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Etudes ethnopharmacologiques, phytochimiques et activités biologiques de trois apiaceae : *Pimpinella anisum* L., *Carum carvi* L. et *Coriandrum sativum* L.

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Dédicaces

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RESUME

Les plantes médicinales et aromatiques ayant une longue histoire dans le domaine culinaire et thérapeutique, qu'il s'agisse de médecine traditionnelle ou moderne. Leur richesse en composés chimiques en fait des ressources importantes, tandis que leur extraction nécessite des recherches pour améliorer l'efficacité, réduire les coûts et accélérer le processus, bien qu'elles aient toujours été utilisées pour leurs propriétés thérapeutiques, l'optimisation de l'extraction des composés bioactifs et le développement des pratiques durables sont essentiels pour répondre aux besoins croissants en médecine. Les progrès dans ce domaine sont cruciaux pour maximiser leurs avantages tout en assurant leur conservation à long terme.

Le présent travail porte sur la valorisation des trois plantes médicinales et aromatiques à savoir *Pimpinella anisum*, *Coriandrum sativum* et *Carum carvi*. Ainsi, des études bibliographiques bien approfondies ont été menées afin de mettre en relief les éléments principaux relatifs aux plantes étudiées. Suivis des études ethnopharmacologiques qui ont été réalisées dans la région Fès-Meknès, auprès des populations locales et des herboristes et ceci afin de déterminer la nature de l'utilisation de ces plantes (Usage thérapeutique, méthode d'emploi, posologie, toxicité, complications et conservation).

Une optimisation de l'extraction des composés chimiques par la méthodologie de conception de mélange, ainsi qu'une partie dédiée aux études des activités biologiques (antioxydantes et antimicrobiennes) ont été réalisées pour mieux comprendre les usages thérapeutiques de nos plantes étudiées.

En termes d'approche d'extraction, la quantité de composés chimiques (TPC et TFC) et l'activité antioxydante variaient selon les plantes et en fonction des solvants d'extraction utilisés. Sur la base des résultats de la méthodologie de conception de mélange, le mélange ternaire contenant 44 % d'eau, 22 % d'éthanol et 34 % de méthanol était le plus approprié pour maximiser simultanément l'activité du TPC, du TFC et de l'antioxydant chez *P. anisum*, tandis que chez *C. sativum* et *C. carvi*, la meilleure combinaison était l'eau (33,33 %), l'éthanol (33,33 %) et le méthanol (33,33 %).Les valeurs des concentrations minimales inhibitrices et bactéricides étaient significativement variables en fonction de l'extrait utilisé, de la plante et des micro-organismes testés.

De l'ensemble des résultats, il ressort que les plantes étudiées sont largement utilisées dans la médecine traditionnelle grâce à leur richesse en composés chimiques et leurs activités biologiques potentielles. En outre, l'utilisation de la conception de mélange comme technique d'optimisation était un excellent moyen de choisir le mélange le plus approprié pour extraire le maximum de composés bioactifs. Cependant, des recherches supplémentaires sont nécessaires pour identifier les molécules contenues dans les extraits et simuler leurs effets sur d'autres maladies.

Mots-clés : Plantes médicinales et aromatiques, ethnopharmacologie, usages traditionnels, extraction, composés chimiques, propriétés biologiques, conception de mélange.

ABSTRACT

Medicinal and aromatic plants have a long history in both culinary and therapeutic domains, spanning traditional and modern medicine. Their richness in chemical compounds makes them significant resources, while their extraction requires research to enhance efficiency, reduce costs, and expedite the process. Although they have always been utilized for their therapeutic properties, optimizing the extraction of bioactive compounds and developing sustainable practices are essential to meet the growing needs in medicine. Progress in this field is crucial to maximize their benefits while ensuring their long-term conservation.

The present study focuses on the valorization of three medicinal and aromatic plants, namely *Pimpinella anisum*, *Coriandrum sativum*, and *Carum carvi*. Thus, comprehensive literature reviews have been conducted to highlight the main elements related to the studied plants.

Following ethnopharmacological studies conducted in the Fès-Meknès region, involving local populations and herbalists, this aimed to determine the nature of the utilization of these plants (method of use, dosage, toxicity, complications, and preservation).

An optimization of chemical compound extraction using mixture design methodology, along with a dedicated section on biological activities studies (antioxidant and antimicrobial), were conducted to better understand the therapeutic uses of the plants under investigation.

In terms of extraction approach, the quantity of chemical compounds (TPC and TFC) and antioxidant activity varied among the plants and depending on the extraction solvents used. Based on the results of the mixture design methodology, the ternary mixture containing 44% water, 22% ethanol, and 34% methanol was found to be the most suitable for maximizing simultaneously the activity of TPC, TFC, and antioxidant in *P. anisum*, while for *C. sativum* and *C. carvi*, the optimal combination was water (33.33%), ethanol (33.33%), and methanol (33.33%).

The values of minimum inhibitory and bactericidal concentrations were significantly variable depending on the extract used, the plant, and the tested microorganisms.

From the entirety of the results, it emerges that the studied plants are widely used in traditional medicine due to their richness in chemical compounds and their potential biological activities.

Furthermore, the utilization of mixture design as an optimization technique was an excellent method for selecting the most appropriate blend to extract the maximum bioactive compounds. However, further research is needed to identify the molecules contained in the extracts and simulate their effects on other diseases.

Keywords: Medicinal and aromatic plants, ethnopharmacology, traditional uses, extraction, chemical compounds, biological properties, mixture design.

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INTRODUCTION GENERALE

La médecine traditionnelle est pratiquée partout dans le monde et s'appuie sur les ressources naturelles de l'environnement local, ainsi que sur les compétences et les connaissances nécessaires à leur utilisation. Depuis les temps anciens l'Homme s'est fortement appuyé sur les plantes comme source de médicaments curatifs.

Les plantes médicinales et aromatiques sont largement utilisées à de multiples fins en tant que matière première dans des systèmes culinaires et thérapeutiques, que ce soit en médecine traditionnelle ou moderne. La composition chimique dense de leurs dérivés étant le principal facteur de leur utilisation dans ces domaines.

En raison de sa localisation géographique et la nature du climat qui y règne, le Maroc présente une diversité florale remarquable. Cette diversité est marquée par la présence de plusieurs familles des plantes médicinales et aromatiques dont la famille des apiacées. Celle-ci, est largement utilisée en médecine traditionnelle depuis l'antiquité. Elle est recommandée pour traiter plusieurs pathologies dont les troubles gastro-intestinaux, neurologiques, cardiaques, les vomissements, la diarrhée et la pneumonie. Dotée de propriétés antispasmodiques, antiseptiques, carminatives, anticancéreuses et anti-ulcéreuses, cette famille est très riche en molécules antioxydantes qui font l'objet de plusieurs recherches scientifiques.

Dans le présent travail, nous nous sommes intéressés à la valorisation de trois espèces de plantes médicinales et aromatiques (*Pimpinella anisum, Coriandrum sativum et Carum carvi*), appartenant à la famille des apiacées. Celles-ci, sont largement utilisées par les populations locales marocaines en médecine traditionnelle.

Pour ce faire, nous avons, dans un premier temps, mené une recherche bibliographique bien approfondie sur les trois espèces étudiées et ceci afin de mettre en relief les éléments principaux relatifs à la thématique abordée. Cette partie d'étude est rédigée sous forme d'articles de synthèse récapitulant un ensemble des recherches réalisées sur les espèces étudiées depuis le début du XIXe siècle jusqu'à la date actuelle, couvrant leur distribution géographique, leur culture, leur composition chimique, leur potentiel agronomique, ainsi que leurs propriétés pharmacologiques "in vitro" et "in vivo". De plus, via cette recherche, nous avons pu identifier les thèmes les plus prometteurs nécessitant des études approfondies, et suggérer ainsi des orientations pour les futures recherches ainsi que des mesures de préservation visant à assurer la conservation des variétés sauvages.

Dans un deuxième temps, nous avons mené des enquêtes ethnopharmacologiques sur les trois plantes étudiées afin de mieux comprendre les méthodes de leur utilisation, les modes de préparation et les usages thérapeutiques.

Ces enquêtes offrent une opportunité précieuse pour l'exploration de nouvelles thérapies et le développement de produits naturels et durables. Cette étude a été réalisée au niveau des différentes régions du Maroc à savoir Taounate et Taza dans la région nord, Meknès et Fès dans la plaine du Saïss (région centrale), ainsi qu'Immouzzer, Sefrou et Ifrane dans la région du Moyen Atlas. Un questionnaire a été élaboré et diffusé dans les sites d'étude ce dernier était divisé en six sections principales : i) détails biographiques des populations ciblées,

ii) informations sur les plantes étudiées, iii) usages thérapeutiques de chaque plante médicinale. iv) formes, modes et parties d'utilisation pour chaque espèce ; v) effets des plantes utilisées sur la santé des participants et enfin vi) conditions de stockage.

Dans un troisième temps, nous avons abordé l'optimisation de l'extraction des composés chimiques par la méthodologie de conception de mélange. Et ceci, afin de définir le mélange de solvants optimal pour l'extraction des composés polyphénoliques des 3 plantes étudiées, en se basant sur la conception « Augmented Simplex-Centroid ». Le choix du mélange idéal repose sur une concentration élevée en polyphénols et flavonoïdes, ainsi qu'une activité antioxydante maximisée.

La formule optimale du solvant a été déterminée à l'aide de courbes de niveau et de surface basées sur des courbes d'iso-réponse. La teneur en composés phénoliques et en flavonoïdes totaux a été quantifiée. L'activité antioxydante des extraits optimisés a été évaluée à l'aide du test DPPH.

Dans un dernier lieu, nous avons étudié les composés chimiques et les activités biologiques des dérivés des trois plantes médicinales et aromatiques étudiées, notamment les polyphénols totaux et les flavonoïdes, ainsi que les activités antioxydantes et antimicrobiennes.

Et enfin, nous avons donné une conclusion générale regroupant les principaux résultats obtenus suivis de perspectives.

GENERALITES

Depuis des temps anciens, les plantes médicinales et aromatiques ont été largement utilisées dans la médecine traditionnelle. Actuellement, celles-ci constituent une source précieuse de composés naturels uniques, utilisés dans le développement de médicaments antidiabétiques, anti-inflammatoires, anticancéreux et antimicrobiens.

Au début du XXIe siècle, les scientifiques ont initié une tendance intéressante dans le développement pharmaceutique : le retour à la nature comme source potentielle de médicaments (Georgiev 2013 ; Lanzotti 2014). Grâce à ces efforts, diverses molécules phytochimiques telles que les flavonoïdes, les alcaloïdes, les acides phénoliques, les anthocyanines, les lignanes, les stilbènes, les polysaccharides et les caroténoïdes, ont suscité un intérêt accru en raison de leur rôle important dans la prévention et la gestion de certaines maladies graves (Andrae-Marobela *et al.,* 2013 ; Shao and Xiao 2013 ; Xiao *et al.,* 2014 ; Xiao, 2015 ; Xiao and Jiang, 2015).

Il existe de nombreuses molécules phytochimiques connues et de nombreuses autres inconnues. Trouvées dans différentes parties des plantes : feuilles, racines, tige, fleur, graine et fruits (Cutter, 2000). Ces substances offrent un mécanisme de défense naturel aux plantes contre les pathogènes et les dangers environnementaux, tels que le stress et la pollution (Gibson *et al.,* 1998). Bien qu'elles ne soient pas des nutriments essentiels pour la plante, la plupart d'entre elles présentent de bonnes activités biologiques, telles que l'activité antioxydante (Seun-Ah *et al.,* 2010), antimicrobienne (Iscan *et al.,* 2002), antifongique (Soković *et al.,* 2009), antibactérienne (Kanatt *et al.,* 2008). Comme elles permettent la modulation des enzymes de détoxification, la stimulation du système immunitaire, la diminution de l'agrégation plaquettaire et la modulation du métabolisme hormonal et des propriétés antitumorales.

Les antioxydants revêtent une grande importance en termes de réduction du stress oxydatif qui est à l'origine de nombreuses maladies chroniques telles que le cancer, le diabète, les maladies neurodégénératives et cardiovasculaires (Bektas *et al.,* 2005). L'action protectrice des plantes médicinales est attribuée aux composés polyphénoliques (Soong et Barlow, 2004), ainsi de nombreux composés phénoliques ont démontré leur activité anticancéreuse, anticarvi nogène ou antimutagène.

Au Maroc, la phytothérapie peut être considérée comme faisant partie intégrante des soins de santé primaires. En effet, la médecine traditionnelle occupe toujours une place importante dans les traditions médicales de cette société. Malheureusement, ce patrimoine culturel et naturel est menacé d'extinction pour deux raisons : tout d'abord, les connaissances autochtones sur

l'utilisation des ressources végétales sauvages disparaissent rapidement des communautés traditionnelles, entraînant la diminution de ces pratiques phytothérapeutiques. Deuxièmement, la dégradation des ressources phytogénétiques due à plusieurs facteurs, dont principalement l'urbanisation, les aléas climatiques ainsi que les actions anthropogéniques, pourrait avoir un impact négatif.

Dans le contexte de la conservation et de l'utilisation durable et équitable des ressources végétales et des pratiques médicales traditionnelles associées, les enquêtes ethnobotaniques peuvent contribuer à la base scientifique de telles décisions de gestion. Cette discipline appelée "ethnobotanique", forgée par Kambu Kabangu, (1988), est un domaine d'investigation émergent qui attire de plus en plus l'attention de la communauté scientifique (McClatchey *et al.,* 2009). Elle offre davantage d'opportunités pour analyser les interactions humaines avec les espèces végétales et de comprendre la relation entre les pratiques locales et les systèmes de connaissance. Cette méthode scientifique vise à décrire et à analyser qualitativement et quantitativement les informations sur les plantes utilisées traditionnellement.

Les produits naturels et leurs dérivés représentent plus de 50 % de tous les médicaments actuellement utilisés dans le monde. Selon l'OMS, 80 % de la population mondiale, principalement celle des pays en développement, dépendent des médicaments dérivés de plantes pour leurs effets thérapeutiques.

Au cours des 40 dernières années, au moins une douzaine de médicaments puissants ont été dérivés de plantes à fleurs, notamment le diosgénine dérivé de Dioscorea spp., à partir duquel tous les agents contraceptifs anovulatoires ont été développés ; la réserpine et d'autres alcaloïdes antihypertenseurs et tranquillisants issus des espèces de Rauwolfia ; la pilocarpine, utilisée pour traiter le glaucome et la sécheresse buccale, dérivée d'un groupe d'arbres sud-américains (Pilocarpus spp.) de la famille des agrumes.

La médecine traditionnelle vise souvent à rétablir l'équilibre en utilisant des plantes chimiquement complexes, ou en mélangeant plusieurs plantes différentes afin de maximiser un effet synergique ou d'améliorer la probabilité d'une interaction avec une cible moléculaire pertinente.

Les populations marocaines possèdent une impressionnante connaissance médicale traditionnelle liée à une flore médicinale diversifiée, avec un total de 905 espèces de plantes utilisées en phytothérapie, appartenant à 116 familles et 726 genres. Les familles les plus représentées sont les Asteraceae, les Fabaceae, les Lamiaceae et les Apiaceae.

1. Modes d'administration

Les plantes médicinales et aromatiques offrent une multitude d'utilisations qui s'étendent à plusieurs domaines, notamment l'alimentation et la médecine. Elles sont administrées de différentes façons : Décoctions, infusions, teintures, sirops et comprimés.

Les huiles essentielles suspendues dans des liquides chauds ou des poudres peuvent être prises par inhalation, de sorte que les composés actifs sont résorbés par la muqueuse.

Des lotions, huiles ou crèmes contenant des extraits de plantes médicinales sont appliquées directement sur la peau, où le composé actif est absorbé.

Les préparations liquides peuvent être administrées sous forme d'irrigations. Les composés actifs sont absorbés par la muqueuse rectale. Dans certains cas, en fonction de la nature des extraits, il peut être souhaitable de contourner l'estomac.

Les plantes ou leurs extraits peuvent être ajoutés à l'eau du bain.

Certains phytomédicaments sont injectés dans le flux sanguin. Un bon exemple de l'utilité de ces composés est fourni par les alcaloïdes de la famille des menispermaceae, souvent utilisés comme relaxants musculaires.

2. Métabolites secondaires des plantes

Une des caractéristiques majeures de végétaux réside dans leur capacité de synthétiser des substances naturelles ou métabolites (Macheix, 2005) connue sous le nom de "métabolites secondaires", en opposition aux métabolites primaires telles que les protéines, les glucides et les lipides (Bouabid *et al.,* 2016 ; Yang *et al.,* 2018 ; Kandar, 2021). Ces métabolites jouent un rôle dans les interactions entre la plante et les organismes vivants de son environnement (Aguirre-Becerra *et al.,* 2021 ; Pang *et al.,* 2021). Ils semblent jouer un rôle essentiel dans la coévolution des plantes avec divers organismes vivants, notamment les parasites, les pathogènes, les prédateurs, ainsi que les pollinisateurs et les agents de dissémination (Zaynab *et al.,* 2018, Divekar *et al.,* 2022). Cette diversification des composés secondaires découle de ses diverses interactions.

La diversité des espèces exploitées et de leurs dérivés suggère l'ampleur ce qui reste à découvrir (Covington *et al.,* 2017). En fait, il est généralement admis que, jusqu'à présent, moins de 10 % des espèces de plantes supérieures qui peuplent notre planète ont été étudiées pour leurs propriétés chimiques et biologiques (Dias *et al.,* 2021, Bhambhani *et al.,* 2021). Ces métabolites secondaires peuvent être classées en plusieurs catégories importantes, notamment les composés phénoliques, les terpènes, les stéroïdes, et les composés azotés tels que les

alcaloïdes (Peters *et al.*, 2019 ; Roaa *et al.*, 2020). Chacune de ces catégories renferme une vaste diversité de composés, tous possédant un large éventail d'activités dans le domaine de la biologie humaine.

2.1. Composés phénoliques des plantes

Les composés phénoliques se distinguent par la présence d'un noyau benzénique, associé à un groupe hydroxyle libre ou lié à une fonction ester, éther ou hétéroside (Bento *et al.*, 2017) (figure 2). De plus, les noyaux aromatiques peuvent être synthétisés soit par la voie du shikimate, soit par celle de l'acétate, ce qui permet de distinguer deux catégories de composés phénoliques (Cutrim et Cortez, 2018 ; Vuolo *et al.*, 2019). Il est également à noter que chez les plantes supérieures, la voie des polyacétates est impliquée dans la formation de composés possédant déjà un noyau aromatique obtenu par la voie de shikimate, conduisant ainsi à des composés mixtes, tels que les flavonoïdes (Avereschs, 2016).

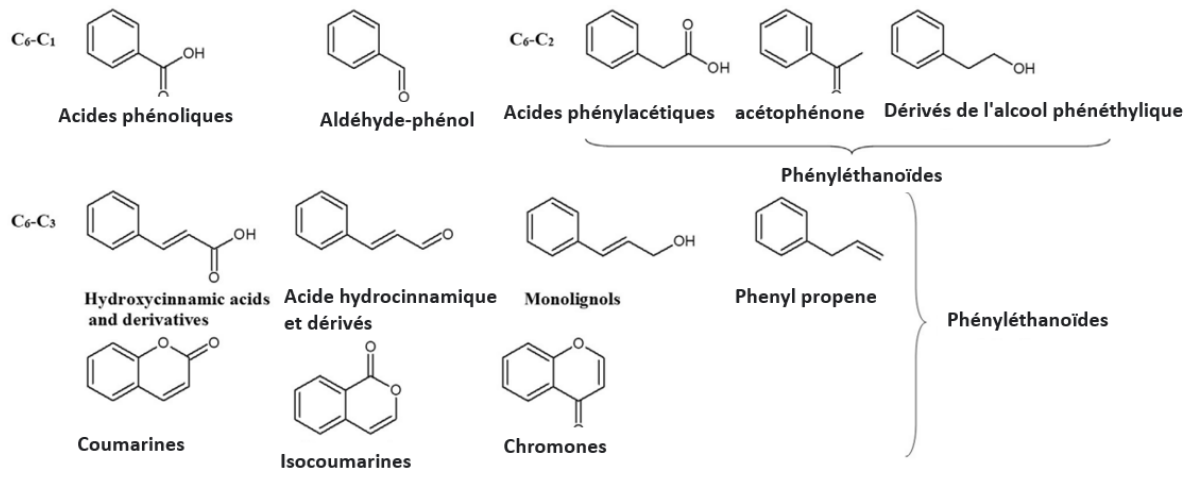


Figure 1: Les squelettes de base des composés phénoliques C6-Cn

2.1.1. Acides phénoliques

Ce sont composés phénoliques possédant un groupe acide carboxylique (Tsimogiannis et Oreopoulou, 2019 ; Kumar et Goel, 2019). Les acides phénoliques ou phénolcarboxyliques (un type de composé phytochimique appelé polyphénol) sont l'une des principales classes de composés phénoliques végétaux (Babenko *et al.*, 2019).

Les acides phénoliques sont principalement divisés en deux sous-groupes : l'acide hydroxybenzoïque et l'acide hydroxycinnamique (Saeed, 2016). Les acides phénoliques possèdent une activité antioxydante in vitro beaucoup plus élevée que les vitamines antioxydantes bien connues (Wojtunik-Kulesza *et al.*, 2020). Les acides hydroxycinnamiques, dérivés de l'acide cinnamique, sont souvent présents dans les aliments sous forme de simples

esters avec l'acide quinique ou le glucose (El-Seedi *et al.,* 2018). L'acide hydroxycinnamique lié soluble le plus abondant présent est l'acide chlorogénique (une forme combinée d'acides caféique et quinique). Les quatre acides hydroxycinnamiques les plus courants sont les acides férulique, caféique, p-coumarique et sinapique (Sova et Saso, 2020). D'autre part, les acides hydroxybenzoïques possèdent une structure commune en C6-C1 et dérivent de l'acide benzoïque (Santos-Buelga *et al.,* 2023). Ils se trouvent sous forme soluble (conjuguée à des sucres ou des acides organiques) et liés aux fractions de la paroi cellulaire sous forme de lignine.

2.1.2. Flavonoïdes

Les flavonoïdes constituent une classe importante de composés naturels ; en particulier, ils appartiennent à une classe de métabolites secondaires végétaux ayant une structure polyphénolique, largement présents dans les fruits et légumes. Ils ont divers effets biochimiques et antioxydants bénéfiques associés à différentes maladies, telles que le cancer, la maladie d'Alzheimer, l'athérosclérose, etc (Burak and Imen, 1999 ; Lee *et al.,* 2009). Les flavonoïdes sont liés à un large éventail d'effets bénéfiques pour la santé et sont un composant indispensable dans une variété de produits nutraceutiques, pharmaceutiques, médicinaux et cosmétiques (Nihel et Terki, 2020).

Ces effets sont attribuables à leurs propriétés antioxydantes, anti-inflammatoires, antimutagènes et anticancérigènes, ainsi qu'à leur capacité à réguler les fonctions enzymatiques cellulaires clés. Ils sont également reconnus comme de puissants inhibiteurs de plusieurs enzymes, telles que la xanthine oxydase (XO), la cyclo-oxygénase (COX), la lipoxygénase et la phosphoinositide 3-kinase.

Ces substances phytochimiques protègent les plantes contre divers stress biotiques et abiotiques. (Takahashi et Ohnishi, 2004), des agents détoxifiants et des composés défensifs antimicrobiens. Ils contribuent à la rusticité et à la résistance à la sécheresse, et peuvent jouer un rôle fonctionnel dans l'acclimatation à la chaleur et la tolérance au gel des plantes (Samanta *et al.,* 2011).

Les flavonoïdes peuvent être subdivisés en différents sous-groupes en fonction du carbone du cycle C auquel le cycle B est attaché, ainsi que du degré d'insaturation et d'oxydation du cycle C.

Les flavonoïdes dont le cycle B est lié en position 3 du cycle C sont appelés isoflavones. Ceux dans lesquels le cycle B est lié en position 4 sont appelés néoflavonoïdes, tandis que ceux dans lesquels le cycle B est lié en position 2 peuvent être subdivisés en plusieurs sous-groupes en

fonction des caractéristiques structurelles du cycle C. Ces sous-groupes sont : les flavones, les flavonols, les flavanones, les flavanonols, les flavanols ou catéchines, les anthocyanes et les chalcones (Figure 2).

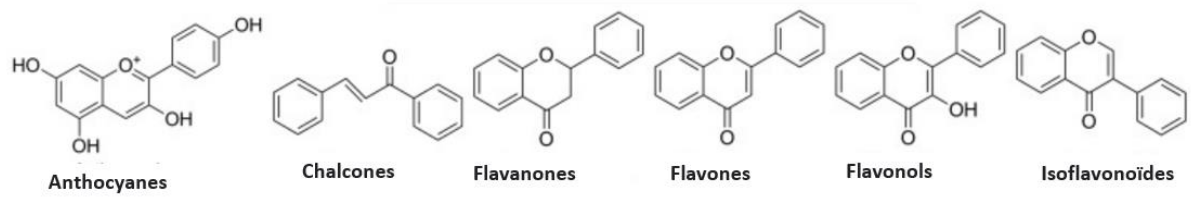


Figure 2: les sous-groupes des flavonoïdes

2.1.3. Coumarines

Les coumarines sont des composés chimiques de type 2H-1-benzopyran-2-ones, qui sont des lactones dérivées des acides ortho-hydroxy Z-cinnamiques (Venkata Sairam *et al.,* 2016 ; Robe *et al.,* 2021). L'isomérisation de la double liaison de type E en Z se produit sous l'effet de la lumière à une longueur d'onde de 320 nm. On a recensé près d'un millier de ces composés, et bien que les plus simples soient largement répandus dans le règne végétal, les coumarines plus complexes sont principalement associées aux familles de plantes Apiaceae et Rutaceae (Küpeli Akkol *et al.,* 2020).

2.1.4. Lignanes et composés apparentés

Les lignanes proviennent de l'accumulation d'unités phénylpropaniques (Cui *et al.,* 2020, Nogueira *et al.,* 2021). On peut identifier quatre groupes de lignanes : les lignanes (qui impliquent la liaison entre deux carbones β des chaînes latérales de deux unités dérivées du phénylpropane), les néolignanes (où un seul carbone β est impliqué), les "oligomères" (résultant de la condensation de 2 à 5 unités phénylpropaniques), et enfin les norlignanes qui ont un squelette en C17 (Ma *et al.,* 2020).

2.1.5. Tanins

Ils sont le plus souvent hydrosolubles et ont la capacité de provoquer la précipitation des protéines, des alcaloïdes et des polysaccharides à partir de leurs solutions aqueuses (Bruneton, 2009).

Leurs propriétés découlent de la capacité à se fixer sur des macromolécules, telles que les fibres de collagène de la peau. Cela explique également pourquoi les tanins précipitent les protéines, la cellulose et les pectines (Soares *et al.,* 2020). De plus, les tanins sont des composés phénoliques hydrosolubles avec une masse moléculaire de 500 à 3000, capables de précipiter la gélatine et d'autres protéines et de colorer les sels ferriques (Smeriglio *et al.,* 2017).

2.1.6. Stilbènes

Les stilbènes sont des composés non flavonoïdes présentant une structure carbonée particulière en C6-C2-C6 (Rivière *et al.,* 2012). Cette structure de base relativement simple donne naissance à un grand nombre de composés qui diffèrent par le nombre et la position des fonctions hydroxyles sur les cycles phénoliques, la conjugaison avec des sucres et divers groupements fonctionnels (méthyles, méthoxyles, etc.), ainsi que par la formation d'oligomères résultant de la condensation oxydative des monomères.

Le resvératrol étant le stilbène le plus reconnu au monde. Celui-ci a été initialement identifié comme une phytoalexine de la vigne (Jeandet *et al.,* 2002). Il a été démontré que ce composé présente de nombreuses activités biologiques potentiellement bénéfiques pour la santé humaine (Vang *et al.,* 2011). Le resvératrol est considéré comme un puissant antioxydant et possède des propriétés anti-inflammatoires.

2.1.7. Quinones

Les quinones sont des composés organiques résultant de l'oxydation de dérivés aromatiques. Ils sont caractérisés par la présence d'un motif 1,4-dicétocyclohexa-2,5-diénique (pour les para-quinones) ou d'un motif 1,2-dicétocyclohexa-3,5-diénique (pour les orthoquinones) (Harrison *et al.,* 2016). Ces structures moléculaires comportent une dione conjuguée avec les doubles liaisons d'un noyau benzénique, formant ainsi différents types de quinones.

- Benzoquinones : ces quinones sont caractérisées par la conjugaison de la dione avec les doubles liaisons d'un noyau benzénique.
- Naphtoquinones : ces quinones résultent de la conjugaison de la dione avec les doubles liaisons d'un système aromatique polycyclique, en l'occurrence le naphtalène.
- Anthraquinones : similaires aux naphtoquinones, mais formées par la conjugaison avec un système aromatique dérivé de l'anthracène.
- Naphtodianthrones : ces composés résultent de la conjugaison d'une dione avec un système aromatique polycyclique de type naphtodianthrène.

2.1.8. Terpènes

Les terpènes subissent une oxygénation, une hydrogénation ou une déshydrogénation pour former des terpénoïdes. La classification des terpènes est basée sur les unités isoprène, proposées par Wallach en 1887 (C5H8), un composé à 5 carbones qui forme le squelette des terpènes (Brahmkshatriya et *al.*, 2013, Trost et Mint, 2020).

Les molécules terpénoïdes possèdent des propriétés antifongiques, antimicrobiennes, antivirales et antiparasitaires. Elles dissuadent les herbivores de se nourrir et sont utilisées comme insecticides pour la conservation des produits agricoles (Abdallah *et al.,* 2018). Cependant, leur plein potentiel n'a pas encore été pleinement exploité.

2.1.9. Alcaloïdes

Les alcaloïdes sont des composés à bases azotées d'origine naturelle. Ils se caractérisent par la présence d'un atome d'azote inclus dans un système hétérocyclique et présentent une activité pharmacologique significative (Roy, 2017). Les alcaloïdes possèdent également la propriété de réagir avec des sels de métaux lourds, ce qui permet leur caractérisation aisée. Ainsi, différents réactifs sont utilisés pour caractériser ces composés, tels que les réactifs de Mayer, de Dragendorf, de Wasicky, de Bouchardat (Dey *et al.,* 2020 ; Verma et Bharadvaja, 2022).

Cette réactivité vis-à-vis des sels de métaux lourds est souvent utilisée en laboratoire pour identifier et caractériser la présence d'alcaloïdes dans divers échantillons, ce qui est crucial pour étudier leur distribution, leur nature chimique et leurs éventuelles applications pharmacologiques.

En dernier lieu, il est important de noter que la compétitivité et la survie des plantes sont fortement influencées par les rôles écologiques de leurs métabolites secondaires. Les approches biotechnologiques participent également à la production de ces métabolites par le biais de processus de génie génétique. De plus, la culture de tissus végétaux peut jouer un rôle significatif dans ce contexte.

3. Techniques d’extraction des dérivés des plantes médicinales et aromatiques

L'extraction est considérée comme l'une des étapes importantes dans la découverte de composés bioactifs potentiels issus de matières végétales, (Prasad *et al.,* 2012 ; Gao et Mazza 1996). Le type et la concentration du solvant, la température, le temps, le pH, les rapports solide-liquide, la pression et la taille des particules de la plante ont été identifiés dans des études antérieures comme des facteurs importants affectant l'efficacité de l'extraction des composés bioactifs de la plante (Aybastıer *et al.,* 2013 ; Prasad *et al.,* 2009).

Parmi les méthodes d’extraction on cite celle Traditionnelle dans laquelle, un seul facteur joue un rôle de variable, tandis que tous les autres facteurs restent constants (Silva *et al.,* 2007). Cependant, cette méthode est considérée comme moins fiable car elle n'inclut pas les effets interactifs entre les facteurs, elle est chronophage et coûteuse. Des conclusions trompeuses et

des conditions fausses peuvent également survenir à la fin de cette méthode expérimentale, en ignorant les véritables facteurs significatifs pendant le processus d'extraction (Prasad *et al.*, 2012).

Pour surmonter ce problème, la méthodologie de surface de réponse (RSM) a été introduite et largement utilisée de nos jours pour optimiser les conditions d'extraction, elle permet d'évaluer les effets entre les multiples facteurs et leurs interactions sur une ou plusieurs variables de réponse simultanément, produisant un nombre moindre de mesures expérimentales (Ilaiyaraja *et al.,* 2015). Elle aide également à localiser la région où l'extraction est optimisée en utilisant une aide visuelle plus claire (Azahar *et al.,* 2017).

3.1. Méthodes d'extraction par solvant

Les caractéristiques d'un solvant découlent de sa structure moléculaire, comprenant des aspects tels que sa miscibilité avec l'eau, sa constante d'acidité (pKa, indiquant son aptitude à former des liaisons hydrogène), sa constante diélectrique (exprimant son caractère dissociant), son moment dipolaire ou sa polarité, sa densité, sa volatilité, et sa toxicité (Emuri Abe *et al.*, 2010).

En fonction de la technique d'extraction et du solvant utilisé, différents types de préparations peuvent être obtenus, notamment des hydrolysats (utilisant de l'eau comme solvant), des alcoolats (utilisant de l'éthanol dilué), des teintures (mélange d'éthanol et d'eau), des résinoïdes (extraits éthanoliques concentrés), et des concrètes (extraits à froid et à chaud utilisant divers solvants). Chaque méthode produit des extraits avec des profils chimiques spécifiques en raison des propriétés uniques des solvants et des conditions d'extraction (Hernandez Ochoa, 2005).

L'utilisation limitée de l'extraction par solvants organiques volatils s'explique par ses coûts élevés, les préoccupations en matière de sécurité et de toxicité, ainsi que la réglementation environnementale. Cependant, cette méthode offre généralement des rendements plus élevés par rapport à la distillation, et elle évite l'action hydrolysant de la vapeur d'eau (Lucchesi, 2005).

3.2. Techniques d'extraction conventionnelle

Les composés bioactifs issus de matières végétales peuvent être extraits par diverses techniques d'extraction classiques. La plupart de ces techniques sont basées sur le pouvoir extractif de différents solvants utilisés et l'application de chaleur et/ou de mélange. Pour obtenir des composés bioactifs à partir de plantes, les techniques classiques existantes sont les suivantes : extraction Soxhlet, Macération et Hydrodistillation.

- Extraction Soxhlet est largement utilisée pour extraire des composés bioactifs précieux à partir de diverses sources naturelles. Elle est utilisée comme modèle pour la comparaison de nouvelles alternatives d'extraction.
- Macération était utilisée depuis longtemps dans la préparation de toniques. Elle est devenue une méthode populaire et peu coûteuse pour obtenir des huiles essentielles et des composés bioactifs. Pour l'extraction à petite échelle, macération facilite l'extraction de deux manières : - augmentation de la diffusion ; - élimination de la solution concentrée de la surface de l'échantillon.
- Hydrodistillation est une méthode traditionnelle d'extraction de composés bioactifs et d'huiles essentielles, aucun solvant organique n'est impliqué et elle peut être réalisée avant la déshydratation des matières végétales. Il existe trois types d'hydrodistillation : distillation à l'eau, distillation à l'eau et à la vapeur, et distillation directe à la vapeur (Vankar, 2004).

L'hydrodistillation implique trois processus physico-chimiques principaux : l'hydrodiffusion, l'hydrolyse et la décomposition par la chaleur. À une température d'extraction élevée, certains composants volatils peuvent être perdus. Cet inconvénient limite son utilisation pour l'extraction de composés thermolabiles.

L'efficacité de l'extraction de toute méthode conventionnelle dépend principalement du choix des solvants (Cowan, 1999). La polarité du composé ciblé est le facteur le plus important pour le choix du solvant. L'affinité moléculaire entre le solvant et le soluté, le transfert de masse, l'utilisation de co-solvant, la sécurité environnementale, la toxicité humaine et la faisabilité financière.

3.3. Techniques d'extraction non conventionnelle

Les principaux défis de l'extraction conventionnelle sont le temps d'extraction plus long, le besoin d'un solvant coûteux et de haute pureté, l'évaporation d'une grande quantité de solvant, une faible sélectivité d'extraction et la décomposition thermique des composés thermolabiles (Luque de Castro et Garcia-Ayuso, 1998).

Pour surmonter ces limitations de méthodes d'extraction conventionnelles, de nouvelles techniques d'extraction prometteuses ont été introduites. Ces techniques sont appelées techniques d'extraction non conventionnelles. Certaines des techniques les plus prometteuses sont l'extraction assistée par ultrasons, l'extraction assistée par enzyme, l'extraction assistée par micro-ondes, l'extraction assistée par champ électrique pulsé, l'extraction par fluide supercritique et l'extraction par liquide pressurisé. Certaines de ces techniques sont considérées

comme des « techniques vertes » car elles sont conformes aux normes établies par l'Agence de protection de l'environnement des États-Unis (EPA)

Ces techniques vertes impliquent l'utilisation d'auxiliaires écologiques, la conception pour l'efficacité énergétique, l'utilisation de matières premières renouvelables, la réduction des dérivés, la catalyse, la conception pour prévenir la dégradation, l'économie atomique, l'analyse du temps pour la prévention de la pollution et une chimie intrinsèquement plus sûre pour la prévention des accidents.

3.3.1. Extraction assistée par ultrasons (UAE)

Le principal avantage de l'UAE peut être observé dans les échantillons végétaux solides car l'énergie ultrasonore facilite la lixiviation des composés organiques et inorganiques de la matrice végétale (Herrera and Luque de Castro, 2005). Le mécanisme probable est l'intensification par ultrasons du transfert de masse et l'accès accéléré du solvant aux matériaux cellulaires des parties de la plante. Le mécanisme d'extraction par ultrasons implique deux types principaux de phénomènes physiques, (a) la diffusion à travers la paroi cellulaire et (b) le rinçage du contenu cellulaire après la rupture des parois (Mason *et al.*, 1996).

Ce processus produit un phénomène appelé cavitation, ce qui signifie la production, la croissance et la rupture de bulles. Une grande quantité d'énergie peut être produite par la conversion de l'énergie cinétique du mouvement en chauffant le contenu de la bulle. Selon Suslick et Doktycz (1990)

3.3.2. Extraction par champ électrique pulsé (PEF)

Le traitement par champ électrique pulsé (PEF) a été reconnu comme utile pour améliorer les processus de pressage, de séchage, d'extraction et de diffusion au cours de la dernière décennie (Barsotti et Cheftel, 1998 ; Angersbach *et al.*, 2000 ; Vorobiev *et al.*, 2005 ; Vorobiev and Lebovka, 2006). Le principe du PEF est de détruire la structure de la membrane cellulaire.

L'efficacité du traitement PEF dépend strictement des paramètres du processus, notamment la force du champ, l'entrée d'énergie spécifique, le nombre d'impulsions, la température de traitement et les propriétés des matériaux à traiter (Heinz *et al.*, 2003).

3.3.3. Extraction assistée par enzyme (EAE)

Certains phytochimiques dans les matrices végétales sont dispersés dans le cytoplasme cellulaire et certains composés sont retenus dans le réseau polysaccharide-lignine par liaison hydrogène ou hydrophobe, ce qui n'est pas accessible avec un solvant dans un processus d'extraction standard. Le prétraitement enzymatique a été considéré comme une manière

novatrice et efficace de libérer les composés liés et d'augmenter le rendement global (Rosenthal *et al.,* 1996).

L'ajout d'enzymes spécifiques telles que la cellulase, l'α-amylase et la pectinase pendant l'extraction améliore le rendement en rompant la paroi cellulaire et en hydrolysant les polysaccharides structuraux et les corps lipidiques (Rosenthal *et al.,* 1996 ; Singh *et al.,* 1999).

3.3.4. Extraction assistée par micro-ondes (MAE)

Les micro-ondes sont des champs électromagnétiques dans la plage de fréquences de 300 MHz à 300 GHz. Ils sont composés de deux champs oscillants perpendiculaires, tels que le champ électrique et le champ magnétique.

Le mécanisme d'extraction assistée par micro-ondes suppose trois étapes séquentielles décrites par Alupului (2012) : premièrement, la séparation des solutés des sites actifs de la matrice d'échantillon sous une température et une pressionaccrues ; deuxièmement, la diffusion du solvant à travers la matrice d'échantillon ; troisièmement, la libération des solutés de la matrice d'échantillon dans le solvant.

