	18	18	16	16	15	15	15	15	14	14	13	13	12	12	10	10	10	8	6	5	3	2	0
16	AM	9	11	12	12	15	16	18	18	16	15	14	10	8	8	6	5	5	4	3	2	0	0	0	0	0	0	0	0

Rats of each group (n= 6) were received the methanolic or aqueous extracts (100 and 200 mg/kg) by oral route (p.o.); Apomorphine (16 mg/kg) was injected via subcutaneous pathway (s.c.); ME: mean methanolic extract; AE: mean aqueous extract; AM: mean Apomorphine; individual quotation stereotypic was calculated as follows: 0 = absence of stereotypes movement and all animals were normal; 1 = rare presence of stereotypes movements; 2 = intense sniffing with rapid movements of the head; 3 = intense and continuous stereotypes movement (displacement of the head, licking). $P< 0.001$ versus the control group.

stereotypes movements in duration and intensity compared with controls (Table 3). Phytochemical studies have identified active components in the methanolic and aqueous extract of this plant, such as flavonoids including diosmetin, diosmin, luteolin, apigenin, quercetin and kaempherol, phenols such as caffeic and rosmarinic acids and terpenoids like, carnosol, carnosic acid, rosmanol and oleonolic and ursolic acids (Altinier et al., 2007; Al-Sereitia et al., 1999; Askun et al., 2009; Barnes et al., 2001; Bentayeb et al., 2007; Erkan et al., 2008; Frankel et al., 1996; Machado et al., 2009; Nolkemper et al., 2006; Okamura et al., 1994; Yesil-Celiktas et al., 2007a,b). Screening of the methanolic and ethanolic extract of *R. officinalis* aerial part has reported the presence of these compounds, but not the presence of alkaloids as detected in an aqueous extract (Hosseinzadeh and Nourbakhsh, 2003; Machado et al., 2009; Maria-José et al., 2003). Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the psychostimulant effect.

In conclusion, the present study indicates that the methanolic and aqueous extracts from stems and leaves of *R. officinalis* possess potential psychostimulants activity. This may justifies its use in traditional medicine as general stimulant.

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Research Paper

Psychostimulant activity of *Rosmarinus officinalis* essential oils

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ABSTRACT

We evaluate the psychostimulant activity of *Rosmarinus officinalis* (*lamiaceae*) on central nervous system (CNS). The effects of the essential oil of the stems and leaves of this plant was investigated in three behavioral models including, observation of animals in free situation, search of stereotypes movement in rats and interaction with thiopental in mouse. The essential oil extracted from stems and leaves by hydrodistillation were characterized by means of gas chromatography-mass spectrometry (GC-MS). *Rosmarinus officinalis* contained α-pinene (15.82%), camphene (6.80%), β-pinene (4.75%), myrcene (1.70%), *p*-cymene (2.16%), 1, 8-cineole (50.49%), camphor (11.61%), broneol (2.58%), and broneol acetate (2.08%). We observed that essential oil (100mg/kg) of *Rosmarinus officinalis* significantly increased the locomotors activity and induced hypermobility and hyperexcitability, as compared to a control group. Treatment with *Rosmarinus officinalis* essential oil at the dose of 100 mg/kg, significantly decreased the duration of thiopental-induced sleeping time from 34 min ± 0.2 to 2 min ± 0.2 (P<0.001). These results suggest that, the essential oil from stems and leaves of *Rosmarinus officinalis* possess potential psychostimulant activity. This may justifies its use in traditional medicine as general stimulant.

Keywords: *Rosmarinus officinalis* L; Psychostimulant activity; Central nervous system; GCMS; Stereotypes movements; Psychiatric disorders.

INTRODUCTION

The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants; divided into 150 families and 940 geniuses (Alnamer, et al., 2011). The term “psychostimulant drugs” refer to a diverse class of psychoactive compound that shares in common the capacity to activate the central nervous system, and subsequently behavior. Physiologically psychostimulants typically increase the functional activities of central

monoaminergic and cholinergic systems (Dalfrerera, et al., 2000). Recently, the search for novel pharmacotherapy from medicinal plants for neurological and psychiatric diseases has progressed significantly owing to their less side effects and better tolerability (Zhang, 2004). Plant extract have been used for the treatment of some psychiatric disorders. Some studies have reported that the extract of *Rosmarinus officinalis* L have a number of pharmacological activities, such as hepatoprodective (Sotelo, et al., 2002), antibacterial (Gachkar, et al., 2007), antithrombotic (Yamamoto, et al., 2005), antiulcereogenic (Dias, et al., 2000), diuretic (Haloui, et al., 2007; Bakirel, et al., 2008), antioxidant (Gonzalez-Trujano, et al., 2007), antinociceptive (Altinier, et al., 2007) and anti-inflammatory (Heinrich, et al., 2006). An ethnopharmacological use of *R. officinalis* in the treatment of depression, among other uses, was reported (Zhang, 2004; Fibiger, et al., 1988; Machado, et al., 2009). However the psychostimulants properties have not yet been studied. Thus, the aim of this study is to evaluate the psychostimulant activity of *Rosmarinus officinalis* essential oil in animal models.

MATERIALS AND METHODS

Plant material: *Rosmarinus officinalis* was collected based on ethnopharmacological information, from villages around the region Rabat-Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V–Rabat–Morocco.

The essential oil was produced from stems and leaves of *Rosmarinus officinalis* by hydrodistillation method. Plant materials (100g) cut into small pieces were placed in distillation apparatus and hydrodistilled for 3h after the oils was dried over an hydrous $K_2 CO_3$, they was stored at +4˚C until used for GC-MS analysis. The yield of extraction (ratio weight of EO/weight of dry plant) was 0.256%.

Phytochemical analysis of Rosmarinus officinalis essential oil by combined gas chromatography-mass spectrometry (GC-MS): The essential oil was submitted to quantitative analysis in a Hewlett-Packard 575, GC condition: carrier gas N_2 (0.5 bar) at flow rat at 1.0m/min, sample size, 0.2µl injected, capillary column (30m siloxane 5% HP EM). The temperature of the injector and detector was set at 250°C. The oven temperature was programmed from 50ºC to 250°C (5min). The MS was taken at 70eV. The components of the essential oil were identified by comparison of their mass spectra with those in the Wiley- NIST 7th edition library of mass spectral data. The percentage composition on the oil sample was calculated from GC-MS peak areas (Wiley and Sons, 2008).

Animals: Male Swiss mice (20-25g) (IOPS Offa) were used in pharmacological test and females of the same strain in the LD_{50} calculation. Animals were obtained from the animal experimental centre of Mohammed V- Souissi University, Medicine and Pharmacy Faculty – Rabat. They were housed three per plastic cage, photoperiod (one light from 6:00 to 18:00h); air changes and room temperature (22±1°C) were controlled. All animals had free access to tap water and at *ad-libitum* feeding; except for short fasting period before the treatment with the single dose of the essential oil. The general behavior of mice was observed continuously for 1h after treatment, intermittently for 4h and over period of 24h (Twaij, 1983). The mice were observed for 14days following treatment, and all signs of toxicity and deaths and their latencies

were recorded. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509. The particular treatment doses were selected depending on the LD_{50} results. The therapeutic doses should be very lesser than the LD_{50} value.

Acute toxicity: LD_{50} (median lethal dose) values were determined as described by Litchfield and Wilcoxon (Leitchfield and Wilcoxon, 1949). Seven groups of mice of both sexes (*n* =10; 5 males and 5 females) received or not oral single doses at different concentration. The control group received only the water or saline solution. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 24h at 6h time interval to detect any eventual side effect. The number of animals, which died during this period, was expressed as percentile, and the LD_{50} was determined by probit test using death percent versus doses log (Alaoui, et al., 1998). Of note, drugs used as control were given to mice in similar conditions.

Drugs: Thiopental (60mg/kg, i.p.) and Apomorphine (16mg/kg, s.c.) were used as reference drugs (positive control). It was dissolved in distilled water prior to administration.

Pharmacological evaluations: The psychostimulant activity of essential oils from *R. officinalis* on the central nervous system (CNS) was then studied, using a battery of behavioral tests used in psychopharmacology.

For testing psychostimulant effects, the effect of *R. officinalis* eeesntial oils on mice was qualified in one of the following tests:

Observation of animals in free situation: Detailed behavioral observations were made on lots of 3 mice per dose, for 15, 30, 60, 120, 180 min and 24h after administration of substances, based on the method that described by Irwin (Axiotis, et al., 1987; Petit, et al., 1990). Especially, the effect of psychostimulant is evaluated after hypermobility and hyperexcitability.

Interaction with thiopental in mice: Mice (6 per dose) were placed in individual boxes and given the substance (essential oil) 30 min before administration of thiopental (a sub-hypnotic dose, 60mg/kg, i.p.). The mice were treated with different dose of essential oil (50 and 100mg/kg, p.o., *n* = 6), the control group (*n*=6) was treated with distilled water (10ml/kg, p.o.). The effects was recorded for disappearance (latency) and reappearance (duration) of the righting reflex (Axiotis, et al., 1987; Petit, et al., 1990).

Search for stereotypes movements in rats: The stereotypes movements, characterized by movements of the head and buckle, sniffing, licking or chewing are scored from 0 to 3 according to the method described by Simon and Chermat (Simon, and Chermat, 1977), every 10 min until their disappearance in all animals.

The essential oil was tested at doses of 50 and 100mg/kg. The control groups have received the solvent under the same conditions. After administration of essential oil of *Rosmarinus officinalis L*, each animal was isolated in individual cages to be observed every 10 min during 210 min. The intensity of stereotypes was assessed according to the quotation of Simon**.** For each lot the sum of individual quotations obtained by fraction of 10 min is calculated. The total number of stereotypes observed during 210 min was also taken into account (Simon and Chermat, 1982).