Plusieurs avantages de l'extraction assistée par micro-ondes ont été décrits par Cravottoa *et al.,* (2008) tels qu'un chauffage plus rapide pour l'extraction de substances bioactives à partir de matériaux végétaux, des gradients thermiques réduits, une taille d'équipement réduite et un rendement d'extraction accru. L'extraction assistée par micro-ondes peut extraire des composés bioactifs plus rapidement et une meilleure récupération est possible par rapport aux processus d'extraction conventionnels.

3.3.5. Extraction liquide sous pression (PLE)

Le concept de PLE repose sur l'application d'une pression élevée pour maintenir le solvant sous forme liquide au-delà de son point d'ébullition normal. La haute pression facilite le processus d'extraction.

La température d'extraction plus élevée peut favoriser une solubilité plus élevée des analytes en augmentant à la fois la solubilité et le taux de transfert de masse, et, en outre, diminuer la viscosité et la tension superficielle des solvants, améliorant ainsi le taux d'extraction (Ibañez *et al.,* 2012).

3.3.6. Extraction par fluide supercritique (SFE)

Le dioxyde de carbone est considéré comme un solvant idéal pour la SFE. La température critique du CO2 (31 °C) est proche de la température ambiante, et la faible pression critique (74 bars) offre la possibilité de fonctionner à des pressions modérées.

Le seul inconvénient du dioxyde de carbone est sa faible polarité, ce qui le rend idéal pour les lipides, les graisses et les substances non polaires, mais inadapté à la plupart des produits pharmaceutiques et échantillons de médicaments. La limitation de la faible polarité du dioxyde de carbone a été efficacement surmontée par l'utilisation d'un modificateur chimique (Lang et Wai, 2001 ; Ghafoor *et al.*, 2010).100 et 450 bars (Temelli et Güçlü-Üstündag, 2005).

Les avantages de l'utilisation de fluides supercritiques pour l'extraction de composés bioactifs peuvent être compris en considérant les points suivants (Lang et Wai, 2001) : - Le fluide supercritique a un coefficient de diffusion plus élevé et une viscosité et une tension de surface plus faibles qu'un solvant liquide, ce qui permet une meilleure pénétration dans la matrice de l'échantillon et un transfert de masse favorable ; - Les fluides supercritiques, tels que le dioxyde de carbone, sont facilement éliminables de l'extrait, laissant un produit propre et sans solvant. Cela simplifie les étapes ultérieures de traitement et de purification ; - Les fluides supercritiques offrent un pouvoir solvant réglable en ajustant la pression et la température, permettant l'extraction sélective de composés spécifiques ; - L'utilisation du dioxyde de carbone supercritique est considérée comme écologique, car il est facilement disponible, non toxique et non inflammable et - L'extraction par fluide supercritique minimise le risque de dégradation thermique des composés thermolabiles, car le processus se déroule généralement à des températures plus basses par rapport à certaines méthodes d'extraction conventionnelles.

Dans le présent travail, nous nous sommes interessés à trois plantes médicinales et aromatiques à savoir : *Pimpinella anisum* L. *Carum carvi* L et *Coriandrum sativum* L. de la famille des apiacées et qui sont utilisées en médecine traditionnelle marocaine.

4. Etude biologique des trois espèces d'apiacées.

La famille des Apiacées regroupe 126 genres répertoriés, les Apiacées constituent l'un des plus grands taxons parmi les plantes supérieures ; le cumin (*Cuminum cyminum L.*), le carvi (*Carum carvi L.*), le fenouil (*Foeniculum vulgare Mill.*), la coriandre (*Coriandrum sativum L.*), l'anis (*Pimpinella anisum L.*), l'aneth (*Anethum graveolens L.)* et le persil (*Petroselinum crispum L.*) sont les membres les plus cultivés de la famille, sur plus de 1,2 million d'hectares dans le monde, leur production annuelle est d'environ 25 millions de tonnes. La famille des Apiaceae est considérée comme l'une des plus anciennes familles parmi les plantes médicinales et aromatiques ; la plupart de ses membres sont indigènes de la région méditerranéenne et du sud-ouest de l'Asie, ils sont introduits et cultivés dans le monde entier en raison de leur utilisation dans l'alimentation, la pharmacie, la parfumerie et la cosmétique. (Hunault *et al.,* 1989, Jazani *et al.,* 2008, Rajamanickam *et al.,* 2013).

Au Maroc, les Apiacées représentent l'une des 20 plus grandes familles de plantes. Avec un total de 1430 rapports d'utilisation.

Utilisée en médecine traditionnelle depuis l'antiquité. La famille des apiacées est recommandée pour les troubles gastro-intestinaux, neurologiques, les vomissements, la diarrhée et la pneumonie (Ghanem *et al.*, 2012). Dotée de propriétés antispasmodiques, antiseptiques, carminatives et anti-ulcéreuses, notamment le cumin, le carvi et le fenouil (Fang *et al.*, 2010 ; Hajlaoui *et al.*, 2010 ; Koppula et Choi, 2011). Tandis que les graines et différentes parties de la coriandre sont utilisées pour soulager les spasmes, les troubles gastriques, la bronchite et la goutte (Rajeshwari et Andallu, 2011). Les graines d'anis sont réputées pour traiter les dysménorrhées, l'épilepsie, les convulsions, les problèmes des voies respiratoires et les crises d'asthme bronchique (Pavlova *et al.*, 2006). L'eau d'aneth a un effet apaisant, elle est utilisée pour traiter les coliques et le hoquet chez les bébés (Said-Al Ahl *et al.*, 2015). Le persil est recommandé pour diminuer l'hypertension artérielle, traiter les maladies cardiaques et urinaires, le diabète, les thromboses et les accidents vasculaires cérébrals (Soliman *et al.*, 2015).

4.1. Espèce *Pimpinella anisum*

Pimpinella anisum L., ou l'anis vert est l'une des plus anciennes plantes médicinales. C'est une herbe annuelle qui pousse jusqu'à 30 à 50 cm de hauteur, avec des fleurs blanches et de petites graines vertes à jaunes. Elle pousse dans la région méditerranéenne orientale, en Asie occidentale, au Moyen-Orient, au Mexique, en Égypte et en Espagne (Baynes, 1878).

4.1.1. Classification botanique

Règne	:	Végétal
Classe	:	Magnoliopsidés
Ordre	:	Apiales
Famille	:	Apiaceae
Genre	:	Pimpinella
Espèce	:	*Pimpinella anisum*



Figure 3 : plante entière de l'anis vert



Figure 6 : feuilles de l'anis vert



Figure 4 : graines de l'anis vert



Figure 5 : fleurs de l'anis vert

L'anis est une plante annuelle à croissance lente qui nécessite un ensoleillement important, un automne chaud et sec pour obtenir un rendement économique significatif et un produit de haute qualité pour d'autres utilisations telles que l'extraction d'huile essentielle) (Hassan et Elhassan 2017). La période de plantation doit se situer entre mars et avril sur un sol bien préparé. Étant donné que les cultures de graines d'anis sont sensibles aux gelées tardives, le semis doit avoir lieu dans les climats chauds (Ullah et Honermeier 2013). Ensuite, il faut environ un mois aux graines pour germer, bien que la croissance végétative commence à augmenter très rapidement dès l'apparition des premières feuilles. Jusqu'à la phase de floraison, qui dure entre juin et juillet, l'arrosage doit être fourni simultanément.

4.1.2. Répartition géographique

L'anis est répertorié comme plante indigène dans les pays méditerranéens, notamment le Maroc, l'Algérie, la Tunisie et l'Égypte en Afrique du Nord, le Portugal, l'Espagne et l'Italie sur la pente nord du bassin méditerranéen. De même, il est signalé comme indigène dans les régions d'Asie centrale telles que l'Inde, l'Iran et la Turquie (Kucukkurt *et al.*, 2009). Elle pousse mieux dans les zones où les températures diurnes annuelles se situent entre 18 et 26 °C, mais peut tolérer 5 à 30 °C. Cependant, l'anis est domestiqué et cultivé dans de nombreuses régions, notamment en Asie du Sud-Est (Chine et Japon), au Moyen-Orient (Iran et Émirats arabes unis) et en Afrique (Soudan et Égypte) (Hassan et Elhassan 2017). De même, la plante se caractérise par une microdistribution spécifique dans chaque région.

Par exemple, au Maroc, la plante pousse naturellement dans les zones nordiques, les régions centrales près des montagnes de l'Atlas et les zones du sud près d'Agadir.

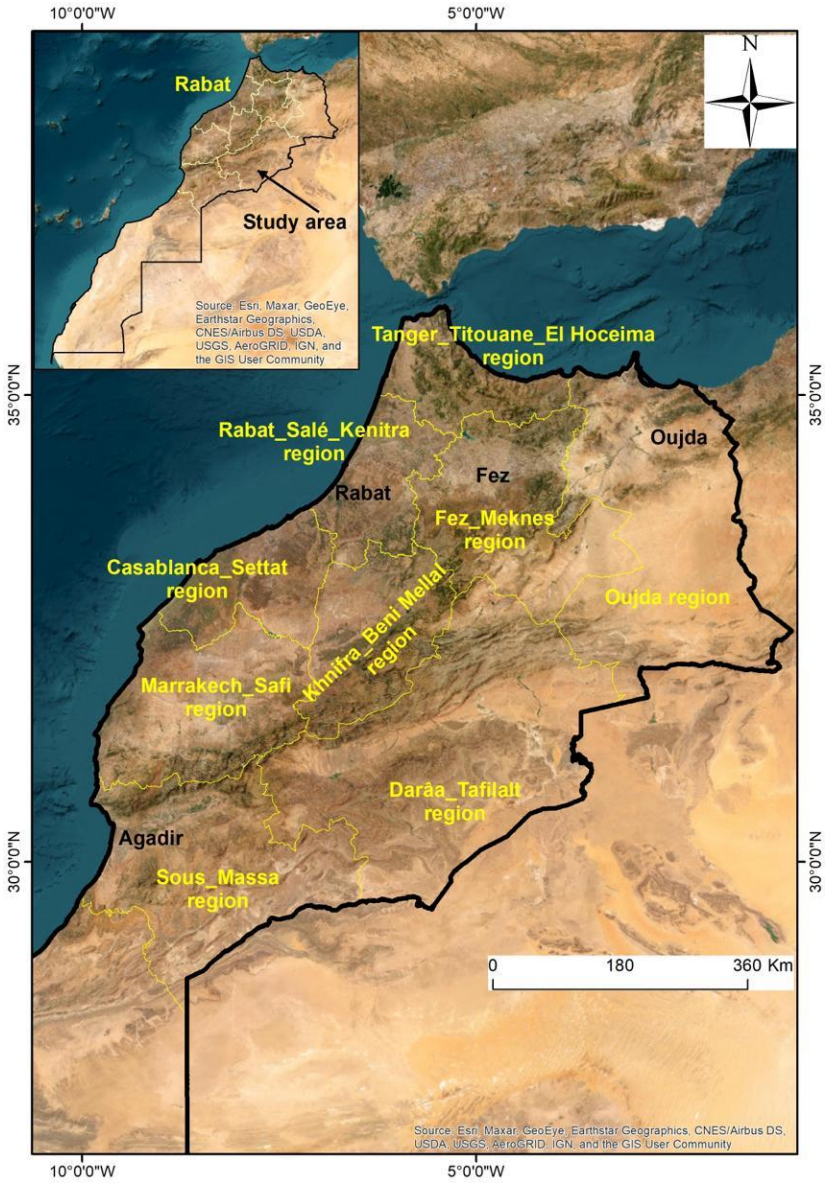


Figure 7 : Répartition géographique de l'anis vert au niveau de Maroc

4.1.3. Usages thérapeutiques

Les graines d'anis contiennent de 1,5 à 5 % d'huile essentielle et sont utilisées comme agent aromatisant (Zargari, 1996). L'anis est décrit pour ses effets bénéfiques sur les troubles gastro-intestinaux et hormonaux en régulant les menstruations. Chez les femmes allaitantes il augmente la production de lait (El Hussein,1983). Les graines d'anis sont utilisées comme analgésique dans la migraine et aussi comme carminatif et diurétique (Salehi Surmaghi, 2010). Il est également efficace pour polir les dents. Dans certains textes traditionnels, l'anis est mentionné pour la mélancolie, les cauchemars, et aussi dans le traitement de l'épilepsie et des convulsions (Özcan et Chalchat, 2006). Cependant, les utilisations médicinales de *Pimpinella anisum* varient selon les populations en fonction de leur emplacement géographique.

4.1.4. Composition chimique

La composition de l'anis varie considérablement en fonction de son origine et de la méthode de culture.

L'huile essentielle obtenue par distillation est généralement d'environ 2 à 3 % et l'anéthole représente 80 à 90 % de l'huile (Özcan et Chalchat, 2006). L'anis contient de l'anéthole, un phytoestrogène (Albert Puleo,1980). L'huile essentielle est utilisée comme insecticide contre les poux et les acariens. Cette huile est très célèbre dans le monde entier. Elle était utilisée dans les confiseries, la pharmacie, le dentifrice et d'autres utilisations industrielles (Guenther, 1975).

Les résultats ont montré la présence d'acide palmitique et d'acide pétrosélinique comme principaux acides gras, ainsi que de l'acide caprique, de l'acide laurique, de l'acide myristique, de l'acide stéarique, de l'acide arachidique, de l'acide oléique, de l'acide linoléique à différentes concentrations dans les populations d'anis, les acides phénoliques ont été étudiés en utilisant la RP-HPLC. Les résultats ont montré la présence d'acide gallique, d'acide chlorogénique, d'acide syringique, d'acide p-coumarique, d'acide rosmarinique et d'acide ellagique (British Pharmacopoeia, 1988). Tandis que les flavonoïdes principaux étaient la naringine, suivie par la quercétine, et d'autres flavonoïdes tels que l'épicatéchine-3-Ɵ-gallate, la coumarine, la rutine et l'apigénine, comme le montre la Figure 2 (British Pharmacopoeia, 1988). En utilisant l'analyse GC-MS, le trans-anéthole a été découvert comme l'ingrédient principal de l'huile essentielle de graines d'anis séchées (BSI, 1976).

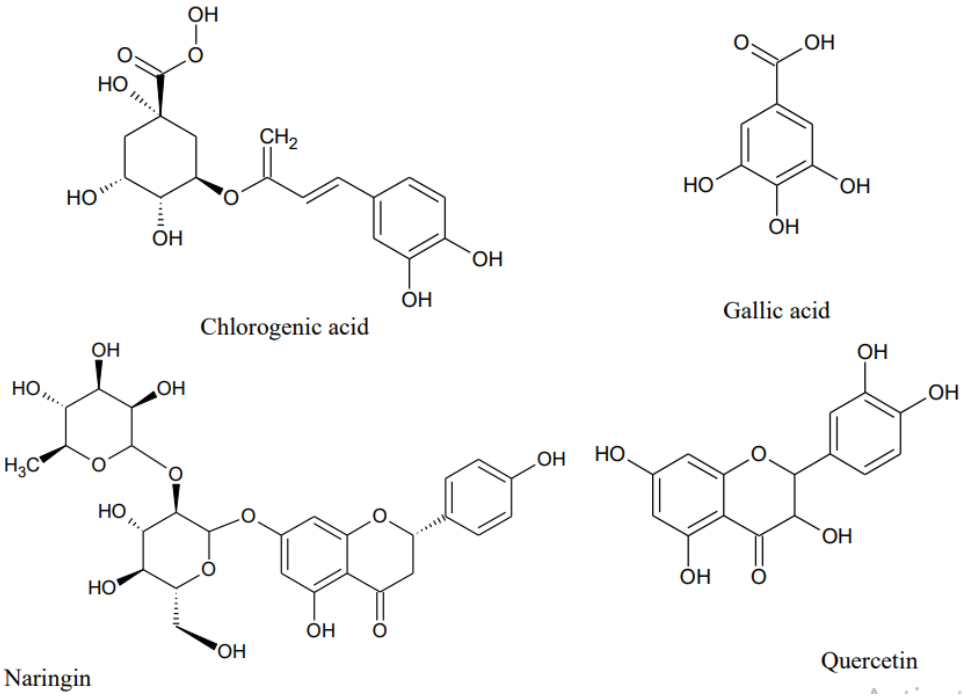


Figure 8: Les principales composés phénoliques identifiées dans l'anis

4.1.5. Activités biologiques de l'anis vert

- **Activité antioxydante**

En termes d'extraits, différents solvants ont été utilisés pour isoler les composés bioactifs et les activités antioxydantes dans différentes parties de *Pimpinella anisum*. Amer et Aly (2019) ont étudié les propriétés antioxydantes des extraits éthanoliques et aqueux préparés à partir de graines et de parties aériennes de *Pimpinella anisum*. Les résultats obtenus ont montré que le pourcentage le plus élevé d'activité de piégeage des radicaux a été enregistré pour l'extrait éthanolique de graines à une concentration de 0,3 mg/ml, suivi par l'extrait aqueux de graines, tandis que l'extrait aqueux de parties aériennes a démontré la plus faible activité de piégeage des radicaux à la même concentration testée.

- **Activité antimicrobienne**

Les activités antimicrobiennes de l'huile essentielle et des extraits du résidu d'hydrodistillation de l'anis ont été étudiées par Lemjallad Lamiaa et *al*. (2019). Les résultats ont montré que l'huile essentielle et l'extrait de *Pimpinella anisum* avait une activité antimicrobienne contre tous les micro-organismes testés. L'huile essentielle a un effet inhibiteur sur certains microorganismes. Cette essence végétale présente une activité antifongique très importante.

En termes de conclusion, en raison des caractéristiques bénéfiques de *Pimpinella asnisum* L., notamment ses activités hypolipidémiques, antidiabétiques, anticancéreuses, antioxydantes et antimicrobiennes, à la fois les graines et les huiles essentielles d'anis sont prometteuses pour être utilisées comme ingrédients bruts et compléments alimentaires dans les industries alimentaires, agronomiques et pharmaceutiques. Les effets pharmacologiques et agronomiques comprennent les activités antimicrobiennes, antifongiques, antivirales, hépatoprotectrices, anti-inflammatoires, anticonvulsivantes, antispasmodiques, œstrogéniques, expectorantes et insecticides, ainsi que des effets cliniques tels que les nausées, la constipation, la bronchodilatation, la ménopause, le diabète, l'obésité et l'action sédatif. Cependant, des investigations cliniques et expérimentales supplémentaires utilisant des techniques d'extraction, des solvants et des agents novateurs sont nécessaires pour comprendre pleinement la myriade de composés et comment ils affectent la protection des cultures et la santé publique. D'autre part, il est conseillé d'étudier comment les plantes s'adaptent aux contraintes abiotiques et biotiques afin de comprendre le statut actuel de l'espèce et d'assurer sa conservation.

4.2. Espèce *Carum carvi*

Le carvi est une plante herbacée bisannuelle, très ramifiée, mesurant de 30 à 80 cm de hauteur, avec des tiges feuillues étroites finement rainurées. Il produit une racine pivotante profonde et

une rosette de feuilles vert foncé finement coupées et plumeuses, qui peuvent atteindre une hauteur allant jusqu'à 75 cm. Les fleurs sont produites en ombelles, sont blanches et mesurent 2 à 3 mm de diamètre, les extérieures étant plus grandes que les intérieures. Elles s'ouvrent à partir de fin avril et sont suivies de fruits qui mesurent de 3 à 6 mm de long, mûrissent à partir de début juillet (Al-Snafi, 2016).

4.2.1. Classification botanique

Règne : Végétal

Classe : Magnoliopsidés

Ordre : Apiales

Famille : Apiaceae

Genre : Carum

Espèce : *Carum carvi*



Figure 9 : plante entière *carum carvi*



Figure 12 : fleurs de *carum carvi*



Figure 11: graines de *carum carvi*



Figure 10: feuilles de *carum carvi*

4.2.1. Répartition géographique

Le cumin persan ou carvi (*Carum carvi L.*) est une plante herbacée bisannuelle de la famille des Apiaceae, originaire d'Europe, d'Asie occidentale et d'Afrique du Nord. En Asie, la plante est cultivée en Iran, au Pakistan, en Inde et dans d'autres pays voisins. Dans ces régions, la plante est cultivée à la fois pour l'alimentation et à des fins médicinales. En Europe, les cultivars de *Carum carvi* sont présents en Italie, en Turquie et dans d'autres pays du nord tels que la Russie, la Pologne et l'Ukraine. En Afrique, les plantes sont largement enregistrées au Maroc, en Algérie et en Tunisie, dans le nord-ouest du continent. À l'est de l'Afrique, le carvi est cultivé en Égypte, au Soudan et en Éthiopie, où il trouve ses exigences écologiques.

Au Maroc, la plante est cultivée dans différentes régions des zones centrales, notamment à Beni Mellal, dans le centre du Moyen Atlas, à Fès-Meknès et dans les montagnes du Haut Atlas.

Cette large gamme de distribution est due aux conditions climatiques et à la disponibilité à la fois de l'eau et des ressources en sol dans le pays.

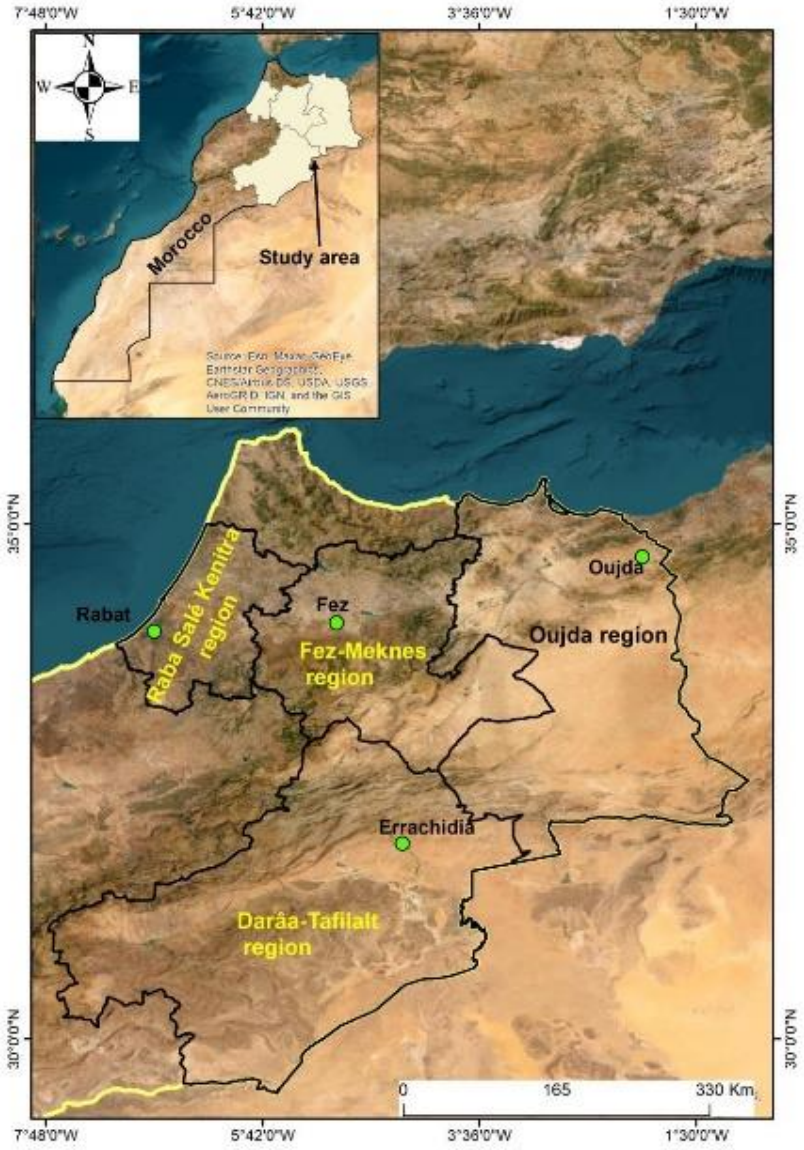


Figure 13: Répartition géographique du carvi au Maroc

4.2.2. Usages thérapeutiques

En raison de ses composés chimiques, les échantillons de carvi (*Carum carvi L.)* sont utilisés contre un large éventail de maladies et à des fins alimentaires. Le carvi est utilisé pour les crampes gastro-intestinales et les sensations de plénitude, ainsi que pour les troubles cardiaques et neurologiques, les indigestions, le manque d'appétit, la dyspepsie chez les adultes et pour soulager les coliques flatulentes des nourrissons.

Il est également utilisé comme tranquillisant, diurétique, et stimulant gastrique, aphrodisiaque, astringent, dans le traitement des nausées matinales, des maux de tête, pour améliorer la fonction hépatique, dans les troubles bronchopulmonaires, la toux et comme analgésique.

L'huile essentielle est utilisée comme constituant dans les bains de bouche et les additifs de bain, ainsi qu'en parfumerie, pour parfumer le savon et comme parasiticide (Iacobellis et al, 2005). Elle est couramment utilisée comme arôme alimentaire (Najda *et al.*, 2008).

Les utilisations traditionnelles de cette plante varient en fonction des populations et des parties de la plante. Par exemple, au Maroc, diverses études ethnobotaniques ont rapporté l'utilisation des différentes parties du carvi à des fins diverses dans la médecine traditionnelle. À Fez-Meknès, la plante est utilisée comme agent antinociceptif par les populations locales. À Agadir, les populations locales des zones urbaines (55,55 %) utilisent le *C. carvi* pour les vertiges, les douleurs d'estomac et d'autres problèmes digestifs. À Errachidia, les populations locales utilisent les plantes pour gérer les troubles gastro-intestinaux, principalement dans les zones rurales. Dans une autre étude, les graines de carvi sont massivement utilisées pour traiter le diabète par décoction et administration orale.

4.2.3. Composition chimique

Les principaux phytochimiques rapportés dans les graines de *C. carvi* sont le limonène, le carvacrol, la carvone, le carvénone, le γ-terpinène, l'α-pinène, le linalol et le p-cymène (Esmaeili et Asgari, 2015). Les compositions en huiles essentielles des différentes parties de la plante de carvi sauvage montrent une proportion élevée de limonène [61-83 %], et celles des inflorescences de limonène [39-62 %] et de germacrène D [23-41 %] (Baananou *et al.*, 2013). Les feuilles et les tiges, faibles en composés volatils, étaient dominées par le germacrène D et le germacrène (Chizzola, 2014).

Hajlaoui et al. (2021) ont étudié les composés chimiques dans les huiles essentielles du *Carum carvi L*. ce qui a permis l'identification de 28 composés volatils, avec les principaux composés étant le γ-terpinène (31,03 %), le β-pinène (18,77 %), le p-cymène (17,16 %) et la carvone (12,20 %).

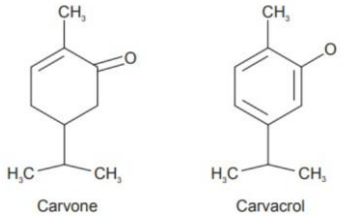


Figure 14 : composés phénoliques majeurs du carvi

4.2.4. Activités biologiques

- **Activité antioxydante**

Dans une étude animale, les effets des extraits de carvi sur la prévention de la septicémie induite par des lésions tissulaires oxydatives ont été étudiés. L'induction de la septicémie a entraîné une augmentation significative de la peroxydation lipidique (LP) dans les reins mais pas dans le cœur, ce qui indique que les reins ont été plus affectés par l'induction de la septicémie que le cœur. La LP rénale et les taux de ratio plasma urée/créatinine ont été rapidement inversés chez les rats traités avec des huiles essentielles mais pas chez ceux traités avec des extraits hydroalcooliques. Ainsi, cela montre que les huiles de carvi ont probablement un rôle protecteur dans les tissus rénaux contre les lésions oxydatives aux stades avancés de la septicémie (Dadkhah & Fatemi 2011).

Dans une autre étude, Trifan *et al.,* (2016) ont étudié l'activité antioxydante de l'huile essentielle de *Carum carvi L.* cultivée dans le nord-est de la Roumanie. L'évaluation de l'activité antioxydante a été réalisée avec des activités de piégeage du DPPH et de l'ABTS, ainsi qu'avec la puissance réductrice. Les résultats obtenus ont montré des effets significatifs.

Dans le cadre d'extraits, l'activité antioxydante de l'extrait phénolique de *C. carvi* a été étudiée par Thippeswamy *et al.,* (2013) en utilisant des tests de piégeage des radicaux DPPH, de piégeage des anions superoxyde et de pouvoir réducteur. Les résultats obtenus ont montré que l'extrait phénolique de *C. carvi* s'est avéré être très efficace pour piéger des radicaux d'anion superoxyde de manière dose-dépendante, avec une valeur de CI50 de 35 µg/ml.

- **Activité antimicrobienne**

En raison de ses composés chimiques, les huiles essentielles et extraits de *C. carvi* sont caractérisés par des effets antimicrobiens intéressants. L'efficacité antimicrobienne des films de pullulane contenant de l'huile essentielle de carvi a été évaluée contre *Salmonella enteritidis, Staphylococcus aureus, Saccharomyces cerevisiae ou Aspergillus niger* (Gniewosz *et al.,* 2013)

En termes d'extraits, diverses études ont abordé l'activité antimicrobienne de différents extraits de différentes parties du carvi et ont enregistré des résultats variables. Par exemple, Ankur Gupta *et al.,* (2011) ont étudié l'activité antibactérienne in vitro des extraits alcooliques et aqueux en utilisant la méthode de diffusion sur disque contre *E. coli*, tandis que l'activité antifongique a été évaluée en utilisant la technique de nourriture empoisonnée contre le champignon *Aspergillus niger*. Les principaux composants de *C. carvi* ont été identifiés comme la carvone, le limonène, le germacrène D et le trans-dihydrocarvone. L'activité était

particulièrement élevée à des concentrations plus élevées, tandis qu'une activité plus faible était observée à des concentrations plus faibles. Le pourcentage d'inhibition variait en fonction de la concentration. Par exemple, dans l'extrait méthanolique, l'inhibition variait entre 38,5% et 47,3% à des concentrations comprises entre 50 mg/mL et 200 mg/mL, tandis que dans l'extrait aqueux, l'inhibition variait entre 36,3% et 45,4% pour les mêmes concentrations.

Dans une autre étude menée par Thippeswamy *et al.,* (2013), l'activité antibactérienne de l'extrait phénolique de *C. carvi* a été testée contre des agents pathogènes d'origine alimentaire et des bactéries responsables de l'altération des aliments, notamment *Bacillus cereus, Escherichia coli, Staphylococcus aureus* et *Salmonella typhimurium,* en utilisant la méthode de diffusion sur agar.

Les espèces bactériennes, à savoir *E. coli, B. cereus, S. aureus et S. typhimurium*, se sont révélées sensibles et ont présenté une inhibition significative de la croissance en présence d'extrait de *C. carvi*. Les données indiquaient que l'inhibition de B. cereus et de *S. aureus* était plus prononcée par rapport à *E. coli* et *S. typhimurium*. Ainsi, les bactéries à Gram positif se sont avérées plus sensibles à l'extrait phénolique de *C. carvi* que les bactéries à Gram négatif. L'effet inhibiteur le plus élevé a été observé contre B. cereus et *S. aureus,* avec une zone d'inhibition enregistrée de 41mm.

Comme conclusion les graines de *Carum carvi* L. sont des sources riches en huiles essentielles contenant divers groupes de phytochimiques. Elles ont un large spectre d'effets pharmacologiques. Au cours des deux dernières décennies, des études approfondies ont été menées sur les graines de *Carum carvi* L. pour explorer leur plein potentiel d'action pharmacologique. Un produit naturel utilisé dans le traitement conventionnel donne un indice de l'existence de phytochimiques. De nouvelles recherches sur le cumin ont prouvé qu'il était une source de nouvelles entités pour réaliser différentes propriétés pharmacodynamiques, responsables de leurs effets pharmacologiques.

4.3. Espèce *Coriandrum sativum*

Coriandrum sativum est une plante herbacée : les tiges ramifiées sont glabres, principalement creuses, et présentent de fines rainures longitudunales distinctives. Elles sont de couleur vert pâle mais couvertes de taches pourpres ou rosées caractéristiques. Les feuilles disposées alternativement sont portées sur de longs pétioles creux qui ont tendance à envelopper la tige à leur base. Elles sont profondément divisées (bi-pinnatisectes), avec des segments dentelés (serrés), et ont un aspect semblable à de la fougère.

4.3.1. Classification botanique

Règne : Végétal

Classe : Magnoliopsidés

Ordre : Apiales

Famille : Apiaceae

Genre : Coriandrum

Espèce : *Coriandrum sativum*



Figure 15 : Plante entière du *coriandrum sativum*



Figure 18 : Feuilles de la coriandre



Figure 17 : Graines de la coriandre



Figure 16 : Fleurs de la coriandre

Ces feuilles (jusqu'à 50cm de long et 40cm de large, mais plus couramment de 12 à 25cm de long). Les tiges et les feuilles dégagent une forte odeur lorsqu'elles sont écrasées ou endommagées. Les fleurs blanches sont portées en grand nombre dans des grappes denses et plates au sommet des branches (en ombelles composées terminales). Les fleurs individuelles sont petites (2-4mm de diamètre).

La floraison se produit principalement au printemps et en été. Le fruit passe du vert à une couleur brune grisâtre en mûrissant et ressemble à une capsule. En réalité, il se compose de deux structures monospermes (méricarpes) qui se séparent facilement lorsque le fruit est complètement mûr. Chacune de ces graines, de 2 à 4mm de long, est glabre.

4.3.1. Répartition géographique

Depuis l'antiquité, *Coriandrum sativum* est cultivé. Originaire de l'est de la Méditerranée et s'est répandu comme plante aromatique au Maroc, en Chine, en Europe centrale, en Russie et en Inde. Néanmoins, il s'est maintenant diffusé dans toute l'Europe et l'Asie.

La coriandre (*Coriandrum sativum* L.) est une plante herbacée annuelle originaire de la Méditerranée et du Moyen-Orient. Elle est cultivée pour ses utilisations culinaires, aromatiques et médicinales de la famille des Apiacées, largement cultivée en Inde, en Russie, en Europe centrale, en Asie et au Maroc. Au Maroc, cette plante est largement répandue et cultivée

principalement pour ses graines, qui sont utilisées dans différents domaines tels que l'alimentation, la médecine, les cosmétiques et les parfums. On la trouve rapportée dans différentes régions, du Nord aux provinces du sud, y compris Dakhla sur l'Atlantique.

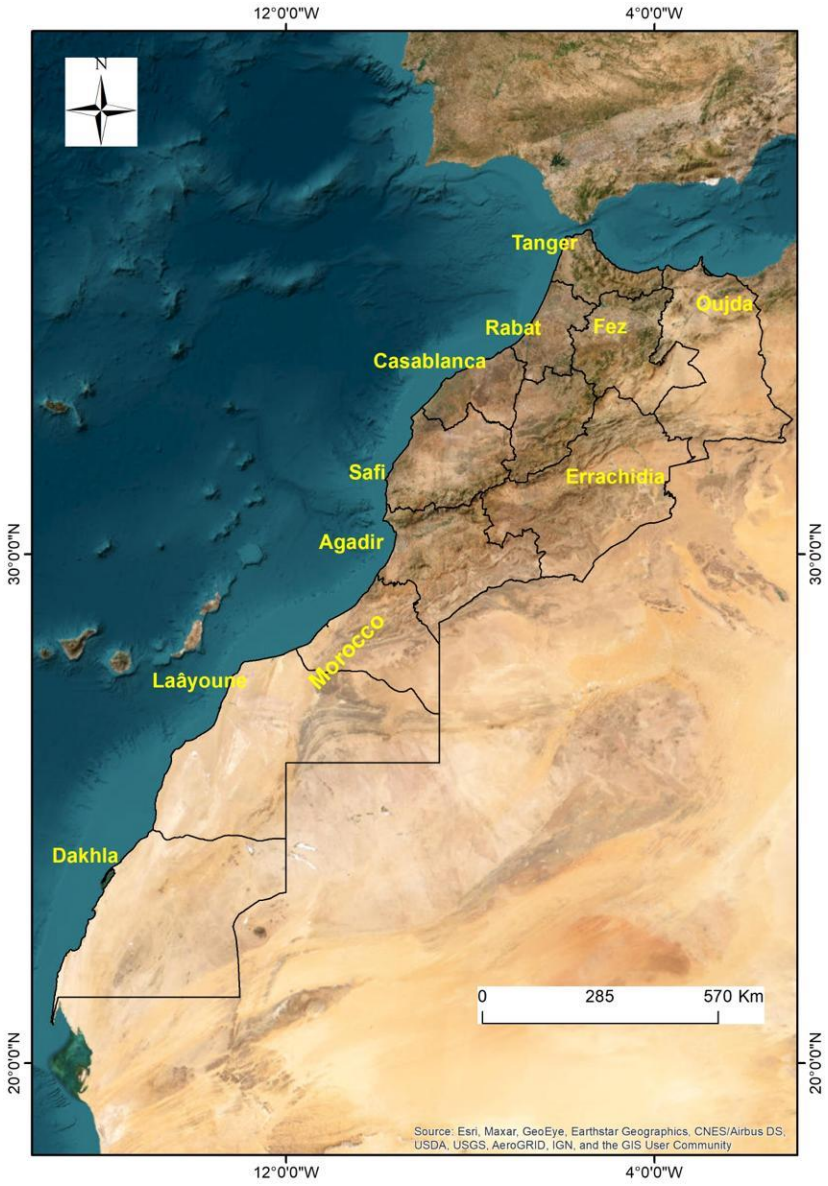


Figure 19 : Répartition géographique de la coriandre au Maroc

4.3.2. Utilisations traditionnelles de la coriandre

L'utilisation de la coriandre remonte à environ 1550 av. J.-C., et c'était l'une des plus anciennes cultures d'épices au monde. Sur le plan médicinal, elle était utilisée comme stimulant, aromatique et carminatif. Le fruit en poudre, l'extrait fluide et l'huile sont principalement utilisés à des fins médicinales comme arômes pour masquer le goût des purgatifs actifs et corriger leurs tendances à provoquer des crampes. Le fruit entier ou moulu était un ingrédient des épices à marinade, également utilisé pour parfumer divers aliments commerciaux, L'huile

essentielle de fruit était un ingrédient courant dans les crèmes, les détergents, les surfactants, les émulsifiants, les lotions et les parfums.

Les graines sont appliquées localement pour soulager les gonflements et les douleurs. Une pâte de coriandre verte est utilisée pour les maux de tête. La coriandre verte en poudre est utilisée pour soulager les sensations de brûlure et les douleurs dans des maladies telles que l'inflammation causée par l'érysipèle et la lymphadénopathie. La décoction de coriandre verte est utilisée dans la stomatite. Les gouttes nasales agissent comme un hémostatique et arrêtent ainsi les saignements dans l'épistaxis. Les graines sont incluses dans de nombreuses prescriptions comme carminatives et pour le traitement de la fièvre, de la diarrhée, des vomissements et de l'indigestion. Le jus frais des feuilles est utilisé en gargarisme dans les maux de gorge et la stomatite.

4.3.3. Diversité des composés chimiques

La coriandre est riche en composés chimiques, y compris des nutriments et des molécules bioactives. En termes de nutriments, les enregistrements les plus fréquents dans les échantillons de la plante (graines, feuilles et racines) sont les suivants : protéines, lipides totaux, glucides, fibres. Suivis de minéraux tels que le calcium, le fer, le phosphore, le magnésium, le potassium, le sodium, le zinc. En ce qui concerne les vitamines, la vitamine C, la thiamine, la riboflavine, la niacine, la vitamine B-12 la vitamine A, et la vitamine D (Coskuner and Karababa, 2007 ; Bhat *et al.*, 2017).

L'analyse de l'huile essentielle réalisée par chromatographie en phase gazeuse couplée à la spectrométrie de masse a révélé 33 composants, représentant 99,99% de l'huile totale des graines de coriandre. Les principaux composants étaient le linalol, l'α-pinène l'acétate de 2,6-octadién-1-ol, 3,7-diméthyl-, le géranol, le 3-cyclohexène-1- méthanol, α, α,4-triméthyl-, l'acide hexadécanoïque, l'acide tétradécanoïque, le 2-α-pinène l'acétate de citronellyle et l'undécanal (Chung *et al.*, 2012).

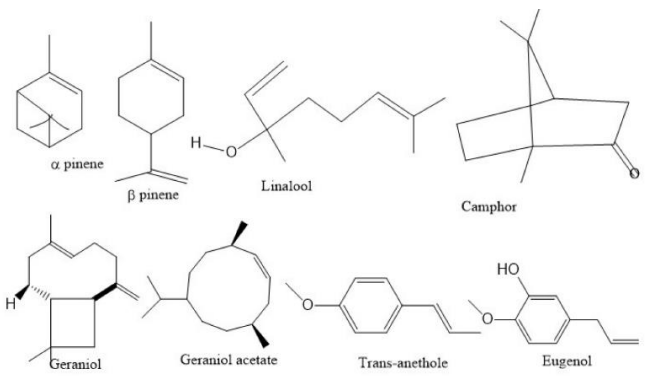


Figure 20 : Structures chimiques des principaux constituants de l'huile de feuilles et de graines de coriandre

4.3.4. Activités biologiques

- **Activité antioxydante**

Plusieurs études ont abordé les activités antimicrobiennes dans *Coriandrum sativum* prélevées à partir de différentes origines. Les résultats obtenus ont montré que l'activité antimicrobienne était variable en fonction de l'origine de la plante, des extraits testés, des parties utilisées et des micro-organismes traités.

Coriandrum sativum présente un profil antioxydant très efficace, montrant une activité de piégeage des radicaux libres de (DPPH), une inhibition de la lipoxygénase, une inhibition de la peroxydation des phospholipides, une activité de chélation du fer, une activité de piégeage des radicaux hydroxyle, une dismutation des superoxydes, une réduction de la glutathion et une antipéroxydation des lipides en raison de sa teneur élevée en composés phénoliques totaux et sa capacité à piéger les radicaux hydroxyle et superoxyde, une forte puissance réductrice, et une protection contre les dommages oxydatifs des macromolécules biologiques en augmentant le niveau de glutathion.

Le potentiel antioxydant in vitro des extraits aqueux de feuilles de *Coriandrum sativum* a été déterminé qualitativement. Les propriétés antioxydantes et de piégeage des radicaux libres des graines ont été évaluées, ainsi que l'investigation sur l'effet de l'administration de graines sur le stress oxydatif dans le rein de rats diabétiques induits par la streptozotocine. L'incorporation de poudre de graines dans l'alimentation a entraîné une baisse marquée de la glycémie et une augmentation des niveaux d'insuline chez les rats diabétiques. Un effet bénéfique parallèle a été observé sur l'équilibre oxydant-antioxydant dans le rein. L'ajout de poudre de graines de coriandre non seulement a inhibé le processus de dommages peroxidatifs, mais a également réactivé de manière significative les enzymes antioxydantes et les niveaux antioxydants chez les rats diabétiques.

Les graines ont également montré une activité de piégeage des superoxydes et des radicaux hydroxyles de manière dépendante de la concentration. L'action maximale de piégeage des radicaux libres et le pouvoir de réduction des radicaux libres de la graine de coriandre ont été observés à une concentration de 50 microgrammes d'équivalent acide gallique (GAE). Les structures histologiques des îlots ont montré une dégénérescence des îlots pancréatiques chez les rats diabétiques, ce qui a également été réduit chez les rats diabétiques traités avec de la poudre de graines.

- **Activité antimicrobienne**

L'effet antibactérien des extraits aqueux et éthanoliques de différentes parties de la coriandre a été étudié contre neuf bactéries pathogènes différentes isolées de l'urine, du sang, des selles et du liquide céphalorachidien de différents patients (*Burkholderia cenocepacia, Escherichia coli, Enterobacter cloacae, Gemella morbillorum, streptocoques α-hémolytiques, Klebsiella pneumoniae, Proteus mirabilis, Streptococcus pneumoniae* et *Salmonella typhi*). L'extrait aqueux froid des graines de coriandre avait un effet inhibiteur contre certaines bactéries testées. En revanche, les extraits éthanoliques des graines, des feuilles et des tiges ont montré une large gamme d'activité antibactérienne et les valeurs les plus élevées de zone d'inhibition ont été enregistrées contre *Klebsiella pneumoniae et Proteus mirabilis*.

En termes d'huiles essentielles, l'effet antibactérien de *Coriandrum sativum* contre les bactéries à Gram positif et à Gram négatif a été évalué en utilisant des techniques microbiologiques classiques, conjointement avec l'utilisation de la cytométrie en flux pour l'évaluation de la physiologie cellulaire. Les résultats ont montré que l'huile de coriandre a une activité antimicrobienne efficace contre toutes les bactéries testées.

L'huile essentielle de *Coriandrum sativum* peut se lier à l'ergostérol de la membrane, augmentant la perméabilité ionique et provoquant des dommages membranaires entraînant la mort cellulaire, mais elle n'agit pas sur les voies de biosynthèse de la paroi cellulaire. L'huile essentielle a également inhibé l'adhérence du biofilm de Candida à un substrat de polystyrène à de faibles concentrations et a diminué l'activité protéolytique de *Candida albicans* à la concentration minimale inhibitrice. De plus, l'huile essentielle et sa fraction active sélectionnée présentaient une faible cytotoxicité sur les cellules humaines. Les graines ont également montré une activité de piégeage des superoxydes et des radicaux hydroxyles de manière dépendante de la concentration. L'action maximale de piégeage des radicaux libres et le pouvoir de réduction des radicaux libres de la graine de coriandre ont été observés à une concentration de 50 microgrammes d'équivalent acide gallique (GAE). Les structures histologiques des îlots ont montré une dégénérescence des îlots pancréatiques chez les rats diabétiques, ce qui a également été réduit chez les rats diabétiques traités avec de la poudre de graines.

Pour conclure la coriandre a été largement utilisée au cours des derniers siècles à la fois à des fins culinaires et traditionnelles. Bien que le linalol ait été identifié dans l'extrait de *C. sativum* comme le principal composant de l'huile extraite, de nombreux flavonoïdes, acides phénoliques et phytostérols ont été identifiés comme les principaux groupes de phytochimiques susceptibles de contribuer aux effets curatifs du coriandre. Son efficacité dans les maladies

cardiovasculaires a été discutée et évaluée en fonction des études, dans lesquelles *C. sativum* a montré des propriétés hypolipidémiques, antioxydantes, antiathérogènes, antihypertensives et antiarythmiques de manière dose-dépendante. Les études antérieures mettent en lumière la capacité cardioprotectrice de la coriandre ; cependant, des études in vitro supplémentaires sont nécessaires pour élucider le mécanisme d'action de ses extraits dans les maladies cardiovasculaires.

REVIEW SUR LES TROIS PLANTES ETUDIEES

Analyse approfondie de *Pimpinella anisum* sous plusieurs angles et suggestions pour les futures études afin de faire face aux conditions climatiques adverses

(A fait l'objet d'un article publié dans le journal Chemistry Africa)

Synthèse d'étude

L'anis vert ‟*Pimpinella anisum*″ est une plante herbacée annuelle, originaire de la région méditerranéenne, appartenant au genre Pimpinella, famille des apiacées (ou ombellifères). Elle est largement utilisée dans le Moyen-Orient et l'Asie.

C'est une plante médicinale et aromatique utilisée dans divers domaines dont l'agroalimentaire, l'industrie pharmaceutique, la médecine traditionnelle et conventionnelle. Riche en molécules bioactives, elle offre des avantages concrets pour préserver la santé humaine.

Au cours de ces derniers décennies, de nombreuses études se sont concentrées sur divers paramètres de développement de l'espèce dont la germination des graines et la croissance des plantules ainsi que la composition chimique.

Dans cette partie d'étude nous avons essayé d'effectuer une synthèse des différentes activités de recherche menées sur l'espèce ‟ *P. anisum*″, depuis le début du XIXe siècle en abordant sa distribution géographique, sa culture, sa composition chimique, son potentiel agronomique, ainsi que ses propriétés pharmacologiques‟ in vitro″ et‟ in vivo″. En outre, nous avons pu identifier les thématiques les plus prometteuses nécessitant des études approfondies, et suggérer des orientations pour les futures recherches, ainsi que des mesures de préservation, visant à assurer la conservation de certaines variétés sauvages.

L'anis vert ‟*Pimpinella anisum*″ est répertorié comme une plante indigène dans les pays méditerranéens, tels que le Maroc, l'Algérie, la Tunisie, l'Égypte, le Portugal, l'Espagne et l'Italie. C'est est une plante annuelle à croissance lente, exigeant un automne ensoleillé, chaud et sec pour atteindre un bon rendement économique.

La composition chimique des dérivés de la plante varie en fonction de nombreux facteurs, dont l'origine géographique, les conditions climatiques, la nature du sol et la méthode d'extraction. Le composé majeur étant l'anéthol qui représente le constituant chimique prédominant et est largement employé dans l'industrie alimentaire, pharmaceutique, dans la parfumerie, les arômes et les pesticides.

Il est noté que les principaux composés de l'huile essentielle extraite des graines de l'anis comprennent l'eugénol, le trans-anéthole, l'anisaldéhyde, le méthylchavicol, l'estragole, le scopoletin, les coumarines, l'ombelliférone, les hydrocarbures terpéniques, les estrols, les polyènes et les polyacétylènes.

En raison des propriétés bénéfiques de *Pimpinella anisum* L., à savoir ses activités hypolipidémiques, antidiabétiques, anticancéreuses, antioxydantes et antimicrobiennes, cette plante présente un fort potentiel en tant qu'ingrédient brut et complément superalimentaire dans les secteurs alimentaires, agronomiques et pharmaceutiques.

Cependant, afin de parvenir à une compréhension approfondie de la multitude de composés présents dans l'anis vert, des enquêtes cliniques et expérimentales supplémentaires s'avèrent nécessaires. En outre, il nous semble important d'utiliser des techniques d'extraction novatrices, des solvants et des agents inédits dans ces investigations.

Chap I: A multidimensional review of *Pimpinella anisum* and recommendation for future research to face adverse climatic conditions

Abstract

Anise, or *Pimpinella anisum,* has a long history of usage in traditional and alternative medicine, as well as in the pharmaceutical sector. The species is native to Southwest Asia, the Middle East, and the Mediterranean region. The current studies have revealed that wild and cultivated *P. anisum* are rich in bioactive compounds, such as phenols, tannins, carotenoids, and fatty acids. Different parts of *P. anisum* plant are used treatment of diseases, the treatment of pathogens for antiviral, antibacterial, and antifungal purposes, and cosmetics. In this paper, we reviewed the current knowledge on geographical distribution, cultivation, chemical compounds, extraction methods, and agronomic and medicinal uses of *Pimpinella anisum*. Equally, we suggested guidelines for future research to fill the gap in less investigated issues. Data for this paper was gathered via electronic literature search on Google, Google Scholar, and databases counting, Science Direct, Scopus, Springer, PubMed, and Hindawi, from 1930 to 2022. Analyzed data show that *Pimpinella anisum* is currently recorded from Portugal on the Atlantic Ocean in the west to China in the East. With new extraction methods, such as Ultrasound-Assisted Extraction, scientists have explored the higher quantities of polyphenols, flavonoids, and antioxidants, and revealed new constituents such as trans-anethole and estragole found in essential oil fruits by gas chromatography-mass spectrometry (GC-MS). These compounds showed significant therapeutic benefits against diseases, pathogens, and cosmetic roles. However, with the current impacts of climate change on natural vegetation cover, more investigations are needed to understand how these affect the geographical distribution, metabolic aspects, and chemical properties of *Pimpinella anisum*. These are suggested to increase the value of the species and ensure its conservation.

Keywords: *Pimpinella anisum,* chemical composition, biological properties, agronomic and pharmaceutical uses, guidelines for future research.

1. Introduction

Anise *Pimpinella anisum* is a Medicinal plant widely used in food and in both vernacular treatments and conventional medicine, as well as, currently in the modern pharmaceutical industry [1–4]. For thousands of years, this plant has been utilized for both culinary and medicinal uses, with clear benefits for preserving human health and enhancing the quality of

life [5]. Several traditional texts mention anise for treating nightmares, melancholy, seizures, and epilepsy [5, 6]. Generally, anise is a dominant spice and traditional medicinal plant used for both food and medicinal purposes due to its biochemical richness with bioactive molecules [5].

Around the Mediterranean basin, the flavor and aroma of *P. anisum* seeds have been used to flavor food, candy, and various alcoholic drinks [7, 8]. The most powerful flavor component of the anise is anethole ($C_{10}H_{12}O$) which is found in both seeds and leaves and has shown powerful inhibition against chronic diseases [9, 10]. Equally, the essential oil of *P. anisum* is rich in polyphenols, flavonoids, terpenes (C_5H_8) with noticeable therapeutic against diabetic, inflammatory, and gastric diseases. Further, different extracts from seeds and leaves have demonstrated powerful inhibitory effects against bacterial, fungal, and viral pathogens. These elements were widely studied and reviewed twice. Moreover, the review papers of 143 articles and most have focused on chemical constituents, and their roles in nutrition, medicine, and pharmacology in a specific area such as Iran. However, with the publication of new papers on new scientific features addressing *P. anisum* an integral review gathering data worldwide is required. However, the richness of chemical components in essential oils and extracts of *Pimpinella anisum* L. varies depending on the type of extract, used part of the plant, geographical location, climate conditions, and utilized extraction methods [11].

Anise is an herbaceous annual plant, cultivated in many areas in the Middle East and Asia, but native to the Mediterranean region [3, 12, 13]. The *Pimpinella anisum* belongs to Pimpinella Genus, the Apiaceae or Ombelliferae Family [14]. It has been reported, it is also cultivated for food, where seeds and fruits constitute the edible parts, as well as for extractions of bioactive compounds that are used in medicine and other industrial purposes [1]. Currently, many studies have addressed growth parameters to increase the productivity of this useful species. They addressed the germination of seeds, the growth of seedlings, and their impact on the quality, Content, and Chemical Composition [3, 5, 15]. The cultivation of this plant aims to respond to increasing agronomic and industrial uses.

In this paper, we highlighted previous reports on *P. anisum* including geographical range, cultivation, chemical composition, agronomic potential, as well as in vitro and in vivo pharmacological properties. Equally, we revealed the most promoting topics that need more advanced studies, and we suggested guidelines for future studies and conservation measures to ensure the conservation of wild varieties. This paper aimed to summarize the entire realized research on *P. anisum* from the early years of 19 century to the date.

2. Methodology

All information and data sources used to formulate this review paper were collected and obtained using electronic search engines, principally Google Scholar, Science Direct, Springer, PubMed, MDPI, and Web of Science depending on the following keywords: *Pimpinella anisum, Pimpinella* genus, distribution range, germination and growth, Phytochemistry, chemical compounds, extraction methods, In vitro and in Vivo studies, inhibitory effects against bacterial, viral and fungal agents of *Pimpinella anisum.* A total of 340 peer-reviewed papers were read, while only 143 peer-reviewed papers have shown a significant relation to our study species and were selected for their scientific quality and relevance to prepare this paper. Non-peer-reviewed papers were eliminated to avoid any low-quality information since this paper is aiming to integrate all addressed topics on *Pimpinella anisum.* We focused on recent research dating from 2015 to 2022, with regard to other dates for support.

3. Nomenclature and classification

Since the species is worldwide, the *P anisum* has local names depending on the geographical area and countries. The Mediterranean basin considered the origin of native species, it is named Anis vert (France), Annesella (Italy) Vigon's Anise (Spain) in the northern slope, while in North Africa (Morocco, Algeria, Tunisia, and Egypt) it is called Petit anise [16–18]. In Asia, it is named Anise seed in Japan and China, while in central zones of Iran and India, it is named Anisa, Kuppi, Badian, Saunf, Muhuri, and Sop. In North America, the species is named Anise and Star anise [5, 19, 20]. The Pimpinella's meaning could be derived from the Latin term "bipenne = bipenne," which was changed to reflect the leaflets' 2 to 2 positioning. Other authors claim that it derives from the diminutive of "pampinus," pampas, for the vine-like similarity of the leaves, or, ultimately, from the Italian name Sanguisorba for the rosacea, again based on the resemblance of the leaves. The word anisum, on the other hand, is of unknown foreign origin and is derived from the Greek word "anison."

In terms of systematic, the *Pimpinella anisum* belongs to Pimpinella Genus, the Apiaceae or Ombelliferae Family, Order of Apiales, Rosidae Subclass, Magnoliopsida Class, Magnoliophyta Division, Tracheobionte Substitution, Kingdom of Plantae, and Eukaryota Domain [21].

4. Range, Cultivation, Growth, and Productivity

Anise (*Pimpinella anisum*) is recorded as a native plant in Mediterranean countries counting Morocco, Algeria, Tunisia, and Egypt in North Africa, Portugal, Spain, and Italy in the Northern slope of the Mediterranean basin. Equally, it has been reported as indigenous to central Asia regions from India, Iran, and Turkey [22, 23]. However, Anise is domesticated and is cultivated in many regions counting Southeast Asia (i.e. China and Japan), the Middle East (i.e. Iran and Emirates), and Africa (i.e. Sudan and Egypt) [24].

Anise is a slow growing annual plant that needs a sunny (Fig. 1), warm, and dry autumn to meet significant economical yield and high quality product for other uses such as extraction of essential oil [24]. In the nineteenth century, there were first attempts at cultivation. The field must be prepared in the fall for planting by being plowed and fertilized with manure or conventional compost. The planting period must be between March and April on well-prepared soil. Because aniseed crops are sensitive to late frost, sowing should take place in warm climates [25]. After then, it takes the seeds about a month to germinate, although vegetative growth starts to increase extremely quickly as soon as the first leaves show. Until the blossoming phase, which lasts between June and July, watering must be provided concurrently. Additionally, it is frequently necessary to manually eradicate wildflower competitors along growth phases. Plants are harvested in traditional bundles between August and September, usually when their umbels are only partially dried and dark in color. They are then dried in airy locations for 7 to 8 days in the shade and 1 to 2 hours in the sun. Then, by shaking the plants, aniseeds may be sorted from the entire batch. [22, 25].

Anise often thrives in dry, rocky terrain, pastures in mountains, fields, meadows, and grasslands, but seed germination is extremely weak in nature due to dormancy [26]. The impacts of pretreatment elements, fertilization, seeding, and agronomic procedures have, however, been the subject of numerous studies recently in an effort to increase anise germination rates, growth, production, and quality. The germination traits (mean time germination, germination percentage, germination rate, and seed vigor index) and growth parameters (coleoptile length, seedling length, and radicle length) were significantly improved with the use of Gibberellic Acid, Benzyladenine, and Kinetin as hormone treatments (0, 15, 30 and 45 days), according to Shahrajabian *et al.*, (2019) [3]. Combining these hormones produced the highest germination. The authors also pointed out that the use of endogenous Benzyladenine+Kinetin and Gibberellic Acid+Kinetin is the most efficient method for releasing seeds from dormancy. Water stress also inhibits anise germination, although the use

of polyethylene glycol (PEG) 6000 and ascorbate (1.4 mM) considerably enhances anise germination percentage (89 and 93%) and, in turn, the tolerance to water stress by lowering osmotic potential [27]. Darzi *et al.*, (2022) [13] considerably increased the umbel quantity per plant, biological yield, and seed production of anise cultivated in vermicompost (0.5 and 10 ton/ha) by using the Phosphate Solubilizing *Bacillus Circulans* (inoculation of seeds and inoculation + spray on base at stem elongation stage). The effects of sowing dates and row spacing on yield-related features, seed yield, growing degree days (GDD), essential oil content, and chemical composition of anise have been studied by Kara (2015) [28] in a study that is intended to promote the crop. The use of delayed sowing treatments (15/April and 1/May) with sowing dates of 1/April, 15/April, and 1/May with row spacings of 15, 20, and 25 cm revealed that seed yield and its components (plant height, the number of umbels, and number of branches per plant) reduced in these treatments. The 15 April planting date produced the best essential oil content values, and row spacing of 20 and 25 cm produced the maximum yield components and essential oil content [28]. Elevated CO_2 (eCO_2) in seeds has an effect on anise seeds' nutritional and biological value, but the effectiveness depends on the seed's maturity and provenance [29]. While essential oil metabolism declines, the decrease can be mitigated by the application of eCO_2, seed maturation increased the antioxidant metabolites and nutrients in eCO_2-treated accessions [29].

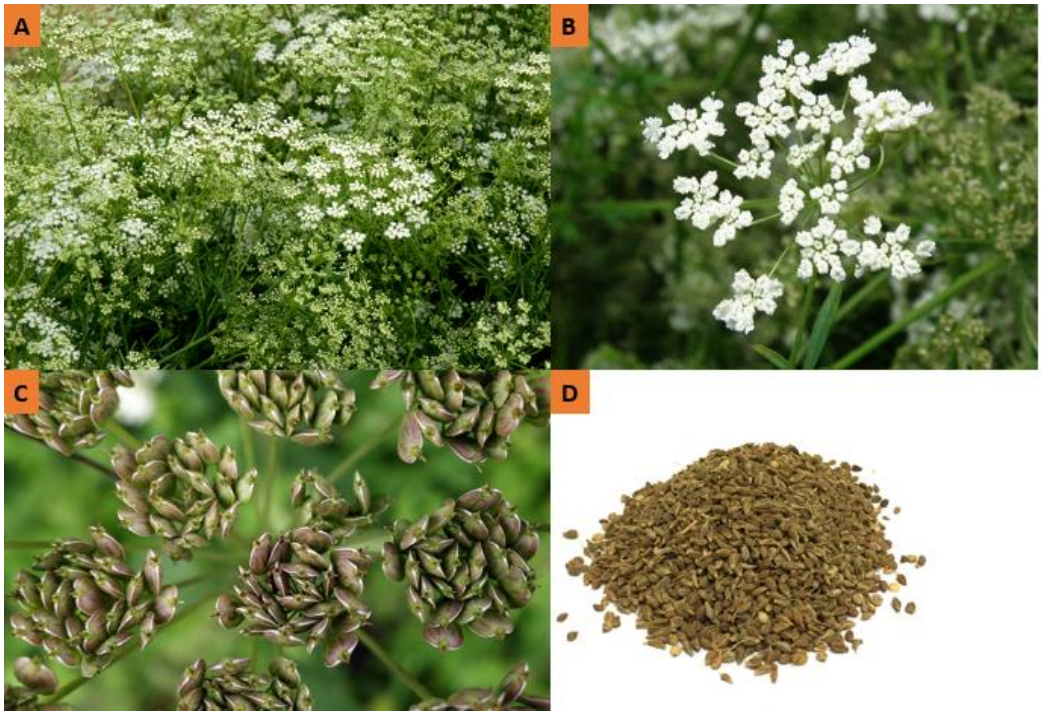


Figure 21: Plant (A), flower (B), and seeds of *Pimpinella anisum* (C: the form of seeds on the plant; D: dried seeds)

5. Diversity and variation of Chemical Components

When the chemical components of anise were studied in the second half of the 20th century, anethole was the first identified element [30–32]. El Sissi and El Sherbeiny (1967) [33] via a micro-screening survey of 600 sampled plants in Egypt, revealed a considerable amount of tannins in 56 plants including *P anisum*. Further, with the development of chemical techniques and screening materials, scientists have described qualitatively and quality of Anise chemicals. However, the chemical composition varies depending on the provenance area, climate conditions, soil characteristics, and used protocols (Table 1) [27, 34].

Table 1 : Variation and diversity of chemical components in *P. anisum* in Europe, Africa, and Asia

Region	Sample origin	Dominant climate	*trans*-Anethole	*p*-Anisaldehyde	*trans*-	Himachalene	Methyl chavicol	cis-anethole	4-allylanisole	Limonene	Fenchone	linalool	estragol	γ-himachalene	zingiberene	anisylacetone	o-isoeugenol	Butanoic Acid, 2-methyl-,4- methoxy -	Oil yield (mL kg^{-1})	References
Europe	France	Mediterranean	76.9	5.4	5.7	3.1	1.5												10	[11]
	Spain	Mediterranean	87.9	2.2	2.3	2.3	1.4												25.4	
	Italy	Mediterranean	92.2	Tr	1.5	2.2	1.3												20.4	
	Greece	Mediterranean	90.2	2.8	0.9	2.6	1.3												48.3	
Baltic	Hungary	Continental climate and the oceanic climate	91.3	0.2	1	3.9	0.5												26.1	
	Russia	humid continental and subarctic	87.7	1.8	1.7	2.5	1.5												23.1	
	Czech	temperate climate	88.4	3.1	2.9	0.7	0.9												23.3	
Mediterranean	Lithuania	humid continental climate	93	0.7	0.7	1.5	2												23	
	Estonia	temperate and mild	78.1	0.1	6.4	8.2	0.6												53.6	
Western Europe	Scotland	temperate maritime climate	93.6	0.7	0.4	1.4	1.9												13.1[a]	
	Germany	temperate annd rainy	93.7	1	1.3	0.4	2.3												25.3	
Northwest Africa	Algeria	Mediterranean and Saharan	92.4					0.5				0.3	1.9	1.1	0.3	0.3	1.9	0.3		[16]
	Tunisia	Mediterranean and Saharan	94.3	0.17		0.12						0.13	0.2	2.32	0.3		0.14			[18]
Northeast Africa	Morocco	Mediterranean and semi-arid	7.4					7.4	76.7	9.75	6.16									[35]
	Egypt	Mediterranean and Saharan	90.41	0.1		0.11						0.04	3.74	1.08	0.25		0.22			[18]
Middle east	Iraq	ontinental and subtropical semi-arid type	71.52	0.19								0.44			1.02					[36]
	Iran	extremely continental	92.9	0.1				0.1							0.4					[37]
Central Asia	Pakistan	extremely continental	82.1			0.44		0.14						7	0.77					[25]

Anethole is the most important chemical constituent of anise and is the most used in food, and medicine, as well as in the industry of pharmaceuticals, perfumery, flavouring, and pesticides [7, 30, 31, 38]. Generally, anise is constituted of protein that occupies 18% of the mass, lipids mainly fatty acids (palmitic and oleic acids) take 8 to 11 % of the mass, volatile oil constitutes 1.5 to 6.0% mass of Anise and is principally composed from *trans*-anethole, and approximately 4% of carbohydrates [39]. The most important proteins and carbohydrates are Lignin-Carbohydrate-Protein Complexes (LC1, LC2, and LC3), isolated from hot water extract of *P. anisum* seeds by a combination of gel filtration, anion-exchange, and hydrophobic interaction column chromatographies [40]. Further, Ghlissi *et al.,* (2020) [41] have currently revealed that Galactose at 33.47%, β-d-Glucose at 26.71%, and α-d-Mannose at 18.21% were the major monosaccharides components presenting in polysaccharide preparation from *Pimpinella anisum* seeds, while β-d-Galactose, d-Fructose, α-d-Glucose, α-l-Galactose, and arabinose were the minor constituents. In essential oil, eugenol *trans*-anethole, anisaldehyde, methylchavicol, estragole, scopoletin, coumarins, umbelliferone, terpene hydrocarbons, estrols, polyenes, and polyacetylenes were the major compounds of *Pimpinella anisum* seed extracts [42]. In fruits, the extracts showed also a higher quantity of *trans*-anethole (93.9%) and minimum fractions of estragole (2.4%), methyleugenol, *α*-himachalene, *α*-cuparene, *β*-bisabolene, *p*-anisaldehyde, and *cis*-anethole (0.06%) [7].

From the analysis of 11 samples of fruit essential oils from11 European countries, Orav *et al.,* (2008) [11] revealed that trans-anethole (76.9–93.7%) was the major component, followed by γ-himachalene with 0.4–8.2%, trans-pseudoisoeugenyl 2-methylbutyrate with 0.4–6.4%, p-anisaldehyde with 5.4%, and methylchavicol with only 0.5–2.3%, while the minor compenents were nonanal with 0–1.4%, isogeijerene, 0–0.9%, cis-anethole (0–2.0%), pregeijerene (0–0.5%, and bourbonene (0–0.9%). These chemicals were variable from country to another and the highest content of trans-anethole with a fraction superior to 90% was found in the samples of Greece, Scotland, Hungary, Italy, Lithuania, and Germany. In Middle East, the analysis (GC-MS) of essential oil extracted from seeds showed 51 components, with the dominance of trans-Anethole or Anisole (71.52 %), followed by benzocycloheptene (6.59 %), and Isoeugenyl acetate (4.63 %) in samples from Iraq [36], and 4-allylanisole (85.28%), Limonene (5.53%), Fenchone (4.12%), Anethole (3.54%), Acide linoléique (trace), and Camphene (trace) in samples from Yemen. In North Africa, 4-allylanisole (76.70%), Limonene (9.75%), Anethole (7.40%), Fenchone (6.16%), linoleic Acide (trace), and Camphene (trace) were the principal chemicals in Moroccan samples, while in Algerian samples, *trans*-anethole (92.4 %) followed

by estragol (1.9%), o-isoeugenol (1.9%), and γ-himachalene (1.1 %) were the major chemical constituents [16].

In terms of variation of chemical constituents according to used methods, via Supercritical fluid extraction using CO2 and GCMS, Rodrigues *et al.,* (2003) [43] obtained anethole (90%), *γ*-himachalene (4%), *p*-anisaldehyde (up to 1%), methylchavicol (up to 1.5%), *cis*-pseudoisoeugenyl 2-methylbutyrate (3%), and *trans*-pseudoisoeugenyl 2-methylbutyrate (1.3%) as major chemical constituents of *P. anisum*. by Silver Ion HPLC, Denev *et al.,* (2011) [44] revealed the composition of fatty acids in of *P. anisum* seed oils, counting the positionally isomeric 18:1 fatty acids, followed by oleic acid (*cis* 9-18:1), petroselinic acid (*cis* 6-18:1), and *cis*-vaccenic acid (*cis* 11-18:1). By Soxhlet, hydrodistillation using Clevenger apparatus (HDC), Ultrasonic-assisted (UAE), subcritical water extraction (SWE), , ethanol ($ScCO_2$-Et), supercritical carbon dioxide extraction ($ScCO_2$) using three solvents (methanol ($ScCO_2$-Met), and hexane ($ScCO_2$-H) and supercritical 1,1,1,2-tetrafluoroethane (ScR134a) methods, Nasir *et al.,* (2022) [45] revealed Anethole as the main component with different values, 94.5% by SWE, 94.5% by Soxhlet respectively. The highest TPC value was 508.5 mg GAE/100 g kb in ScR134a extract, while the lowest value was 27.77 mg GAE/100 g kb in HDC extracts, the highest antioxidant capacity was $IC_{50} = 1.58$ mg/mL in $ScCO_2$-H and the lowest was $IC_{50} = 0.07$ mg/mL in Soxhlet extracts. Also, as we mentioned above, three lignin-carbohydrate-protein complexes were purified from hot water seed extracts of *Pimpinella anisum* by a combination of anion-exchange, gel filtration, and hydrophobic interaction column chromatographies [40, 46].

6. Nutritional and industrial uses

Various ethnobotanical studies have shown that *Pimpinella anisum* plants, seeds, roots, and/or leaves are used in cuisine for food, while their derivatives couting essential oils and extracts serve as aroma for different receipts and drinks [17, 47, 48]. In North Africa from Morocco to Egypt, *P. anisum* is mainly used as a fragrance and spice in cooking of Cakes and dishes (46.1%) [17]. It is often used as a “licorice” flavor substitute or added to licorice candy, while the the flavoured components are used alcohols, liqueurs, dairy products, gelatins, puddings, meats, and candies. Generally, seeds are used as a breath freshener, and it is sold as a spice.

Nath *et al.,* (1996) and Sharaf and Goma (1965) [49, 50] were among the author that studied the nutritional uses of *P. anisum* . Sharaf and Goma (1965) [50] demonstrated that extract and *Pimpinella anisum* oil mixed with food have positive oestrogenic effects in immature rabbits and ovariectomized rats. Further, Nath *et al.,* (1996) [49] demonstrated a significant nutritional

potential of the residue resulting after the distillation of steams for essential oil in Kumauni bullocks of about 200 kg liveweight. Equally, the use of anise seed (*Pimpinella anisum* L.) powder supplemented to the drinking water (0, 500, 750, and 1000mg/L) for one-day-old Hubbard Classic chicks for 56 days, significantly improved blood RBC, WBC, Hgb, HCT, TP, albumin, globulin, GLU, P, and Ca levels of broilers, and the highest levels of anise seed powder, i.e., 1000 and 750mg/L, had a stimulating effect on the physiological traits of the birds [51].

During last decades, Cabaroglu et Yilmaztekin, 2011 [52] have proven one of *P. anisum's* productive uses in industry. In order to make the traditional Turkish alcoholic beverages known as "Raki," aniseed *P. anisum* is used. The beverages are made from raw materials such as raisins, grapes, and molasses by double or triple distilling either (1) suma, a distillate of raw materials that have undergone fermentation, or (2) suma combined with agricultural ethanol and aniseed flavoring [53]. The beverage is aromatized by adding 6% to 10% (w/v) of aniseed [52]. Turkey produces several different varieties of raki (the most preferred spirit among other distilled spirits) [54], and are consumed by the people in Turkey and exported to other countries, and the most preferred spirit among other distilled spirits in Turkey. Sales of these alcoholic drinks have more than doubled since 2000, with 614 million liters to 1.127 billion liters in 2012.

Currently, Das *et al.,* (2021) [1] have demonstrated a new way to substitute artificial preservatives to control the contaminations that causing significant loss in nutritive value of stored food commodities, with derivatives of *P. anisum* plants. The authors encapsulated essential oil of *P. anisum* in chitosan nanobiopolymer (CS-PAEO-Nm) to improve its sensorial suitability and bioefficacy for application in food system. Obtained results showed a higher inhibitory effect against fungal infestation and aflatoxin B1 contamination, which make the essential oil of *P. anisum* a green food preservative against deterioration of nutritional qualities. Similarly, the co-encapsulate binary synergistic formulation of *P. anisum* and *C. sativum* essential oils (0.75:0.25) into chitosan nanoemulsion (Nm-PC) showed effective inhibition against fungal proliferation, aflatoxin B1 secretion (AFB1), and peroxidation of lipids in stored rice [1]. The encapsulated essential oils caused irreversible destruction of plasma membrane in cells of toxigenic *Aspergillus flavus* and significant diminution of methylglyoxal [1]. Poly-lactic acid films comprising diverse percentage of anise essential oil (0, 0.5, 1 and 1.5% v/v) showed significant antibacterial inhibitory *L. monocytogenes* and *V. parahaemolyticus* in a

concentration-dependent manner, which suggests the significant potency of anise essential oil to be used as an antibacterial agent in active food packaging [55].

7. Agronomic uses

7.1. Uses against pathogens

7.1.1. Insecticidal potential

Extracts of seeds and leaves of *Pimpinella anisum* have widely tested against pathogen insects from the seventies. These tests were realized in fields and laboratory on different phytopathogen insects that impact most important crops. Obtained results were variable depending on used quantities, type of extracts, and pathogens (Table 2).

First study of toxicology of anise bioactive compounds was realized by Marcus et Lichtenstein (1979) [56] who tested the impacts of 9 anise compounds counting anethole, estragole, anisyl alcohol, anisic acid, *p*-creosol, *p*-cresol, eugenol, anisaldehyde, acetaldehyde, and hydroquinone combined with insecticides counting parathion, paraoxon, carbofuran, carbaryl, DDT, or pyrethrum against houseflies. In results, anethole at 0.5% of diet of the insect combined with insecticide was effective.

Further, essential oil nanoemulsions of *P. anisum* were tested against the red flour beetle, (*Tribolium castaneum* Herbst) considered as one of the most an economically important Coleoptera pest of stored crops [57]. The nanoemulsion of essential oil containing 81.2% of (*E*)-anethole exhibited poisonousness on *T. castaneum* (LC_{50} = 9.3% v/v), with important effect on its progeny and scanning electron microscopy (SEM) revealed Morphological and histological damages against insects. Tunç *et al.,* (2000) [58] also tested the insecticidal activity of essential oil vapours from anise *Pimpinella anisum,* eucalyptus *Eucalyptus camaldulensis,* cumin *Cuminum cyminum,* rosemary *Rosmarinus officinalis,* and oregano *Origanum syriacum* var. *bevanii* against another two stored-product pathogen insects, the Mediterranean flour moth (*Ephestia kuehniella*) and the confused flour beetle (*Tribolium confusum*). Further, 100% mortality of the eggs was obtained in essential oils from anise and cumin, compared to 77 and 89% in Oregano against *T. confusum* and *E. kuehniella*, respectively and 45 and 65% in eucalyptus and rosemary respectively. At a concentration of 98.5 µl anise essential oil/l air, the LT_{99} values were 60.9 for *E. kuehniella* and 253.0 h for *T. confusum*, respectively. In another study, essential oils of *Pimpinella anisum* and other 39 plant species were tested against larvae of *Lycoriella ingenua* that cause serious crop loss, using a fumigation bioassay. In results, among all tested essential oils, anise and garlic were the most

effective with insecticidal activity against the larvae. The constituents counting allyl isothiocyanate followed by *trans*-anethole, diallyl disulfide, and *p*- nisaldehyde were the most toxic agents against larvae of *L. ingenua* [59]. Against three mosquito species *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus,* essential oils of *Pimpinella anisum* and *Juniperus macropoda* showed effective insecticidal activity against larvae and eggs. icidal and ovicidal [60]. Against another mosquito *Culex pipiens,* the essential oil of *Pimpinella anisum* showed the highest insecticidal activity as compared to *Eucalyptus camaldulensis, Mentha piperita, Ocimum basilicum,* and *Laurus nobilis* [61]. We investigated the acaricidal activity of p-anisaldehyde produced from anise seed oil and commercially available components of anise seed oil against *Dermatophagoides farina* and *D. pteronyssinus,* two species of home dust mites. The findings indicated that p-anisaldehyde and benzyl benzoate were the two substances that were most poisonous to these dermatophagoides, and as a result, p-anisaldehyde may be beneficial as a lead drug for the selective control of house dust mites [62].

In current studies, the insecticidal activity of *Pimpinella anisum* essential oils were test with positive results against different stages (eggs, larvae and adults) of a wide range of pathogens counting aphids (i.e. *Brevicoryne brassicae, Myzus persicae)* on kale plants [63], moskitos (i.e. *Culex quinquefasciatus*) [64], insect vectors (i.e., the common housefly *Musca domestica* and the lymphatic filariasis and Zika virus vector *Culex quinquefasciatus*) [65], non-target organism such as *Daphnia magna* [64], and beneficial organisms such as the earthworm *Eisenia fetida* and the aphid predator *Harmonia axyridis* (Benelli et al 2018). For examples, essential oils of *P. anisum* were effective on larvae of *Spodoptera littoralis* (LC_{50} = 57.3 µg/Larvae) and *Culex* quinquefasciatus (LC_{50} = 25.4 µl/L), while they were definitely non-toxic effects on predators of aphids counting *Harmonia axyridis* and *Eisenia fetida* [65]. These findings represent a milestone to product and commercialize eco-friendly or green formulations or bio pesticide to be used in crop protection.

7.1.2. Acaricid potential

As the insects, Acarians cause also huge losses in crops as well as diseases to human health unpleasant odours, allergies and stains. The most problematic issue is the development of resistance to the available chemicals. However, essential oils of *Pimpinella anisum* showed significant effects against some of these pathogens, including resistant ones (Table 2).

Lee, (2004) [62] have compared the effectiveness of two types of home dust mites, *Dermatophagoides pteronyssinus* and *D. farina*, on the commercially available components of anise seed oil and p-anisaldehyde derived from anise seed oil. According to the findings, p-

anisaldehyde was more successful in killing these dermatophagoides than chemical and commercial chemicals (i.e. benzyl benzoate) [62]. Currently, El-Sayed *et al.*, 2022 [66] investigated how the two-spotted spider mite (*Tetranychus urticae*), one of the most dangerous pests in agriculture and a major source of significant losses of various crops (including green bean plants), would react to the essential oil from *P. anisum* seeds. In the experiment, spiders were applied to green bean seedlings for a week before the plants were treated with various quantities of *P. anisum* essential oil for 24, 48, and 72 hours. The findings demonstrated that anise oils have considerable, dose- and time-dependent acaricidal effects against *T. urticae*. Anise oil inhibited acetylcholinesterase at all concentrations, and when the plants were treated with 30 or 40 L/L of anise oil, the spiders' protease activity decreased. The protease activity of *T. urticae* mites was unaffected by the concentrations of 10 and 20 L/L.