Results are expressed as mean ± S.D (standard deviation). Statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by student's *t*- test. A level of significance (P<0.05 or 0.01) was considered for each test.

RESULTS

Chemical composition of the essential oil: The results obtained by GC-MS analyses of the essential oils of *Rosmarinus officinalis* are presented in table 1. Thirteen compounds were identified in this essential oil by GC-MS analyses (Fig 1); *Rosmarinus officinalis* contained three major compounds *i.e.* 1, 8-cineole (50.49%), α-pinene (15.82%) and camphor (11.6%).

Acute toxicity of Rosmarinus officinalis essential oil: Following oral administration of *R. officinalis* essential oil at the dose 500 mg/kg, neurological deficit and abdominal contraction were observed by 20-25 min after the administration in 60% and might be due to the route of administration of *R. officinalis* essential oil. In addition to the abdominal contraction; the essential oil reduced spontaneous motor activity and exploratory behavior. The mortality was recorded 24h after administration at the doses 850, 900, 950, and 1000mg/kg. The severity of these effects was increased within 6h, and mortality was 100% (Table 4). Boniface et al., have established a program that calculate the percentage of mortality according to the administration dose (Boniface, et al., 1972). This calculation gives the following results: LD_{50} = 897.85mg/kg, 885.90 < LD_{50} < 909.97, with confidence limits at 95%. This result indicates that this essential oil had low toxicity (Table 2).

Psychostimulant activity of the essential oil on central nervous system: The results of psychostimulant effects of essential oil were determined by comparison with control group. Pharmacological tests were then performed at non toxic doses *i.e.* 50 and 100 mg/kg. These doses did not induce severe neurological side effects.

Observation of animals in free situation: We observed that oral administration of the essential oil of *Rosmarinus officinalis* L at the dose of 100mg/kg caused the appearance of rapid movements of the head, sniffing the buckle (gnawing, biting the cage walls) suggesting an increasing locomotors activity and curiosity, induction of spontaneous aggression, hypermobility and hyperexcitability of the central nervous system (stereotypes movements).

Interaction with thiopental in mice: The administration of the therapeutic doses (50 and 100mg/kg, p.o.) of the essential oil of *Rosmarinus officinalis*, 30 min before the injection of the hypnotic dose of thiopental (60mg/kg, i.p.), significantly increased sleep latency and caused a decrease in sleep time. The effect was significantly higher at the dose 100mg/kg than at the dose of 50mg/kg (Table 3).

Search for stereotypes movements in rats: From Table 4, Apomorphine (16mg/kg, s.c.) induces on the control group the appearance of stereotypes movements after just 10 min, after the end of 80 min, the total of quotation being 18. The stereotypes movements decreased gradually until disappear at 210 min. The stereotypes Phenomena are increased in duration and intensity. However, the essential oil (100mg/kg) produced a significant (P<0.001) potentiating of the effects of Apomorphine, but at the dose 50mg/kg the agonist of stereotypes movements was partial in duration and intensity compared with control group. Indeed, the stereotypes movements appeared at 30 min, reaching a peak at 40 min (total quotations = 18) remain at 260 min for disappearance at 280 min (Table 4).

DISCUSSION

It should be noted that, this type of work has not been done on same plant species or family. Also the papers that published previously are very few.

Aromatherapy provides a potentially effective treatment for a range of psychiatric disorders (Perry and Perry, 2006; Sarris, 2007). In aromatherapy, the essential oils are believed to possess antidepressive effects and to be useful for treating nervous breakdown and depression (Tisserand, 1993; Fibiger, et al., 1988; Machado, et al., 2009). Oil compositions of *R. officinalis* have already been reported (Tisserand, 1993; Gachkar, et al., 2007; Costa, et al., 1972). Thus, it has been shown that α-pinene, 1,8-cineole, camphor, verbenone and broneol account for 80% of *R. officinalis* essential oils, but in our study, these compounds represent 97.6%. These differences in chemical composition of essential oil may be due to both developmental and environmental factors that influences plant metabolism. We analyzed the effects of different doses of essential oil from stems and leaves of rosemary (*Rosmarinus officinalis*) for their acute toxicity and psychostimulant activities. In the current study, the psychostimulant activity was investigated by recording the spontaneous locomotors activity in mice. In the test of observation in free situation, the essential oil (100mg/kg) produced an increase in motor activity and curiosity, by the induction of spontaneous aggression, hypermobility and hyperexcitability. These observations suggest that, the psychostimulant action of this plant is mediated by dopaminergic pathway, since dopaminergic transmission can produce profound stimulant effects in mice (Simon and Chermat, 1982; Boissier, et al., 1972). It is possible that, the dopaminergic transmission is due to the releasing of catecholamine (nor epinephrine and dopamine) neural storage vesicles (Costa, et al., 1972; Samanin, et al., 1977). The essential oil of *R. officinalis* has considerable dopaminergic potentiating mechanisms. In the test of thiopental-induced sleep, the essential oil (100mg/kg) produced a significant ($P<0.001$) highest increase in the time of onset of sleep as well as highest reduction of sleep induced by thiopental (Table 3).

The prolongation in the time of onset of sleep and the highest reduction in the sleep time and the induction of exploratory behavior indicate a central nervous system stimulant activity of *Rosmarinus officinalis* essential oil. Further chemical and pharmacological analysis of the essential oil will be conducted to isolate and characterize the active principles responsible for the psychostimulant effects.

Thus present study indicates that essential oil from stems and leaves of *Rosmarinus officinalis* possess potential psychostimulant activity. This may justifies its use in traditional medicine as general stimulant.

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Table -1: Chemical composition of *Rosmarinus officinalis* essential oil.

Peak N	Identified compound	Rt (retention time)	Relative percentage%
1	α-pinene	8.37	15.82
2	Camphene	8.83	6.80
3	β-pinene	9.79	4.75
4	Myrcene	10.40	1.70
5	p-cymene	11.54	2.16
6	1,8-cineole	11.80	50.49
7	Camphor	15.75	11.61
8	Borneol	16.57	2.58
9	Bornyl acetate	24.98	2.08
10	Tans-caryophyllene	29.85	Tr
11	δ-cadinene	35.59	Tr
12	Unknown	39.13	Tr
13	Bisabolol	43.35	Tr

Table -2: Mortality of *Rosmarinus officinalis* essentials oil by oral route (p.o.).

	Doses (mg/kg) p.o.	Mortality %
EO	500	0
	750	10
	850	30
	900	40
	950	60
	1000	100

- Mice of each group (*n*=10) were received single dose and they were examined for 14 days to determine LD_{50} and any behavioral changes.
- EO: mean essential oil; p.o.: mean oral route.

Table -3: Influence of essential oil of *Rosmarinus officinalis* on sleep latency and duration of sleep induced in mice by an hypnotic dose of thiopental (60mg/kg).

Groups	Dose (mg/kg)	Sleep latency (min)	Sleeping time (min)
Control	60	18 ± 1	34 ± 2
EO	50	20 ±1	6 ± 0.1*
EO	100	42 ±1*	2 ± 0.2*

- Data are expressed as mean ± SD; *n*= 5 per group; **P*< 0.001 versus the control group.
- EO: mean essential oil; i.p.: mean intraperitonial route; n: mean number of animals; p.o.: mean oral

Table -4: In**fluence of essential oil of *Rosmarinus officinalis* L on stereotypes movements induced by Apomorphine (16 mg/kg, s.c.) in rats.**

Sum of individual quotation stereotypic																												
Time (min)	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280
AM 16 mg/kg	9	11	12	12	15	16	18	18	16	15	14	10	8	8	6	5	5	4	3	2	0	0	0	0	0	0	0	0
EO 50 mg/kg	8	10	12	13	15	16	16	14	14	12	11	10	10	8	8	6	5	3	2	1	0	0	0	0	0	0	0	0
EO 100 mg/Kg	15	15	16	18	18	18	16	16	14	15	15	15	14	13	12	10	10	9	8	8	6	6	5	5	3	2	1	0

- (*n*= 6)= number of animals per lots; EO= essential oils; AM: mean apomorphine; s.c. = subcutaneous.
- The individual quotation stereotypic was calculated as follows:
- 0 = absence of stereotypes movements and all animals were normal; 1 = rare presence of stereotypes movements; 2 = intense sniffing with rapid movements of the head; 3 = intense and continuous stereotypes movements (displacement of the head, licking) ($P< 0.001$).