7.1.3. Antibacterial potential

The anti-bacterial capacity of *P. anisum* is not widely investigated. Currently, Rastgou *et al.*, 2022 [67] have investigated the effect of twenty-nine plant extracts, including *Pimpinella anisum*, and twenty-seven essential oils against *Pectobacterium betavasculorum* isolate C3, the Causative Agent of Bacterial Soft Rot and Vascular Wilt of Sugar Beet [68]. The author extracted and the antibacterial activities of these plants against *P. betavasculorum*, using the agar diffusion method at 0.01%, 0.1%, and 100% (*v*/*v*). This was the first report of essential oils and extracts having antibacterial action against Pectobacterium betavasculorum (Table 2). Pure *Pimpinella anisum* L. oil had the strongest antibacterial properties among three different concentrations of essential oils and extracts, followed by *Thymus vulgaris L.* oil and *Rosa multiflora Thunb*. extract. The impact of advantageous essential oils and extracts on the in vivo germination and growth of sugar beet seedlings of the Ic1 cultivar was also studied. The Ic1 cultivar's seed germination was impaired at every concentration of essential oil used. Only the plant extracts of *Pimpinella anisum* L., *Brassica oleracea* L., *Salvia rosmarinus Spenn., Eucalyptus globulus Labill., Rosa multiflora Thunb., Lactuca serriola* L., *Syzygium aromaticum* (L.) *Merr.* and *L.M. Perry*, and essential oils of Mentha pipe and *Ocimum basilicum* L. are permitted.

7.1.4. Antiviral potential

In agronomy, only Rastgou *et al.*, 2022 [69] have tested the inhibition capacity of *P. anisum* against phytopathogen viruses (Table 2). The authors applied different concentrations of *P. anisum* and *Foeniculum vulgare* essential oils against three viruses counting tobacco mosaic virus (TMV), potato virus X (PVX), and tobacco ring spot virus (TRSV) and obtained results

showed that at a concentration of 3000 ppm both essential oils totally inhibited the formation of local lesions on the hypersensitive host *Chenopodium amaranticolor*.

Table 2 : The inhibition capacity of *P. anisum* against phytopathogens

Type of Pathogens	Crop	Treatment substances	Treated parts	Treated species	Used concentration	Effectivess	Ref°
Insects	Various stored crops	Essential oil nanoemulsions	Individual	Houseflies	0,50%		[56]
		Essential oil nanoemulsion	Individual	Red flour beetle, (*Tribolium castaneum* Herbst)	14%, v/v of the total coarse emulsion), ethanol (3%, v/v), and Tween 80 (3%, v/v), representing 20% (v/v) of the total emulsion	LC_{50} = 9.3% v/v)	[57]
Acarians	Green bean plants	Essential oil nanoemulsion	Adult Individual	House dust mites, *Dermatophagoides pteronyssinus* and *D. farina*	Concentrations of anise oil (10, 20, 30, or 40 µL/L)	LD50 values, to D. farinae was p-anisaldehyde (1.11 µg/cm2), 3-carene (42.10 µg/cm2), and estragol (43.23 µg/cm2). Against D. pteronyssinus, p-anisaldehyde (0.98 µg/cm2), 3-carene (39.84 µg/cm2), and estragol (40.11 µg/cm2).	[62]
			Adults	The two-spotted spider mite, Tetranychus urticae		40 µL/L killed 96.0% of the red spiders after 72 h	[66]
Virus	*Chenopodium amaranticolor*	Essential oils		Tobacco mosaic virus (TMV), potato virus X (PVX), and tobacco ring spot virus (TRSV)	Agar diffusion method at 0.01%, 0.1%, and 100% (*v/v*)	Concentration of 3000 ppm totally inhibited the formation of lesions	[69]
Bacteria	Sugar Beet	Essential oil and Extracts		*Pectobacterium betavasculorum*	Agar diffusion method at 0.01%, 0.1%, and 100% (*v/v*)	Diameter of the Inhibition Zone (mm) in Pure Concentration 12.87 ± 0.83 and 5.08 ± 0.49 in 10-1 Concentration	[67]

7.2. Improvement of productivity

7.2.1. Improvement of Broiler Performance

(Sharaf et Goma, 1965; Nath *et al.*, 1966) [49, 50] were among the author that studied the nutritional uses of *P. anisum* . Sharaf et Goma, (1965) [50] demonstrated that extract and *Pimpinella anisum* oil mixed with food have positive oestrogenic effects in immature rabbits

and ovariectomized rats. Further, [49] demonstrated a significant nutritional potential of the residue resulting after the distillation of steams for essential oil in Kumauni bullocks of about 200 kg liveweight. Equally, the use of anise seed (*Pimpinella anisum* L.) powder supplemented to the drinking water (0, 500, 750, and 1000mg/L) for one-day-old Hubbard Classic chicks for 56 days, significantly improved blood RBC, WBC, Hgb, HCT, TP, albumin, globulin, GLU, P, and Ca levels of broilers, and the highest levels of anise seed powder, i.e., 1000 and 750mg/L, had a stimulating effect on the physiological traits of the birds [51]. In another study, Ciftci *et al.,* (2005) [70] added 400 mg/kg of anise oil to the diet of day-old broilers which improved feed conversion ratio by approximately 6% compared to antibiotic group. So anise oil can be considered as a natural growth promoter for poultry [70].

7.2.2. Improvement of Milk Production

The effects of adding aniseed and fenugreek seeds to the diet on the effectiveness of the does and kits were investigated. Findings showed that kits in the anisum-fenugreek group consumed the same amount of milk every day as control rabbits. Additionally, there was no discernible difference in the two groups' body weights after 17 days. The differences in litter size, litter weight, kit weight, and 1-35-day weight gain between the anisum-fenugreek group and the control groups at 35 days of nursing were not statistically significant. In conclusion, additional research is required to determine the ideal dosage and taste of these spices in the feed for breastfeeding rabbits [71].

8. Traditional Medicine uses

For thousands of years ago, *Pimpinella anisum* have been used for in traditional medicine, with significant roles in maintaining human health and improving their life quality [72–74]. In modern era, many populations, such as in Iranian, North African, and Turkish, are yet using similar old treatments based on *Pimpinella anisum* and its derivatives. However, these uses very between countries, used parts of plant, and targeted diseases [3, 5].

In Iran, seed extracts of *Pimpinella anisum* are habitually as aromatic, carminative, and disinfectant for hands, while against illnesses, they are used as analgesic in migraine and in diuretic [75]. Further, Aniseed plant has dry and warm nature and is used to increase menstruation, sweat, and urine secretion, as well as to make good complexion. It is also used in polishing of teeth and mentioned for nightmare, melancholy, and in epilepsy and seizure treatment [6, 76]. In central areas of Morocco, *Pimpinella anisum* plant and extracts are used for therapeutic treatments for neurologic, digestive, gynecologic, and respiration disorders [17, 35, 77, 78]. In Southern areas (i.e. Tan-Tan), *Pimpinella anisum* is used in phytotherapy mainly

in urinary tract infections principally against pyelonephritis and cystitis [79]. In Algeria, plant parts counting seeds, leaves are habitually used against respiratory disorders, chronic uremic in Algerian inflammatory-malnutrition, and currently against COVID19 infection [80–82]. In Turkey, seeds of *Pimpinella anisum* are mixed in herbal tea and used for weight loss [83], while in Egypt, it is used for treatment Kidney Stones [84, 85]. In Italy, seeds of *Pimpinella anisum* are used as flavoring in bread, sweet and meat dishes for carminative, digestive treatments [86–88].

9. Pharmacological Properties

9.1. Antiviral activity

The anti-viral activity is widely tested in various extracts of *Pimpinella anisum* on different viruses. The first test was realized in Iraq when Alwan *et al.,* (1988) [89] studied the antiviral activity of aqueous and ethanolic extract of 17 plant species including *Pimpinella anisum* against rinderpest virus (RPV). However, during antiviral in vivo and in vitro tests, the inhibitory effects were variable depending on the viruses and used *Pimpinella anisum* materials.

In medicine, many tests were conducted by researchers to evaluate the effects of essential oils and other extracts on viruses that infect human health. Sokmen *et al.,* (1999) and SÖKMEN, (2001) [90, 91] tested the effects of crude extracts from 24 callus and 11 cell suspension counting *P. anisum* against herpes simplex viruses, HSV-I and HSV-II, but the results were negative. In huge Sudanese screening, one hundred fifty-two water and methanol extracts of 71 plants frequently used in traditional medicine were tested against C virus (HCV) protease (PR), and in results, 34 extracts counting those of *P. anisum* showed significant inhibitory activity superior to 60% at 100 µg/mL [92]. Further, three Lignin-Carbohydrate-Protein Complexes were isolated from *Pimpinella anisum* by the mixture of gel filtration, anion-exchange, and hydrophobic interaction column chromatographies, and tested against human cytomegalovirus (HCMV), herpes simplex virus types 1 and 2 (HSV-1 and -2) and measles virus [40]. All LCs were found to inhibit the adsorption of viruses to the host cell surface and disable them via induction of iNOS mRNA of cytokines including IL-1β and IL-10. Currently, seed extracts of *P. anisum* showed significant effects against the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) considered a serious health hazard internationally [93]. Preparation of green synthesis combining zinc oxide nanoparticles, seed extracts of *P. anisum* and *Nigella sativa* loaded nanostructured oil carriers (NLC) (composed from black seed and olive essential oils) were extensively characterized (high docking energy scores (ranging from

−7.9 to −9.9 kcal/mol) and tested against SARS-CoV-2 main protease (3CLpro or Mpro). Obtained results showed no conformational change in the protein during simulations.

9.2. Antibacterial effect

The anti-bacterial activity of *P. anisum* was tested against different strains counting pathogen and non-pathogen agent [94, 95]. The scientists used the plant and its derivatives to demonstrate the inhibition effects against this king and microorganism [95]. Equally, *P. anisum* was combined with other plants, industrial substances and materials to increase their effectiveness against resistant pathogens [94].

Using the disc diffusion method, the aqueous 50% (v/v) acetone, methanol, and petroleum ether extracts of *P. anisum* fruits were found to have antibacterial effects against four pathogenic bacteria, including *Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumonia, and Escherchia coli* [96]. The obtained results demonstrated that acetone and petroleum extracts were unable to suppress the growth of the same harmful test bacteria, while both methanol and aqueous extracts shown fair inhibition effects against all tested bacteria. The aqueous extract demonstrated noticeably more efficacy than the methanolic extract.

In another study, Gülçın *et al.,* (2003) [42] used the disc diffusion method to examine the antibacterial properties of ethanolic and aqueous extracts of *P. anisum* against 10 bacterial species, including *Candida albicans*. As a result, water extract was found to have an inhibitory impact against *Candida albicans* but not against Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* [42]. The ethanolic extract, in contrast hand, exhibited a strong inhibitory effect against all of the examined microorganisms but was ineffective against *Candida albicans*. Alcoholic extracts from *P. anisum* seeds have demonstrated positive antibacterial activity against *Mycobacterium smegmatis* and *Micrococcus luteus* [97]. Based on the disc diffusion method, the antibacterial potential of four plants' aqueous decoctions against 176 microbial isolates from 12 genera, including anise, coriander, black pepper, and bay leaf, was discovered [98]. As a result, although an aniseed decoction only displayed 18.1% antibacterial activity against the studied microorganisms, black pepper had greater inhibitory effects with 75% antibacterial activity. *Micrococcus roseus* was the target of *P. anisum's* strongest antibacterial effects. Dental plaque samples from kids aged 3 to 5 years old were gathered after detecting the bacterial colonies of *Streptococcus mutans, S. sanguinis,* and *S. salivarius* in an in vitro investigation. Then, using macrodilution and microdilution techniques, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic and methanolic extracts of *P. anisum* and Oregano vulgare were determined [99].

As a consequence, there was a statistically significant difference between the mean MIC and MBC of *P. anisum* and *O. vulgare* extracts alone and in combination against *S. sanguinis*, *S.s mutans*, and *S. salivarius*. These extracts together revealed the lowest MIC and MBC levels.

In terms of combination with other substances, Al-Bayati, (2008) [100] have studied the synergic antibacterial effects between essential oil and methanol extract of both Thymus vulgaris and *P. anisum* against nine pathogenic bacteria. The combination of methanol extract and essential oil of these species exhibited significant inhibitory activity against most tested pathogens, with superior inhibition levels against three bacteria *Bacillus cereus, Staphylococcus aureus,* and *Proteus vulgaris*. Moreover, this combination showed an improved effect against most tested bacteria, particularly *Pseudomonas aeruginosa* [100]. Zayed *et al.*, (2020) [94] used the disk diffusion method to assess the in-vitro antibacterial properties of metal nanoparticles biosynthesized using optimized *P. anisum* extract against Escherichia coli, *Staphylococcus aureus*, and *Candida albicans*. Comparing biosynthesized AgNPs to AuNPs and *P. anisum* extract, they demonstrated greater antibacterial activity.

9.3. Antifungal effects

The essential oils of *P. anisum* were also tested againd fungi, with a positive result [69]. In a promoting study, Kosalec et al have evaluated the antifungal effects of essential oil and fluid extract of *P. anisum* fruits on four dermatophytes species and on seven yeast species using broth dilution method and the diffusion technique with cylinders. The obtained results showed that the fluid extract of *P. anisum* showed antimycotic inhibition against *Candida albicans, C. tropicalis, C. parapsilosis, C. krusei, and C. pseudotropicalis,* and the biggest inhibition zone was detected for *C. albicans*. Similarly, the fluid extract of *P. anisum* exhibited inhibitory activities against all dermatophyte species, *Trichophyton mentagrophytes, T. rubrum, Microsporum gypseum,* and *M. canis*, and the superior inhibitory zone was measured for *T. mentagrophytes*. Similarly, the essential oil of *P. anisum* showed robust antifungal effects against dermatophytes and yeasts, and the superior inhibition zone was recorded against *C. parapsilosis* with 30 mm, followed successively by *C. albicans, C. glabrata,* and then *Geotrichum* spp. Generally, essential oil of anise demonstrated robust antifungal effects rather than extracts against both yeasts and dermatophytes [101]. Inhibitory activity of anise essential oil also was mentioned by Özcan et Chalchat, 2006 [7] against *Aspergillus niger, Aspergillus parasiticus,* and *Aternaria alternata,* and the most effected fungus was *A. parasiticus*. The results of other studies utilizing methanolic extracts of anise against four dermatophyte species and a saprophyte fungus revealed that the extract only suppressed the growth of *Trichophyton*

mentagrophyte, *Candida albicans*, and *Microsporum canis*. On the other hand, saprophyte *A. niger* was not inhibited by aniseed's methanolic extracts in any way [102].

Özcan et Chalchat, 2006 [94] used the disk diffusion method to assess the in-vitro antibacterial properties of metal nanoparticles biosynthesized using optimized *P. anisum* extract against *Aspergillus flavus*. Comparing biosynthesized AgNPs to AuNPs and *P. anisum* extract, they demonstrated greater antifungal activity.

9.4. Muscle Relaxant Effect

Boskabady et Ramazani-Assari, 2001 [103] initiated the investigations about the relaxant effects of *P. anisum* . These authors have studied the relaxant roles of *P. anisum* on isolated tracheal chains of guinea pig and the possible involved mechanism. They used ethanol and aqueous extracts, and essential oil of *P. anisum* on precontracted isolated tracheal chains (10 mM methacholine) under two different conditions; group 1of non-incubated tissues (1 mM propranolol) and and group 2 of 1mM chlorpheniramine incubated tissues. The results revealed that all treatments including ethanol and aqueous extracts, essential oil, and theophylline displayed significant relaxant effects compared to those of under control. Further, the relaxant effects of essential oil were significantly lower than those of theophylline, while the relaxant effects were similar between aqueous and ethanol extracts, and theophylline. Equally, the relaxant effects were similar between examined groups. As possible mechanism, the results of Boskabady et Ramazani-Assari, (2001) [103] showed that the inhibitory effects on muscarinic receptors is the potential cause of the relaxant effect of this plant. The effects of three hydroalcoholic extracts from the aerial portions of *P. anisum*, comprising ethanol/water; 40/60, 60/40, and 80/20, on the rat anococcygeus smooth muscle were examined in a different study by Tirapelli *et al.,* (2007) [104]. As a consequence, all three hydroalcoholic extracts reduced the contraction caused by acetylcholine. This study also noted that, in contrast to the other two hydroalcoholic extracts, only the 60% ethanol extract produced concentration-dependent relaxation of acetylcholine-precontracted tissues. The authors hypothesized that the NO-cGMP pathway's activation is mostly responsible for the relaxing effect.

9.5. Anticonvulsant Effect

The anticonvulsant effects of an essential oil from the fruits of *Pimpinella anisum* against seizures induced by pentylenetetrazole (PTZ) or maximal electroshock were investigated in male mice (MES). This study shows that *P. anisum* decreases the threshold for inducing clonic seizures by intravenous PTZ infusion and can stop tonic convulsions by intraperitoneal PTZ injection. In addition, *P. anisum* contains anticonvulsant qualities that protect against tonic

seizures caused by MES [105]. The effects of an anise methyl alcohol extract on a mouse model of picrotoxin-induced epilepsy were also the subject of his research by Heidari et Ayeli, 2005 [106]. The results showed that mice pretreated with various dosages of anise extract experienced a delayed onset of seizures, with 200 mg/kg being the most effective amount (P 0.05). In addition, when compared to phenobarbital (40 mg/kg), this dose (P 0.01) significantly prolonged the time to death in mice [106]. The results of the other study, which examined the effects of the leaves and stems of some Arab medicinal plants, including *Pimpinella anisum,* on picrotoxin-induced seizures in mice, revealed that *Rosmarinus officinalis, Pimpinella anisum, Matricaria chamomilla,* and *Artemisia vulgaris* extracts delayed the onset of the seizures and decreased mortality [107]. Janahmadi *et al.,* 2008 [108] conducted research on the cellular mechanisms underlying the antiseizure effects of anise fruits en 2008. In this study, it was evaluated if anise fruit essential oil affected snail neurons' bioelectrical activity under control conditions or following the induction of epileptic activity by pentylenetetrazol (PTZ). The findings suggest that augmentation of Ca^{2+} , K^{+} channel activity or suppression of voltage and/or Ca^{2+} dependent K^{+} channel activity underpinning post-hyperpolarization potential are likely candidate cellular processes underlying the hyperexcitability caused by anise oil [108].

9.6. Effect on Gastrointestinal System

9.6.1. Effect on Gastric Ulcer

Indomethacin and other toxic substances were used to induce acute stomach ulceration in rats in order to examine the effectiveness of anise's aqueous suspension against these lesions. The findings demonstrated that anise considerably reduced the damage to the stomach mucosa brought on by necrotizing agents and indomethacin. Histological evidence further supported the antiulcer efficacy [109].

9.6.2. Palliation of Nausea

In a case study, 25 patients in a hospice and palliative care program with nausea symptoms received an aromatherapy treatment that included *P. anisum, Foeniculum vulgare*, var. dulce, *Anthemis nobilis,* and *Mentha piperita*. Most patients who received aromatherapy treatments reported feeling better. However, all study participants were also receiving a range of additional therapies for their problems [110].

9.6.3. Effect on Constipation

In a randomized clinical trial with 20 patients who met the criteria for chronic constipation according to the American Association of Gastroenterology, the laxative efficacy of a

phytotherapeutic compound containing *P anisum* L., *Foeniculum vulgare* Miller, *Sambucus nigra* L., and *Cassia augustifolia* was examined. Colonic transit time (CTT), which was assessed radiologically, served as the main endpoint. The quantity of evacuations each day, how well the bowels appeared to be working, side effects, and quality of life were considered secondary objectives. When compared to a placebo, the study's findings showed that phytotherapic substance had considerable laxative effects. A reduction in colonic transit time and an increase in daily evacuations served to highlight this effect. This substance can be a safe alternative for the treatment of constipation even though there were no appreciable differences in quality of life across the research periods [111].

9.7. Effect on Morphine Dependence

We investigated the effects of *Pimpinella anisum* essential oil on the expression and development of conditioned place preference (CPP), a morphine-induced behavior in mice. The results demonstrated that subcutaneous injections of morphine (2–5 mg/kg) produced place preference in a dose-dependent manner and that injections of *P. anisum* essential oil may cause conditioned place aversion in mice, i.e., the essential oil has some unpleasant effects as investigated by a place conditioning paradigm. Additionally, the oil possesses GABAergic effects [112].

9.8. Anti-Inflammatory and Analgesic Effect

The extracts of *Tribulus terrestris* and *Pimpinella anisum* shown considerable analgesic effect against benzoquinone-induced writing and in thermal testing, according to a screening of several Iraqi medicinal plants for analgesic action [113]. *P. anisum* essential oil demonstrated a significant analgesic effect in a study by Tas that was comparable to morphine and aspirin [114]. Additionally, mice were used to test the analgesic and anti-inflammatory effects of fixed oil of anise. The results demonstrated that the fixed oil of anise has an anti-inflammatory impact equivalent to indomethacin's strength, as well as analgesic effects comparable to morphine and aspirin at the 30-minute mark [115].

9.9. Effect on Menopausal Hot Flashes

Anise extract's impact on menopausal hot flashes in 72 postmenopausal women was investigated in a double-blind clinical experiment. In this study, postmenopausal women who took three capsules of anise extract daily for four weeks saw a significant decrease in the frequency and intensity of their hot flashes [116].

9.10. Effect on Dysmenorrhea

In a research by Karami et al. (2008) [117], 180 female students (aged 17 to 28) with primary dysmenorrhea had their response to a herbal capsule containing dried extracts of celery, saffron, and anise compared to a capsule containing mefenamic acid. Both the herbal and the mephnamic acid groups significantly reduced their levels of pain when compared to the placebo group, according to the data. Additionally, the outcomes showed that herbal capsules were more successful in relieving pain than mephnamic acid and could be a good substitute for primary dysmenorrhea [117].

9.11. Antioxidant Activity

In a study by [42] antioxidant activities of natural and synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and -tocopherol, were compared. The antioxidant properties of water and ethanolic extracts of aniseeds were evaluated using various antioxidant tests. In comparison to BHA, BHT, and -tocopherol, both aniseed extracts demonstrated strong antioxidant activity, reducing power, DPPH radical and superoxide anion scavenging, hydrogen peroxide scavenging, and metal chelating activities. Water extract also demonstrated a higher antioxidant capacity than ethanolic extract [42]. Additionally, research on the in vivo and in vitro antioxidant capability of aniseeds revealed that the aniseed's ethanolic extract has concentration-dependent scavenging activity against nitric oxide, superoxide, and 1,1-diphenyl, 2-picryl hydrazyl (DPPH) radicals as well as reducing power [118]. Anise seed oleoresins and essential oil were investigated for their antioxidant properties. Through the suppression of linoleic acid peroxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Fe^{3+} reducing power, and different lipid peroxidation assays, the antioxidant properties were evaluated. The results demonstrated that anise oil and its methanol oleoresin had the strongest antioxidant activity, surpassing both BHA and BHT. Other oleoresins had somewhat lower antioxidant activity[119]. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test was used to examine the antioxidant capacity of some Umbelliferae fruits from Iran, including *P. anisum.* It revealed that all the extracts had antioxidant capacity, with *P. anisum* extract having the strongest activity (IC_{50} = 614 109.80) and the highest activity and flavonoid content.

The strength of the antioxidants and the flavonoid content of the fractions were shown to be positively correlated [120]. In a different study, the antioxidant activity of anise seeds, dill seeds, and water and alcohol extracts of chamomile flowers was examined. In both linoleic acid and liposome model systems, the extracts demonstrated noticeable antioxidant activity,

while the water extracts displayed greater antioxidant activity than the comparable alcohol extracts. Additionally, aniseeds' antioxidant activity was lower than that of chamomile flowers and dill seeds [121]. Thirteen herbal teas, including anise tea, had their antioxidant capacities tested in vitro. The results suggest that compared to other herbal teas, anise tea had poor antioxidant activity in the Troloxequivalent Antioxidant Capacity (TEAC) assay and a modest impact on the hypochlorite and peroxynitrite quenching assays [122]. At different phases of ripening, Rebey *et al.,* (2019) [18] looked at the bioactive components and antioxidant activity of four populations of *P. anisum* , including accessions from Egypt, Tunisia, Serbia, and Turkey. As a result, all aniseed accessions had their peak antioxidant activity, DPPH scavenging activity, chelating capacity, and reducing power at full maturity. These results suggest that it may be crucial for certain industries to determine the best times and provenances for antioxidant buildup in order to assess the quality of aniseeds. In-vitro antioxidant activity of metal nanoparticles biosynthesized based on optimized *P. anisum* extract was investigated by Zayed *et al.,* (2020) [94]. The authors evaluated the free radical scavenging abilities of silver and gold nanoparticles against 1,1-diphenyl-2-picryl-hydrazyl in order to accomplish this goal (DPPH). Comparing biosynthesized AgNPs to AuNPs and *P. anisum* extract, they demonstrated better antioxidant activity. According to our findings, *P. anisum* seed extract can be widely exploited in the environmentally friendly manufacture of prospective antioxidant and antibacterial AgNPs for secure biomedical applications.

9.12. Effects on Diabetic Patients

Aniseeds and coriander seeds were evaluated in type 2 diabetes patients for their antidiabetic, hypolipidemic, and antioxidant properties [118, 123]. Two groups of type 2 diabetic patients received the seed powders (5 g/day) for 60 days. The results showed an 11% increase in fasting blood sugar in controls, a 36% decrease in type 2 diabetics receiving anise treatment, and a 13% decrease in those receiving coriander treatment. Additionally, a significant reduction in blood cholesterol and triglycerides was seen in patients who received either aniseed or coriander seed treatment. Both treated groups showed a reduction in protein oxidation in serum and lipid peroxidation in erythrocytes and plasma when compared to baseline levels. Both groups demonstrated an increase in serum levels of -carotene and vitamin A, which may have led to a marked reduction in the amount of lipid peroxidation in RBC and plasma. Both the coriander and anise groups also demonstrated an increase in vitamin C. In a diabetic patient, both seeds have antidiabetic, hypolipidemic, and antioxidant properties [118, 123]. To test *P. anisum* 's ability to heal wounds, Hashemnia *et al.,* (2019) [124] produced full-thickness

excisional wounds on the backs of male Sprague-Dawley rats with diabetes. The rats were divided into four treatment groups at random: a control group, *P anisum* 10% for 14 days, doxycycline (3%), and 1ml basal cream. Five animals from each group were put to death at days 7, 14, and 21 after the injury, and the wounds were examined grossly, histopathologically, and for oxidant/antioxidant levels. In addition, the skin sample's dry matter and hydroxyproline contents were examined. As result, *P. anisum* -treated animals showed a considerable reduction in wound size compared to other groups. Additionally, *P. anisum* therapy improved early fibroblast counts, lowered lymphocyte counts, and increased late-stage fibrocyte counts in the process of wound healing. When compared to the control, other variables like re-epithelialization, tissue alignment, more mature collagen fibers, and big capillary-sized blood vessels showed substantial modifications. Malondialdehyde, glutathione peroxidase, and total antioxidant capacity alterations brought on by diabetic wounds were all considerably reversed by *P. anisum*. At various stages of wound healing, it also markedly enhanced the dry matter and hydroxyproline contents.

9.13. Effect on Glucose Absorption

Aniseed oil's impact on the absorption of glucose from the jejunum and water from the colon and renal tubules was examined by Kreydiyyeh et al. in 2003 [132], along with the oil's mode of action [125–127]. The findings showed that aniseed oil significantly increased the rate of glucose absorption in the rat jejunum. The sodium gradient that drives mucosal glucose transport was increased as a result of the oil's stimulation of the Na^+-K^+ ATPase activity. However, adding anise oil at a concentration of 0.05% had no appreciable effect on the colon's ability to absorb water, indicating that the colon's ATPase is immune to the oil's action. Aniseed oil was also added to drinking water to reduce the amount of urine the rats generated and to increase the activity of the renal Na^+-K^+ ATPase even at extremely low doses [125].

9.14. Immunomodulatory effects

The ability of aniseed (*Pimpinella anisum*) and ginger (*Zingiber officinale*) extract mixture to enhance growth and immunomodulatory in broiler chicks with dose dependent has been studied by Raziq *et al.*, (2022) [128]. The dose dependence trials (2gm + 4gm, 2.5gm + 5gm, and 3gm + 6gm for both plants in drinking water) were conducted by the authors. Two hundred forty (240) day-old broiler chicks were separated into two subgroups for each of the four groups, which were randomly assigned the letters A, B, C, and D. Ten checks per duplicate for various treatments were performed for each of the three replications of each subgroup. One of the subgroups received the recommended doses of the vaccines against infectious bronchitis, new

castle disease, and infectious bursal disease. Group B, C, and D were treated with an extract mixture of aniseed and ginger at the rates of (2gms + 4gms), (2.5gms + 5gms), and (3gms + 6gms)/litter of drinking water, respectively. Group A was kept as a control. In terms of mean feed intake and water intake, there were substantial differences between body weight and feed conversion ratio (FCR). Comparing group D and the vaccination groups to other groups, the mean antibody titter against ND, IB, and IBD was considerably higher in group D. Conclusion: Adding an extract mixture of aniseed and ginger to the water that poultry birds consume is better for promoting body weight gain, achieving the best FCR, and enhancing immunological response. Similar to that, it is rather safe, has no adverse effects, is environmentally friendly, and can be found nearby.

10. Guidelines for future research

10.1. Extraction methods

Variation in extraction approaches is habitually dependent on the particle size of the plant tissues, solvent used, the solvent-to-sample ratio, temperature, length of the extraction period, and pH of the solvent. The fundamental principle is to grind the herb dry or wet material finer, which rises the surface space for extraction thus increasing the amount of extraction.

In *Pimpinella anisum,* various extraction methods were used, such as the sprouts of Glycine max, Water, and ethanol Extraction. These traditional extraction methods involve several drawbacks such as low selectivity for biomolecules, high extraction time, and low extraction efficiency. Therefore, more advanced methods are needed to increase the efficiency, reduce time, solvents, and cost of extractions. In this point, we suggest new methods such as Supercritical Fluid (SFE) and Pressurised Liquid Extractions (PLE) techniques known for their high selectivity toward targeted biomolecules, short extraction times, and use the of non-toxic solvents [129]. In fact, Effective identification of bioactive molecules from plant material is basically reliant on the nature of solvent used in the extraction method [129]. Generally, good solvent for extractions must be ease of evaporation at inferior temperatures, promote of fast physiologic absorption of the extract, low toxicity, preserve nature, action mod, and ability of extracted molecules [130]. As the end product in extraction will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay [131]. In our case, we suggest to use optimization simulators that enable to select more suitable solvent, or a mixture of solvent with optimised fractions. At this point, we suggest Mixture Designs Techniques as a good tool to explore needed solvents, quantities, time of extraction for extraction of higher quantity of molecules in *Pimpinella anisum* [132]. Equally, these

approaches permit to combine between solvents which allows to extract large spectre of molecules based on solubility ability of solvents [133].

10.2. Tests against more devastating agents

A huge number of agents impact human life, productivity of crops and animals. The most devastating agents to human life are pathogenic bacteria are *Serratia marcescens, Pseudomonas aeruginosa, Vibrio cholerae, Francisella tularensis, Lactobacillus plantarum, Salmonella Typhimurium, Mycobacterium marinum* [134], fungi are *fungus Cryptococcus, Ajellomyces dermatitidis, Ajellomyces capsulatus, Paracoccidioides lutzii, Lacazia loboi, Coccidioides immitis, Coccidioides posadasii* [135, 136], and viruses are influenza viruses, and HIV. These pathogens and their vectors (i.e insects) infect humans and cause neurologic, immunity, and other metabolic disorders. In crops insects, fungi, viruses are causing every year losses of cereals, rice, potato, and other important crops that ensure the food security of humans. Further, the majority of cited agents have developed resistance to the available chemical treatments. Therefore, the selections of biological materials to treat them will be of great importance. In this case, we suggest applying in vitro, in vivo, and in the field the extracts, essential oils, purified molecules of *Pimpinella anisum* against these pathogens, then select promoting results to produce plant-based treatments for vulnerable agents.

10.3. Climate change and adaptation

Counting aromatic and medicinal plants *Pimpinella anisum* are an essential part of human health care because they represent the primary source of medicine for about 95% of inhabitants, largely in underdeveloped nations, and they are increasingly used by many people living in wealthy countries, as many authors have noted [39, 76]. Millions of people also make a living as traditional healers, herb collectors, or sellers. As the value of the global export trade for herbal compounds was recently projected to be over US$32.6 billion per year, the gathering and trade of medicinal plants serve as a significant source of income for both rural and urban people [137]. However, the life of many plants as well as their chemical makeup are threatened by climate change, which disrupts temperature regimes and precipitation profiles. According to a recent study, over 600 plant species, including APMs, have vanished from the planet in the last 250 years [138].

In *Pimpinella anisum,* all cited studies didn't include any aspect of climate change. Determining the effects of climate change on the plant morphology, metabolism, and genetic is suggested to clarify, how this plant react physiologically, morphologically to this change. Equally, it will allow to identify the impact of climate change on synthesis of biomolecules. At

the end, if the climate change impact the wild populations of the species, scientists and responsible will know how to ensure the conservation of it.

11. Conclusion

Due to *Pimpinella asnisum* L.'s beneficial characteristics, including its hypolipidemic, antidiabetic, anticancer, antioxidant activities, and antimicrobial properties, both the seeds and essential oils of anise are promising for use as raw ingredients and superfood supplements in the food, agronomy, and pharmaceutical industries. The main constituents of anise seed oil include anethol, eugenol, pseudisoeugenol, estragole, methyl chavicol, anisaldehyde, and coumarins. Other important constituents include umbelliferon, scopoleting, terpene hydrocarbons, estrols, and polyacetylenes. The antimicrobial, antifungal, antiviral, hepatoprotective, anti-inflammatory, anticonvulsant, antispasmodic, estrogenic, expectorant, and insecticidal effects are among the pharmacological and agronomic effects, as are clinical effects like nausea, constipation, bronchodilation, menopause, diabetes, obesity, and sedative action. However, further clinical and experimental investigations using novel extraction techniques, solvents, and agents are required to fully understand the myriad compounds in ginseng and how they affect crop protection and public health. On the other hand, it is advised to investigate how plants adapt to abiotic and biotic stresses in order to understand the current status of the species and assure its conservation.

12.References

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Propriétés ethno-pharmacologiques et phytochimiques de l'espèce *Carum carvi* L : Une revue de littérature

(A fait l'objet d'un article soumis pour publication)

Synthèse d'étude

Le carvi *(Carum carvi* L.*)* de la famille des ombellifères, apiacées, est l'une des plantes les plus courantes et bien connues qui se trouvent naturellement en Europe, Asie occidentale, Sibérie, Turquie, Iran, Inde et en Afrique du nord. Cette espèce est répertoriée dans différentes régions du Maroc. Les graines de carvi demeurent appréciées en tant qu'épices culinaires, elles ont également été largement utilisées dans la thérapie populaire depuis l'antiquité.

Les substances aromatiques présentes dans cette plante ont fait l'objet de recherches rigoureuses, à travers le monde entier, pour valider expérimentalement leurs utilisations thérapeutiques.

L'objectif de cette partie d'étude est d'analyser les composants chimiques et les effets pharmacologiques du *Carum carvi*, plus précisément, étudier la variation des composés chimiques dans les huiles essentielles, les extraits et les parties volatiles de la plante.

Pour la rédaction de cet article de revue, nous nous sommes servis des moteurs de recherches électroniques, afin d'obtenir toutes les sources de données nécessaires : Web Of Science, MDPI, Springer, PubMed, Science Direct, Google Scholar, tout en utilisant un ensemble de mots clés dont : *Carum carvi*, genre carum, aire de répartition, germination et croissance, substances chimiques, techniques d'extraction et phytochimie.

Parmi les 130 publications évaluées, seules 78 présentaient un lien significatif avec l'espèce étudiée et ont été retenues en raison de leur haute qualité de recherche et leur pertinence.

Les articles analysés ont révélé diverses utilisations ethno-médicinales en fonction des pays. Dans la médecine traditionnelle de la Tunisie par exemple, le carvi est considéré comme une plante abortive, galactagogue, antiseptique et antihypertensive. Par contre, en Italie, il est utilisé comme tonique amer, carminatif et purgatif. Alors que dans les médecines indigènes arabes, les graines sont considérées comme stimulantes, carminatives et dotées d'un effet rafraîchissant. Elles font partie de la plupart des prescriptions contre la gonorrhée, la diarrhée chronique et la dyspepsie.

Sous forme de cataplasme, l'utilisation apaise la douleur et l'irritation. Dans la plupart des pays, les graines sont fortement utilisées pour les indigestions et les douleurs abdominales. Les

huiles essentielles et les extraits de différentes parties du carvi ont montré une activité antioxydante notable, selon plusieurs méthodes, notamment en neutralisant les radicaux hydroxyles, les radicaux 2,2-diphényl 1-picrylhydrazyle (DPPH) et les peroxydes lipidiques.

L'action antimicrobienne a été évaluée contre diverses souches bactériennes, qu'elles soient gram-positives ou gram-négatives. L'huile essentielle de graines et l'extrait alcoolique ont inhibé la croissance de Klebsiella Pneumoniae et de ses isolats cliniques, entraînant une amélioration de la morphologie cellulaire. Cela est attribué à des composés chimiques démontrant des propriétés préventives contre la formation de biofilm, spécifiquement observées avec Streptococcus mutans et streptococcus pyogenes.

Les effets antidiabétiques des produits de carvi sont largement documentés, l'huile essentielle a présenté une activité anti-hyperglycémique chez des rats diabétiques, avec une augmentation du poids corporel. Tandis que l'extrait aqueux a une activité anti-lipidique (hypotriglycéridémiques et hypocholstérolémique).

L'usage traditionnel du carvi, comme diurétique, a été confirmée par une étude expérimentale dans laquelle, le traitement peroral d'un extrait aqueux de cette plante a montré une augmentation de la production d'urine chez les rats 24h après administration. Les niveaux urinaires de sodium et potassium ont été augmentés, tandis que dans le plasma, ils n'ont pas été affectés. Aucune toxicité rénale, ni autre effet indésirable n'a été observé avec l'extrait de carvi.

Globalement, dans les essais cliniques sur l'homme, certaines préparations à base principalement du carvi, ont démontré leur efficacité dans le soulagement des symptômes dyspeptiques. L'extrait alcoolique présente des effets inhibiteurs sur les contractions des muscles lisses induites par les spasmogènes, acétylcholine et histamine. Les extraits ont aussi, montré un effet anti-ulcérogène et une réduction de la production d'acide et de leucotriènes, ainsi que d'une augmentation de la sécrétion de la mucine et de la libération de la prostaglandine.

Il a été souligné que les graines de *Carum carvi* L. représentent une richesse très importante, dotées de diverses qualités pharmacodynamiques et pharmacologiques, soutenant leur utilisation massive dans la phytothérapie.

Chap II: Ethnopharmacological and phytochemical properties of *Carum carvi* L: A literature review

Abstract

Caraway (*Carum carvi* L.) is a biennial plant in the family Apiaceae. Its seeds (fruits) which are used in food, cosmetics, beverage and pharmaceutical industries. Processed products from caraway whole seed, such as seed powder, essential oil, fatty oil, oleoresins and caraway carvone are widely used in the food and medicinal industries. Caraway is one of the earliest cultivated herbs in Asia, Europe and Africa. The caraway seeds are considered as antispasmodic, carminative, astringent and used in the treatment of stomach stimulant, diarrhea, dyspepsia, hysteria, flatulent indigestion, colic, dyspeptic headache and improve liver function. In ancient Indi an ayurvedic literature use of *C. carvi* seeds is well documented Scientist working in different parts of the world has now experimentally proved the validity of ancient therapeuti c uses of *C. carvi* seeds. This will strengthen our the use of caraway seeds in traditional system of ayurvedic treatments by *C. carvi* seeds.

Keywords: Caraway, bioactivity, antioxidant activity, limonene

1. Introduction

Medicinals plants play a vital role in the development of new drugs and the forests of India have been a source of traditional medicines for millennia. Of the 17,000 species of higher plants described in India, 7500 are known for their medicinal uses [1-3]. The Charak Samhita, a document on herbal therapy written about 300 BC, reports on the production of 340 herbal drugs and their indigenous uses [4]. The Indian Himalayan Region is well known to have a great range of plant diversity. The state of Uttarakhand is a part of north-western Himalaya, and still maintains a dense vegetation cover (65%). The maximum species of medicinal plants have been reported from Uttarakhand [5-6]. This region alone supports about 18,440 plant species (Angiosperm: 8,000 spp., Gymnosperm: 44 spp., Pteridophytes: 600 spp., Bryophytes: 1,736. Lichens: 1,159 spp. and fungi: 6,900 spp [7]. According to Samant et al. out of the total vascular plant species, 1,748 species are of medicinal uses [8].

Carum carvi belonging to the family Apaiaceae, are one of the earliest cultivated herbs in Asia, Africa, and Europe. Caraway seeds, respectively, have remained popular as culinary spices and are also overwhelmingly used in folklore therapy since antiquity in diverse geographical areas. The aromatic substances present in these herbs have attracted enormous attention of researchers

worldwide to experimentally validate the therapeutic uses of cumin and caraway seeds, which are documented in several indigenous healing systems. It is known as Caraway in English, Krishna jiraka in Ayurved. It used as Carminatives, antispasmodic, antimicrobial etc. Its seed oil is used in dyspeptic problems, such as mild, spatic conditions of gastrointestinal tract. The seed oil contains a volatile oil consisting of carvone and limonene with other constituents; flavonids mainly quercetin derivatives as a fixed oil.

Heartburn, bloating, gas, appetite loss, and minor stomach and intestinal spasms are among the digestive issues that caraway can help with [6, 7]. Additionally, caraway oil is used to treat constipation, enhance urine control, help patients cough up phlegm, and destroy microorganisms in the body. Caraway oil is used by women to induce menstruation and soothe cramps; it is also used by nursing moms to boost milk production [8–10]. Mouthwashes and skin rubs containing caraway are used to increase local blood flow. *Carum carvi*, or caraway, seeds are used to treat gastrointestinal issues such as diarrhea, dyspepsia, hysteria, flatulent indigestion, colic, dyspeptic headache, and stomatitis stimulant. Additionally, they are claimed to have antibacterial, anticonvulsant, antispasmodic, carminative, and astringent properties as well as effects on obesity, hyperglycemia, and Reno protection [6, 11–18]. A common herb, carumcarvi has long been used in traditional medicine as a medicinal and as a food additive [19]. Its possible biological effects include those related to hypothyroidism, analgesics, anti-inflammatory, anti-anxiety, anti-obesity, and antispasmodic properties [20–24].

This review highlights the significance of caraway as a potential source of diverse natural products and their medicinal applications. Equally, we discussed ethno-medicinal uses of the plant.

2. Data collection

A bibliography on the circulated studies of *Carum carvi* was gained using electronic exploration engines, principally Google Scholar, Science Direct, and the WSU online database (PubChem) (Figure 22). The keywords comprised *Carum carvi*, Caraway phytochemicals, medicinal plants, effect of climate on chemical contents, the effect of geographical location, genotypes, altitude, bioactivity, ethnopharmacology, and medical potentials, were used to collect data. To summarize *Carum carvi* L.'s chemical components and medical potential, as well as their variation under climatic, soil, geographical, extraction conditions, and genetic factors, a thorough study of the literature covering the last two decades, from 1950 to 2023, was conducted. All authors selected and reviewed twenty-nine (29) papers that related to the

areas of our emphasis. The search results were meticulously arranged according to broad knowledge, the review questions, and the associated goals.

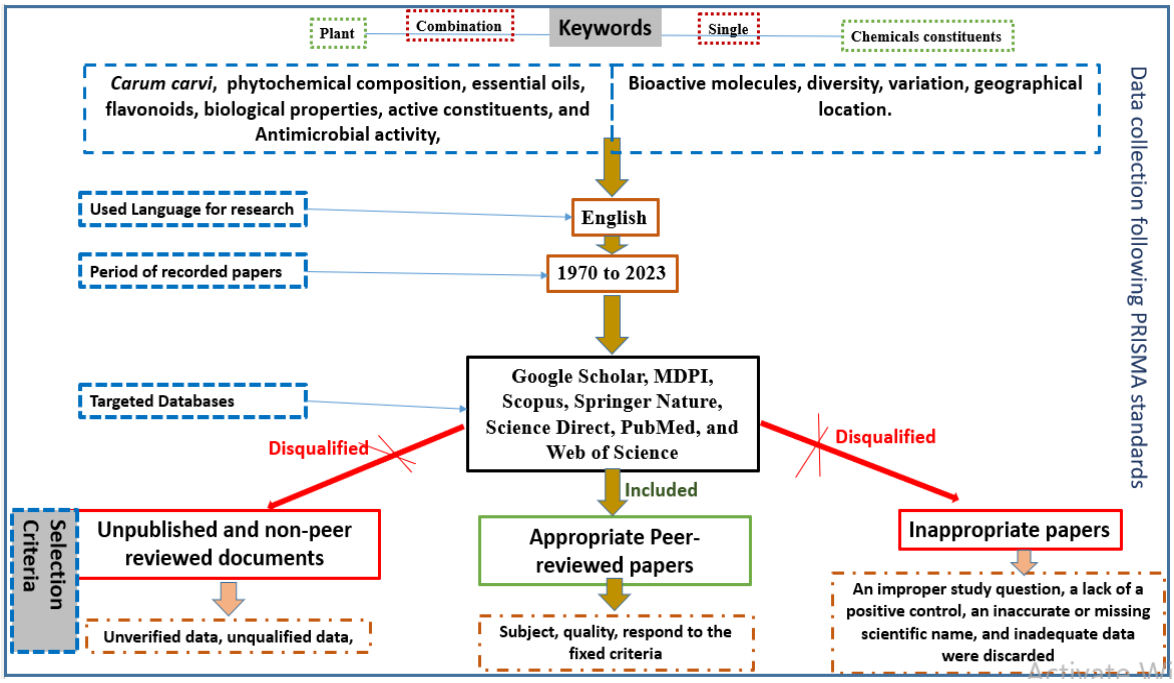


Figure 22: Summary of research approach followed to collect bibliographic data on *Carum carvi*

3. Description of the plant

Typically growing to a height of 30 to 80 cm, caraway is a biennial plant with numerous branches and thin, finely grooved green stems. In the establishment year, it produces a rosette of feathery, dark green, finely cut leaves as well as a deep taproot. In order to start producing flowering stems in the second year, which can reach a height of 75 cm, it needs a high vernalization requirement. The white, 2-3 mm in diameter blooms are produced in umbels, with the outside flowers being larger than the inner ones. They begin to blossom in late April and are followed by light brown, 3-6 mm long fruits that ripen in early July [7].



Figure 23 : Plant (A), flowers (B), and seeds (C) of Carum carvi

4. Ethno-medicinal uses

In Indian therapeutic system a large number of medicinal herbs of various taxonomic genera are included in many forms in this traditional therapy. Literature survey revealed that cumin as well as caraway seeds are prominently considered carminative, eupeptic, antispasmodic, astringent and used in the treatment of mild digestive disorders, diarrhea, dyspepsia, Flatulence, morning sickness, colic, dyspeptic headache and bloating, and are said to promote the assimilation of other herbs and to improve liver function [23-24]. Essential oils from caraway seeds are reported to give relief in patients suffering from lumbago and rheumatism [25]. Caraway water finds use as a vehicle for paediatric medicines. As a mixture with alcohol and castor oil, it has been used for the treatment of scabies [26]. In fact, medicinal usage of cumin and caraway seeds has also been immensely widespread in diverse ethno-medicinal systems from Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia and North America, where these have remained as an integral part of their folk medicines. In Iranian traditional medicine, cumin is considered stimulant, carminative and astringent and its therapeutic effects have been described on gastrointestinal, gynaecological and respiratory disorders, and also for the treatment of toothache, diarrhea and epilepsy [27]. In the Moroccan traditional medicine, caraway seeds are used as diuretics [28] and given to treat diabetes and hypertension [29].

In traditional medicine of Tunisia, caraway is considered abortive, galactagogue, antiseptic, antihypertensive herb, while in Italy, it is used as bitter tonic, carminative, and purgative [30]. In indigenous Arabic medicines, the seeds are documented as stimulant, carminative, and attributed with cooling affect and therefore form an ingredient of most prescriptions for gonorrhea, chronic diarrhea and dyspepsia; externally, they are applied in the form of poultice to allay pain and irritation of worms in the abdomen. Seeds reduced to powder, mixed with honey, salt and butter are applied to scorpion bites [31]. In Poland, caraway is recommended as a remedy to cure indigestion, flatulence, lack of appetite, and as a galactagogue. In Russia, it is also used to treat pneumonia. In Great Britain and USA, it is regarded a stomachic and carminative. In Malay Peninsula, caraway is one important medicinal herb, and in Indonesia, it is used in the treatment of inflamed eczema [32].

5. Chemical composition

Caraway (*Carum carvi* L.) seeds contain several components; major phytochemicals of *C. carvi* essential oil were 37.98% (R)-carvone, 26.55% D-limonene, 5.21% apinene, 5.01% cis-carveol and 4.67% ß-myrcene [71] (Table 3). The essential oil of *C. carvi* was also

characterized by high contents of oxygenated monoterpenes (62. 17%), monoterpenes (36.08%) and sesqui terpenes (0.41%), saturated and unsaturated fatty acids, ketones, aldehydes and esters [72-73]. Earlier reports showed that this plant contain a variety of secondary metabolites such as mono and sesquiterpenoids [74], steroids and coumarins [75], flavonoids [76], aromatics and their glucosides, alkaloids and aliphatic compounds [75]. High proportion of sesquiterepene hydrocarbons, limonene, g-cadinene, b-selinene and belemene were observed in GC/MS analyzed flower volatiles of *C. carvi* seeds [74]. Kunzemann et al. [77]. In samples from Tunisia, gas chromatography coupled with flame ionization detectors (GC-FID) and mass spectrometry (GC-MS), resulting in the identification of 28 volatile compounds for *C. carvi* EO, representing 99.1% of the total oil composition, with the major compounds being γ-terpinene (31.03%), β-pinene (18.77%), *p*-cymene (17.16%), and carvone (12.20%). It has been reported that EOs from three Tunisian ecotypes were dominated by carvone (76.8–80.5%) and limonene (13.1–20.3%) [35]. Chinese caraway seed EO contain limonene (43.5%), carvone (32.6%), and apiole (15.1%) as major components [36], however only carvone (65.77 to 78.8%) and limonene (19.38 to 31.64%) are very abundant in other EOs [37]. EO cultivated from *C. carvi* in the high hills of the Uttarakhand Himalayas mainly contains carvone (44.5–95.9%), limonene (1.5–51.3%), β-myrcene (0–0.4%), trans-dihydrocarvone (0–0.5%), and trans-carveole (0–0.2%) [38]. Based on the results of the chemical composition of *C. carvi* EO from different ecotypes, it has been found that carvone (61.6–77.4%) and limonene (16.2–29.1%) were the major components of caraway oils. German caraway EO mainly contains carvone (77.3%) and limonene (16.2%), while the corresponding values were 76.3% and 19.5% for other Tunisian chemotypes. Carvone (61.6%), limonene (29.1%), β-myrcene (3.9%) and α-selinene (10.9%) were the main components of Egyptian chemotype [39].

Table 3 : Chemical composition (%) of the EOs isolated from the seeds of C. carvi.

Nº	**Compounds Identified**	**RI [a]**	**RI [b]**	**(%) *C. carvi***	**(%) *C. sativum***	**Identification**
1	α-thujene	921	927	0.34	tr	RI,MS
2	α-pinene	932	935	0.62	4.44	RI,MS,CS
3	Camphene	946	950	-	0.40	RI,MS
4	Sabinene	969	974	-	0.21	RI,MS
5	**β-pinene**	979	981	**18.77**	0.66	RI,MS
6	Myrcene	992	991	1.00	0.56	RI,MS
7	Octanal	1004	1002	-	tr	RI,MS
8	α-phellendrene	1002	1006	0.22	-	RI,MS
9	δ-3-carene	1011	1011	0.06	-	RI,MS
10	α-terpinene	1014	1018	0.16	tr	RI,MS,CS
11	***p*-cymene**	1027	1026	**17.16**	1.74	RI,MS
12	Limonene	1032	1034	0.42	1.23	RI,MS
13	**γ-terpinene**	10,599	1064	**31.03**	5.35	RI,MS,CS
14	Cis-linalool oxide	1072	1076	-	0.5	RI,MS
15	Octanol	1070	1085	-	0.30	RI,MS
16	**Linalool**	1095	1089	Tr	76.41	RI,MS,CS
17	Cis-sabinene hydrate	1098	1099	0.25	-	RI,MS
18	Trans-allo-ocimene	1128	1141	Tr	-	RI,MS
19	**Camphor**	1146	1151	-	2.20	RI,MS
20	Citronellal	1148	1153	-	0.49	RI,MS
21	p-mentha-1,5-dien-8-ol	1166	1158	Tr	-	RI,MS
22	Trans-pinocarveol	1145	1161	0.19	-	RI,MS
23	Borneol	1169	1179	0.18	0.23	RI,MS
24	Terpinen-4-ol	1177	1193	-	0.23	RI,MS
25	α-terpeniol	1195	1204	-	.026	RI,MS
26	Cryptone	1193	1195	0.41	-	RI,MS

Nº	Compounds Identified	RI [a]	RI [b]	(%) *C. carvi*	(%) *C. sativum*	Identification
27	Myrtenal	1196	1204	Tr	-	RI,MS
28	Decanal	1205	1227	0.12	-	RI,MS
29	Citronellol	1230	1243	-	0.58	RI,MS
30	**Carvone**	1242	1247	**12.20**	-	RI,MS
31	**Cuminaldehyde**	1247	1254	**-**	0.90	RI,MS
32	Geraniole	1265	1261	-	0.15	RI,MS,CS
33	**1-phenylbutanol**	-	1289	**3.29**	-	RI,MS
34	Decanol	1277	1287	-	0.10	RI,MS
35	Safrole	1285	1293	-	0.27	RI,MS
36	**Bornyl acetate**	1292	1298	**12.84**	-	RI,MS,CS
37	(E)-anethole	1282	1302	Tr	-	RI,MS
38	Undecanal	1308	1305	-	tr	RI,MS
39	δ-elemene	1334	1330	Tr	-	RI,MS
40	α-terpenyl acetate	1346	1350	-	0.14	RI,MS
41	**Geranyl acetate**	**1379**	**1382**	0.14	1.81	RI,MS
42	α-copaene	1385	1383	0.15	-	RI,MS
43	Trans-caryophyllene	1417	1425	0.10	-	RI,MS
44	γ-selinene	-	1437	Tr	-	RI,MS
Total identification				**99.10**	**99.46**	

6. Biological activities

Literature survey revealed that Cumin (*Cuminum cyminum*) and Caraway (*Carum carvi* L.) products (oils as well as their aqueous and solvent derived extracts) have shown significant number of biological activities in many ways. A summary of all those given in table x.

6.1. Antioxidant activity

Essential oils and extract of different parts of cumin (*Cuminum cyminum*) have shown significant antioxidant activity in several test methods, as quench hydroxyl radicals, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals and lipid peroxides. The other assays employed were ferric thiocyanate method in linoleic acid system, Fe2+ ascorbate-induced rat liver

microsomal lipid peroxidation (LPO), soybean lipoxygenase dependent lipid peroxidation and ferric reducing ability. Caraway (*Carum carvi* L.) root extract has also shown significant antiDPPH radical activity.

6.2. Antimicrobial activity

Investigations revealed that antimicrobial activity of cumin (*Cuminum cyminum*) and caraway (*Carum carvi* L.) products (essential oils as well as their aqueous and solvent derived extracts). This antibacterial action was assessed against a range of useful and pathogenic gram-positive and gram-negative bacterial strains. Cumin seed oil and alcoholic extract inhibited the growth of Klebsiella pneumoniae and its clinical isolates and caused improvement in cell morphology, capsule expression and decreased urease activity. This property was attributed to cuminaldehyde biofilm-formation preventive properties were found against Streptococcus mutans and Streptococcus pyogenes.

The results of antimicrobial potential are reported via the determination of IZ (inhibition zone), MIC, MBC, and MFC values of *C. carvi* and *C. sativum* EOs alone and in combination. Furthermore, antibiotics against eighteen human pathogenic strains were also used as standards in the present study, and are outlined in Table 3 and Table 4. Table 3 showed that IZDs are in the range of 11.00 ± 00 mm to 25.00 ± 0.00 mm for *C. carvi* EO, 8.33 ± 0.57 mm to 21.66 ± 1.15 mm for *C. sativum* EO, 12.00 ± 1.00 mm to 25.66 ± 0.57 mm for the mixture, and 17.00 ± 1.00 mm to 32.67 ± 0.58 mm for the standards (tetracycline and gentamycin).

Table 4 : Inhibition zones of growth (IZ mm), showing the qualitative antibacterial activity of two EOs (*C. carvi* and *C. sativum*) and their mixture against human pathogenic bacteria compared to standard antibiotics (gentamycin and tetracycline).

	IZ (mm)			
Strains	*C. carvi*	*C. sativum*	Mixture (*v/v*)	Antibiotics
Gram positive bacteria				Gentamycin
S. epidermidis CIP 106510	16.33±0.57	14.00±1.00	18.66±0.57	21.33±0.58
S. aureus ATCC 25923	15.66±0.57	11.66 ± 0.57	18.00±0.00	32.67 ± 0.58
M. luteus NCIMB 8166	25.00± 0.00	21.66 ± 1.15	25.66 ± 0.57	27.67 ± 1.53
E. feacalis ATCC 29212	16.33± 0.57	12.33 ± 0.57	18.33±0.57	26.00 ± 1.00
B. cereus ATCC 11778	18.33± 0.57	16.00 ± 1.00	20.66 ± 0.57	26.00 ± 1.00
B. cereus ATCC 14579	19.00± 1.00	17.33± 0.57	22.33± 0.57	28.00 ± 0.00
Gram negative bacteria				
E. coli ATCC 35218	12.00±1.00	13.00± 0.00	14.66±0.57	22.00±1.00
L. monocytogenes ATCC19115	15.33±0.57	12.00 ± 0.00	18.00 ±0.00	23.00± 0.00
P. aeruginosa ATCC 27853	9.66 ± 1.15	8.33 ± 0.57	12.00 ± 1.00	17.00 ± 1.00
S. typhimurium LT2 DT104	11.00± 0.00	9.33 ± 1.15	14.33±1.15	20.33 ± 0.57
Vibrio strains				Tetracycline
V. parahaemolyticus ATCC17802	13.33±0.57	12.33 ± 0.57	16.66±0.57	18.33 ± 0.57
V. alginolyticus ATCC 33787	14.66±0.57	12.00 ± 1.00	15.66±0.57	20.67± 0.57
V. proteolyticus ATCC15338	11.00± 1.00	9.66 ± 1.54	13.00 ± 1.54	18.00 ± 1.00
V. furnisii ATCC 35016	14.33 ± 0.57	12.66±0.57	16.33±0.57	22.67±0.57
V. mimicus ATCC 33653	12.33±0.57	9.66 ± 0.57	15.33± 0.57	21.00± 0.00
V. natrigens ATCC 14048	13.33±0.57	12.00 ± 1.00	11.33± 9.81	23.67 ± 0.57
V. carhiaccae ATCC 35084	14.66±1.15	13.00±1.00	18.33± 0.57	23.33± 0.58
V. fluvialis ATCC 33809	14.33±0.57	14.66 ± 0.57	18.66± 0.57	23.67 ± 0.57

In addition, *C. carvi* exhibited potent antifungal activity (Table 4) when compared to *C. sativum* against all strains (except equipotent activity with *C. glabrata*), however the mixture displayed more potent activity as compared to *C. carvi* and *C. sativum* alone for all strains. The combined EOs displayed the same IZDs as the reference drug except for *C. parapsilosis* and *S. cerevisae* strains. IZDs, meaning a comparison of all tested microorganisms (bacteria and yeast), showed that the mixture has the highest activity ($p < 0.05$) compared to the two oils alone. Moreover, mixture IZDs for yeast strains were similar to amphotericin B except against *C. parapsilosis* and *S. cerevisiae.*

The results of MIC and MBC values against all strains ranged from 0.059 mg/mL (*M. luteus, E. feacalis*) to 1.875 mg/mL (*P. aeruginosa, V. natrigens*) and 0.234 mg/mL (*M. luteus*) to 3.750 mg/mL (*P. aeruginosa, V. parahaemolyticus, V. furnisii, V. mimicus, V. natrigens*) for *C. carvi* EO, and from 0.234 mg/mL (*S. aureus, M. luteus, V. parahaemolyticus*) to 1.875 mg/mL (*P. aeruginosa, V. alginolyticus, V. proteolyticus, V. furnisii, V. mimicus, V. natrigens, V. carhiaccae*) and 0.938 mg/mL (*S. aureus, M. luteus, E. feacalis*) to 7.500 mg/mL (*V. alginolyticus, V. furnisii, V. mimicus, V. carhiaccae*) for *C. sativum* EO. Interestingly, in the case of the EO mixture, MIC and MBC values have been reduced and were found to be in the range of 0.029 mg/mL (*S. epidermidis*) to 0.469 mg/mL (*P. aeruginosa, V. natrigens*) and 0.059 mg/mL (*M. luteus*) to 0.938 mg/mL (*P. aeruginosa, V. mimicus, V. natrigens*), which are very close to the used antibiotics.

Table 5 : Minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC), and MBC/MIC ratio showing quantitative antibacterial activity of two EOs (*C. carvi* and *C. sativum*) and their mixture against human pathogenic bacteria compared to standa

Microorganisms Tested	*C. carvi* EO			*C. sativum* EO			Mixture (*v*/*v*) EOs			Antibiotics		
	MIC mg/mL	MBC mg/mL	MBC/MIC (Interpretation)	MIC mg/mL	MBC mg/mL	MBC/MIC (Interpretation)	MIC mg/mL	MBC mg/mL	MBC/MIC (Interpretation)	MIC mg/mL	MBC mg/mL	MBC/MIC
Staphylococcus epidermidis CIP 106510	0.117	0.469	4 (Bactericidal)	0.469	1.875	4 (Bactericidal)	0.029	0.117	4 (Bactericidal)	0.009	0.039	4
Staphylococcus aureus ATCC 25923	0.117	0.469	4 (Bactericidal)	0.234	0.938	4 (Bactericidal)	0.059	0.234	4 (Bactericidal)	0.004	0.019	4
Micrococcus luteus NCIMB 8166	0.059	0.234	4 (Bactericidal)	0.234	0.938	4 (Bactericidal)	0.015	0.059	4 (Bactericidal)	0.004	0.019	4
Enterococcus faecalis ATCC 29212	0.059	0.469	8 (Bacteriostatic)	0.469	0.938	2 (Bactericidal)	0.117	0.234	2 (Bactericidal)	0.004	0.019	4
Bacillus cereus ATCC 11778	0.117	0.469	4 (Bactericidal)	0.938	1.875	2 (Bactericidal)	0.059	0.117	2 (Bactericidal)	0.004	0.039	8
Bacillus cereus ATCC 14579	0.117	0.469	4 (Bactericidal)	0.938	1.875	2 (Bactericidal)	0.059	0.234	4 (Bactericidal)	0.004	0.039	4
Escherichia coli ATCC 35218	0.469	1.875	4 (Bactericidal)	0.938	3.750	4 (Bactericidal)	0.117	0.469	4 (Bactericidal)	0.009	0.039	4
Listeria monocytogenes ATCC19115	0.469	1.875	4 (Bactericidal)	0.938	1.875	2 (Bactericidal)	0.059	0.234	4 (Bactericidal)	0.019	0.078	4
Pseudomonas aeruginosa ATCC 27853	1.875	3.750	2 (Bactericidal)	1.875	7.500	4 (Bactericidal)	0.469	0.938	2 (Bactericidal)	0.019	0.15	4

Microorganisms Tested	*C. carvi* EO			*C. sativum* EO			Mixture (*v*/*v*) EOs			Antibiotics		
	MIC mg/mL	MBC mg/mL	MBC/MIC (Interpretation)	MIC mg/mL	MBC mg/mL	MBC/MIC (Interpretation)	MIC mg/mL	MBC mg/mL	MBC/MIC (Interpretation)	MIC mg/mL	MBC mg/mL	MBC/MIC
Salmonella typhi *typhimurium* LT 2 DT104	0.234	0.938	4 (Bactericidal)	0.938	1.875	2 (Bactericidal)	0.059	0.117	2 (Bactericidal)	0.019	0.039	2
Vibrio parahaemolyticus ATCC17802	0.938	3.750	4 (Bactericidal)	0.234	1.875	8 (Bacteriostatic)	0.117	0.234	2 (Bactericidal)	0.039	0.078	2
V. alginolyticus ATCC 33787	0.234	1.875	8 (Bacteriostatic)	1.875	7.500	4 (Bactericidal)	0.059	0.469	8 (Bacteriostatic)	0.019	0.078	4
V. proteolyticus ATCC15338	0.469	1.875	4 (Bactericidal)	1.875	3.750	2 (Bactericidal)	0.117	0.469	4 (Bactericidal)	0.009	0.039	4
V. furnisii ATCC 35016	0.469	3.750	8 (Bacteriostatic)	1.875	7.500	4 (Bactericidal)	0.117	0.469	4 (Bactericidal)	0.009	0.039	4
V. mimicus ATCC 33653	0.938	3.750	4 (Bactericidal)	1.875	7.500	4 (Bactericidal)	0.234	0.938	4 (Bactericidal)	0.039	0.078	2
V. natrigens ATCC 14048	1.875	3.750	2 (Bactericidal)	1.875	3.750	2 (Bactericidal)	0.469	0.938	2 (Bactericidal)	0.019	0.039	2
V. carhiaccae ATCC 35084	0.938	1.875	2 (Bactericidal)	1.875	7.500	4 (Bactericidal)	0.117	0.234	2 (Bactericidal)	0.039	0.078	2
V. fluvialis ATCC 33809	0.469	1.875	4 (Bactericidal)	0.469	3.75	8 (Bacteriostatic)	0.117	0.469	4 (Bactericidal)	0.019	0.039	2

Table 6 : Minimal inhibition concentration (MIC), minimal fungicidal concentration (MFC) and ratio MFC/MIC showing antifungal activity for two EOs (C. carvi and C. sativum) and their mixture against human pathogenic fungi compared to standard antifungals (Ampho

	C. carvi EO			*C. sativum* EO			Mixture EO (*v/v*)			Amphotericin B		
Strains	CMI	CMF	CMF/CMI (Interpretation)	CMI	CMF	CMF/CMI (Interpretation)	CMI	CMF	CMF/CMI (Interpretation)	CMI	CMF	CMF/CMI (Interpretation)
C. albicans ATCC90028	0.059	0.469	8 (Fungistatic)	0.469	1.875	4 (Fungicidal)	0.029	0.117	4 (Fungicidal)	0.078	0.31	4 (Fungicidal)
C. glabrataATCC 90030	0.059	0.234	4 (Fungicidal)	0.234	0.938	4 (Fungicidal)	0.029	0.117	4 (Fungicidal)	0.009	0.078	9 (Fungistatic)
C. parapsilosis ATCC2219	0.059	0.234	4 (Fungicidal)	0.469	0.938	2 (Fungicidal)	0.029	0.117	4 (Fungicidal)	0.039	0.078	2 (Fungicidal)
C. kruseiATCC 6258	0.059	0.469	8 (Fungistatic)	0.234	1.875	8 (Fungistatic)	0.029	0.117	4 (Fungicidal)	0.009	0.019	2 (Fungicidal)
S. cerevisae	0.029	0.234	8 (Fungistatic)	0.234	0.938	4 (Fungicidal)	0.015	0.117	8 (Fungistatic)	0.009	0.039	4 (Fungicidal)

6.3. Antidiabetic activity

A methanolic extract of cumin (*Carum carvi* L) seeds reduced the blood glucose and inhibited glycosylated hemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen (liver and skeletal muscle) content in alloxan and streptozotocin (STZ) diabetic rats [49-50]. The anti-diabetic effects of cumin (Cuminum cyminum) and caraway (*Carum carvi*

L.) products are amply documented [51]. In a glucose tolerance test conducted in rabbits, caraway significantly increased the area under the glucose tolerance curve and hyperglycemic peak [52]. In another study, an aqueous extract of caraway prevented in vitro glycation of total soluble protein, α-crystallin, and delayed the progression and maturation of STZinduced cataract in rats. caraway prevented loss of chaperone activity in diabetic rats and also attenuated the structural changes of α-crystallin in lens, which is a long-lived protein and is susceptible to several posttranslational modifications in certain diabetic conditions [53]. Caraway oil (*Carum carvi* L.) exhibited antihyperglycemic activity in alloxan-induced diabetic rats and increased the body weight [54]. The biologically active constituent of caraway seed oil was characterized as cuminaldehyde which inhibited aldose reductase and alpha-glucosidase isolated from rat [55]. Caraway suppressed alcohol and thermally oxidized oil induced hyperlipidemia. It decreased aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) activities and decreased the tissue (liver and kidney) levels of cholesterol, triglycerides and phospholipids and prevented the changes in the composition of fatty acids in the plasma of rats administered with alcohol and/or thermally oxidized oil. The activity of phospholipase A and C decreased significantly [56- 57]. Hypocholesterolemic effect of methanolic extract of caraway is also documented in ovariectomized rat in relation to its anti-osteoporotic effect [58]. Aqueous extract of caraway showed potent lipid lowering activity (hypotriglyceridemic and hypocholesterolemic) in both normal and STZ-diabetic rats after single and repeated oral administration [59]. Caraway added to a hypercholesterolemia diet decreased serum and liver cholesterol in rats [60].

To reduce the high levels of glucose in the blood, enzyme inhibitors are a key. An anti-diabetic bioassay was carried out for the first time to test *C. carvi, C. sativum,* and a mixture of both EOs as α-glucosidase inhibitors. It was obvious that *C. carvi* (IC_{50} = 6.83 ± 0.76 mg/mL) and *C. sativum* (IC_{50} = 6.24 ± 0.86 mg/mL) EOs demonstrated similar antidiabetic activity, which is about nine-fold lower than the standard drug, acarbose (IC_{50} = 0.73 ± 0.10 mg/mL); however, the EO mixture (IC_{50} = 0.75 ± 0.15 mg/mL) displayed equipotent activity to acarbose. Our finding justifies the increased dietary intake of coriander seeds in decreasing the oxidative burden in DM. Terpenes have been provided for their antidiabetic potential. Studying the EO from *Harita cheirifolia* L., Majouli et al. [64] have reported that among the active monoterpenes, p-cymene and γ-terpinene revealed potent inhibitory effects. The strongest α-glucosidase inhibition effect was also displayed by *Sideritis galactic* EO containing high levels

of β-pinene (32.2%), and the activity was ascribed to the high level of monoterpene hydrocarbons.

6.4. Diuretic activity

The traditional use of caraway as a diuretic was confirmed in an experimental study in which peroral treatment of an aqueous extract of caraway (in acute and sub-chronic mode) was shown to increase the urine output during and after 24 hours in rat. The urinary levels of sodium and potassium were found to be increased, while in plasma these were not affected. Carum (*Carum carvi* L.) extract did not produce any renal toxicity or any other adverse effects during the study period [61].

6.5. Gastrointestinal activity

In human trial studies, some herbal preparations consisting predominantly caraway (*Carum carvi* L.) have shown efficacy in relieving dyspeptic symptoms [62]. The antispasmodic effect of an alcoholic extract of caraway has shown inhibitory effects on smooth muscle contractions induced by the spasmogens, acetylcholineand histamine [63-64]. Extracts from caraway produced dose-dependent antiulcerogenic effect against indomethacin-induced gastric ulcers, accompanied by reduction in acid and leukotrienes′ output, and increased mucin secretion and prostaglandin E2 release. The antiulcerogenic activity was also confirmed histologically and was attributed to its flavonoid content and free radical scavenging properties [65]. Aqueous and solvent derived of caraway increased amylase, protease, lipase and phytase activities [66].

6.6. CNS activity

Anti-epileptic activity of fruit essential oil of (*Carum carvi* L) is documented. It decreased the frequency of spontaneous activity induced by pentylenetetrazol (PTZ). This protection was measured in a time- and concentration-dependent manner as increased duration, decreased amplitude of hyperpolarization potential, the peak and firing rate of action potential and excitability of nerve cells [67]. Cumin oil was found to attenuate seizures induced by maximal electroshock and PTZ in mice [68]. Cumin oil has also been found to possess significant analgesic action in a chemical model (formalin test) of nociception in rat [69].

6.7. Immunomodulatory activity

In a recent study, oral treatment with cumin showed immunomodulatory properties in normal and immunesuppressed animals via modulation of T lymphocytes′ expression in a dose-dependent manner. It stimulated the T cells (CD4 and CD8) and Th1 cytokines′ expression in normal and cyclosporine-A induced immunesuppressed mice. In restraint stress-induced

immune-suppressed animals, the active compound of cumin countered the depleted T lymphocytes, decreased the elevated corticosterone levels and size of adrenal glands and increased the weight of thymus and spleen [70].

7. Conclusion

Carum carvi L. seeds are rich sources of essential oils containing diverse group of phytochemicals. It has abroad spectrum pharmacological effect in treatment of traditional healing systems in different parts of the world. In last two decades extensive studies has been carried on *Carum carvi* L. seeds to explore its full potential of pharmacological action. A natural product used in conventional treatment provides a clue for the existence of phytochemicals. New researches on caraway proved it as a source of new entities to perform different pharmacodynamic properties, responsible for their pharmacological effects. The present review is a step to promote it for cultivation in large scale in Uttrakhand for improvement of economy of peoples living in hill areas.

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La diversité et la variation des composés chimiques de *Coriandrum sativum* favorisant un large éventail d'activités pharmacologiques

(A fait l'objet d'un article soumis pour publication)

Synthèse d'étude

La coriandre, est une plante annuelle, appartenant à la famille des Apiacées (Ombellifères). Elle est l'une des plantes médicinales et aromatiques ayant une histoire culinaire très ancienne, elle constitue une source importante de composés aromatiques et médicinales. Approuvée pour une utilisation alimentaire aux États-Unis et en Europe, la coriandre joue un rôle essentiel dans l'industrie alimentaire et pharmaceutique.

Cette partie d'étude a fait l'objet d'un article de synthèse qui est rédigé en se basant sur différents moteurs de recherche et qui ont été consultés afin de collecter les données nécessaires pour la revue actuelle, dont, Scopus, Google Scholar, PubMed et ScienceDirect, tout en utilisant un ensemble de mots-clés : *Coriandrum sativum*, propriétés bénéfiques, composés phytochimiques et effets pharmacologiques, un total de 195 articles téléchargés ont été examinés pour leur pertinence et leur nouveauté afin de sélectionner 127 articles utilisés pour structurer le présent article.

Les articles analysés ont montré, la richesse de la plante étudiée en composés chimiques responsables des effets pharmacologiques pertinents et des activités biologiques très importantes (Activité antioxydante, anticonvulsivante, antidiabétique, anticancéreuse, antifongique et autres). En outre cette plante a une saveur légère et fraîche et, est utilisée à des fins culinaires. Les feuilles de coriandre ont un goût qui diffère de celui de ses graines. Elles constituent une source de micronutriments et d'éléments nutritionnels. Elles sont très faibles en matières grasses saturées, cependant, la plante contient une bonne quantité d'acide linoléique, qui est une bonne source d'α-tocophérol et de vitamine K. D'où l'intérêt du présent travail qui consiste à mener une étude de synthèse permettant de mettre en évidence les caractéristiques phytochimiques des produits dérivés de la coriandre.

Par la présente étude, nous avons pu souligner que le goût de la coriandre est lié à son huile essentielle qui comprend une quantité significative d'acide linoléique et de furanocoumarines (coriandrine et dihydrocoriandrine). L'huile essentielle de *C. sativum* est utilisée de différentes manières, notamment dans les aliments (comme agent aromatisant et conservateur) et dans les produits pharmaceutiques (action thérapeutique), ainsi que dans les parfums (fragrances et lotions).

Il a été noté que les graines de *Coriandrum sativum* L. sont une source précieuse, offrant une gamme étendue de qualités pharmacodynamiques et pharmacologiques. Cette richesse soutient largement leur utilisation répandue en phytothérapie.

Chap III : The diversity and variation of *Coriandrum sativum*'s chemical compounds support a wide range of pharmacological activities

Abstract

Coriandrum sativum was shown to include flavonoids, phenolics, tannins, terpenoids, reducing sugars, essential oil, fatty acids, sterols, and glycosides through phytochemical screening. High nutritional values were also present, including proteins, fats, carbs, fibers, and a variety of vitamins, minerals, and trace elements. Anxiolytic, antidepressant, sedative-hypnotic, anticonvulsant, memory enhancement, improvement of orofacial dyskensia, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antioxidant, cardiovascular, hypolipidemic, anti-inflammatory, analgesic, antidiabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, detoxification, and many other pharmacological effects were found in the preceding pharmacological studies. The purpose of this paper is to provide an overview of the pharmacological effects and chemical makeup of *Coriandrum sativum*.

Keywords: *Coriandrum sativum*, chemical compounds, essential oils, biological activities.

1. Introduction

The World Health Organization estimates that about 80% of the developing countries 51 inhabitants rely on the traditional medicine for their primary health care needs, and that most 52 of these therapies involve the use of plant extracts or their active components (WHO, 2000). 53 Not only in developing countries but all over the world the use of medicinal plants has been 54 playing a significant role in maintaining human health and improving the quality of human 55 life. Thus, Fruits have become important for human nutrition due to their nutrients and 56 potential beneficial health effects (Albuquerque et al. 2016).

Traditional medicine is practiced around the world and is reliant on the local environment's natural resources as well as the knowledge and skills required to use them (Elujoba et al. 2005). Since ancient times, man has relied heavily on plants as a source of curative medications, particularly in impoverished homes (Olajuyigbe 2012). There are still many groups all over the world that practice herbal medicine (Kassaye et al. 2006). Additionally, 80% of the African population still relies only on medicinal plants for treatment, according to the World Health Organization (2001). Many nations have recently invested a ot of time and energy into the research of medicinal plants. Chemistry, pharmacology, and botany must all be integrated in

the development of new herbal medicines (Wyk 2011; Yusupova et al. 2023). Identification and documentation of medicinal plants can be done effectively through ethnobotanical surveys and ethnopharmacological research (Schultz & Garbe 2023).

Herbal medicine is the oldest form of medicine known to mankind. It was the mainstay of many early civilizations and still the most widely practiced form of medicine in the world today. Plants generally produce many secondary metabolites which are bio-synthetically derived from primary metabolites and constitute an important source of many pharmaceutical drugs [1-30].

The phytochemical screening of *Coriandrum sativum* showed that it contained essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and glycosides. It also contained high nutritional values including proteins, oils, carbohydrates, fibers and wide range of minerals, trace elements and vitamins. The previous pharmacological studies revealed that it possessed anxiolytic, antidepressant, sedative-hypnotic, anticonvulsant, memory enhancement, improvement of orofacial dyskinesia, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antioxidant, cardiovascular, hypolipidemic, anti-inflammatory, analgesic, antidiabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, detoxification and many other pharmacological effects.

The current review gives an overview of the chemical constituents and pharmacological effects of *Coriandrum sativum*. We reviewed the chemical compounds and their variation depending on the geographical location, extraction techniques, and environmental factors. Then we discussed the pharmacological properties of the plant and its compounds. Equally, we presented data on the morphological characteristics and geographical distribution of the plant and its varieties.

2. Research approach

Different search engines were checked to collect data used to prepare the current review such as Scopus, Google Scholar, PubMed, and ScienceDirect on the basis of the following keywords: *Coriandrum sativum*, beneficial properties, flavonoids and pharmacological effects, phytochemical (Figure 24). A total of 195 articles downloaded were examined for their relevance and novelty to select 127 articles used to structure the present manuscript.