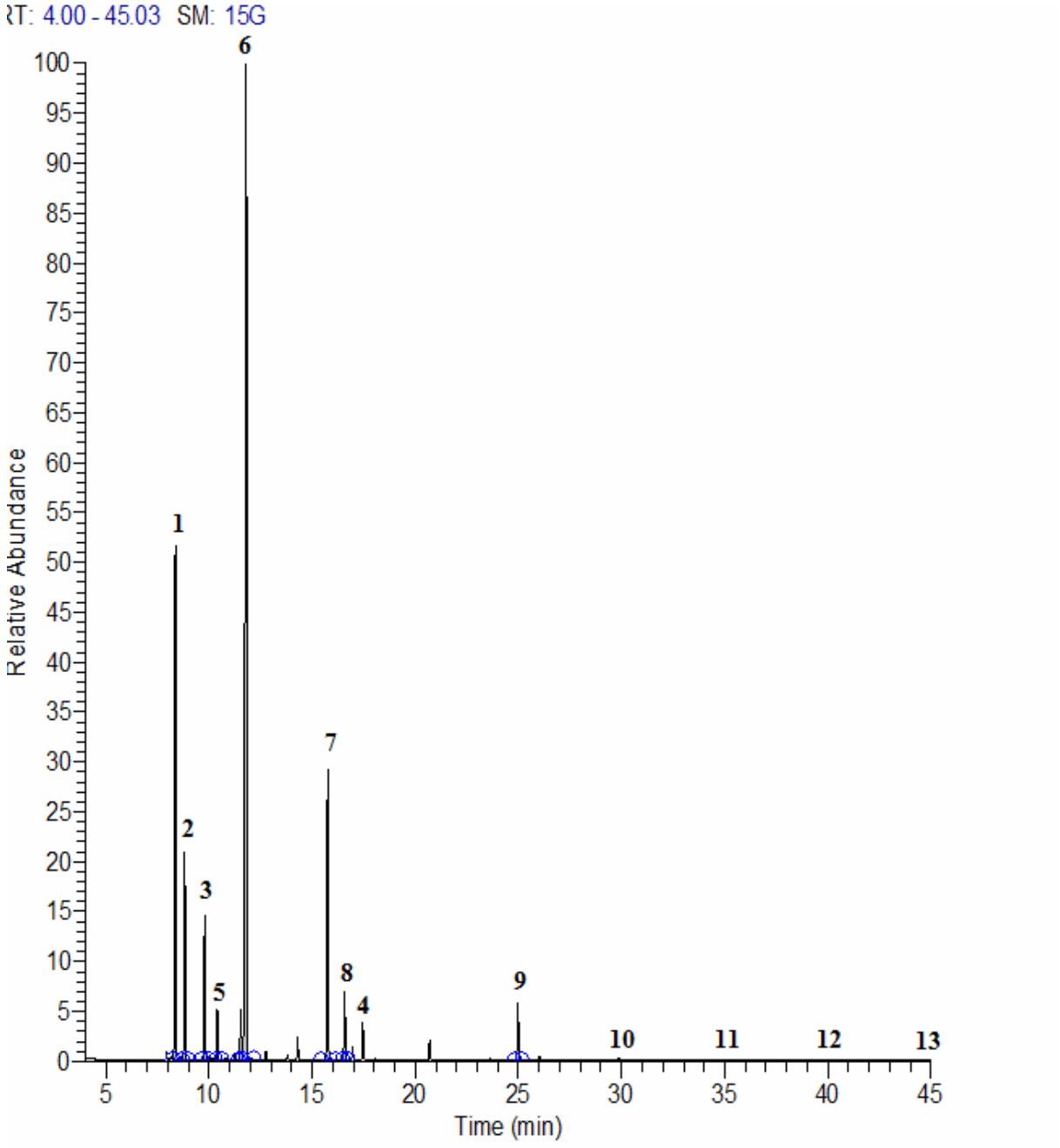

Figure-1: Gas chromatography- mass spectrometry (GC-MS) of *Rosmarinus officinalis* essential oil.

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Analgesic and Anti-inflammatory Activities of *Boswellia elongata balf* Methanolic Extracts, as Endemic Plants in Yemen

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Abstract: We evaluate the analgesic and anti-inflammatory activities of leaves of *Boswellia elongata balf* methanolic extract an experimentally induced acute inflammation in rats. This study was carried out by using male and female Swiss mice (20-30 g) and Wister male rats (180-220 g). The methanolic extracts were prepared by using maceration at room temperature (25°C) over period 48 h. The effects of methanolic extracts of *B. elongata balf* was investigated for anti-inflammatory properties by using carrageenan and experimental trauma-induced hind paw oedema in rats. The analgesic activities were performed by using chemical and thermal models which will induce acute pain in animal models. The anti-inflammatory properties oral administration of extract at the dose of (50, 100 mg/kg p.o., respectively) showed significant reduction ($P \leq 0.001$) and inhibition of oedema induced by carrageenan and experimental trauma in hind paw of rats when compared with control and standard drug (Indomethacin). We can conclude that, *B. elongate balf* methanolic extract have not central analgesic effect and have peripheral analgesic as anti-inflammatory activities, supporting the traditional application of this plant in treating various diseases associated with inflammation and pain.

Key words: Medicinal plants; *Boswellia elongate balf*; Methanolic extracts; Anti-inflammatory activity; Analgesic activity.

Introduction

Generally, the inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischaemia, antigen-antibody interaction and thermal or physical injury [1, 2]. Inflammation is usually associated with pain as a secondary process resulting from the release of algesic mediators [2, 3]. Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressant drugs, which have been used usually in the relief of inflammatory diseases by the people of the world for a long time.

However, these drugs were often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcers [4]. Recently, many natural medicines derived from medicinal plants, were considered as the effective and safer for the treatment of various diseases including inflammation and pain [5].

The genus Boswellia (Family *Burseraceae*) consists of many species widespread throughout the world. It includes approximately 23 species of small trees that grow mainly in Arabia, in eastern coast of Africa and in India, Oleogum-resin that exudes from tapings in the bark of

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boswellia trees [6]. The genus is represented in Soqotra Island by 8 species; the oleogum resin is used traditionally by the native inhabitants for relieving the pain in cavities, sweetening the breath, or to sooth a disturbed stomach [7]. *Boswellia elongata* is a species of plant in the *Burseraceae* family. Different parts of the plant especially have been mentioned to be useful in a variety of diseases like diarrhea, dysentery, urinary disorder, gonorrhea, bronchitis. The composition of *B. elongata* oil was dominated by the diterpene verticiol (52.4 %), the sesquiterpene caryophellene (39.1 %) and methyl cycloundecane carboxylate (7.8 %). Antioxidant, anti cholinesterase activity of essential oils obtained from the oleogum resin of boswellia [8]. Previous biological studies on the bark of *Boswellia elongata* reported a significant anti microbial and antiviral activities for this plant [9, 10].

However the analgesic and anti-inflammatory properties of the leaves of *B. elongata balf* methanolic extract have not yet been studied. Thus, the aim of this study is to evaluate the analgesic and anti-inflammatory activities of *Boswellia elongata balf* methanolic extract in animal models.

Materials and methods

Plant material

Fresh leaves of *Boswellia elongata balf* (Family *Beurceaceae*) were collected from Socotra Island in Yemen on January 2008 and identified by prof. Ahmed Said and botanist of Soqotra. A voucher specimen (N°RAB76712) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat.

Preparation of extract

Leaves of *boswellia elongate* were successively extracted with methanol by maceration at room temperature (25°C) over period of 48h. 300g of plant material and one litre of methanol were used in extraction. Methanol, containing the extract was then filtered through Whitman paper and the solvent was vacuum distilled at 65°C in rotary evaporator. Final extract was a drake brown semi-solid in percentage dray weight 7 %, this methanolic extract was kept in deep freezer at -20°C until use.

Animals

Male and female Swiss mice (20-30g) (Offa-credo, France) were used in the acute toxicity from estimation of the LD50, and wistar male rats (180-220g) in the pharmacological tests (anti-inflammatory and analgesic activities). The animals were acquired from the animal centre of Mohammed V-Souissi University, Medicine and Pharmacy Faculty-Rabat. All animals were kept in a room maintained under environmentally controlled conditions of 23±1°C and 12 h light-12 h dark cycle. All animals had free an access to water and standard diet. They were acclimatized at least one week before the experiments were started. The animals submitted to oral administration of the extracts or drugs were fasted for 18 h before the experiment (water was available). All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509.

Analgesic activity

The evaluation of the analgesic activity of the methanolic extract of the leaves of *Boswellia elongata balf* was carried out by using two different methods that used thermal stimuli (Tail Flick test), and chemical stimuli (koster test).

Acetic acid-induced writhing response in mice

The method used in this test has been described by Koster *et al.*,[11]. The total number of cramps followings intraperitneal administration of acetic acid solution (3 % at 300 mg/kg i.p) was recorded over a period of 20 min, starting 5 min after acetic acid injection, the mice were treated with methanolic extracts of *Boswellia elongate balf* (50 and 100 mg/kg) or standard drug (aspirin, 200 mg/kg), 30 min before administration of acetic acid, the number of cramps and stretching was recorded and permitted to express the percentage

of protection using the following ratio (control mean-treated mean) x 100/control mean [12-14].

Tail Flick test

The procedure is based on the observation that morphine like drugs are selectively prolonging the reaction time of the typical tail withdrawal reflex in rats induced by immersing the end of the tail about (4-5cm) in warm water of 55°C [15,16]. Morphine (5 mg/kg; s.c), was used as positive control and *Boswellia elongata balf* methanolic extract was administrated (50, 100 mg/kg; p.o.). The tail withdrawal reflex was recorded before and after 15, 30, 60 and 120 min following oral route administration of the methanolic extract of *B. elongata balf* to different groups of six animals.

Anti - inflammatory activity

The evaluation of the anti-inflammatory activity of *Boswellia elongate balf* was carried out by using two different methods that used chemical stimuli [17, 18], and mechanical stimuli [19] induced paw oedema in rats. In both methods, all animals were fasted 18h before testing and received 5 ml of distilled water by gavages to minimize individual variations in response to the swelling of the paws. The right hind paw (RP) is not treated, and it is taken as control.

% of inhibition = mean [v Left_v Right] control - [v Left_v Right] treated D [v Left_v Right] control ×100.

V Left means volume of oedema on the left hind paw and v Right means volume of oedema on the right hind paw.