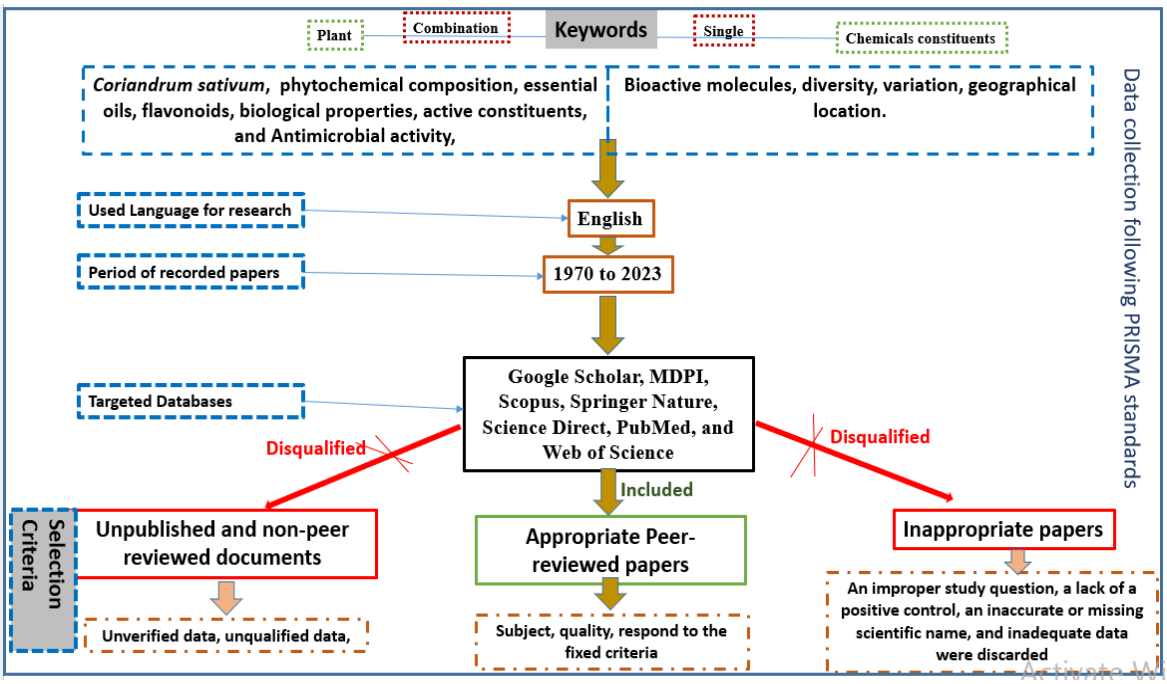


Figure 24: Research approach followed to gather the data on Coriandrum sativum

3. Description of the plant

Coriandrum sativum (Figure 25) is an upright and short-lived herbaceous plant usually growing 1-2 m. Stems and leaves: The branched stems are hairless (i.e. glabrous), mostly hollow, and have fine lengthwise (i.e. longitudinal) grooves. They are pale green in colour but covered in distinctive purplish or pinkish blotches. The alternately arranged leaves are borne on long hollow leaf stalks (i.e. petioles) that tend to sheath the stem at their bases. They are deeply divided (i.e. bi-pinnatisect), with toothed (serrate) segments, and are ferny in appearance.



Figure 25 : Plant (A), flowers (B), and seeds (C) of Coriandrum sativum

These leaves (up to 50 cm long and 40 cm wide, but more commonly 12-25 cm long) are hairless (glabrous). The upper leaf surfaces are dark green in colour, while their undersides are a paler green or greyish-green. The stems and leaves give off a strong odour when crushed or damaged. Flowers and Fruit: The white flowers are borne in large numbers in dense flat-topped clusters at the tips of the branches (in terminal compound umbels). Individual flowers are small

(2-4 mm across), have five incurved petals and five stamens, and are borne on stalks (pedicels) up to 5 cm long. Many (about 15) of these stalks (pedicels) radiate from the same point and form a small cluster of flowers (an umbel), with several (6-20) of these smaller clusters (often called rays) being grouped together into a much larger cluster (a compound umbel) that is subtended by several small leafy bracts (about 5 mm long).

Flowering occurs mostly during spring and summer. The fruit turns from green to greyish-brown in colour as it matures and resembles a capsule. It actually consists of two one-seeded structures (mericarps) that readily split apart when the fruit is fully mature. Each of these (seeds), 2-4 mm long, hairless (glabrous), but has five prominent yellowish-coloured ribs. There are many varieties of *Coriandrum sativum* which differ in the fruit size and oil yield [36-39].

4. Geographical distribution

Since human antiquity, *Coriandrum sativum* has been cultivated. It is thought to have originated in the Eastern Mediterranean and spread as a spice plant to Morocco, China, Central Europe, Russia, and India, [36]. Nevertheless, it was now diffused throughout Europe (the Netherlands, Poland, Switzerland, Belarus, Estonia, Latvia, Denmark, Finland, Ireland, Norway, Sweden, UK, Austria, Belgium, Czechoslovakia, Germany, Hungary, Lithuania, Moldova, Ukraine, Albania, Bulgaria, Greece, Italy, Romania, Yugoslavia, France, Portugal and Spain), Northern Africa (Algeria, Morocco, Tunisia and Ethiopia), Asia (Afghanistan, Iran, Iraq, Palestine, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, southern Russia, Kyrgyzstan, Kazakhstan, Turkmenistan, Tajikistan, Uzbekistan, China, India and Pakistan) [35, 37].

5. Traditional uses of *Coriandrum sativum*

The use of coriander dated back to around 1550 BC, and it was one of the oldest spice crops in the world. Medicinally, it was used as stimulant, aromatic and carminative. The powdered fruit, fluid extract and oil are chiefly used medicinally as flavouring to disguise the taste of active purgatives and correct their griping tendencies. The whole or ground seed (fruit) was an ingredient of pickling spices, also used to flavor various commercial foods, particularly, to prepare some instant soups and dishes, in many cakes, breads and other pastries, alcoholic beverages, frozen dairy desserts, candy, and puddings. The fruit essential oil was a common ingredient in creams, detergents, surfactants, emulsifiers, lotions, and perfumes [40].

Seeds were applied locally to alleviate swelling and pains. A paste of green coriander was used for headaches. Externally, powdered green coriander was used to alleviate burning sensation and pain in diseases like inflammation caused by erysipelas and lymphadenopathy. Decoction of green coriander was used in stomatitis. Nasal drops of green coriander act as a haemostat and thus stop bleeding in epistaxis. Juice or decoction of green coriander was used in conjunctivitis. The seeds were included in many prescriptions as carminative and for the treatment of fever, diarrhoea, vomiting and indigestion. Coriander was used internally as tonics. It was also used for syncope and memory loss. Fresh juice of leaves was used as gargle in sore throat and stomatitis. Paste of leaves were locally applied for swellings and boils and were applied over forehead and temples for headache [41-44].

6. Diversity of chemical compounds

Coriander is rich in chemical compounds including nutrients and bioactive molecules. In terms of nutrients, protein 21.93-12.37 g, total lipid (fat) 4.78-17.77 g, carbohydrate 52.10-54.99 g, fiber 10.40-41.9 g were the most recorded in samples of the plant (seeds, leaves, and roots). Followed by minerals such as calcium 1246-709 mg, iron 42.46-16.32 mg, phosphorus 481-409 mg, magnesium 694-330 mg, potassium 4466-1267mg, sodium 211-35 mg, zinc 4.72-4.70 mg. in terms of vitamins, vitamin C 566.7-21 mg, thiamin 1.252-0.239 mg, riboflavin 1.500-0.290 mg, niacin 10.707-2.130 mg, vitamin B-120.00-0.00 µg, vitamin A, RAE 293-0.00 µg, vitamin A, IU 5850 IU and 0 IU and vitamin D (D2 + D3) 0.00-0.00 µg, were also mentioned by[40, 46-47] in the plant.

The phytochemical screening of plant showed the presence of essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and glycosides [48-50]. The most important constituents of coriander fruits were the essential oil and fatty oil. The essential oil content of dried coriander fruits varies between 0.03 and 2.6%, while the fatty oil content varies between 9.9 and 27.7% [40].

Table 7 : The chemical groups and their constituents in the essential oil of coriander [27].

Chemical Group	Constituents
Alcohols	Linalool (60–80%), geraniol (1.2–4.6%), terpinen-4-ol (3%), α-terpineol (0.5%)
Hydrocarbons	Limonene (0.5–4.0%), g-terpinene (1–8%), *p*-cymene (3.5%), α-pinene (0.2–8.5%), camphene (1.4%), myrcene (0.2–2.0%)
Ketones	Camphor (0.9–4.9%)
Esters	Geranyl acetate (0.1–4.7%), linalyl acetate (0–2.7%)

Table 8 : Chemical composition of coriander seed and leaf oil along with its biological significance

Constituent	Concentration (%)		Function	References
	Seed	Leaf		
Linalool	40.9–79.9	0–13	Antimicrobial, anti-inflammatory, anticancer, antioxidant properties	[30,31]
Geraniol	0.5–3.0	-	Insecticidal and repellent properties	[32]
Terpinen-4-ol	0.43–1	0.2	Promotes anti-inflammatory cytokine production, enhances the effect of several chemotherapeutic and biological agents	[33]
α-terpineol	0.5–1.5	-	Anticancer, anticonvulsant, antiulcer, antihypertensive	[34]
γ-terpinene	0.3–11.2	0.9–3.1	Potential biofuel alternative	[35]
p-cymene	0.5–1.5	-	Natural antioxidant, antimicrobial activity	[36]
Limonene	2.0–5.0	-	Anticancer activity	[37]
α-pinene	0.2–10.9	1.9–2.5	Antimicrobial, apoptotic, antimetastatic, and antibiotic	[38]
Camphene	1.3–2	-	Antileishmanial, hepatoprotective, antiviral and anticancer activity by inducing apoptosis in cancer cells	[39]
Myrcene	0.5–1.5	-	Anxiolytic, antioxidant, anti-ageing, anti-inflammatory, analgesic properties	[40]
Camphor	0.9–4.9	-	Insecticidal, antimicrobial, antiviral, anticoccidial, antinociceptive	[41]
Geranyl acetate	0.2–5.4	-	Antinociceptive activity	[17,42]
Linalyl acetate	0–2.7	-	Flavoring agent, antimicrobial and anti-inflammatory activity	[43]
Eucalyptol	0.1–1%	0.5–2	Anti-inflammatory and antioxidant mainly via the regulation on NF-κB and Nrf2 pathway	[44]
β-phellandrene	0–1.5	-	Antimicrobial activity	[45]
Borneol	4.5	-	Acesodyne, sedation, anti-inflammation, antibiosis effect	[46]
β-caryophyllene	3.26	-	Antibacterial, antioxidant, gastroprotective, anxiolytic, anti-inflammatory	[47]
Citronellol	0.15–0.25	8.1–10	Anti-inflammatory, analgesic and anticonvulsant effects	[48]

Constituent	Concentration (%)		Function	References
	Seed	Leaf		
Caryophyllene oxide	3.12	-	Cytotoxic activity, analgesic activity	[49]
Thymol	2.4–3	-	Antioxidant and antimicrobial properties	[50]
Decanal	-	5.1–8.8	Antioxidant and antimicrobial properties	[17]
(E)-2-Dodecenal	-	12–14	Anthelmintic activity	[51]
Tridecanal	-	0.3–0.4	Antibacterial, antifungal and antioxidant activities	[52]
1-Decanol	-	5–10%	Bactericidal activity and membrane-damaging activity	[53]
(E)-2-Tetradecenal	-	4.1–8.2	Antimicrobial, and antioxidant activity	[54]
(E)-2-Decenal	-	20–35	Antimicrobial, and antioxidant activities	[55]
2-decenoic acid	-	30.8	Antimicrobial activity	[56]
Capric acid	-	12.7	Antibacterial and anti-inflammatory activity	[57]

The variations in the oil constituents of *Coriandrum sativum* leaves and seeds could be attributed to the variations in the cultivar and not due to geographic divergence and ecological conditions. However, the compounds isolated from Coriander essential oil were included: Monoterpene hydrocarbons (p-cymene, camphene, Δ-3-carene, limonene (dipentene), myrcene, cis- and trans-ocimene, α-phellandrene, β-phellandrene, α- pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, terpinolene, α-thujene); Monoterpene oxides and Carbonyls (Camphor, 1,8- cineole, linalol oxide, carvone, geranial); Monoterpene alcohols (Borneol, citronellol, geraniol, linalool, nerol, α-terpineol, 4-terpinenol); Monoterpene Esters (Bornyl acetate, geranyl acetate, linalyl acetate, α-terpinyl acetate); Sesquiterpenes (β-Caryophyllene, caryophellene oxide, elemol, nerolidol); Phenols (Anethole, myristicin, thymol); Aliphatic hydrocarbons (Heptadecane, octadecane); Aliphatic alcohols (Decanol, dodecanol); Aliphatic aldehydes (Octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, tetradecanal, 3-octenal, 2-decenal, 5-decenal, 8-methyl-2-nonenal, 8- methyl-5-nonenal, 6-undecenal, 2-dodecenal, 7- dodecenal, 2-tridecenal, 8- tridecenal, 9-tetradecenal, 10-pentadecenal, 3,6-undecadienal, 5,8-tridecadienal) and Miscellaneous compounds: Acetic acid, α-pdimethyl styrene) [51-52].

The analysis of the essential oil conducted by gas chromatography-mass spectroscopy, revealed 33 components, representing 99.99% of the total oil from the seeds of coriander. The major components were linalool (55.09%), α-pinene (7.49%), 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)- (5.70%), geraniol (4.83%), 3-Cyclohexene-1- methanol, α,α,4-trimethyl- (4.72%), hexadecanoic acid (2.65%), tetradecanoic acid (2.49%), 2-α-pinene (2.39%), citronellyl acetate (1.77%), and undecanal (1.29%) [53-54]. Sudanese *Coriandrum sativum* oils contained seventy eight compounds with sabinene (17.63%), camphor (15.5%), cis-beta-ocimene (10.11%), trans-beta-ocimene (5.64%), alpha pinene (4.69%) and norboreneolacetate (4.09%) as the main constituents [55].

The essential oils obtained from the coriander fruits, from Iran, by hydrodistillation (HD) and Microwaveassisted hydrodistillation (MAHD) then, the oils were analyzed by GC and GC-MS. The results indicated that the HD and MAHD isolated essential oils (EO) were dominated by monoterpenoids such as linalool, geranyl acetate and γ-terpinene. The major compound in both EO was linalool, its amount in HD and MAHD was 63 % and 66 %, respectively. The total amount of monoterpenes hydrocarbons isolated by HD was differ significantly with the amount isolated by MAHD (12.56 % compare to 1.82 %) [56].

Coriander (*Coriandrum sativum* L.) seeds, from Germany, were extracted with chloroform/methanol (2:1, v/v) and the amount of total lipid was 28.4% of seed weight. The major fatty acid was petroselinic acid (65.7% of the total fatty acid methyl esters) followed by linoleic acid, palmitoleic acid, arachidicacid, γ -linolenic acid, linolenic acid, gadoleic acid, cetoleic acid and docosahexanoic acid. Chromatographic analysis yielded 93.0% neutral lipids, 4.14% glycolipids, and 1.57% phospholipids. Six triacylglycerol molecularspecies were detected but one component (C54:3) corresponding to tripetroselinin, and/or dipetroselinoyloleoyl glycerol comprised more than 50% of the totaltriacylglycerols. Sterol content was estimated to be at ahigh level (5186 µg /g oil). Stigmasterol, β -sitosterol, Δ 5-avenasterol, and campesterol were found to be the sterol markers. The major phospholipid subclasses were phosphatidylcholine followed by phosphatidylethanolamine, phosphatidy-linositol and phosphatidylserine [57].

The leaves and stems of Korean *Coriandrum sativum* were extracted and the essential oil composition were studied. Thirty-nine components representing 99.62% of the total oil were identified from the leaves. The major components were cyclododecanol (23.11%), tetradecanal (17.86%), 2-dodecenal (9.93%), 1-decanol (7.24%), 13-tetradecenal (6.85%), 1-dodecanol (6.54%), dodecanal (5.16%), 1-undecanol (2.28%), and decanal (2.33%). On the other hand,

thirty-eight components representing 98.46% of the total oil were identified from the stems of the coriander. The major components were phytol (61.86%), 15-ethyltricyclo[6.5.2(13,14),0(7,15)]-pentadeca1,3,5,7,9,11,13-heptene (7.01%), dodecanal (3.18%), and 1-dodecanol (2.47%) [58].

The leaf oil of *Coriandrum sativum* from Bangladesh contained 44 compounds mostly of aromatic acids, the major were 2-decenoic acid (30.82%), E-11-tetradecenoic acid (13.4%), capric acid (12.7%), undecyl alcohol (6.4%) and tridecanoic acid (5.5%). Other major constituents in the leaf oil were undecanoic acid (2.13%), 2-dodecanal (1.32%), 2-undecenal (3.87%), cyclododecane (2.45%), decamethylene glycol (1.15%), decanal (1.35%) and dodecanoic acid (2.63%). The seed oil contains 53 compounds of which the major compounds are linalool (37.65%), geranyl acetate (17.57%) and γ-terpinene (14.42%). Other major compounds in the fruit oil are β- pinene (1.82%), m-cymene (1.27%), citronellal (1.96%), citronellol (1.31%), citral (1.36%), geraniol (1.87%), citronellyl acetate (1.36%), α-cedrene (3.87%), and α- farnesene (1.22%) and β-sesquiphellandrene (1.56%). The seed oil contained 53 compounds, the major compounds were linalool (37.7%), geranyl acetate (17.6%) and γ-terpinene (14.4%). However, the components isolated from seeds oil and their percentage were: γ-Terpinene 14.42, Camphene 0.14, E-Verbenol 0.27, Sabinene 0.23, β-Pinene 1.82, 2-Oxabicyclo[2.2.2]0ctan-6-l,1,2,3-trimethyl 0.02, β-Myrcene 0.55, Cyclooctanol 0.02, α-Thujene 0.04, m- Cymene 1.27, Limonene 0.40, E-Ocimene 0.05, Z-Ocimene 0.04, Lilac alcohol 0.11, α-Terpinene 0.04, ZVerbenol 0.11, Linalool 37.65, Isothujol 0.04, α-Campholenal 0.22, Citronellal 1.96, Umbellulone 0.11, Borneol 0.32, 4-Terpineol 0.06, Terpinyl acetate 0.31, Decanal 0.14, Z-verbenone 0.10, Citronellol 1.31, Citral 1.36, Geraniol 1.87, Eugenol 0.90, Carveol 0.15, Undecanal 0.58, Methyl geranate 0.17, Myrtenyl acetate 0.43, Citronellyl acetate 1.36, Geranyl acetate 17.57, Z-myrtenyl acetate 0.10, β-Elemene 0.06, Dodecenal 0.15, Caryophyllene 0.33, β-Farnesene 0.07, 2-Dodecenal 0.18, Curcumene 0.98, α-Cubebene 0.13, α-Cedrene 3.87, α-Farnesene 1.22, β-Bisabolene 0.80, β-Sesquiphellandrene 1.56, E-Nerolidol 0.13, Artumerone 0.04, 8-Hexadecenal, 14-methyl-, (Z) 0.22, α-Bisabolol 0.15 and n-Hexadecanoic acid 0.08 [59]. Many isocoumarins were isolated from the aerial parts of *Coriandrum sativum*, including coriandrone A, coriandrone B, isocoumarins, coriandrin and dihydrocoriandrin [60].

Caffeic acid, protocatechinic acid, and glycitin were characterized as the major polyphenolics of coriander aerial parts [61]. However, Rajeshwari and Andallu found that the ethanolic extract of coriander seeds contained many flavonoids including caffeic acid, chlorogenic,

quercetin and rutin [62]. However, the total polyphenolic content of the seeds was found to be 12.2 gallic acid equivalents (GAE)/g while total flavanoid content was found to be 12.6 quercetin equivalents/g [63]. The amount of flavonoids in 70% ethanol extract was found to be 44.5 µg and that of the total phenols was 133.74 µg gallic acid equivalents per mg of the hydro-alcohol extract of *Coriandrum sativum* leaves [64].

Table 9 : Variation of coriander essential oil among different varieties of coriander and growing areas

Location	Varieties	Essential Oil Content (%)	Linalool Content (%)	References
Turkey	Arslan	0.30	89.46	[60]
	Gürbüz	0.33	89.44	
	Erbaa	0.38	91.77	
	Gamze	0.35	89.77	
Pakistan	Native	0.15	69.64	[20]
Egypt	Russian	0.19	69.50	[61]
	Balady	0.2	62.53	
	Selected	0.09	65.48	
India	Acr1	0.25	78.22	[62]
	Sindhu	0.32	81.3	
	Swathi	0.34	73.2	
	Sadhna	0.29	79.2	
	Sudha	0.32	85.3	

Table 10 : Variation of some important phytochemicals in coriander seed EO across different geographical locations.

	Percentage of Important Phytochemicals in Coriander Seed Oil from Different Geographical Locations									
Constituent	Bangladesh [28]	Bulgari[77]	India [78]	Iran [58,71]	Morocco [79]	Pakistan [20]	Poland [80]	Romania [81]	Russia [82]	Turkey [60,83]
α-Thujene	-	-	-	-	0.10	0.02	-	-	-	-
α-Pinene	-	7.14	2.81	3.3	7.69	1.63	5.03	1.62	6.44	3.06
α-Terpineol	-	0.66	-	-	0.54	-	0.63	-	0.81	-
β-Pinene	1.8	1.15	0.48	0.4	0.93	0.23	0.48	0.71	0.34	0.25
β-caryophyllene	0.3	0.17	-	-	0.14	0.07	-	0.44	-	-
p-Cymene	1.3	2.67	0.42	2	0.03	1.12	0.54	8.000	7.44	0.39
γ-Terpinene	14.4	7.35	0.15	9.3	11.59	4.17	3.80	5.236	-	0.20
Borneol	0.3	0.44	0.14	-	0.42	0.18	-	-	-	-
Camphor	-	8.30	-	0.2	6.98	0.38	3.90	6.01	7.94	3.56
Camphene	-	2.37	-		1.07		0.64		1.09	-
Citronella	1.3	-	-	0.3	-	0.65	-	-	-	-
Decanal	0.1	-	0.20	0.4	0.13	0.14	-	-	-	0.54
Geraniol	1.9	-	24.51	1.9	2.79	-	1.07		0.11	2.37
Geranyl acetate	17.6	5.05		4.0	-	4.99	2.13	1.423	3.19	2.77
Limonene	0.4	5.19		0.3	3.24	0.26	2.58	9.628	3.29	0.27
Linalool	37.7	50.16	57.52	70.1	48.41	69.60	78.45	45.387	59.92	75.26
Myrcene	0.6	1.78	0.37	0.2	1.16	0.18	0.47	1.504	0.20	0.42
Neryl acetate	-	-		6.9	6.47	-	-	-	-	-
Sabinene	0.2	0.73		0.2	0.58	0.12	-	-	-	0.10
Thymol	-	-		0.2	0.06	0.41	-	0.376	-	-
Undecanal	0.1	-	0.13	-	0.06	0.41	-	-	-	-

7. Pharmacological effects

7.1. Anxiolytic effect

The anxiolytic effect of aqueous extract (50, 100, 200 mg/kg, ip) was examined in male albino mice using elevated plus- maze as an animal model of anxiety. In the elevated plus-maze, aqueous extract at 200 mg/kg showed an anxiolytic effect by increasing the time spent on open arms and the percentage of open arm entries, compared to control group [65].

The anxiolytic effect of *Coriandrum sativum* (CS) aqueous extract was evaluated in mice. The antianxiety effect was assessed by elevated plus maze (EPM). In EPM, 50, 100, and 200 mg/kg of CS were significantly ($P<0.001$) increases the number of entries in open arms compared to control. The time spent in open arms also increased in all the doses of CS extract significantly [66].

The anti-anxiety activity of hydroalcoholic extract of *Coriandrum sativum* was studied using different animal models (elevated plus maze, open field test, light and dark test and social interaction test) of anxiety in mice. Diazepam (0.5 mg/kg) was used as astandard drug and hydroalcoholic extract of *Coriandrum sativum* fruit was used in dose of (50, 100 and 200 mg/kg) to study the antianxiety effect. Results revealed that the extract of *Coriandrum sativum* at 100 and 200 mg/kg dose produced anti-anxiety effects almost similar to diazepam, while, at 50 mg/kg dose, it did not produce anti-anxiety activity in all models [67].

The anxiolytic effect of the aqueous extract of *Coriandrum sativum* seed and its effect on spontaneous activity and neuromuscular coordination were evaluated in mice. The anxiolytic effect of aqueous extract (10, 25, 50, 100 mg/kg, ip) was examined in male albino mice using elevated plus-maze as an animal model of anxiety. The effects of the extract on spontaneous activity and neuromuscular coordination were assessed using Animex Activity Meter and rotarod. In the elevated plus-maze, 100 mg/kg of the aqueous extract showed an anxiolytic effect by increasing the time spent on open arms and the percentage of open arm entries, compared to control group. Aqueous extract at 50, 100 and 500 mg/kg significantly reduced spontaneous activity and neuromuscular coordination, compared to control group [68-69].

The effect of the hydroalcoholic extract of *Coriandrum sativum* leaves on the exploratory behaviour pattern and locomotor activity was investigated in mice. Elevated plus maze (EPM) and open field test (OFT) were used to assess the anxiolytic activity of the extracts. Diazepam (1 mg/kg) was used as standard anxiolytic agent. The 200 and 400 mg / kg body weight of the crude dried extract and diazepam produced highly significant ($P<0.01$) anxiolytic effects, in a

dose-dependent manner, by increasing the time spent on, and the number of entries into the open arms of the EPM and by an increase in the locomotion by mice in the OFT. However, in lower doses the extract did not affect the locomotor activity [70].

The effects of fresh *Coriandrum sativum* leaves (CSL) on cognitive functions, total serum cholesterol levels and brain cholinesterase activity was investigated in mice. CSL (5, 10 and 15% w/w of diet) was fed orally with a specially prepared diet, for 45 days consecutively to mice. Elevated plus-maze and passive avoidance apparatus were used as the exteroceptive behavioral models for testing memory. Diazepam, scopolamine and ageing-induced amnesia were used as the interoceptive behavioral models. CSL (5, 10 and 15% w/w of diet) produced a dose-dependent improvement in memory scores of young as well as aged mice. CSL also reversed successfully the memory deficits induced by scopolamine (0.4 mg/kg, ip) and diazepam (1 mg/kg, ip). Brain cholinesterase activity and serum total cholesterol levels were considerably reduced by CSL administration in daily diets for 45 days [71-72].

7.2. Sedative-hypnotic effects

The aqueous, hydroalcoholic extracts and essential oil of coriander seeds possessed sedative-hypnotic activity. The aqueous, hydroalcoholic extracts and essential oil of coriander seeds (100, 200, 400 and 600 mg/kg) were intraperitoneally administered to male albino mice, 30 minutes before pentobarbital injection (40 mg/kg). Latency to sleep and sleep duration were recorded. Aqueous extract prolonged pentobarbital-induced sleeping time at 200, 400 and 600 mg/kg. Hydroalcoholic extract at doses of 400 and 600 mg/kg increased pentobarbital induced sleeping time compared to saline-treated group. The essential oil increased pentobarbitalinduced sleeping time only at 600 mg/kg [74].

The sleep-prolonging effect of *Coriandrum sativum* was investigated in mice. The hydroalcoholic extract (HAE) and its three fractions, water (WF), ethyl acetate (EAF) and N-butanol (NBF) were prepared from *Coriandrum sativum* aerial parts and administrated to mice. The HAE, EAF and NBF significantly prolonged sleep duration. Only the NBF was significantly decreased sleep latency. No decrease in the neuronal surviving was observed either by HAE or by its fractions. The data indicated that *Coriandrum sativum* exerted sleep prolonging action without major neurotoxic effect [75].

7.3. Anticonvulsant effect

The effects of hydroalcoholic extract of aerial parts of the plants (100, 500 and 1000 mg/kg) on brain tissues oxidative damages following seizures induced by pentylenetetrazole (PTZ) was investigated in rats. The extract significantly increased the MCS (latencies to the first

minimal clonic seizures) and GTCS (latencies to the first generalized tonic-clonic seizures) ($P<0.01$, $P<0.001$) following PTZ-induced seizures. The malondialdehyde (MDA) levels in both cortical and hippocampal tissues of PTZ group were significantly higher than those of the control animals ($P<0.001$). Pretreatment with the extract prevented elevation of the MDA levels ($P<0.010$ - $P<0.001$). Following PTZ administration, a significant reduction in total thiol groups was observed in both cortical and hippocampal tissues ($P<0.050$). Pre-treatment with the 500 mg/kg of the extract caused a significant decreased in total thiol concentration in the cortical tissues ($P<0.010$). Accordingly, the hydroalcoholic extract of the aerial parts of *Coriandrum sativum* possessed significant antioxidant and anticonvulsant activities [75].

Intraperitoneal injection of decoction and maceration extracts increased the latency of the convulsions induced by PTZ in albino mice, but failed to produce complete protection against mortality. The anticonvulsant activities of high dose extracts were similar to that of phenobarbital at a dose of 20 mg/kg in the PTZ test. In the maximal electroshock seizures, the aqueous extracts of seeds (at a dose of 0.5 g/kg) and the ethanolic extract (at doses of 3.5 and 5 g/kg) decreased the duration of tonic seizures by 22.30%, 30.43% and 36.96%, respectively [76-77].

7.4. Anti-microbial effect

7.4.1. Inhibition of Bacteria

The antibacterial effect of aqueous and ethanolic extracts of different coriander parts was studied against nine different pathogenic bacteria isolated from urine, blood, stool and cerebraspinal fluid of different patients (Burkhella capacia, Escherichia coli, Enterobacter cloacae, Gamella morbillorum, α-Haemolytic streptococci, Klebsiella pneumonia, Proteus mirabilis, Streptococcus pneumonia, and Salmonella typhi). Cold aqueous extract of coriander seeds had inhibitory effect against some tested bacteria. On the other hand, ethanolic extracts of seeds, leaves and stems showed wide range of antibacterial activity and the highest values for inhibition zone was recorded against Klebsiella pneumoniae and Proteus mirabilis [83].

Essential oils from commercial samples of coriander were assayed for their antibacterial and antifungal activities. Twenty-five genera of bacteria and one fungal species (Aspergillus niger) were used as test organisms. The essential oils showed a high degree of inhibition against all the tested microorganisms [84].

The antimicrobial activity of ethanol, methanol, acetone, chloroform, hexane and petroleum ether extracts of *Coriandrum sativum* was investigated against infectious pathogenic bacteria such as *E. coli*, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella Pneumonia;

and many fungi including Aspergillus niger, Candida albicans, Candida kefyr and Candida tropicalis using agar well diffusion method. The methanol extract of *Coriandrum sativum* showed more antibacterial activity against Staphylococcus aureus (zone of diameter 12.17±0.29mm) and Klebsiella pneumonia zone (12.17±0.15mm), while, it showed more antifungal activity against Candida albicans (zone of diameter 14.20±0.20mm) and Aspergillus niger (10.10±0.10mm). It appeared that methanol extract showed a varying degree of antibacterial and antifungal effects more than ethanol, acetone, chloroform, hexane and petroleum ether extracts [85].

The antibacterial potential of the leaf essential oil, petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Coriandrum sativum* were studied against human pathogenic bacteria (Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa and Serratia marcescens) by agar well diffusion method. Leaf essential oil as well as leaf ethyl acetate, chloroform and methanol extracts of *Coriandrum sativum* exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity was quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions [86].

The antibacterial effect of *Coriandrum sativum* essential oil against Gram-positive and Gram-negative bacteria was evaluated using classical microbiological techniques concomitantly with the use of flow cytometry for the evaluation of cellular physiology. The results showed that coriander oil has an effective antimicrobial activity against all tested bacteria. Propidium iodide incorporation and concomitant loss of all other cellular functions such as efflux activity, respiratory activity and membrane potential seem to suggest that the primary mechanism of action of coriander oil was membrane damage, resulted in cell death [87].

Aliphatic (2E)-alkenals and alkanals isolated from the fresh leaves of the *Coriandrum sativum* were found to possess bactericidal activity against Salmonella choleraesuis ssp. choleraesuis ATCC 35640. (2E)-Dodecenal (C12) was the most effective against this food-borne bacterium with the minimum bactericidal concentration (MBC) of 6.25 microg/ml (34 microM), followed by (2E)-undecenal (C11) with an MBC of 12.5 microg/ml (74 microM). The time-kill curve study showed that these alpha, beta-unsaturated aldehydes were bactericidal against S. choleraesuis at any growth stage and that their bactericidal action came in part from the ability to act as nonionic surfactants [88-89].

Twelve essential oils were tested in vitro for antimicrobial activities against several strains of Campylobacter jejuni, a pathogen causing food-borne diseases worldwide. Coriander oil exhibited the strong antimicrobial activity against all tested strains. In evaluating the antimicrobial potency of coriander oil against C. jejuni on beef and chicken meat at 4 degrees C and 32 degrees C, it reduced the bacterial cell load in a dosedependent manner. The type of meat and temperature did not influence the antimicrobial activity of the oil [90]. Antimicrobial effect of essential oils from the seeds of *Coriandrum sativum* was studied against gram-positive bacteria, gram-negative bacteria and Saccharomyces cerevisiae. Essential oil appeared effective against Listeria monocytogenes [91].

The antibacterial potential of two commercial essential oils (EOs) from *Coriandrum sativum* was studied against vaginal clinical strains of bacteria and yeast. Antimicrobial activities were determined using macro-diffusion (disc, well) and micro-dilution method against twelve clinical strains of bacteria: Escherichia coli, Proteus mirabilis, *S. aureus* and Enterococcus sp., *S. aureus* ATCC 25923, ATCC 6538 and Escherichia coli 25922 and two clinical Candida albicans ATTC 10231 strains. An antimicrobial effect of EOs was strain specific. Bactericidal activity was higher for coriander EO (MICs 0.4 – 45.4 µl/ml) against almost all tested bacteria, except multiple resistant strains of Eneterococcus sp. and Proteus sp. It showed low fungicidal activity [92].

Antimicrobial activities of essential oils were evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* by microdilution method. The essential oils of *Coriandrum sativum* fruits obtained by hydrodistillation (HD EO) showed greater activity against *Staphylococcus aureus* and *Candida albicans* than that obtained by microwave-assisted hydrodistillation (MAHD EO). Moreover, their activities against *E. coli* and *P. aeruginosa* were the same with minimum inhibitory concentration, MIC 0.781 and 6.25 µl/ml, for HD EO and MAHD EO respectively [56].

7.4.2. Inhibition of Fungi

The antifungal activity of essential oil from *Coriandrum sativum* fruits was evaluated against Microsporum canis and Candida spp. by the agar-well diffusion method and the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were established by the broth microdilution method. The essential oil induced growth inhibition zones of 28 ± 5.42 and 9.25 ± 0.5mm for M. canis and Candida spp. respectively. The MICs and MFCs for M. canis strains ranged from 78 to 620 and 150 to 1.250 µg/ml, and the MICs

and MFCs for Candida spp strains ranged from 310 to 620 and 620 to 1.250 μg/ml, respectively [97].

The antifungal activity of coriander essential oil was studied on germ tube formation, and the potential synergism with amphotericin B were also studied. Coriander essential oil has a fungicidal activity against the Candida strains tested, with MLC values equal to the MIC value and ranging from 0.05 to 0.4% (v/v). Flow cytometric evaluation of BOX, PI and DRAQ5 staining indicated that the fungicidal effect was a result of cytoplasmic membrane damage and subsequent leakage of intracellular components such as DNA. Also, concentrations bellow the MIC value caused a marked reduction in the percentage of germ tube formation for C. albicans strains. A synergetic effect between coriander oil and amphotericin B was also recorded against C. albicans strains, while for C. tropicalis strain only an additive effect was observed [98].

The antifungal activity and mode of action of the essential oils (EO) from *Coriandrum sativum* leaves were evaluated against Candida spp. In addition, the molecular targets affected in whole-genome expression in human cells was also studied. The EO showed anticandidal effects. *Coriandrum sativum* EO may bind to membrane ergosterol, increasing ionic permeability and causing membrane damage leading to cell death, but it did not act on cell wall biosynthesis-related pathways. The EO also inhibited Candida biofilm adherence to a polystyrene substrate at low concentrations, and decreased the proteolytic activity of Candida albicans at the minimum inhibitory concentration. In addition, the EO and its selected active fraction had low cytotoxicity on human cells [99]. *Coriandrum sativum* essential oil possessed antifungal activity against Candida species isolates from the oral cavity of patients with periodontal disease. 2-hexen-1-ol, 3-hexen-1-ol and cyclodecane were determined as the active constituents in the oil [100].

7.5. Effects against invertebrates

The efficacy and tolerability of 6% coriander oil was tested in unguentum leniens in the treatment of interdigital tinea pedis. The study was performed on 40 participants. 6% coriander oil showed highly significant improvement of the clinical signs in unguentum leniens ($p < 0.0001$) during the entire observation period. The number of positive fungal cultures also decreased ($p = 0.0654$). The tolerability of the tested substances was good [101].Commercial essential oils from 28 plant species were tested for their nematicidal activities against the pine wood nematode, Bursaphelenchus xylophilus. The best nematicidal activity against B. xylophilus was achieved with essential oils of coriander (*Coriandrum sativum*) [102].In vitro anthelmintic activities of crude aqueous and hydro-alcoholic extracts of the seeds of

Coriandrum sativum were investigated on the egg and adult nematode parasite Haemonchus contortus. The aqueous extract of *Coriandrum sativum* was also investigated for in vivo anthelmintic activity in sheep infected with Haemonchus contortus. Both extracts of *Coriandrum sativum* inhibited hatching of eggs completely at a concentration less than 0.5 mg/ml. ED50 of aqueous extract of *Coriandrum sativum* was 0.12 mg/ml while that of hydroalcoholic extract was 0.18 mg/ml. There was no

statistically significant difference between aqueous and hydroalcoholic extracts ($p>0.05$). The hydroalcoholic extract showed better in vitro activity against adult parasites than the aqueous one. For the in vivo study, sheep were artificially infected with Haemonchus contortus, crude aqueous extract of *Coriandrum sativum* was given at 0.45 and 0.9 g/kg dose levels. Efficacy was tested by faecal egg count reduction (FECR) and total worm count reduction (TWCR). On day 2 post treatment, significant FECR was detected in groups treated with higher dose of *Coriandrum sativum* ($p<0.05$) and albendazole ($p<0.001$). Significant ($p<0.05$) TWCR was detected only for higher dose of *Coriandrum sativum* compared to the untreated group. Reduction in male worms was higher than female worms. Treatment with both doses of *Coriandrum sativum* did not help the animals to improve or maintain their PCV, while those treated with albendazole showed significant increase in PCV ($p<0.05$) [103].

The antiparastic efficacy of *Coriandrum sativum* essential oils was studied by two in vitro assays on Haemonchus contortus using egg hatch test (EHT) and larval development test (LDT). *Coriandrum sativum* essential oils exhibited a dose-dependent effect in the EHT, inhibiting 81.2% of H. contortus larvae hatching, at a concentration of 2.5 mg/ml. The effective concentration to inhibit 50% (EC50) of egg hatching was 0.63 mg/ml. In LDT, *Coriandrum sativum* at concentration of 10 mg/ml inhibited 97.8% of H. contortus larval development [104].

The in vitro effect of fractions from *Coriandrum sativum* (coriander) on promastigotes and amastigotes of *Leishmania infantum* was studied in addition to its toxicity against the murine monocytic cells RAW 264.7. All fractions were effective against *L. infantum* promastigotes and did not differ from the positive control pentamidine ($p>0.05$). However, the *Coriandrum sativum* methanol fraction, was the most effective against amastigotes and did not differ from the positive control amphotericin B ($p>0.05$) [105].

The biological activity of essential oil of *Coriandrum sativum* seeds was tested against adult Tribolium confusum Duval (Coleoptera: Tenebrionidae) and Callosobruchus maculatus F. (Coleoptera: Bruchidae) in a series of laboratory experiments. The mortality of 1-7 day old

adults of the insect pests increased with concentration from 43 to 357 µl/l air and with exposure time from 3 to 24 h. In the probit analysis, LC50 values showed that C. maculatus (LC50 = 1.34 µl/l air) was more susceptible than T. confusum (LC50 = 318.02 µl/l air) to seed essential oil of *Coriandrum sativum* [106].

The essential oil (EO) of the fruits of *Coriandrum sativum* was evaluated for its larvicidal and repellent activities against Aedes albopictus Skuse (Diptera: Culicidae). *Coriandrum sativum* EO exerted toxic activity against A. albopictus larvae: LC50 was 421 ppm, while LC90 was 531.7 ppm. Repellence trials highlighted that *Coriandrum sativum* EO was a good repellent against A. albopictus, RD50 was 0.0001565 µl/cm2 of skin, while RD90 was 0.002004 µl/cm2 . At the highest dosage (0.2 µl/cm2 of skin), the protection time achieved with *Coriandrum sativum* essential oil was higher than 60 min [107].

The leaf oil had significant toxic effects against the larvae of Aedes aegypti with an LC_{50} value of 26.93 ppm and an LC_{90} value of 37.69 ppm, and the stem oil has toxic effects against the larvae of A. aegypti with an LC_{50} value of 29.39 ppm and an LC_{90} value of 39.95 ppm [58]. The seed oil had significant toxic effects against the larvae of Aedes aegypti with an LC50 value of 21.55 ppm and LC90 value of 38.79 ppm. The major components in the essential oil of coriander play an important role as immunotoxicity on the A. aegypti [53].

7.6. Antioxidant effect

Coriandrum sativum has a very effective antioxidant profile showing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, lipoxygenase inhibition, phospholipid peroxidation inhibition, iron chelating activity, hydroxyl radical scavenging activity, superoxide dismutation, glutathione reduction and antilipid peroxidation due to its high total phenolic content [61, 108-110]. The fresh juice exhibited high antioxidant activities, evidenced by its ability to scavenge hydroxyl- and superoxide-radicals, high reducing power, and protection against biological macromolecular oxidative damage and by increasing the level of glutathione [111]. Among the leaf essential oil and leaf petroleum ether, chloroform, ethyl acetate and methanol extracts of *Coriandrum sativum* studied, methanol extract and leaf essential oil showed potent scavenging activity on 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical [86].

The in vitro antioxidant potential of aqueous leaf extracts of *Coriandrum sativum* leaves was determined qualitatively. Enzymatic antioxidant analysis in the extract of *Coriandrum sativum*: Catalase (µ/moles of H2O2 decomposed/min/g protein, 1 unit = µ/moles of H2O2 decomposed/min/g protein) = 3.135; peroxidase (Unit/mg protein, 1 unit = mg of GSH utilized

/ min)= 2.508×10 3 ; ascorbate oxidase (μ mole/ml, 1 unit = 0.01 O.D change/min)= 100.262 [48].

The amount of the total phenolic contents (TPC) in the aqueous extract of Sudanese *Coriandrum sativum* was 1654 ± 3.4 mg GAE/L. The results of the in vitro antioxidant activity by β -carotene/linoleic acid assay, showed that *Coriandrum sativum* aqueous extract had strong antioxidant activity (84.6% at 400ug/ml), when compared with the standard reference Tert-butyl hydroquinone (TBHQ) (99.5% at the same concentrations). The aqueous extract showed strong DPPH free-radical scavenging activity (88.5% at 400ug/ml), compared with the standard reference (TBHQ) (99.73% at the same concentration) [55]. The pre-feeding of rats with coriander seed powder (CSP) at 10% level was found to reduce the experimentallyinduced (HCH-induced) rise in conjugated dienes, hydroperoxide and malondialdehyde (MDA) contents in the liver [112].

The antiperoxidative effect of coriander seeds was studied in rats administered high fat diet. Significant decrease in the levels of lipid peroxides, free fatty acids and glutathione were observed when compared to control group whereas the activity of antioxidant enzymes was increase [113].

Phenolic content and antioxidant activity were evaluated in the coriander leaves and seeds. The results revealed that serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were significantly increased in thioacetamide (TAA)-induced hepatotoxicity groups compared to the normal control. Oxidative stress was manifested by a significant rise in nitric oxide (NO), thiobarbituric acid reactive substance (TBARS) levels and myloperoxidase (MPO) activities in the liver tissues, in induction group compared with the control. Rats fed with coriander leaves and seeds showed a decrease in the serum ALT, AST and ALP activities and in the liver NO and TBARS levels as compared to the induction group. Histopathological study revealed that coriander feeding attenuated TAA-induced hepatotoxicity in rats [114].

The hydroalcohol extract of *Coriandrum sativum* leaves at the dose of 1 mg/ml was subjected to a series of in vitro assays (2, 2'- diphenyl-1-picrylhydrazyl, lipid peroxidation by thiobarbituric acid, reducing power and nitric oxide (NO) radical scavenging) in order to study its antioxidant efficacy. The amount of flavonoids in 70% ethanol extract was found to be 44.5 μg and that of the total phenols was 133.74 μg gallic acid equivalents per mg extract. The extracts of the leaves showed metal chelating power, with IC50 value of 368.12 μg/ml, whereas that of standard EDTA was 26.7 μg/ml. The IC50 values for 2, 2'-azino-bis (3-

ethylbenzothiazoline-6-sulphonic acid radical scavenging was 222 µg/ml, whereas that of standard ascorbic acid was 22.6 µg/ml. The NO scavenging activity of the extract of the leaves showed IC50 value of 815.6 µg/ml; at the same time the standard BHA had 49.1 µg/ml. All the plant extracts provided DNA damage protection; however, the protection provided at the dose of 8 µg/ml was comparable to that of standard gallic acid. The *Coriandrum sativum* leaf extract was able to prevent in vitro lipid peroxidation with IC50 value of 589.6 µg/ml, whereas that of standard BHA was 16.3 µg/ml. The results also showed significant ferric reducing power indicating the hydrogen donating ability of the extract [64].

Table 11 : Antioxidant profile of C. sativum seed extract.

Metabolite	Amount in mg/g Dry Weight of Sample	Method of Analysis	References
Caffeic Acid	0.08	HPLC method	[**111**]
Ellagic Acid	0.162	HPLC method	[**111**]
Gallic Acid	0.173	HPLC method	[**111**]
Kaempferol	0.233	HPLC method	[**111**]
Oxidized Ascorbate	0.15	Spectrophotometric method	[**112**]
Reduced Ascorbate	0.136	Spectrophotometric method	[**112**]
Riboflavin	0.0046	Spectrophotometric method	[**113**]
Tocopherol	0.181	Spectrophotometric method	[**113**]
Total Ascorbate	0.287	Spectrophotometric method	[**112**]
Total Polyphenol	18.7	Folin-Ciocalteau method	[**114**]
Quercetin	0.608	HPLC method	[**111**]

7.7. Anti-inflammatory and analgesic effects

The anti-inflammatory and anti-granuloma activities of *Coriandrum sativum* hydroalcoholic extract (CSHE) was studied in experimental models. The anti-inflammatory activity of CSHE was evaluated using carrageenan-induced paw edema model and the anti-granuloma activity of CSHE was evaluated using the subcutaneous cotton pellet implantation-induced granuloma formation and stimulation of peritoneal macrophages with complete Freund's adjuvant. Serum tumor necrosis factor-α (TNF-α), IL-6, IL-1 β levels, and peritoneal macrophage expression of TNF-R1 were evaluated as markers of global inflammation. CSHE at the highest dose (32 mg/kg) produced a significant reduction ($p<0.05$) in paw edema after carrageenan administration. CSHE treatment also reduced dry granuloma weight in all treated animals.

Serum IL-6 and IL-1 β levels were significantly ($p<0.05$) lower in the CSHE (32 mg/kg)-treated group as compared to control. Although there was an increase in serum TNF-α level in the CSHE-treated group as compared to control, but TNF-R1 expression on peritoneal macrophages was reduced [124].

The anti-inflammatory and analgesic effects of *Coriandrum sativum* seeds were evaluatedin animal model. Carrageenan test was used for evaluation of anti-inflammatory effect, while, writhing and formalin tests were used for evaluation of analgesic effects. The results showed that coriander had no anti-inflammatory effect in carrageenan test. In writhing test, only the essential oil (4ml/100g, po) had a significant effect ($p<0.01$). Total extract, polyphenolic extract and essential oil of coriander, had significant effect in both phases of formalin test [125].

The role of opiate system in the antinociceptive effects of *Coriandrum sativum* (CS) was studied in acute and chronic pain in mice using hot plate (HP), tail flick (TF) and formalin (FT) tests, and its effects were compared with dexamethasone (DEX) and stress (ST). CS (125 250, 500 and 1000 mg/Kg IP), DEX (0.5, 1 and 2 mg/Kg IP), vehicle (VEH) or swim stress were used 30 min before the pain evaluation tests. Acute and chronic pain was assessed by HP, TF and FT models. In addition, Naloxone (NAL, 2 mg/Kg, IP) was injected 15 min before the CS extract administration in order to assess the role of opiate system in the antinociception of CS. Results indicated that CS, DEX and ST have analgesic effects ($p<0.01$) in comparison with the control group and higher dose of CS was more effective ($p<0.001$). Pretreatment with NAL modulated the antinociceptive effects of CS in all models ($p<0.001$). The findings showed an interaction between antinociceptive effects of CS and opiate system [126].The analgesic effects of the extract were assessed using hot plate method. Aqueous extract at 50, 100 and 200 mg/kg significantly produce analgesic activity compared to control group [65].

The anti-inflammatory activity of ethanolic extract of *Coriandrum sativum* was studied using carrageenan induced paw edema in albino rats. Ethanolic leaf extract of *Coriandrum sativum* was used as 200 and 400mg/kg. Oral administration of *Coriandrum sativum* ethanolic leaf extract of 400mg/kg/ip was more effective anti-inflammatory than 200mg/kg/ip [127].The antiarthritic activity of *Coriandrum sativum* seed hydroalcoholic extract (CSHE) was evaluated in adult rats by using two experimental models (formaldehyde and complete Freund's adjuvant (CFA) induced arthritis). The expression of pro-inflammatory cytokines (predominantly contributed by macrophages) was also evaluated. TNF- α level was estimated in serum. TNF-R1, IL-1 β and IL-6 expression were also analysed in the synovium. CSHE produced a dose dependent inhibition of joint swelling as compared to control animals in both formaldehyde

and CFA induced arthritis. Although there was a dose dependent increase in serum TNF-α levels in the CSHE treated groups as compared to control, the synovial expression of macrophage derived pro-inflammatory cytokines/cytokine receptor was found to be lower in the CSHE treated groups as compared to control [128]. The protective effects of *Coriandrum sativum* on acetic acid-induced colitis was investigated in rats. Treatment was carried out using three increasing doses of extract (250, 500, 1000 mg/kg) and essential oil (0.25, 0.5, 1 ml/kg) of coriander started 2 h before colitis induction and continued for a five-day period. Colon biopsies were taken for weighting, macroscopic scoring of injured tissue, histopathological examination and measuring myeloperoxidase (MPO) activity. Colon weight was decreased in the groups treated with extract (500 and 1000 mg/kg) and essential oil (0.5 ml/kg) compared to the control group. Regarding MPO levels, ulcer severity and area as well as the total colitis index, the results indicated that the treatment with extract and essential oil induced meaningful alleviation of colitis [129].

The anti-inflammatory ability of the aerial parts (stem and leaf) of *Coriandrum sativum* was investigated on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. The molecular mechanism underlying the pharmacological properties of *Coriandrum sativum* was also investigated. Ethanolic extracts from both stem and leaf of *Coriandrum sativum* (CSEE) significantly decreased LPS-induced nitric oxide and prostaglandin E2 production as well as inducible nitric oxide synthase, cyclooxygenase-2, and pro-interleukin1beta expression. Moreover, LPS-induced IkappaB-alpha phosphorylation and nuclear p65 protein expression as well as nuclear factor-kappaB (NF-kappaB) nuclear protein-DNA binding affinity and reporter gene activity were dramatically inhibited by aerial parts of CSEE. Exogenous addition of CSEE stem and leaf significantly reduced LPS-induced expression of phosphorylated mitogen-activated protein kinases (MAPKs). The authors concluded that CSEE had a strong anti-inflammatory property which inhibited pro-inflammatory mediator expression by suppressing NF-kappaB activation and MAPK signal transduction pathway in LPS-induced macrophages [131].

The anti-inflammatory potency of coriander oil was investigated in the ultraviolet (UV) erythema test in vivo. 40 volunteers were enrolled in this monocentric, randomized, placebo-controlled double-blind study. The test areas on the back were irradiated with the 1.5 fold minimal erythema dose UV-B. Subsequently, the test areas were treated under occlusion for 47 hours with a lipolotion containing 0.5% or 1.0% essential coriander oil. Hydrocortisone (1.0%) and betamethasone valerate (0.1%) in the vehicle were used as positive controls. The

vehicle was used as placebo. The effect of the test substances on the UV-induced erythema was measured photometrically after 48 hours. Additionally, the skin tolerance of the test preparations was assessed on nonirradiated skin. Compared to placebo, the lipolotion with 0.5% coriander oil significantly reduced the UVinduced erythema, but it was not as effective as hydrocortisone. The skin tolerance of both coriander oil concentrations was excellent [132].

7.8. Antidiabetic effect

Administration of coriander seeds (5g/day) to NIDDM patients for 60 days significantly decreased lipid peroxidation, protein oxidation, decreased activity of erythrocyte catalase (CAT), increased serum β carotene, vitamin A, E and C in NIDDM diabetics. The treatment was also increased the activity of erythrocyte antioxidant enzyme i.e. glutathione-S-transferase (GST) and reduced glutathione content (GSH) in the treated diabetics [134].

The hypoglycemic effect of *Coriandrum sativum* was studied clinically in patients with type-2 diabetes mellitus. After assaying fasting plasma and urinary glucose, 10 patients of type-2 diabetes mellitus with no previous medication, 10 patients of type-2 diabetes mellitus taking oral hypoglycemic agents with history of inadequate control and six control subjects were given low (2.5 g tid) and high (4.5 g tid) doses of aqueous and alcoholic extracts of *Coriandrum sativum* for 14 days. On 15th day, blood and urine samples were taken for glucose estimation. *Coriandrum sativum* has significant hypoglycemic activity in high dose and can be successfully combined with oral hypoglycemic agents in type-2 diabetic patients whose diabetes was not controlled by oral hypoglycemic drug alone [135-136].

The hypoglycemic activity of methanolic extracts of leaves of *Coriandrum sativum* was evaluated in rats. The methanolic extract showed significant dose dependant decrease in blood glucose level at a dose of 200 and 400 mg/kg. It also decreased the lipid parameters such as total cholesterol, LDL, HDL, VLDL and TG when compared with diabetic control. SGOT and SGPT were reduced dose dependently [137].

Coriander incorporated into the diet (62.5 g/kg) and drinking water (2.5 g/l, prepared by 15 min decoction) reduced hyperglycaemia of streptozotocin-diabetic mice. An aqueous extract of coriander (1 mg/ml) increased 2-deoxyglucose transport (1.6-fold), glucose oxidation (1.4-fold) and incorporation of glucose into glycogen of isolated murine abdominal muscle (1.7-fold) comparable with 10-8 M-insulin. In acute 20 min tests, 0.25-10 mg/ml aqueous extract of coriander evoked a stepwise 1.3-5.7-fold stimulation of insulin secretion from a clonal B-cell line. This effect was abolished by 0.5 mM-diazoxide. The effect of extract was potentiated by 16.7 mM-glucose and 10 mM-L-alanine but not by 1 mM-3-isobutyl-1-methylxanthine. Insulin

secretion by hyperpolarized B-cells (16.7 mM-glucose, 25 mM-KCl) was further enhanced by the presence of extract. Activity of the extract was found to be heat stable, acetone soluble and unaltered by overnight exposure to acid (0.1 M-HCl) or dialysis to remove components with molecular mass<2000 Da. Activity was reduced by overnight exposure to alkali (0.1 M-NaOH). Sequential extraction with solvents revealed insulin-releasing activity in hexane and water fractions indicating a possible cumulative effect of more than one extract constituent [138].

The ethanol extract of *Coriandrum sativum* seeds was investigated for its effects on insulin release from the pancreatic beta cells in streptozotocin-induced diabetic rats. Pancreatic sections of 5 microm were processed for examination of insulin-releasing activity using an immunocytochemistry method. The results showed that administration of the ethanol extract (200 and 250 mg/kg, ip) exhibited a significant reduction in serum glucose. On the other hand, administration of streptozotocin decreased the number of beta cells with insulin secretory activity in comparison with intact rats, but treatment with the coriander seed extract (200 mg/kg) increased significantly the activity of the beta cells in comparison with the diabetic control rats [140].

The antidiabetic and antioxidant effects of *Coriandrum sativum* (CS) were studied in alloxan-induced diabetic rats. The extracts of CS in alloxan-induced diabetic rats were found to significantly lower blood glucose levels. Antidiabetic activity of the CS extracts was comparable with the clinically available drug glibenclamide. The levels of serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol were lower in the extract treated group and high-density lipoprotein cholesterol was higher than the diabetic control rats. The extracts of CS exhibited strong scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl free radical and inhibited lipid peroxidation. The free radical scavenging effect of the extracts was comparable with that of the reference antioxidants. Furthermore, it also showed an improved antioxidant potential as evidenced by decreased lipid peroxidation and a significant increase in the activity of various antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase in the liver of diabetic rats [50].

7.9. Anticancer effect

Brine shrimp lethality bioassay revealed that coriander LC50 was 2.25 mg/ml [92]. The anticancer activities of *Coriandrum sativum* root, leaf and stem, as well as its effect on cancer cell migration, and its protection against DNA damage, with special focus on the roots was evaluated. The ethyl acetate extract of *Coriandrum sativum* roots showed the highest

antiproliferative activity on MCF-7 cells (IC50 = 200.0 ± 2.6 µg/ml), had the highest phenolic content and FRAP and DPPH scavenging activities among the extracts. Ethyl acetate extract of *Coriandrum sativum* root inhibited DNA damage and prevented MCF-7 cell migration induced by H2O2, suggesting its potential in cancer prevention and metastasis inhibition. The extract exhibited anticancer activity in MCF-7 cells by affecting antioxidant enzymes possibly leading to H2O2 accumulation, cell cycle arrest at the G2/M phase and apoptotic cell death by the death receptor and mitochondrial apoptotic pathways [147].

The antitumor and immunomodulating activities of aqueous and methanol extracts of *Coriandrum sativum* (leaf and seed) was investigated in vitro. The aqueous extract of *Coriandrum sativum* (leaf), caused significant (P<0.05) 24, 39 percent L5178Y-R lymphoma cells toxicity at 31.2 µg/ml (MIC), whereas the methanol extract of *Coriandrum sativum* (seed and leaf) caused 40 and 31 percent cytotoxicity at 7.8, 62.5 µg/ml (MICs), respectively. In addition, *Coriandrum sativum* leaf aqueous extract stimulated significant (P<0.01) 14 to 45 percent splenic cells lymphoproliferation at 7.8 to 125 µg/ml respectively. The methanol extracts of *Coriandrum sativum* leaf extract caused significant (P<0.01) 43 to 59 percent lymphoproliferation at the tested concentrations. Furthermore, *Coriandrum sativum* aqueous extracts were significantly (P<0.01) reduce up to 100% nitric oxide production by LPS-stimulated macrophages [148].

Three different cell lines BMK (kidney), KHOS-2405 (bone), and WRL-68 (liver) were used to determine cytotoxicity. Cells were treated with different *Coriandrum sativum* (Cilantro) concentrations (0.125%, 0.25%, 0.5%, 1%, 1.5%, 2% and 2.5%), for 24 hours. After this time, cytotoxic studies were performed. An embryotoxicity study was done using fertile chicken eggs (Gallus gallus) inoculating with *Coriandrum sativum* concentrations (0.125%, 0.25%, 0.5%, 1%, 1.5%, 2% and 2.5%); incubated for 48 hours and observe them using an electronic microscope to check the effects. The three lines showed decreased proliferation and number of cells proportional to the concentrations. The cell cycle analysis showed that *Coriandrum sativum* arrested the WRL-68 cells in the (S) phase; the BMK cells were arrested in the G2 and M phase, and the KHOS cells in the G1 phase. *Coriandrum sativum* produced important morphologic effects on chicken embryos. The use of *Coriandrum sativum* produces toxicological effects on the embryos only in high doses [149].

7.10. Cardiovascular effects

Coriander crude extract (1-30 mg/ml) caused fall in arterial blood pressure of anesthetized animals which partially blocked by atropine. Coriander crude extract produced vasodilatation

against phenylephrine and K+ (80 mM)-induced contractions in rabbit aorta and caused cardio-depressant effect in guinea-pig atria. Bioassaydirected fractionation revealed the separation of spasmogenic and spasmolytic components in the aqueous and organic fractions respectively. Furthermore, Coriander crude extract produced diuresis in rats at 1-10mg/kg [150].

Aqueous extracts of coriander seeds inhibited the electrically- evoked contractions of spiral strips and tubular segments of isolated central ear artery of rabbit [151]. The water extract of coriander seed had hypotensive effects in rats [152]. The preventive effect of *Coriandrum sativum* (CS) on cardiac damage was evaluated by isoproterenol induced cardiotoxicity model in male rats. Rats were pretreated with methanolic extract of CS seeds at a dose of 100, 200 or 300 mg/kg orally for 30 days and they were subsequently administered (sc) with isoproterenol (85 mg/kg body weight) for the last two days. Isoproterenol treated rats showed increased LPO, decreased levels of endogenous antioxidants and ATPases in the cardiac tissue together with increased plasma lipids and markers of cardiac damage. TTC staining showed increased infarct areas while HXE staining showed myofibrillar hypertrophy and disruption. CS (200 and 300 mg/kg body weight) pretreatment significantly prevented or resisted all these changes. The results showed that methanolic extract of CS is able to prevent myocardial infarction by inhibiting myofibrillar damage. It is also postulated that, the rich polyphenolic content of CS extract was responsible for preventing oxidative damage by effectively scavenging the isoproterenol-generated ROS [153].

7.11. Diuretic effect

The acute diuretic activity of aqueous extract of the seed of *Coriandrum sativum* was evaluated in rats. The aqueous extract of coriander seed was administered by continuous intravenous infusion (120 min) at two doses (40 and 100mg/kg) to anesthetized Wistar rats. Furosemide (10mg/kg), a standard diuretic was used as the reference drug. Excretion of water and electrolytes (sodium, potassium and chloride) in urine, and glomerular filtration rate (equal to creatinine clearance) were determined. The crude aqueous extract of coriander seeds increased diuresis, excretion of electrolytes, and glomerular filtration rate in a dose-dependent way. Furosemide was more potent as a diuretic and saluretic. The authors concluded that the mechanism of action of the plant extract appears to be similar to that of furosemide [168].

8. Conclusion

Coriander has been widely used over the past centuries for both culinary and traditional purposes. Although linalool has been identified in *C. sativum* extract as the main component in the extracted oil, many flavonoids, phenolic acids, and phytosterols were found to be the

major groups of phytochemicals that are expected to be accountable for the cardioprotective effects of coriander. In this review, the efficacy of coriander in CVDs has been discussed and evaluated according to the previous studies and reports, in which *C. sativum* is shown to exhibit hypolipidemic, antioxidant, antiatherogenic, antihypertensive, and antiarrhythmic properties in a dose-dependent manner. The included studies in this systematic review shed the light on the cardioprotective ability of coriander; however, more in vitro studies are needed to elucidate the mechanism of action of its extracts in cardiovascular diseases. Clinical trials are also warranted for fresh and extracted *C. sativum* to confirm the results presented in this review in an endeavor to consider it as a functional food and nutraceutical agent that can be consumed for therapeutic and nutritive purposes.

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ETUDE ETHNOBOTANIQUE ET USAGES THERAPEUTIQUES DES TROIS PLANTES ETUDIEES

Enquête ethnobotanique approfondie sur l'anis vert (*Pimpinella anisum* L.) au Maroc : variation des usages thérapeutiques, des sources d'information et de l'efficacité

(A fait l'objet d'un article publié dans le journal Ethnobotany research and applications)

Synthèse d'étude

La présente étude consiste à mettre en évidence un certain nombre de paramètres relatifs à l'utilisation de l'anis vert "*pimpinella anisum*" par les populations locales dans la région "Fès-Meknès". Pour ce faire, une étude ethnopharmacologique a été menée au niveau de différents sites (Fès, Meknès, Azrou, Ifrane, Immouzare, Sefrou, Taounate et Taza).

Ainsi, nous avons essayé : i) De déterminer les utilisations médicinales et culinaires des graines d'anis dans les régions d'étude ; ii) De caractériser les méthodes d'emploi et les parties utilisées ; iii) De comprendre les critères de sélection pour cette plante ; iv) Et enfin de comprendre le mode d'utilisation thérapeutique par les populations locales.

Pour mener à bien cette étude, le questionnaire élaboré est rempli sur le terrain. Il était divisé en deux parties distinctes : la première pour recueillir les informations personnelles des participants (nom, prénom, âge, sexe, etc.), et la seconde axée sur la plante en question (usages thérapeutiques, origine des connaissances, parties utilisées, maladies traitées, etc.). Selon les directives de Daget et Godron (1982), les enquêtes ethnopharmacologiques adoptent une méthode d'échantillonnage aléatoire et stratifié, garantissant ainsi un échantillon représentatif pour obtenir une collecte d'informations exhaustive. Pour l'analyse des données, des méthodes statistiques descriptives et multivariées ont été employées.

Au total nous avons, 85 herboristes et 600 informateurs. Selon les herboristes, la plante est utilisée comme laxatif, antitussif, remède aux dysménorrhées, amélioration de la lactation, traitement des troubles digestifs et aussi comme épice caulinaire. Les participants interrogés ont principalement souligné que le plante est utilisée pour traiter des affections, notamment les troubles gastro-intestinaux, dysménorrhées, la constipation et comme antitussif.

La principale source d'information sur les bienfaits de la plante pour les herboristes était les médias et les formations éducatives. Parmi les 600 informateurs les données sur les vertus

thérapeutiques de l'anis provenaient principalement des herboristes, les autres sources, incluant la famille et d'autres utilisateurs.

Dans chacun des sites étudiés, les personnes interrogées emploient exclusivement l'anis sous forme séchée, sans le mélanger avec d'autres plantes ou substances. La méthode d'administration la plus courante est la tisane. L'infusion reste la seule technique de préparation, les utilisateurs employant l'anis à des posologies imprécises.

Cette étude constitue une exploration ethnopharmacologique approfondie sur l'usage thérapeutique de la graine d'anis dans les régions d'étude. Nous avons abordé les applications thérapeutiques et culinaires de la graine auprès des populations des zones étudiées. De plus, nous avons identifié les principales sources d'information concernant les vertus médicinales de l'anis et évalué son efficacité. Il ressort de cette partie d'étude que la plupart des informés exploitent la graine d'anis à la fois pour ses qualités alimentaires et thérapeutiques, traitant diverses affections comme la dysménorrhée, les troubles digestifs et plus encore. Les femmes constituent la majorité des utilisateurs, avec des nuances selon les zones d'étude. La totalité des participants l'utilisent séchée et sans mélange, généralement en infusion.

Chap I: Deep ethnobotanical survey on anise (*Pimpinella anisum* L.) in Morocco: variation in therapeutic uses, sources of information and efficacy

Abstract

Background: An extensive ethnomedicinal analysis of anise (*Pimpinella anisum* L.) in Morocco was conducted as part of this study. We aimed to compile information on its medicinal uses and the amounts consumed by locals.

Methods: Interviews were conducted in seven Moroccan sites during 2019 and 2020. To examine the gathered data, descriptive and multivariate statistics were employed. We sought participant agreement on the specified therapies for each disorder group.

Results: 85 herbalist males and 600 participants ranging from 20 to 60 years old were interviewed in the study zone that uses or sells it for therapeutic purposes. The plant was primarily utilized by herbalists for food (18.93%), digestive disorders (18.93%), and laxatives (18.93%), while users use it for Dysmenorrhea (34.85%), anti-constipation (33.02%), antitussive (21.31%), and to promote Milk flow (10.82%). Other users used anise as an antitussive (12.47%) and a treatment for Dysmenorrhea (15.81%), while 14.92% of participants used it to encourage milk flow. Participants used the plant in dried form alone, and the usage form was like a Tisane or infusion in all investigated sites. However, all studied parameters varied among the tested sites.

Conclusions: The new study could be of great use in expanding knowledge of the medicinal flora and conserving ancestors' knowledge in Morocco's Middle Atlas and the Saiss Plain.

1. Introduction

Anise, *Pimpinella anisum* is a medicinal plant that is being employed in the modern pharmaceutical sector as well as in cuisine, traditional medicine, and folk remedies (Abouzid and Mohamed 2011; Ibrahim *et al.* 2017; Das *et al.* 2021). This plant has been used for thousands of years for both culinary and medicinal purposes, with clear advantages for maintaining human health and boosting quality of life (Sun *et al.* 2019). Anise is mentioned in several ancient literature as a remedy for epilepsy, depression, nightmares, and seizures (Mirheydar 2001; Andallu and Rajeshwari 2011; Abdollahi Fard and Shojaii 2013; Sun et al. 2019; Sarheed and Jaffat 2022). Due to its biochemical richness in bioactive compounds such as *trans*-anethole, naringin, palmitic, and petroselinic acids (Shobha et al. 2013; Rebey et al.

2019), anise is typically a major spice and traditional medicinal plant used for both food and medicine (Sun *et al.* 2019).

Anise is an annual herb that is native to the Mediterranean region and is widely farmed in the Middle East and Asia (Faravani *et al.* 2013; Shahrajabian *et al.* 2019; Darzi *et al.* 2022). According to Giordano *et al.* (2022) the *Pimpinella anisum* is a member of the Pimpinella Genus and Apiaceae or Umbelliferae Family. According to reports, it is also grown for food, with the edible parts being the seeds and fruits, as well as for the extraction of bioactive substances for use in medicine and other industrial applications (Das *et al.* 2022). The perfume and flavour of anise are attributed to its well-known essential oil. Despite trans-anethole making up between 75 and 90 percent of anise oil, there are other components including coumarins (umbelliprenine, umbelliferone, bergapten, and scopoletin), flavonoids (flavonol, glycosides, isoorientin, flavone, rutin, and isovitexin), lipids (betaarmyrin, stigmasterol, fatty acids, and its salts), carbohydrates, and proteins (Picon *et al.* 2010; Sun *et al.* 2019; Soussi *et al.* 2023). These compounds are behind the therapeutic and food benefits of anise.

Anise seeds are used in traditional medicine as a diuretic, carminative, antiseptic, and migraine analgesic (Amin 2005). Anise is also recommended as a treatment for epilepsy and seizures, as well as for sadness, nightmares, and other conditions (Mirheydar 2001). Rajeshwari *et al.* (2011) observed that aniseed has a wide range of uses and exploitations in the food and pharmaceutical industries since it is a powerful antiperoxidative and antidiabetic agent. In another study, (Ibrahim *et al.* 2017) found that waste residues of anise and star anise are prospective new sources of phenolic antibacterial chemicals, providing the pharmaceutical industry with new business opportunities. They claim that mixing some medicines with anise waste extracts provides a novel option for treating infectious disorders and that waste extracts may change the activity of antibiotics.

In Morocco, many studies have addressed the chemical composition and biological activities of anise seed. The phytochemical components and the antibacterial activity of *Pimpinella anisum* fruits obtained from various cultural zones in Morocco have been discussed by Ajebli *et al.* (2017). Al Maofari *et al.* (2013) evaluated the chemical makeup and antibacterial effects of *Pimpinella anisum* L. essential oils produced in Yemen and Morocco and found notable differences in the biocompounds present in the tested kinds. Despite the importance of results recorded in laboratory investigations, only one study has addressed the ethnobotany of *Pimpinella anisum* in Morocco. However, this study was limited in space and objectives. It targeted only one city (El Hajeb) and only fruits of cultivated varieties.

In the Middle Atlas, the Saiss plain, and the Northern areas, a number of ethnomedicinal surveys were carried out to determine the culinary and medicinal uses of anise seed in Morocco. The main goals of this paper were to: i) identify the medicinal and culinary use of anise seed in Morocco; ii) characterize the uses methods and used parts; iii) understand the selection criteria for this plant; iv) understand how therapeutic uses vary depending on sampling site; and v) assess anise seed toxicity cases.

2. Materials and Methods

2.1. Study Site



Figure 26 : Geographical location of sampled sites in Morocco

The Middle Atlas, the Saiss Plain, and the North of Morocco were the three areas of Morocco where this study was conducted (Fig. 1). The selected areas have various contexts. The Middle Atlas is a mountainous region with a semi-arid climate. The winter is bitterly cold and snowy with an average annual rainfall of 250 mm. The Saiss plain occupies an area of 40.075 km2, or 5.7% of the entire nation. Summers are hot and dry and winters are chilly and damp, which are characteristics of the continental climate that dominates in the northern regions. The average annual rainfall is about 500 mm. In Northern regions, the continental climate, is typified by dry and hot summers, while winters are chilly and humid. Over 700 mm of rain falls on average each year, and the downpours are often accompanied by hail and flooding.

Six different locations served as the basis for the data gathering, including Azrou, Ifrane, Immouzar, and Sefrou in the Middle Atlas; Fez and Meknes on the Saiss plain; and Taounate and Taza in the north of Morocco. These sites were selected to present a representative sample of various populations in Morocco.

2.2. Methodology and resource person interviews

This ethnomedical study was carried out between the academic years 2019 and 2020. A questionnaire form was designed and distributed across the area to accomplish the study's objectives. Aiming to learn as much as possible about anise seed's culinary and medicinal applications, as well as its methods of preparation, was expected at each research location. The questionnaire was broken down into two sections: one for the respondent's biographical information (surname, first name, age, sex...) and one for the plant (therapeutic uses, source of knowledge, the part used, pathologies treated...). According to Daget and Godron (1982), the ethnomedical surveys were carried out using a random and stratified sampling strategy, which allowed for the use of a representative sample and is a good technique for obtaining the most thorough inventory possible (Lahsissene *et al.*, 2009).

In order to determine the groupings of therapeutic uses of anise, Microsoft Word, Excel, and statgraphics applications were used to collect, process, and statistically analyze the data. All species' scientific names were reviewed and updated using the Plant List website (Rivera *et al.* 2014). Equally, the WHO's global illness classification was used (Staub *et al.* 2015). The questionaire was distributed among herbalists (herbalists that sell anise and users (populations that use anise).

2.3. Statistics

We verified that all analyzed parameters were normal before beginning the statistical analysis. We also estimated the percentages of all the factors, such as gender distribution (men and

women), ages ([20–30], [31–40], [41–50], [51–60], and > 60), educational attainment (primary, college, high school, university, and illiterate), family circumstances, therapeutic purposes, and style of use. All parameters were compared between herbalists and users to understand the traditional uses of Anise in the studied areas. The independent T-test was used to compare the use of traditional medicine between women and men, between traditional and modern medicine users, between the form of plant usage, and the preparation modes. Further, we used an ANOVA one-way test to compare the ages of participants, education level, family situation, health problems, diseases treated with Anise, and sources of information about the therapeutic benefits of Anise. Study locations (n = 7) were regarded as independent variables, whereas age categories (n = 5) were regarded as dependent variables, and then factor Analysis (FA) was used to elucidate the difference in responses. The distribution of age, education level (n = 5), therapeutic applications (n = 6), and study sites (n = 7) was clarified using a similar methodology. Therefore, only axes with eight values more significant than one were considered. The use of primary and multivariate statistics aimed to clarify the variation in response between questioned participants and among studied sites.

3. Resultats et Discussion

2.4. Participants' demographic details

In the study zone, 85 herbalists and 600 informants were questioned; the findings are shown in Table 12. The participants in the study ranged in age from 20 to 60. Among herbalists, the age category [41–50] was the most dominant, with 38.82%, followed by [51–60] with 24.70%, and [31–40] with 22.35% (f = 9.505, df = 4, P = 0.000). In contrast, the categories of ages >60 and [20–30] were the least investigated herbalists, with 2.35% and 11.76%, respectively. Further, age categories were variable among sample sites (Fig. 27). In Immouzer, the ages 20 to 30 were the most dominant among the investigated participants. In Sefrou and Meknes, age >60 was the most dominant, while in Ifrane, 51–60 was the most dominant among participants. In Fez, the participants were dominated by those aged 31–40. In Taounate and Taza, the age category 41–50 was the most dominant among participants (Fig. 28). Further, the totality (100%) of the investigated participants were male, while females were absent. Regarding the family situation, the married participants were dominant with 69.41%, followed by single participants with 21.18% (f = 35.918, df = 2, P 0.001). In contrast, divorced participants presented only 9.41%. Interviewed people were dominated by participants with college levels (75.29%), followed by participants with High school education (16.27%), and participants with Primary education

levels (8.24%) (f = 75.626, df = 2, P = 0.000). On the other hand, participants with a university education level and Illiterates were absent among the interviewed people.

Among the 600 participants (users of anise), the age also ranged from 20 to up to 60, while the dominant ages were between 51 and 60 with 26%. The age sections 41–50 and 31–40 presented 22.67% and 22.33%, respectively. The ages > 60 and 20–30 were the least represented among the informants (f = 5.151, df = 4, P = 0.003). The gender ratio was 57.33% for men and 42.67% for women. Further, 32.25% of participants were single, 67.67% were married, and only 10.83% were divorced (f = 66.455, df = 2, P< 0.001). The education of participants was 35.33% illiterate, 23.5% primary, 29.33% college, and 12.33% high school degrees (f = 10.179, df = 4, P<0.003). The distribution of age sections was variable among the investigated sites. In Immouzer, 41–50 was the most dominant; in Sefrou, Taounate, and Ifrane, sections 31–40 and 51–60 were the most recorded, while in Meknes, Taza, and Fez, 20–30 and >60 were the most observed. Equally, the education level of participants varied among the investigated participants. Illiterates and participants with secondary levels were the most recorded in Immouzer, Ifrane, Sefrou, and Taounate; the university level was in Taza; and the Primary level was not dependent on any site.

Table 12 : : Participants' demographic details (age, gender, education, occupation, and therapeutic techniques)

		Herborists		Users	
	Categories	Total of participants	Percentage %	Total of participants	Percentage %
Age	[20-30]	2	2.35	78	13
	[31-40]	19	22.35	134	22.33
	[41-50]	33	38.82	136	22.67
	[51-60]	21	24.70	156	26
	> 60	10	11.76	96	16
Gender	M	85	100	344	57.33
	F	0	0	256	42.67
Familial situation	Single	18	21.18	129	32.25
	Married	59	69.41	406	67.67
	Divorced	8	9.41	65	10.83
Education	Illiterate	0	0	212	35.33
	Primary	7	8.24	141	23.5
	College	64	75.29	176	29.33
	High school	14	16.27	74	12.33
	University	0	0	0	0

Despite the large number of ethnobotanical studies conducted in Morocco (Jouad *et al.* 2001; El Hassani 2020; Fakchich and Elachouri 2021), this is the first study to analyze the therapeutic uses of anise seed among local populations in central Morocco. This study targeted populations

in central Morocco with ages varying from young citizens 20 to up to 60 years old. Similar results were cited in other surveys on medicinal plants in Morocco. In a Large-scale inventory on the therapeutic applications of medicinal plants used by the local population in the plain of Saiss and the middle atlas of Morocco, Yaagoubi *et al.* (2023) investigated populations with ages ranging from 35 to 74 years old, with a dominance of 25–34 and 35–44 categories and males compared to females. In another study, (Idm'hand *et al.* 2020) conducted an Ethnobotanical investigation to assess the biodiversity of medicinal plants used in the Tarfaya Province, located in the south of Morocco. The research targeted 600 citizens dominated by the age range [51–60] and up to 60 years (25% each). As a result, 64% of the interviewed men use medicinal plants, compared to 73% of the surveyed women. In our case, the interviewed populations were dominated by males (100%) and the age categories [41–50], [51–60], and [31–40], while the ages >60 and [20–30] were the least recorded. However, the age of the investigated participants varied depending on sampling sites, which is in agreement with the results recorded currently by El Yaagoubi *et al.* (2023). These authors revealed the dominance of 55–64 ages in Ifrane, 65–74 in Meknes, and 25–54 in Immouzer, compared to 20–30 in Immouzer, >60 in Meknes and Sefrou, and 41–50 in Taounate and Taza in our study.

Concerning the education of participants, college level was the most dominant, followed by high school education, while university education level and illiterate people were absent. In another ethnobotanical study conducted in Tarfaya Province (South of Morocco), illiterate people dominated participants with 50%, followed by primary education level with a rate of 42% (Idm'hand *et al.* 2020), which partially agrees with our results. In another study on medicinal plants of the Agadir Ida Ou Tanane (southwest Morocco), (Ouhaddou *et al.* 2014) revealed the dominance of illiterates (52%) and the primary level of education (35%) compared to secondary and university (9%) and 4 %, respectively. These results confirm the variation in educational levels among users of medicinal plants. Equally, in our case, the educational level of participants varied from site to site; the Illiterate were absent in all sampled areas, University level in Taza and Taounate, Primary in Immouzer, Secondary in Meknes, Ifrane, and Sefrou. This variation in terms of participants' age, education level, and family situation is related to the demographic and educational structure of the interviewed populations. Equally, the sampling sites could interfere with the structure of the investigated participants.