Carrageenan-induced rat paw oedema

Carrageenan-induced paw inflammation was produced according to previously described method [17, 18]. One hour after oral administration of the extract (50 and 100 mg/kg, p.o), reference drug (indomethacin, 10 mg/kg, p.o), an injection of 0.05 ml of carrageenan (1 % carrageenan suspended in 0.9 % NaCL) was made in to the left hind paw of each rat under the sub-plantar aponeuvrosis. Measurement of paw size was done by mean of volume displacement technic using plethysmometer Digitals 7500 immediately before carrageenan injection and 1h30, 3h and 6 h after carrageenan injection.

Experimental trauma induced paw oedema in rat

The anti-inflammatory activity was evaluated according to the method described by the Riesterer *et al.*,[19]. For the experiment the male wistar rats (180-220 g) were divided into different groups (n= 6). The animals were fasted overnight (18 h) prior to the start of the experiment, and water *ad libitum*. The control group received (5 ml/kg of distilled water p.o.), the standard group received the reference drug (indomethacin 20 mg/kg, p.o.), and the test group received different concentration of methanolic extract of *Boswellia elongate balf* (50 and 100 mg/kg, p.o). One hour after oral administration of different substances, dropping a weight of 50 g to all animals on the left hind paw [20, 21]. The right hind paw is not treated; it is taken as a witness. The difference in the foot pad volume between the left and right foot was measured and taken as the edema value by using a plethysmometer Digitals 7500 at 1h30, 3h and 6h after induction of inflammation [20].

Statistical analysis

The results were reported as mean ± S.E.M and analyzed by one-way Analysis of Variance (ANOVA) followed by student's t-test. A value of $P<0.05$ or 0.01 was considered significant.

Results

Effect of methanolic extract on acetic acid-induced writhing reflex in mice

As shown in (Fig. 1 and 2) the methanolic extract (50 mg/kg and 100 mg/kg) of *B. elongate balf* restrained the writhing reflex induced by acetic acid with an inhibition percentage of (37.68 %, 58 %, 34 %) respectively. The positive drug (Aspirin) also significantly inhibited the writhing response by (44.11 %).

Effect of methanolic extract on Tail Flick test

In the TAIL Flick test, the analgesic effects were observed (Fig. 3), indicating that the methanolic extracts of *B. elongata balf* could not increase the

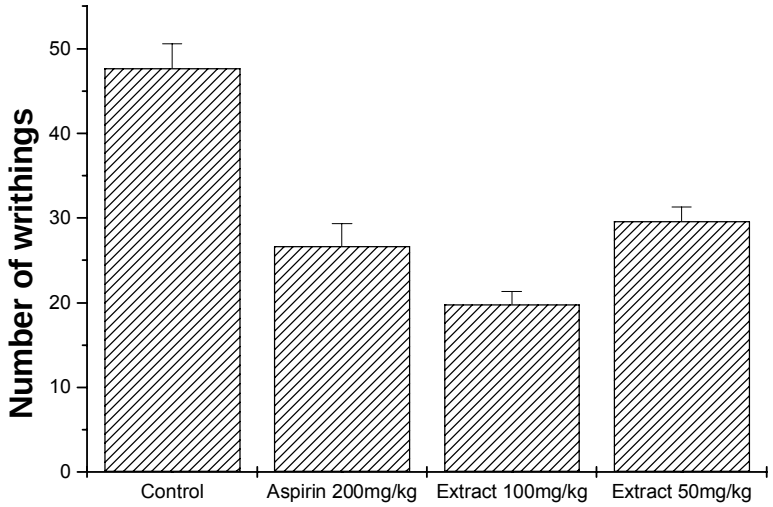

Fig. 1. Effect of methanolic extract of *Boswellia elongate balf* and aspirin on acetic acid-induced writhing response in mice. Comparisons are made with control group. Values are expressed as mean ±S.D. (n6). Symbols represent statistical significance at $P<0.001$.

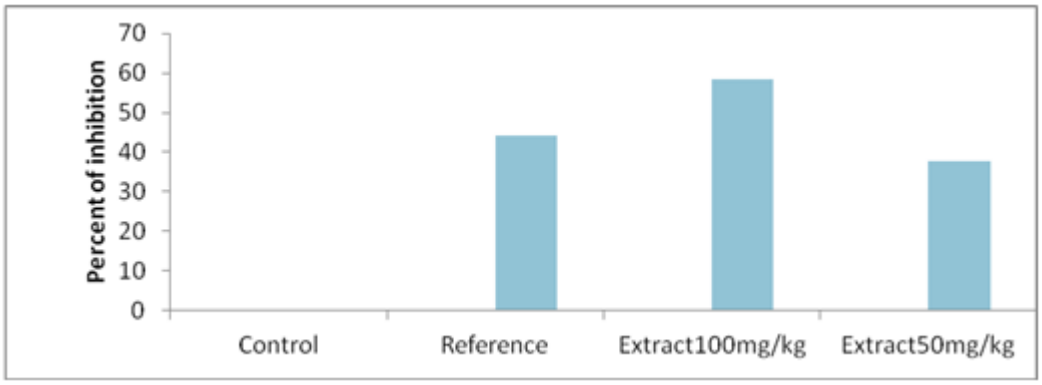

Fig. 2. Effect of methanolic extract of *Boswellia elongata balf* and aspirin on acetic acid-induced writhing response in mice.

animal reaction time to the heat stimulus and showed weak analgesic activity with 50 mg/kg and 100 mg/kg as compared to the control. While the latency time was significantly increased by morphine (5 mg/kg) at 45 and 60 min.

Effects of methanolic extract on carrageenan -induced rat paw oedema

The results are presented in (Table 1). The methanolic extract showed reduction in the carrageenan-induced paw oedema in rat at the dose (100 mg/kg) at 1h30; 3; 6 h after carrageenin injection with (41.84 %, 48.20 %, 47.70 %). Indomethacin (10 mg/kg) also inhibited paw oedema in rat after carrageenin injection with (69.74 %, 75.21 %; 63 %) of the inhibition rat, respectively (Table 1).

Effect of methanolic extract on experimental-induced rat paws oedema

The results are presented in Table 2. The methanolic extract showed reduction in the experimental -induced paw oedema in rat at the dose (100 mg/kg) at 1h30, 3, and 6h after experimental-induced rat paw oedema with (36.12 %, 47.56 %, 45.63 %) of inhibition rat, respectively. Indomethacin (20 mg/kg) also inhibited paw oedema in rat after experimental-induced rat paw oedema with (81.52 %, 84.81 %, 74.50 %) of the inhibition rat respectively (Table 2).

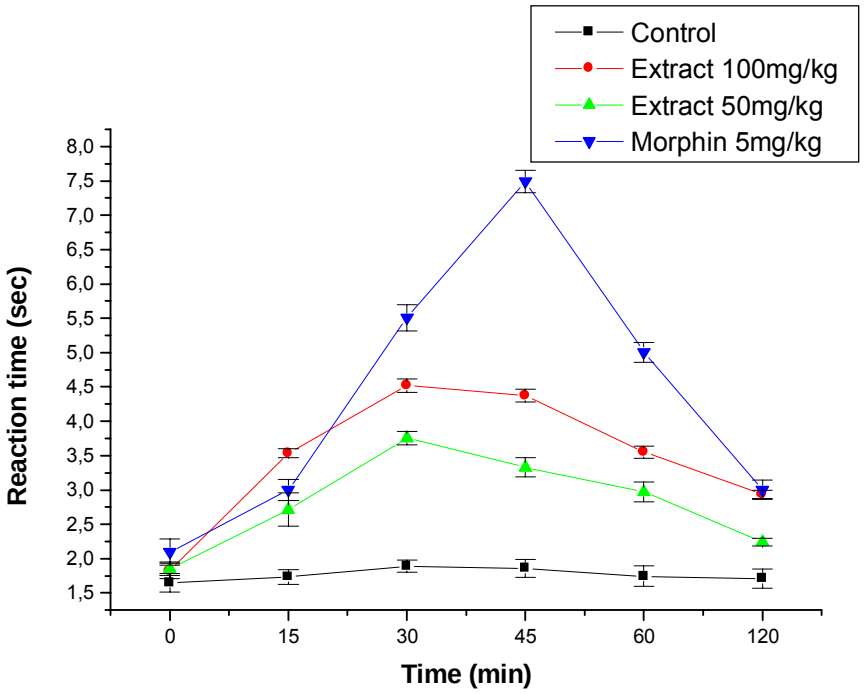

Figure. 3. Central Analgesic activity of methanolic extract of *Boswellia elongata balf* by TAIL Flick test. Each value represents the mean ± SEM (n = 6) *P*<0.001, statistically significant relative to control at 45 min.

Table 1. Effects of methanolic extracts of *Boswellia elongata balf* on carrageenan-induced rat paw oedema. Each value represents the mean ± SEM (n = 6) *P*<0.001, statistically significant relative to control at 3h.

Group	Doses (mg/kg)	Mean oedema volume 1h30	3h	6h	Percent inhibition (%) 1h30	3h	6h
Control		0.38±0.010	0.585±0.004	0.471±0.014			
Reference	10	0.116±0.006	0.145±0.006	0.17±0.008	69.74	75.21	63.90
Extract	100	0.218±0.009	0.285±0.010	0.221±0.007	42.63	51.28	53.29
Extract	50	0.315±0.008	0.463±0.008	0.345±0.008	18.42	20.85	26.75

Table 2. Effect of methanolic extract of *Boswellia elongata balf* on experimental-induced rat paw oedema .Each value represents the mean ± SEM (n = 6) *P*<0.001, statistically significant relative to control at 3h.

Group	Doses (mg/kg)	Mean oedema volume 1h30	3h	6h	Percent inhibition (%) 1h30	3h	6h
Control		0.433±0.010	0.698±0.012	0.561±0.001			
Reference	10	0.08±0.006	0.10±0.012	0.143±0.006	81.52	84.81	74.50
Extract	100	0.276±0.008	0.366±0.010	0.305±0.007	36.12	47.56	45.36
Extract	50	0.358±0.007	0.58±0.008	0.471±0.007	17.32	16.90	16.04

Discussion

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilizations. Driven by their intuition and their sense of observation, they were able to find answer to their health problems in the plant environment [22-24]. Recently, the search for novel pharmacotherapy from medicinal plants for inflammation diseases has progressed significantly owing to their less side effects and better tolerability. Aromatherapy is currently used worldwide in the management of chronic pain [25].

The analgesic and anti-inflammatory effects of the methanolic extract of *Boswellia elongata balf* were investigated in this study. The analgesic activities were evaluated using two animal models. The Tail Flick test was selected to investigate the central analgesic activity. Acetic acid-induced writhing response was selected to evaluate the peripheral analgesic effects.

In the acetic acid-induced writhing test, the methanolic extract demonstrated a significant analgesic effects, inhibiting pain by 37.68 %, 58.34 % at the doses 50 mg/kg and 100 mg/kg, respectively as compared to the control group (Fig.1 and 2).

In the TAIL Flick test, the analgesic effects were observed (Fig.3), indicating that the methanolic extracts of *B. elongata balf* could not increase the animal reaction time to the heat stimulus and showed weak analgesic activity with 50 mg/kg and 100 mg/kg as compared to the control group. It is known that, abdominal constriction response is very sensitive and able to detect anti-nociceptive effect of extracts or compound that may appear inactive in other methods like tail-flick test [26, 27]. Local peritoneal receptors are postulated to be partly involved in abdominal writhing response [26, 27]. The mechanisms of the reaction to this nociceptive stimulus seem to be related with prostanoid system. Experimental results obtained by several researchers indicated increased levels of lipoxygenase product [28]. As well as increase in the peritoneal fluids of PGE2 and PGF2α, serotonin and histamine [29, 30]. Pro-inflammatory mediators like prostaglandins and bradykinins were suggested to play an important role in analgesia [31]. So the results show that the extracts have a significant inhibitory activity in inflammation pain, and this activity may be related with the suppression of synthesis and/or release of endogenous pro-inflammatory substance. It suggests that methanolic extract of *B. elongata balf* has some inhibitory action on the cyclo-oxygenase pathway which is actually involved in the synthesis of prostaglandin biosynthesis [32]. Carrageenan and experimental trauma-induced paw oedema in rats are a suitable experimental animal model to evaluate the anti-edematous effect of natural products and is believed to be biphasic: the first phase (1h) involves the release of serotonin and histamine and the second phase (over 1h) is mediated by prostaglandin, cyclo-oxygenase products, and the continuity between the two phases is provided by kinins [33, 34]. We observed that animals treated with *B. elongata balf* methanolic extract (50 mg/kg p.o.) low reduced and inhibited of oedema in rats. By increasing the doses to 100 mg/kg, showed that, reduced and inhibited significantly ($P \leq 0.001$), the oedema in the early and late phases of an acute inflammation with maximum significant during (3 h) after induction of oedema, these results were compared with control and indomethacin (10 mg/kg, p.o.) ($P \leq 0.001$). In the experimental trauma induced oedema in rats, the extract (100 mg/kg p.o) was reduction and inhibition significant ($P \leq 0.001$) of oedema in the different phases of inflammatory response, these results were compared with control and indomethacin (20 mg/kg, p.o.) ($P \leq 0.001$).

These results indicate that the methanolic extract of *B. elongata balf* exert significant anti-inflammatory activity, especially in the acute phase of inflammation. The activity may be related to the inhibition of inflammation's chemical mediators. Previous pharmacological studies on the bark of *B. elongata* reported a significant analgesic and anti-inflammatory activities at the doses of 200 and 400 mg/kg via oral route [46]. These results clearly confirmed our results on the leaves of *B. elongata* methanolic extract (50 and 100 mg/kg, p.o.). Previous studies on the oleogum resin (olibanum) of other boswellia species such as *B. carteri* and *B. serrata* confirmed possessing a significant anti-

inflammatory effect [35-37].The research for the active principles of the resin resulted in the isolation of several boswellic acids [38, 41]. The resin of *B. serrate* contains a mixture of terpenoids made up of four pentacyclic triterpene acids: β-boswellic acid (the most abundant), 3-O-acetyl β (ABA), 11-keto-ßboswellic acid, and 3-O-acetyl-11- keto-β-boswellic acid. The triterpenoids are the active constituents and are collectively called boswellic acids [42]. As such, the observed analgesic and anti-inflammatory properties of methanolic extract of *B. elongata balf* in the present study may be due to the presence of phytochemical compound like boswellic acids. It is reported that *Boswellia* species contain in addition to essential oils, triterpenic boswellic acids. Boswellic acids are considered to be the ingredients responsible of the plant anti-inflammatory activity, since these compounds inhibit leukotriene biosynthesis by impairing the lipoxygenase activity [43, 45]. Thus, we concluded that, *B. elongata balf* methanolic extract are capable of inhibiting inflammatory reaction as well as pain via oral route. These results clearly confirmed the traditional anti-inflammatory and antinociceptive of *B. elongata* and suggest that *B. elongata* could be a potential source for anti-inflammatory and antinociceptive agents.

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IN VITRO CYTOTOXICITY AND FREE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC AND ALKALOIDIC EXTRACTS OF *DELPHINIUM STAPHISAGRIA*

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ABSTRACT

In this study, the *in vitro* cytotoxicity and antioxidant properties of the ethanolic and alkaloidic extract of *Delphinium Staphisagria* seeds were assessed. The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity. The *in vitro* cytotoxicity was carried out by using the 3-(4,5- dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, against two animals cancer cell lines; Vero cell line, initiated from the kidney of a normal, adult African green Monkey and Neuro-2a (N2a) from a spontaneous tumor of a strain A albino mouse. The DPPH scavenging activity of both extracts was concentration-dependent (increasing from 15.62 µg/ml to 500 µg/ml), exhibited considerably ($P<0.05$) DPPH radical-scavenging activity and was able to inhibit the formation of DPPH radicals with a percentage inhibition of 62.19 and 94 % respectively at the highest concentration. The results showed that the alkaloidic extract (1000-31.25 µg/ml) of *Delphinium Staphisagria* possesses significant IC_{50} compared to the drug positive control on all cancer cell lines used. The lower IC_{50} represent the highest potency of a compound to inhibit the growth of cells and cause toxicity and death of cells. The result obtained in this work demonstrated a high activity at low alkaloids extracts doses (500µg/ml). In conclusion, the results of the present study suggest that *Delphinium Staphisagria* seeds plant have various secondary metabolites and has good quantity of alkaloidic compounds (diterpenoid alkaloids). Plant has potent free radical scavenging activity. Detailed studies on chemical composition, isolation of active constituents and pharmacological evaluation are essential to characterize them as biological antioxidants. The present findings of this study support the view that *Delphinium Staphisagria* seeds are a promising source of potential antioxidant which can be used in treatment of various ailments.

Keywords: *Delphinium Staphisagria*; DPPH; Radical-scavenging; Cytotoxic activity; Diterpenoid alkaloids.

INTRODUCTION

Phytochemicals found in plants have beneficial effect on health or play active role in curing diseases. Plants have the ability to synthesize many secondary metabolites of which only 10% are identified. These metabolites serve remedy for various alemeties like antimicrobial, anti-inflammatory, antihypertensive anti diabetic ect.. [1, 2]. Since these constituents influence human health in beneficial way, analyses of the constituents become important in view of pharmaceutical and health care products.

Certain chronic and degenerative diseases are induced by oxidative stress [3]. Free radical, one of the factors to cause stress, are generated due to

environmental pollutants, radiations, chemicals, toxins, metabolic process especially oxidation, make body cell to deteriorate and cause depletion of innate immunity, change in gene expression, if not used up. Antioxidants take up these free radicals and protect body against pathogenic conditions like anemia, arthritis, inflammation, neruo-degeneration, Parkinson's disease [4, 5]. Hence antioxidant property is important for study.

Antioxidant properties in medicinal plants are attributed to the wide range of amphipathic molecules broadly termed "Polyphenolic compounds". Phenolic products act mainly due to its redox properties, which make them to act as hydrogen donor, reducing agents and singlet oxygen quencher. They also act as metal chelators [6]. In addition to pharmaceutical industry, in the food industry also, these phenolic compounds are of interest because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Natural phenolic compounds are of interest to scientists, food manufacturers, and consumers due to its beneficial health effects [7].

Due to depletion of INNATE antioxidants, it may be necessary to consume antioxidants as free radical scavenging. Synthetic antioxidants are being replaced by naturally occurring antioxidants, mainly due to low solubility and moderate antioxidant activity [7]. Hence, there is a demand to explore the antioxidant potential of plant species [15].

Medicinal plants presents a potential source of drugs or molecular models for new drugs, in fact, in developing countries, including Morocco, the use of herbal medicine is for a majority of the population an alternative therapy due to the high cost of western pharmaceuticals, health care and the cultural, spiritual point of view of the people of the country [16]. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used in traditional medicine become necessary and led researchers to enhance scientifically the possible beneficial effects of medicinal plants. Natural compounds derived from plants may contribute on the mechanisms behind pathogenesis involved in some diseases like cancer. The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants; divided into 150 families and 940 geniuses [8-14]. *Delphinium staphisagria* is an endemic annual or biennial herb from the Mediterranean Basin belongs to the family Renonculaceae, some studies have reported that the extract of *Delphinium staphisagria* have a number of pharmacological activities such as antispasmodic, cathartic, emetic and vermifuge. The seed is used to make a potent insecticide, parasiticide and to destroy vermin [17-19, 20]. Thus, the aim of this study is to evaluate the in vitro cytotoxicity and antioxidant properties of the ethanolic and alkaloidic extract of *Delphinium Staphisagria* seeds.

MATERIALS AND METHODS

Plant material: Plant materials were collected based on ethnopharmacological information, from villages around the region Chefchaoun, northern Morocco with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. A voucher specimen n (N° RAB 65077) was deposited in the Herbarium of Scientific Institute of Rabat in Morocco.

Extraction procedure: Seeds of *Delphinium Staphisagria* were extracted with 80% Ethanol by maceration at room temperature (25°C) over the period of 24 hours. 250 g of seeds material and one liter of 80% Ethanol were used in the extraction. Ethanol, containing the extract, was then filtered through Whatman paper and the solvent was vacuum distilled at 50°C in rotary evaporator. The ethanolic extract was then treated with 0.5 M H_2SO_4 and filtered. The acid solution was extracted with CH_2Cl_2 to give a crude material (6.44g). Acid aqueous phase was neutralized to pH 7 and extracted with CH_2Cl_2 to give a crude materiel (5.36g). Neutral aqueous phase was basified with 20% NaOH to pH 12 and extracted with CH_2Cl_2 to give a crude alkaloidic material (1.45g). Ethanolic and Alkaloid extracts were kept in deep freezer at –20°C until use [20, 21].

Median Lethal Dose Determination: LD_{50} values were determined as described by OECD 423 [18, 20, 23]. Median lethal dose (**LD_{50}**) of the alkaloid extract was determined using female Swiss mice (Animal Center of Mohammed V-Souissi University, Medicine and Pharmacy Faculty-Rabat), weighing 25 ± 2 g. All animals had free access to food and water; they were housed under standard environmental conditions on a 12/12h light/dark cycle. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509. Ethanolic extracts was dissolved in distilled water and given by orally way in a single dose (300mg/kg, p.o.) of body weight. Mice were observed for clinical effects and mortality. The LD_{50} of extract was determined using a

modified up and down method known as Globally Harmonized System (GHS) [20]. Using this method for acute toxicity testing, animals are dosed one at a time. The test consists on a stepwise procedure with the use of three female mice per step. If the first animal survives, then the next animal receives a larger dose, while if the first animal dies the next animal receives a smaller dose. The dose for each successive animal is adjusted up or down depending upon the outcome for the previous animal. This causes the doses to be rapidly adjusted toward the LD_{50} and then be maintained in the region of the LD_{50}. This method is preferred because fewer animals are required; however, it can result in unbalanced numbers in each group [20, 22, 40].

Cytotoxic activity

Cell line and culture conditions: The cell lines tested in this study were Vero cell line, initiated from the kidney of a normal, adult African green Monkey and Neuro-2a (N2a) from a spontaneous tumor of a strain A albino mouse, this tumor line was obtained from the Jakckson Laboratory, Bar Harbor, Maine. Neuro-2a cells produce large quantities of microtubular protein which is believed to play a role in a contractile system which is responsible for axoplasmic flow in nerve cells. Cells were cultured in MEM media (Sigma, USA) supplemented with 10% heat-inactivated FBS 2% antibiotics and L-glutamine. The cells were grown at 37°C in a humidified incubator set at 5% CO2. Cells were subcultured after they formed a monolayer on the flask. The cells were detached by treating them with trypsin-EDTA for 10 minutes and then by adding complete medium to inhibit the reaction.

In Vitro Cytotoxicity Assay: Cytotoxicity of sample on tumor cells was measured by microculture tetrazolium (MTT) assay. For the assays, 96-well microplates were seeded with 100 µl medium containing 2.10^5 cells in suspension. After 24 h incubation and attachment, the cells were treated with dilutions of crude Ethanolic and alkaloidic extracts. Exactly from the stock solution (10 mg/ml), the extract sample was applied in a series of 6 dilutions (final concentrations ranging from 31.25 to 1000 µg/ml) with a final DMSO concentration of 0.1% and was tested in quadruplicate. After 48 h incubation, cell viability was determined by adding (Sigma) tetrazolium salt as cytotoxicity indicator and by reading absorbance at 590 nm with a scanning multiwell spectrophotometer (Spectra Count, Packard, Ont., Canada). Tetrazolium salts are cleaved to formazan dye by cellular enzymes (only in the viable cells). The level of absorbance directly correlates to the metabolically active cells [23, 24]. Mytomicin C (~ 95 % HPLC, sigma-Aldrich) was used as a positive control. All determinations were conducted in triplicate. The inhibition of cell growth was calculated by the following formula:

Percentage inhibition = (**OD** of treated cells **/ OD** of untreated cells) **x** 100

IC_{50} values were calculated as the sample concentration that caused 50% cell death.

Free Radical Scavenging Activity: The free radical scavenging activity of the ethanolic and alkaloidic extracts of *Delphinium Staphisagria* were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) [Sanchez-Moreno C, 2002]. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of plant extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = [(ADPPH−AExtr) / ADPPH] × 100. Where ADPPH is the absorbance value of the DPPH blank sample, and AExtr is the absorbance value of the test solution. AExtr was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank [25, 26].

Statistical analysis: The results are expressed as mean ± standard error of mean (S.E.M). Significant differences between control and experimental groups were assessed by Student's t test. . The IC50 values were obtained by nonlinear regression using the (Delta graph ….Canada article). Dose-response curves between cell viability percentages and extract concentrations were constructed. The cell viability (%) was calculated as follows:

Cell viability (%) = (Mean OD of treated cells/Mean OD of control cells) x100

RESULTS AND DISCUSSION

The results of *in vivo* acute toxicity during the 14 days show that the treated groups at the administered dose of 300 mg/kg body weight appear normal, presented a significant weight gain in mice group. Weight loss was significant ($P< 0.05$) in the 2000 mg/kg group; the reduction in body weight gain is a simple and sensitive index of toxicity after exposure to toxic substances [27, 28]. There was 100 % mortality recorded in the group treated by the extract at dose 2000 mg/kg body weight. Results showed that treatment with the extract at dose 2000 mg/kg induced some significant changes like skin effects, breathing, and impairment in food intake and water consumption, abdominal contraction, salivation and

hair loss. These results showed that, the ethanolic extract was evaluated as less toxic extract for seeds of *Delphinium staphisagria* (LD_{50}=300 mg/kg, p.o.). However, the alkaloid extract to be the most toxic extract with LD_{50} equate to 200 mg/kg. Under the system of global harmonization of chemicals (GHS), these products are classified category 3 and 4, respectively, due to the LD_{50} was lower than 2000mg/kg. The results from this acute toxicity study indicate that Delphinium staphisagria containing large amounts of MDL-type alkaloids, in addition to high MSAL-type alkaloid content, should be considered potentially **less** dangerous to human than plants with only high MSAL-type alkaloids [40, 41]. Therefore, even though the concentration of the MSAL-type alkaloids is the most important factor, these results suggest that the MDL-type alkaloids play an important role in the toxicity of larkspur plants by exacerbating the toxicity of the MSAL-type alkaloids [41].

The cytotoxicity studies were carried out on the crude extract against a panel of animal cancer cell lines with the MTT bioassay. Cells were treated for 48h by the alkaloidic extract at different concentrations ranging from 1000 to 31.25µg/ml. The results showed that the extract of *Delphinium Staphisagria* possesses significant an IC_{50} value of 31.25µg/ml compared to the positive drug control with an IC_{50} value of 30.40µg/ml on all cancer cell lines used. The lower IC_{50} represent the highest potency of a compound to inhibit the growth of cells and cause toxicity and death of cells [29]. The ethanolic extract doesn't have a cytotoxic effect against Vero and N2a cell lines; the IC_{50} of the extract is more or equal to 1 mg/ml. *Delphinium Staphisagria* is known for its anti-inflammatory, cathartic, emetic, narcotic, nerving, pediculicide and antispasmodic activities [30-32] and these have been attributed to components such as flavonoids, alkaloids (Isoazitine, 19-oxodihydroatisine, and 22-O-acetyl-19-oxodihydroatisine, Azitine, dihydroatisine, delphinine, Neoline, bullatine C (14-acetylneoline), Chasmanine, 14-acetylchasmanine, and the quaternary base atisinium chloride) [32, 33, 41]. Diterpenoid alkaloids, found in plant of the genera Delphinium, are of the C_{18}, C_{19} and C_{20} diterpenoides types have been the targets of considerable interest of medicinal chemists for a broad range of demonstrated pharmacological properties: arrhythmogenic (neurocardiotoxic), local anesthetic, antiarrhythmic, curariform, analgesic, hypotensive, anti-inflammatory, spasmolytic, neurotropic and psychotropic[33-36]. It is possible that this result is related to the tumor origin of animal cancer cell line used. Indeed, the NCI (National Cancer Institute) recommends testing new anticancer agent of 60 strains belonging to seven categories tissue: leukemia, melanoma, lung, colon, kidney, brain and ovary [37, 38]. Thus, we are tempted in perspective, to test the ethanolic and alkaloidic extract on other cell lines, to examine the specific effect on cancer of the different tissue. It is useful and necessary to carry out other investigations to better assess the cytotoxic effect of the extracts of *Delphinium Staphisagria*.

The radical scavenging activity of the extract of *Delphinium Staphisagria* was estimated by comparing the percentage inhibition of formation of DPPH radicals by the alkaloidic and ethanolic extract and those of Trolox. The DPPH scavenging activity of both extracts was concentration-dependent (increasing from 15.62 µg/ml to 500 µg/ml), exhibited considerably ($P<0.05$) DPPH radical-scavenging activity and was able to inhibit the formation of DPPH radicals with a percentage inhibition of 62.19 and 94%, respectively, at the highest concentration (Fig. 1 and 2). In general, the ethanolic extracts showed greater antioxidant activity than alkaloidic extract (Fig. 1 and 2). It can be noted that extract from *Delphinium Staphisagria* show average inhibitory values that give an idea of the interesting antioxidant activity of the extracts of this species and that it may be useful therapeutic agents for treating radical-related pathological damage. Some Further more researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the plants and their above mentioned functional properties [38]. From these results it can be concluded that the major components of *Delphinium Staphisagria* extract (Alkaloides, Flavonoides, Glycosides Anthraniliques in ethanolic extract and Delphinine, 14-acetylneoline, Chasmanine, 14-acetylchasmanine Neoline in alkaloidic extract) could have key roles for their functional properties [39-41]. The result obtained in this work demonstrated a high activity at low alkaloids extracts doses (500µg/ml).

Alkaloids are commonly found to have antioxidant and cytotoxic activities they are detected in the ethanolic and alkaloidic seeds extracts. It is also possible that the minor compounds might be involved in some type of synergism with the other active compounds [40, 41]. Several studies show a very strong correlation between antioxidant activity and alkaloidic compounds. Since alkaloidic compounds are effective hydrogen donors, they are good antioxidants. Alkaloidic compounds have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. Thus, therapeutic

properties of the *Delphinium Staphisagria* can be attributed to the alkaloidic compounds present [40].

CONCLUSION

In conclusion, the results of the present study suggest that *Delphinium Staphisagria* seeds plant have various secondary metabolites and has good quantity of alkaloidic compounds (diterpenoid alkaloids). Plant has potent free radical scavenging activity. Detailed studies on chemical composition, isolation of active constituents and pharmacological evaluation are essential to characterize them as biological antioxidants. The present findings of this study support the view that *Delphinium Staphisagria* seeds are a promising source of potential antioxidant which can be used in treatment of various ailments.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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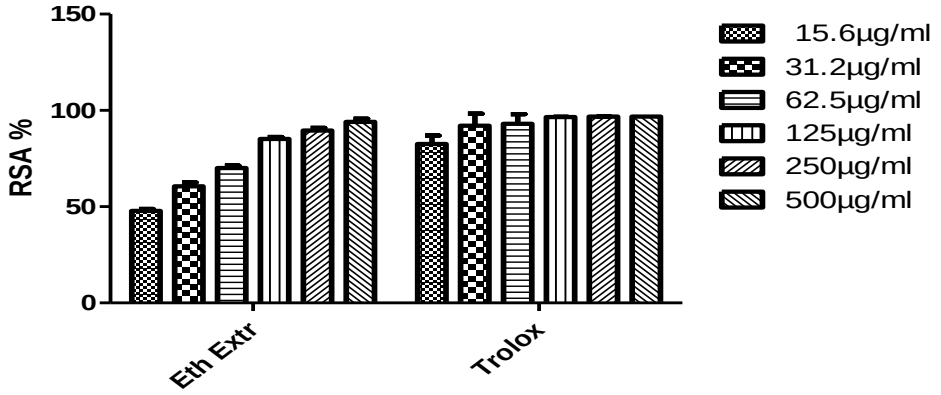

Fig. 1: Free-radical scavenging activity of *Delphinium Staphisagria* ethanolic extract measured using the DPPH assay.

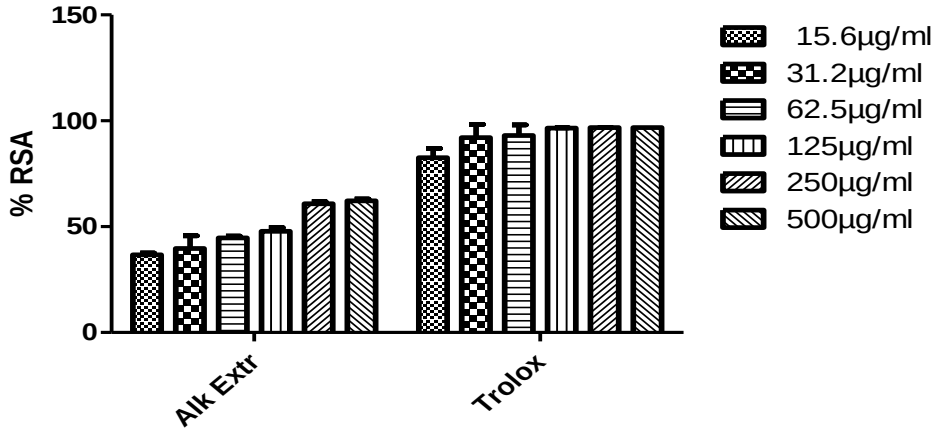

Fig. 2: Free-radical scavenging activity of *Delphinium Staphisagria* alkaloidic extract measured using the DPPH assay.

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INVESTIGATION OF METHANOLIC AND AQUEOUS EXTRACT OF *LAVANDULA OFFICINALIS* FOR TOXICITY AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

This study is aimed at determining the toxicity and antibacterial activity of *Lavandula officinalis* flowers against pathogenic microorganisms by determination the minimal inhibitory concentration and to serve as criteria to recommend the ethno pharmacological uses of the plant. Plant flowers were dried, powdered and extracted by cold maceration with methanol for 48h. The extracts were screened against 24h broth culture of bacteria seeded in Muller Hinton Agar at concentration 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56mg/ml in sterile distilled water and incubated at 37°C, for 18h and measuring the inhibition zone diameter (IZD). Minimum inhibitory concentrations (MICs) against three Gram-positive bacteria (*Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus subtilis*), three Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) were determined for the methanolic and aqueous extracts of *Lavandula officinalis*. The aqueous extract showed pronounced antibacterial than the Methanolic extract against all of the tested microorganisms. Aqueous extract inhibited with minimal inhibitory concentration of 1.56,

1.56, 3.13, 3.13, 6.25 and 6.25 mg/ml against *Micrococcus luteus*, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*, *and Pseudomonas aeruginosa,* respectively, while the methanolic extract inhibited with minimal inhibitory concentration of 6.25 mg/ml against all tested bacterial strains both Gram-positive and Gram-negative bacteria. The methanolic and aqueous extracts of *L. officinalis* demonstrated activities against certain bacteria confirming the use of the plant in ethno pharmacology.

Keywords: *Lavandula officinalis*; acute toxicity; minimal inhibitory concentration; antibacterial screening; bacterial strains; medicinal plant.

INTRODUCTION

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population.[1] This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations. [2] Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some cases) and the cultural, spiritual point of view of the people of the countries.[2] In western developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited.[3] Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine become necessary. [4]

Lavandula officinalis L., is a common dense, evergreen, aromatic shrub grown in many parts of the world. [5] The fresh and dried flowers are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods. A tisane can also be made from them. They are extensively used in cooking, and a

distinct mustard smell gives off while they are burned, therefore, they often are used to flavor foods while barbecuing.

Essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. [6,7] Particularly, the antimicrobial and antivirus activities of plant oils and extracts have formed the basis of applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. [8] Because of the possible multiple resistances and side effects of the synthetic antimicrobial, increasing attention has been directed towards natural antimicrobial. [9] There are many studies on the antimicrobial activity of secondary metabolites in recent years, but to our knowledge, fewer comparative studies on antimicrobial activity of *L. officinalis* L. methanolic and aqueous extracts have been reported. [10]

To the best of our knowledge, this is the first study to provide data that the methanolic and aqueous extracts of *Lavandula officinalis* evaluated against a wide range of bacteria. Thus, the aim of this study is to evaluate the toxicity and antimicrobial activity of lavender (*Lavandula officinalis*) methanolic and aqueous extract, and to, therefore, determine the scientific basis for its use in traditional medicine in the treatment of infection diseases.

MATERIALS AND METHODS

Plant material: *Lavandula officinalis* was collected based on ethnopharmacological information, from villages around the region Rabat-Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco.

Preparation of extract

Methanolic extract: Flowers of *L. officinalis* were successively extracted with methanol by maceration at room temperature (25°C) over period of 48h. 500 g of plant material and one liter of methanol were used in the extraction. Methanol containing the extract was then filtered through Whatman paper and the solvent was vacuum-distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for 2h to ensure the

removal of any residual solvent. Final extract was a dark green powder in percentage dry weight 21.8%.; this methanolic extract was kept in deep freeze at -20°C until use.

Aqueous extract: 500 g of plant material was extracted by infusion in boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration and then lyophilized (Free Zone® Dry 4.5, USA). For each study, the lyophilized aqueous extract was carefully prepared under the same condition used throughout the studies (time, temperature and the amount of plant material and water used for extraction under reflux and lyophilization) and each time the quality of extraction was checked by the yield of the lyophilization material. The final crude extract was obtained as yellow greasy powder in percentage from dry weight (15.7% d.w). For assuring stability, the lyophilized material was stored at -20°C.

Animals: Male and females Swiss mice (20-25g) (IOPS Offa) were used in the LD_{50} calculation. Animals were obtained from the animal experimental centre of Mohammed V-Souissi University, Medicine and Pharmacy Faculty – Rabat. They were housed three per plastic cage, photoperiod (one light from 6:00 to 18:00h); air changes and room temperature (22±1°C) were controlled. All animals had free access to tap water and at *ad-libitum* feeding; except for short fasting period before the treatment with the single dose of the methanolic and aqueous extract. The general behavior of mice was observed continuously for 1h after treatment, intermittently for 4h and over period of 24h. [11] The mice were observed for 14days following treatment, and all signs of toxicity and deaths and their latencies were recorded. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509. The particular treatment doses were selected depending on the LD_{50} results. The therapeutic doses should be very lesser than the LD_{50} value.

Acute toxicity: LD_{50} (median lethal dose) values were determined as described by Litchfield and Wilcoxon. [12] Seven groups of mice of both sexes (n =10; 5 males and 5 females) received or not oral single doses at different concentration. The control group received only the water. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 24h at 6h time interval to detect any eventual side effect. The number of animals, which died during this period, was expressed as percentile,

and the LD_{50} was determined. Of note, drugs used as control were given to mice in similar conditions.

Antibacterial Activity Test

Bacterial strains: The following 6 microorganisms (three Gram-negative and three Gram-positive bacteria), were used in this study: *Escherichia coli* ATCC 54127, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 53153, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633 were used for antibacterial testing. The cultures of bacteria were maintained in their appropriate agar slants at +4°C throughout the study and used as stock cultures. The bacteria were obtained from the **L**aboratory of **M**icrobiology, **N**ational **L**aboratory of **V**eterinary **D**rugs **C**ontrolled, Rabat, Morocco.

Determination of antibacterial activity by the paper disc diffusion method: The methanolic and aqueous extracts of *L. officinalis* were tested for antibacterial activity by the paper disc diffusion method. Molten (4 5°C) sterile Muller Hinton Agar (10ml) in a flask was inoculated with a broth culture (1%, containing 10^6-10^7 cfu/ml) of the respective bacterial strains and poured over plates containing 10ml Muller Hinton Agar in sterile 9cm Petri dishes. Fifty microliters of dilutions of the methanolic and aqueous extracts were pipette on sterile filter paper disc (Whatman No.1. 5mm in diameter), which were allowed to dry in an open sterile Petri dishes in a biological safety cabinet with vertical laminar (Nuaire Petri dishes Laminar Flow Products, USA). 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56mg/ml of the methanolic and aqueous extracts solutions were applied to the discs. Discs were placed on the surface of the inoculated plates and incubated at 37°C for 18h. Zones of inhibition of microbial growth around the paper disc containing the extracts were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (mm) from the outside margin of the paper disc to the initial point of the microbial growth. All analyses were applied in triplicate. [13] Discs impregnated with sterile distilled water served as negative control. Penicillin and Ampicillin which purchased from Sigma (St. Louis, USA) was used as a positive control. [14-16]

Determination of Minimum Inhibitory Concentration (MIC): Test strains were suspended in Muller Hinton Agar (MHA). The suspension was adjusted spectrophoto-metreically to match the turbidity of a McFarland 0.5 scale (*i.e.* to give a final density of

10^7cfu/ml). The viability indicator MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide, Sigma, Aldrich) and the quick microplates method were used for the determination of the minimum inhibitory concentration (MIC). [17-20] Serial dilutions of extracts were dissolved in distilled water and made in a concentration range from 1.56mg/ml to 200mg/ml in sterile test tubes. Each test tube was inoculated with 20µl from each various dilutions of the methanolic and aqueous extracts of *L. officinalis* were added to 5ml of Muller Hinton Agar both in tubes containing 10^7cfu/ml of live bacterial cells. The tubes were then incubated under optimal conditions at 37°C for 18h. After incubation, as an indicator of bacterial growth 1 mg/ml of MTT was added to each well, after incubation periods ranging from 3 to 5h at 37°C, control without plant extracts with MTT and bacterial inoculums were used as negative control. The bacterial suspension changed to blue when bacterial growth occurred. The highest dilution (lowest concentration), showing no visible growth was regarded as the MIC. Cell suspension (0.1ml) from the tubes showing no growth was sub cultured on Muller Hinton Agar plates in triplicate to determine if the inhibition was reversible or permanent. All tests were performed in triplicate.

Statistical analysis: Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Following oral administration of *Lavandula officinalis* extract at the doses of 500, 1000, 1500, 2000, 3000, and 5000 mg/kg, p.o., no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at these doses. This result indicates that, the LD50 was higher than 5000 mg/kg. These results were previously reported by Alnamer et al.,. [21] The methanolic and aqueous extracts were evaluated for antimicrobial activity against Gram positive (*S. aureus, M. luteus* and *B. subtilis*), Gram negative (*P. aeruginosa, K. pneumoniae* and *E. coli*) bacteria. Lavender aqueous was found to be the most active against all of the bacterial strains. *L. officinalis* aqueous extract displayed good activities, inhibiting the growth of *E. coli*, *P. aeruginosa* and *K. pneumoniae* with IZD of 28, 24 and 20 mm respectively but with less activity against three Gram positive bacteria (*S. aureus, M. luteus* and *B. subtilis*), with inhibition zone diameter of 20 mm (P>0.001). The inhibition zone diameter for the lavender aqueous extract ranged from 20 to 48 mm, while IZD for methanolic extract ranged from 18 to 30 mm (P>0.01) (Table 2 and 3). The results of the paper disc diffusion support and extend previous finding that rosemary contains numerous

biologically active compounds and some of these have been frequently used in folk medicine for their antibacterial properties. In addition, the MIC values support the finding of the paper disc diffusion method (Table 1, 2 and 3). The biological activity of lavender against the tested bacteria could be attributed to the presence of flavonoids, phenolic acids (caffeic, chorogenic and rosmarinic). [21-25] Biologically active components are believed to disturb permeability of the cytoplasm membrane and thereby facilitate the influx of antibiotic. [26] The results presented in this report highlight the potential of rosemary extract as a source of antibiotic resistance modifying compounds. The MICs for the rosemary aqueous extract ranged from 1.56 to 6.25 mg/ml for all test microorganisms (P>0.001), while MICs for methanolic extract was 6.25 mg/ml (P<0.01) (Table 1). These differences in the susceptibility of the test microorganisms to the test samples could be attributed to variation in the rate of samples' penetration through the cell wall and cell membrane structures. [27] In general, the aqueous extract showed greater antimicrobial activity than methanolic extract (Table 1). Taking the least IZD of the standard (Penicillin and Ampicillin) as the breaking point, most of the extracts passed the breaking point (Table 2 and 3).

Table 1: Determination of Minimal Inhibitory Concentration (MIC) of *Lavandula officinalis* methanolic and aqueous extracts (mg/ml).

	Minimal Inhibitory Concentration (mg/ml)	
Bacterial strains	**Aqueous extract**	**Methanolic extract**
E. coli	3.13 ± 0.00	6.25± 0.00
K. pneumoniae	6.25 ± 0.00	6.25± 0.00
P. aeruginosa	6.25± 0.00	6.25± 0.00
M. luteus	1.56± 0.00	6.25± 0.00
S. aureus	1.56± 0.00	6.25± 0.00
B. subtilis	3.13± 0.00	6.25± 0.00

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341. Values are means±SD of three determinations. Data indicate significant difference (P < 0.05) with respect to positive control (Penicillin and Ampicillin).

Table 2: Inhibition by aqueous extract of *Lavandula officinalis* (zone size, mm).

Different concentration of the aqueous extract (mg/ml)									Positive control	
Bacterial strains	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	48	40	30	28	26	24	20	19	32	28
K. pneumoniae	40	32	28	26	22	22	20	18	28	24
P. aeruginosa	42	26	22	20	19	18	18	17	18	18
M. luteus	52	52	34	30	28	26	24	24	33	30
S. aureus	46	34	28	26	24	24	22	20	26	24
B. subtilis	36	34	32	28	24	22	20	20	32	28

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341, Am, Ampicillin, Pe, Penicillin. Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive control (Penicillin and Ampicillin).

Table 3: Inhibition by methanolic extract of *Lavandula officinalis* (zone size, mm).

Different concentration of the methanolic extract (mg/ml)									Positive control	
Bacterial strains	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**	**Am (20 µg/ml)**	**Pe (20 µg/ml)**
E. coli	36	24	22	20	18	18	16	14	32	28
K. pneumoniae	28	22	22	20	18	18	14	12	28	24
P. aeruginosa	26	24	20	20	18	16	14	12	18	18
M. luteus	24	18	18	18	16	16	14	12	33	30
S. aureus	28	20	20	19	19	18	17	14	26	24
B. subtilis	30	28	26	26	24	22	20	20	32	28

Bs, Bacillus subtilis ATCC 6633, Pa, Pseudomonas aeruginosa ATCC 15442, Kb, Klebsiella pneumoniae ATCC 53153, Ec, Escherichia coli ATCC 54127, Sa, Staphylococcus aureus ATCC 6538, Ml, Micrococcus luteus ATCC 9341, Am, Ampicillin, Pe, Penicillin. Values are means±SD of three determinations. Data indicate significant difference ($P < 0.05$) with respect to positive control (Penicillin and Ampicillin).

It is quite difficult to attribute the antimicrobial effect of an extract to one or a few active principles, because extracts always contain a mixture of different chemical compounds. In addition to the major components, also minor components may make a significant contribution to the antimicrobial activity of extracts. Following the results above, we could infer that the antimicrobial activity of lavender methanolic and aqueous extract is the synergistic effect of their compositions. It provided evidence that lavender methanolic and aqueous extracts may become the potential natural antimicrobial in the field of food and pharmaceutical industries.

CONCLUSION

The data indicate that the extracts of the *Lavandula officinalis* species inhibit efficaciously some resistant bacterial strains which make serious sanitary problems worldwide and confirm that there is a good correlation between the reported traditional uses of the plant for infectious diseases. This indicates that this plant may be useful for developing alternative compounds to treat infections caused by these antibiotic resistant pathogens. According to these results, it is possible to conclude that *Lavandula officinalis* had a strong and a broad spectrum of antibacterial activity.

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