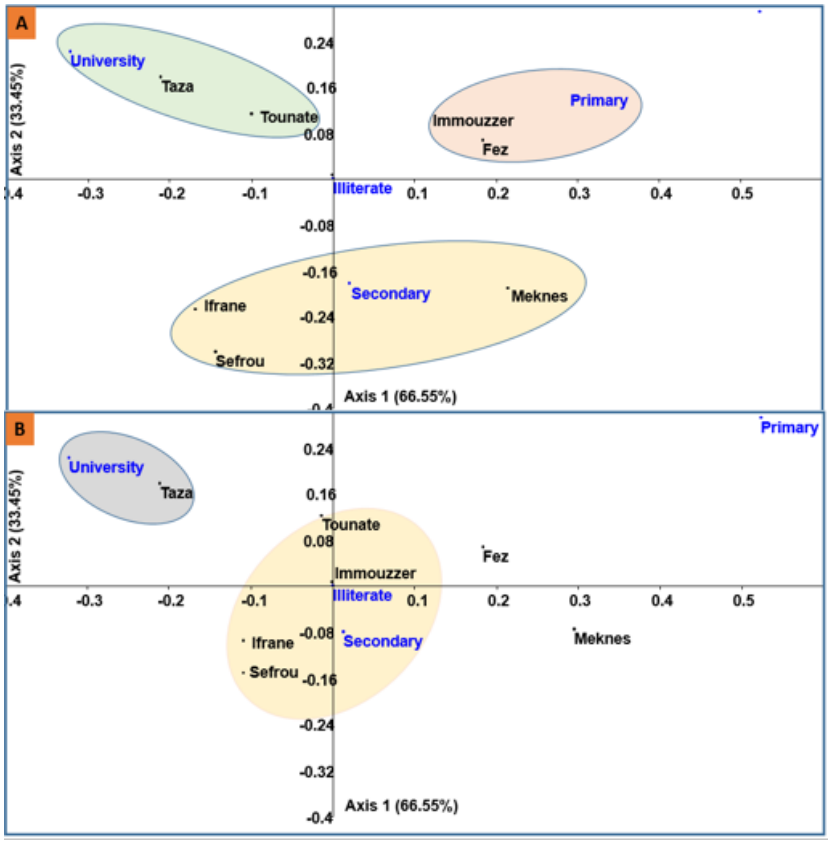


Figure 27: Geographical distribution of educational level among sampled cities in central Morocco (A: Herbalists; B: Users)

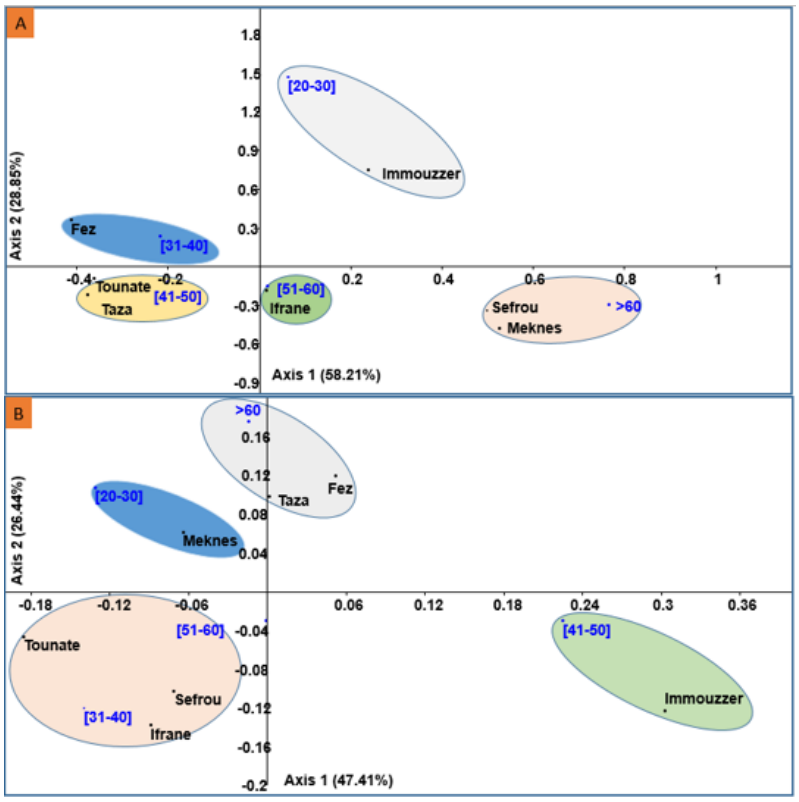


Figure 28 : FCA plot showing the geographical distribution of age categories

3.1. Health and use of medicinal plants

Health and diseases among interviewed participants are presented in the Fig.29. As a result, 34.64% of participants were unaffected by any diseases in the study sites, compared to 55.46% with various diseases. Diabetes was the most recorded disease, with 26.14%, followed by High blood pressure at 16.99% and digestive troubles at 14.38% (f=75.626, df=4, P=0.009). In contrast, only 7.84% of participants were affected by cardiovascular diseases. However, the geographical distribution of recorded diseases varied among sampled sites (Fig.30). The absence of diseases was primarily recorded in Ifrane and Meknes. In Taounate, participants were affected by cardiovascular and High blood pressure diseases. In Fez and Sefrou, participants were mainly affected by diabetes, while in Immouzer and Taza, participants were mostly affected by digestive disorders.

Of the participants, 29.66% were unaffected by any disease compared to 69.44% of unhealthy people. High blood pressure was the most recorded disease, with 21.83%, followed by Diabetes at 20.17% and digestive disorders at 19.5% (f=2.737, df=3, P=0.066). In contrast, cardiovascular problems were less recorded among tested populations. The recorded diseases showed dissimilar distribution among investigated sites. In Taza, digestive disorders were the most recorded, while in Immouzer, High blood pressure was the most observed among participants. Diabetes and cardiovascular diseases were recorded most in Taounate, Ifrane, and Meknes. In contrast, the healthy people were not related to a specific site since these people were dominant in all sites.

Many studies have investigated the diseases among the users of medicinal plants. This study demonstrated that diabetes was the most recorded disease among participants. In comparison, Idm'hand *et al.* 52020) recorded the dominance of gastrointestinal diseases (31.96%) and genito-urinary disorders (12.19%) among 600 participants (users of medicinal plants) in the Tarfaya Province, which is different from our results. Briguiche and Zidane 52016) have reported that most used plants in traditional medicine in Jadida City (Northwest Morocco) were mentioned to treat gastrointestinal disorders. In another study, (El-Hilaly *et al.* 2003) have found that medicinal plants recorded in Taounate region (North Morocco) are used principally in the treatment of digestive problems. However, the laboratory investigations showed that the anise seed has significant anti-diabetic effects thanks to its bioactive compounds. Aniseed (*Pimpinella anisum* L.) fractions obtained from the progressive fractionation of methanolic extract by benzene, hexane, n-butanol ethyl, acetate, and water were investigated for their antidiabetic properties (in vitro) by Shobha & Andallu, (2018). As a result, ethyl acetate

fraction of aniseeds showed the strongest anti-diabetic activity in terms of -glucosidase and -amylase (0.15mg/ml and IC50 0.12mg/ml, respectively). Methanolic extract and all investigated fractions of aniseeds also showed anti-diabetic activities. Equally, Shobha & Andallu, (2018) proved the Anti-Diabetic Effects of Aniseeds (*Pimpinella anisum* L.) in in vitro and in in vivo tests. All cited papers indicates the beneficial roles of anise seeds against diabetes and could help the interviewed participants to fight against this disease considered as chronic and was listed in 2016 as the seventh leading cause of death (Meerza *et al.* 2013; WHO).

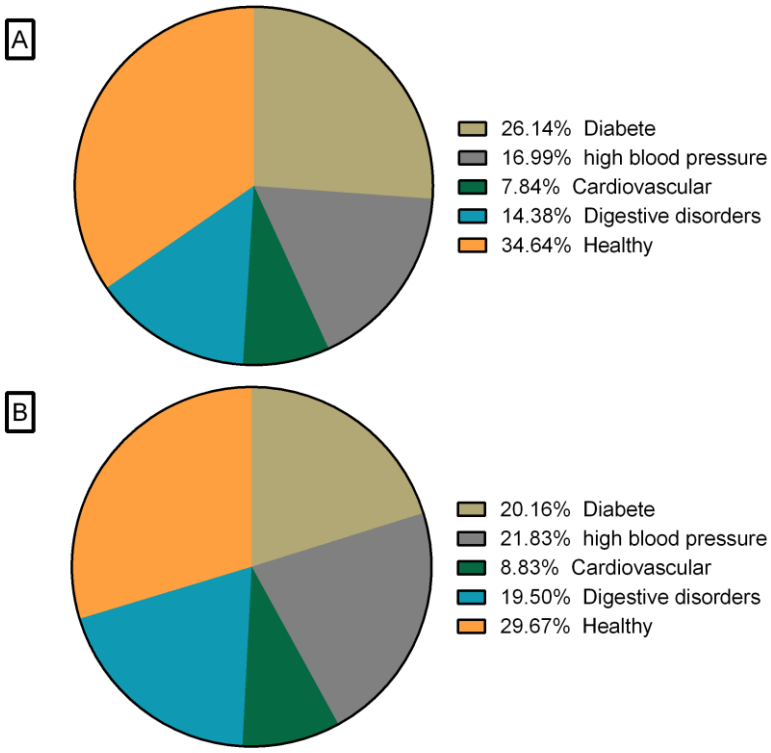


Figure 29: Health and diseases among interviewed herbalists (A) and participants (B)

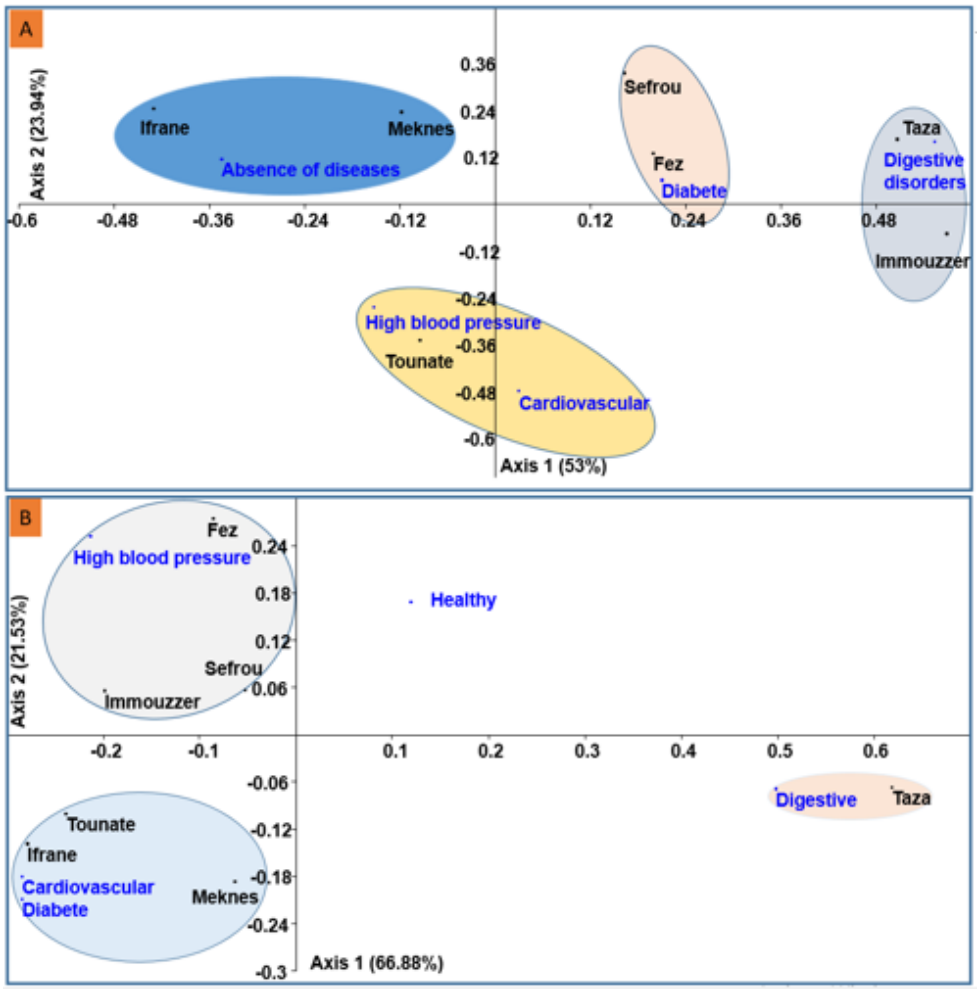


Figure 30: Distribution of recorded diseases among sampled sites analysed with FCA (A: Herbalists; B: Users)

3.2. Use of traditional medicine

The use of traditional medicine depending on gender of participants, in result, 87% of females use the traditional medicine compared to 13% of males in the study sites (t=2.089, df=12, P= 0.059). In terms of the selection of traditional medicine based on medicinal plants, 77.65% of participants (herbalists) mentioned the effectiveness and the low cost as reasons to select this approach. On the other side, 22.35% of participants cited the easy acquisition of medicinal plants and their derivatives as the main reason for the selection of traditional medicine.

Among tested participants (users), 84.33% use traditional medicine, while only 15.77% use only modern medicine. Females used traditional medicine more than men (t=2.089, df=12, P= 0.059). These users buy medicinal plant Anise from various sources counting herbalists (57.33%), local markets named Souk (38.66%), and Supermarkets (4.00%). The source of medicinal plant was variable among tested sites. In Immouzar, the principal source of Anise is the local market Souk. On the contrary, in Sefrou, Meknes, Ifrane and Taounate the principal source was the herbalists, while the supermarket was the principal source of medicinal in Fez

and Taza. On the other hand, 83.33% of users mentioned the low price and efficacy as the principal reasons behind the use of traditional medicine compared to easy acquisition cited by 16.67% of the population.

Many ethnobotanical studies in Morocco and other North African countries revealed the use of traditional medicine based on medicinal plants to treat various health diseases (Abouri *et al.* 2012; Idm'hand *et al.* 2020; Karous *et al.* 2021; Bencheikh *et al.* 2021; Souilah *et al.* 2022). For instance, Idm'hand *et al.* (2020) found that in the Tarfaya Province, 73% of the women surveyed used medicinal plants, compared to 64% of the men interviewed. This finding is consistent with our research. Similar conclusions were reported in Mechraâ Bel Ksiri (Northwest Morocco) and High Atlas (Central Morocco), respectively, by (Benkhnigue *et al.* 2010; Benlamdini *et al.* 2014). On the other hand, the selection of medicinal medicine was controlled by effectiveness and the low cost of this approach, which is similar to the reasons cited by Idm'hand *et al.* (2020) in the South of Morocco.

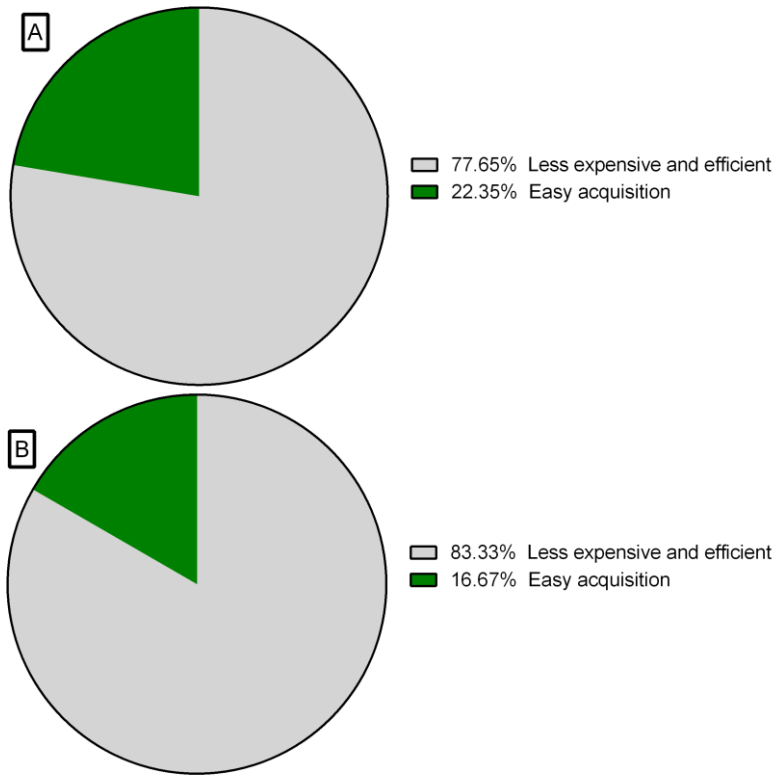


Figure 31: Reasons for the selection of traditional medicine (use of medicinal plants)

3.3. Therapeutic uses of anise seed

The therapeutic uses of Anise seed are presented in Fig.32. Based on herbalists, the plant is used to treat five diseases in all sampled sites, counting laxative, dysmenorrhea, promote milk flow, antitussive, digestive disorders, and food. Investigated participants used the plant mostly against laxative (18.93%), digestive disorders (18.93%), and for food purposes (18.93%). Other participants used the Anise seed to treat dysmenorrhea (15.81%) and as antitussive (12.47%) (f=2.906, df=3, P=0.055). Moreover, 14.92% of participants used the plant to promote milk flow. However, the uses of the Anise seed differed depending on sampling sites (Fig.33). In Fez, the majority of participants used seeds of Anise to promote milk flow, while in Ifrane and Sefrou the plant was used as antitussive. In Meknes and Immouzer, the plant was used for tree purposes, including treatment of laxative and digestive disorders, and for food purposes. In Taza and Taounate, participants used the plant only to treat Dysmenorrhea.

Based on responses of user participants, the anise is used to treat three diseases counting dysmenorrhea (34.85%), anti-constipation (33.02%), and as antitussive (21.31%), as well as a promoter of milk flow (10.82%) (f=26.213, df=3, P=0.000). The therapeutic uses of anise varied also among tested sites. In Meknes, dysmenorrhea is the principal therapeutic use, while in Taza anise is mostly used to as promoter of milk flow. In Ifrane and Immouzer, the anise was mostly used as anti-constipation, while in Taounate and Sefrou the plant is used as antitussive.

Anise (*Pimpinella anisum* L.) is a traditional medicinal herb and a dominant spice for medicinal and nutrition purposes. Recent research has demonstrated that anise seeds have antiviral, antifungal, antibacterial, anti-inflammatory, anticonvulsant, analgesic, and anti-diabetic properties (Sun *et al.* 2019; Ghosh *et al.* 2019; El-Rokiek *et al.* 2020). These activities are offered by the diversity and quantity of chemical compounds recorded in this plant's essential oils and extracts (Sun *et al.* 2019; Soussi *et al.* 2022, 2023). For example, Anise is rich in anethole, used in the food, pharmaceutical, flavoring, and perfumery industry (Özcan and Chalchat 2006; Tuncturk and Yildirim 2011). In our case, Anise is also used for food and therapeutic purposes, principally against laxatives, dysmenorrhea, antitussive, and digestive disorders. Other studies revealed the use of anise seeds are an analgesic in migraine and an anti-diuretic in traditional medicine (Amin 2005).

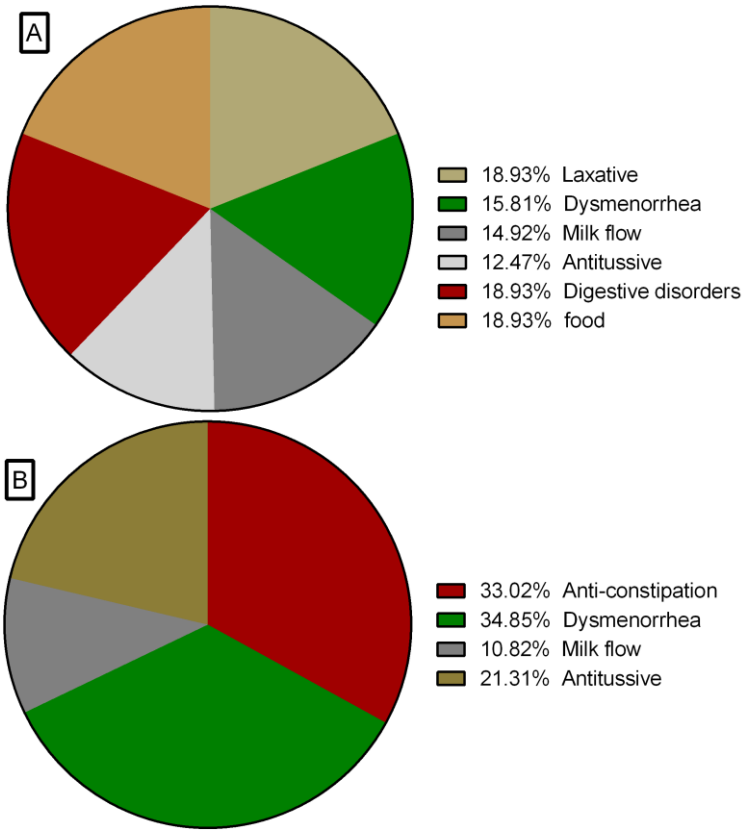


Figure 32: Therapeutic uses of Anise seed among herbalists (A) and participants (B)

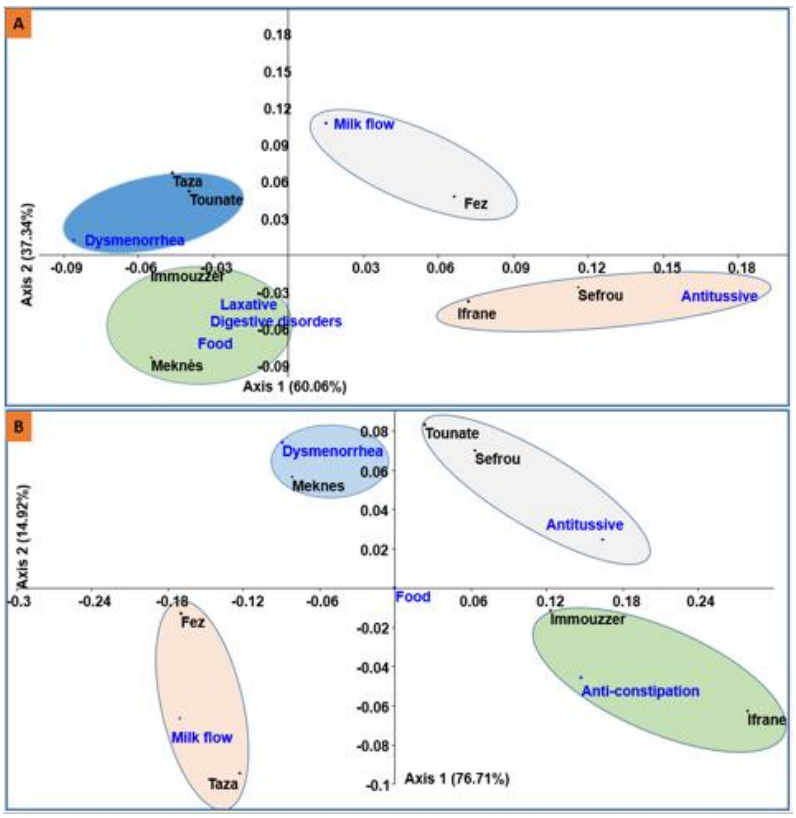


Figure 33: Therapeutic uses of anise seed among herbalists (A) and participants (B) in sampled sites

3.4. Sources of information about the therapeutic roles of anise

The sources of the information about anise seed among tested participants is presented in Fig.34. The principal source of information about the therapeutic benefits of the plant among herbalists were medias (68.24%) counting TV, radio, social medias, followed by educational trainings with 15.29%. Other sources such as families and between friends' discussions, books represented only 16.47% of source about the medicinal uses of anise. On the other hand, these sources differed among visited sites (Fig.35). In Ifrane and Sefrou the sources of information about the plant were Medias. In Fez and Meknes, the knowledge of medicinal effects of anise were acquired from trainings. In Taounate, Taza, and Immouzer, the information about the medicinal effects of anise were acquired from other sources.

Among the 600 users, the source of information about the therapeutic benefits of anise were trainings, media, herbalists, and other sources. Herbalists were the most important source with 55.5%, followed by other sources (family and the other users) with 30.5%, and media with 14.5% (f=31.117, df=2, P=0.000). The cited sources varied among monitored sites. In Taza, the media were the principal source of information about the therapeutic uses of anise, while the herbalists were the principal sources in Meknes, Taounate, Ifrane, and Immouzer. In Sefrou, the professional training was the principal source compared to other sources in Fez.

Dissimilar results were mentioned in other ethnobotanical studies conducted in Morocco. For example, in Tarfaya, sources of information on the use of medicinal plants was the ancestry experience with a rate of 70.60% (Idm'hand *et al.* 2020). In the case of Tarfaya, the oral image of the traditional know-how transmission from one generation to the next was very important, while in our case, the media take place.

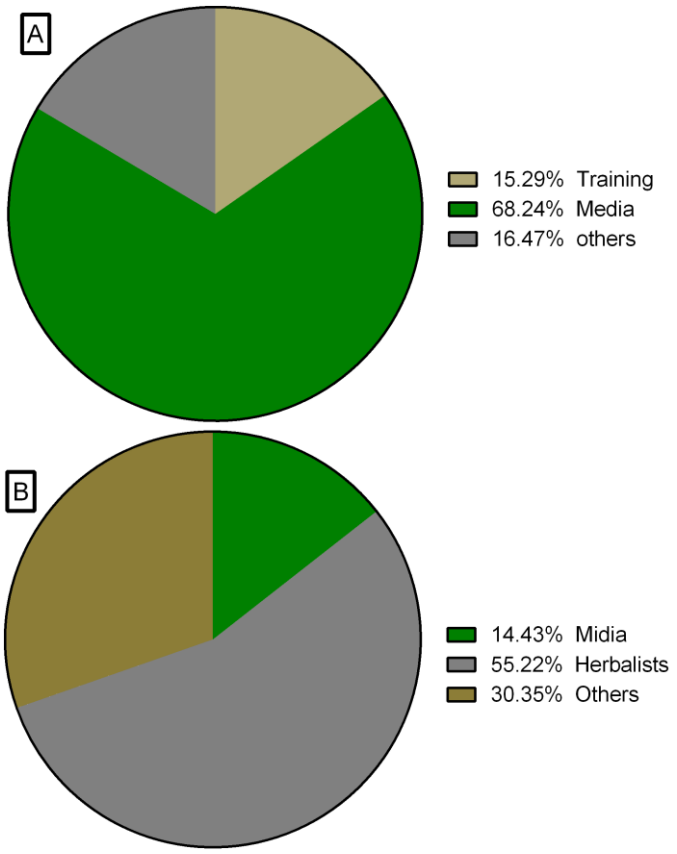


Figure 34: Sources of information about the therapeutic uses of anise among herbalists (A) and participants (B)

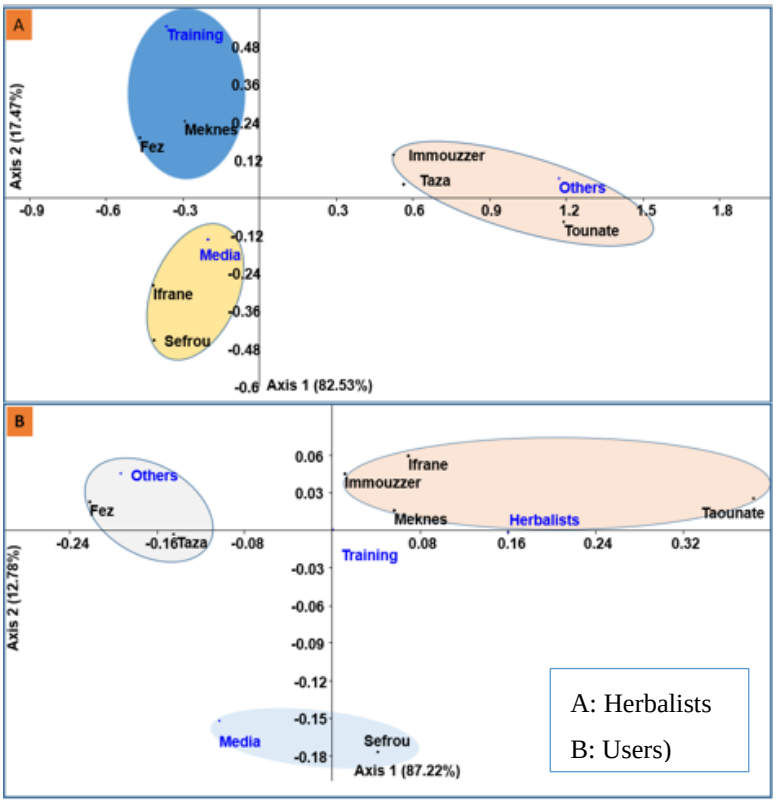


Figure 35 : Sources of information about the therapeutic usages of anise seed in sampled sites

3.5. Usage mode of anise seed

In all investigated sites, herbalists use the plant in dried form. Further, they used it alone, while none has used anise seed in a mixture with other plants or products. The usage form was Tisane in all investigated sites. The preparation mode was 100% Infusion in all investigated cities. The anise seed was used with 100% when needed, and the results were 100% amelioration of treated cases. All investigated participants mentioned the absence of any cases in terms of toxicity and secondary effects. As precautions for use, all herbalists used the plant alone with no mixture with other products to avoid toxicity or secondary effects. Equally, 100% of herbalists have conserved the plant and its infusions away from light and room temperature.

In participants (user population), dried from is the only used (100%). The users used anise plants, principally seeds alone (100%), without mixing with other products. Further, Tisane is the principal form of usage with 77.83% compared to powder with 22.17% ($t=6.302$, $df=12$, $P= 0.000$). The infusion is the only preparation mode, and 89.67% of users use imprecise quantities of anise compared to 10.33% of users that use accurate spoonfuls ($t=-10.127$, $df=12$, $P< 0.001$). As precautions for use, all participants used the plant alone with no mixture with other products to avoid toxicity or secondary effects. Equally, 100% of participants have conserved the plant and its infusions away from light and room temperature. All investigated participants mentioned the absence of any cases in terms of toxicity and secondary effects.

Currently, Yaagoubi *et al.* (2023) conducted an extensive study on medicinal plants used in traditional medicine in the same areas. As a result, anise seed was among the recorded plants and was used with three modes Decoction, brewing, and cataplasm. The most used part was fruit, which is different from our result. Since both studies were realized in the same sites, the variation could be attributed to users' knowledge and source of information about the used parts and preparation mode. On the other hand, all investigated participants mentioned the effectiveness and absence of toxicity cases after using anise; In contrast, Sun *et al.* (2019) mentioned the toxicity of estragole, anethole, and veranisatin (A, B, and C) from anise when used at high doses. The absence of toxicity in our case is attributed to the excellent conservation conditions and the use of adequate quantities of the anise.

Table 13 : Mode of use, result and effects of anise seed

	Herbalists	Users
Dosage	100% as needed	100% as needed
Results	100% improvements	100% improvements
Side effects	None	None
Toxicity	In no case	None
Precautions for use	100% no mixture with other plants	100% no mixture with other plants
Storage conditions	100% protected from light and ambient temperature	100% protected from light and ambient temperature

4. Conclusion

The current study presents the first and deep ethnobotanical investigation of the therapeutic uses of Anise seed in Morocco and Northwest Africa. We investigated the therapeutic and food uses of anise seed among populations of central and North Morocco depending on the age and education level and their variation depending on sampling sites. Equally, we investigated the sources of information on this plant's medicinal uses and assessed its effectiveness in therapeutic uses. The most important conclusion was that populations of ages (20 to 60) use medical medicine, including anise seed, for both food and therapeutic uses. The plant treats diseases, counting laxatives, dysmenorrhea, digestive disorders, and antitussives in food, such as promoting milk flow. Women dominated the users, which were variable depending on the sampling site. The principal source of information about the therapeutic benefits of the anise were media counting TV, radio, and social media. In all investigated sites, participants used the plant in dried form and alone. The usage form was Tisane, and the preparation mode was infusion. The anise seed was used when needed, and the results were 100% amelioration of treated cases, and all investigated participants mentioned the absence of toxicity cases. The outcomes showed the importance of anise seed in the traditional medicine of Moroccan populations. However, it is essential to broaden this kind of research to numerous areas of the country to transform intuition into knowledge and verify the cures discovered by precise scientific methods experimentally. This scientific valuation is believed to raise the economic significance of natural resources and, as a result, the incomes of rural and underprivileged populations.

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Etude ethnobotanique comparative de *Pimpinella anisum*, *Coriandrum sativum* et *Carum carvi* dans trois zones du Maroc : Utilisations thérapeutiques, sources de connaissances et efficacité

(A fait l'objet d'un article publié dans le journal Ethnobotany research and applications)

Synthèse d'étude

Dans cette partie d'étude, notre objectif était de valoriser les trois plantes médicinales et aromatiques : (*Pimpinella anisum* L.), (*Carum carvi*) et (*Coriandrum sativum* L.). Récoltés dans différents sites de la région Fès- Meknès (Taounate, Taza, Meknès, Fès, Immouzzer, Sefrou et Ifrane).

L'enquête ethnopharmacologique a été menée au cours de l'année 2019 /2020. Ainsi, un questionnaire a été élaboré et diffusé dans les sites d'étude. Celui-ci est divisé en six sections principales : i) Informations biographiques des populations ciblées (nom, prénom, sexe, âge, éducation, famille...), ii) Les plantes étudiées (connaissances et source d'information), iii) usage thérapeutique de chaque plante médicinale, iv) Modes et parties d'utilisation ; v) effets des plantes utilisées sur la santé des participants (guérison, toxicité et effets secondaires), et vi) conditions de stockage des plantes.

Au total, 600 personnes ont été interrogées. Les participants ont des âges compris entre 20 et 60 ans, la catégorie d'âge la plus représentée est 51-60 ans, les autres tranches d'âge sont 31-40 et 41-50 ans.

La plupart des informateurs sont familiers avec les plantes médicinales examinées, *Pimpinella anisum, Coriandrum sativum* et *Carum carvi*.

Pour les trois plantes étudiées, les herboristes étaient la principale source d'information, tandis que les médias ne représentent qu'un faible pourcentage (14,5% pour *Pimpinella anisum* ; 12,16% pour *Carum carvi* et 12,16% pour *Coriandrum sativum*). 34,85 % utilisent l'anis vert pour traiter les dysménorrhées, 33,02 % pour lutter contre la constipation, 21,31 % comme antitussif et seulement 10,82 % pour favoriser la montée du lait.

Le carvi a été utilisé par 44,51% des participants pour traiter les ballonnements et les crampes intestinales, 43,97% pour traiter les troubles digestifs, et 11,51% pour favoriser la lactation et réguler les menstruations.

En revanche, la coriandre a été utilisée par 42,5 % de la population cible pour traiter à la fois les troubles digestifs et les spasmes intestinaux, suivi par les antidiarrhéiques pour 38,25 % des participants, tandis que les rôles alimentaires ont été limités à 19,25 %.

Il semble que les graines constituent la partie utilisée pour l'anis et le carvi. Par contre, la coriandre est utilisée à la fois sous forme entière et ses graines. Par ailleurs, toutes les plantes ont été utilisées à l'état sec plutôt que frais.

Pour *Pimpinella anisum*, et *Carum carvi,* la plupart des sujets interrogés les utilisent principalement sous forme de tisane. Par contre la plante *Coriandrum sativum*, est utilisée sous forme de poudre. La majorité utilise des doses imprécises des préparations médicinales à partir de toutes les plantes.

Les résultats obtenus ont montré que les populations des trois zones, utilisent ces plantes pour traiter diverses affections, telles que les troubles digestifs, les dysménorrhées, la constipation, la diarrhée, la régulation des menstruations. De plus, ces plantes sont employées à des fins alimentaires et cosmétiques.

L'utilisation de chaque plante varie en fonction du site d'étude. Les principales sources d'information sur les bienfaits thérapeutiques de ces plantes sont les herboristes, ainsi que les médias. Les populations utilisent les graines ou les plantes entières sous forme séchée, principalement en infusion ou en poudre. Pour éviter tout risque de toxicité ou d'effets secondaires, les plantes sont conservées à l'obscurité et à température ambiante. Les informés ont souligné que traitement s'est avéré efficace contre les maladies traitées, bien que les doses ne soient pas précises.

Chap II: Comparative ethnobotanical study of *Pimpinella anisum*, *Coriandrum sativum*, *Carum carvi* in three areas of Morocco: Therapeutic uses, sources of knowledge and efficacy against diseases and for food purposes.

Abstract

Background: This study aimed to compile data on the variation of medicinal uses of three medicinal plants (*Pimpinella anisum, Coriandrum sativum,* and *Carum carvi*) among local populations in three Moroccan regions.

Methods: Interviews were conducted in seven sites belonging to three regions of Moroccan from 2019 to 2020. To analyze recorded data, graphic, descriptive indicators, and multivariate tests were employed. We investigated the response of participants about therapeutic uses and the factors influencing their responses.

Results: In the research zone, interviews were conducted with 600 persons ranging in age from 20 to 60 years who use *Pimpinella anisum, Coriandrum sativum, Carum carvi* for therapeutic purposes. The selected plants were used by participants for food purposes (milk flow), cosmetic interests, and therapeutic roles counting digestive disorders, intestinal spasms, laxative, dysmenorrhea, bloating, intestinal cramps, and antitussive. The majority of people only utilize the plants in their dried form, while Tisane, powder, and infusion were the most popular usage forms across all analyzed sites. The surveyed sites varied in terms of all studied factors, though.

Conclusions: This study could be very helpful in preserving ancestors' knowledge and advancing our understanding of the medicinal plants of Morocco, including the Middle Atlas and the Saiss plain.

1. Introduction

Traditional medicine is practiced around the world and is reliant on the local environment's natural resources as well as the knowledge and skills required to use them (Elujoba *et al.* 2005). Since ancient times, man has relied heavily on plants as a source of curative medications, particularly in impoverished homes (O. O. Olajuyigbe 2012). There are still many groups all over the world that practice herbal medicine (Kassaye *et al.* 2006). Additionally, 80% of the African population still relies only on medicinal plants for treatment, according to the World Health Organization, (2001). Many nations have recently invested a lot of time and energy into the research of medicinal plants. Chemistry, pharmacology, and botany must all be integrated

in the development of new herbal medicines (Van Wyk 2011; Z.A.Yusupova *et al.* 2023). Identification and documentation of medicinal plants can be done effectively through ethnobotanical surveys and ethnopharmacological research (Schultz and Garbe 2023).

Due to its geographic location and the effects of several climate types, Morocco boasts a diverse range of flora. Vascular plants are classified into almost 5200 species and subspecies, which are further split into 155 families and 981 genera. There are 900 indigenous species in the flora, which constitutes endemism. On the other hand, there are more than 600 species of medicinal plants in Morocco (Tahraoui *et al.* 2023). These plants are employed for a variety of industrial and medical reasons. In the North of Morocco, the vegetal biodiversity is characterized by the presence of multiple plants in the plains, mountains, and rivers. In addition to their ecological and forage importance, these plants have many uses, traditionally practiced by the local population.

In many Moroccan provinces, particularly in rural areas, the health care system has not yet reached the expected level. Several constraints hinder effective medical supervision of the rural population, due to their poverty, harsh geographical location, and unbalanced spatial distribution of health facilities, which are concentrated in urban areas. Faced with this situation, and due to the poverty of the major households in the area, the rural population is depending more and more on this traditional medicine. Equally, the abundance of medicinal plants in rural areas, their low cost, and their curative efficacy promote their usage for therapeutic purposes. However, in some cases, this traditional knowledge is monopolized by a small part of the population who doesn't share it with the rest of the community, which is currently threatening the continuity and guarantee of this knowledge transmission (Salawu *et al.* 2023). Therefore, the documentation of medicinal plants via laboratory and ethnobotanical studies is crucial in order to transmit this knowledge and traditional techniques to a large spectrum of scientists and informants.

In this study, we aimed to explore the culinary and medicinal uses of three medicinal plants, including anise seed (*Pimpinella anisum* L.), caraway (*Carum carvi*) and Coriander (*Coriandrum sativum L.*), in different regions of Morocco. In detail, we aimed to i) identify the medicinal uses of these plants depending on the plants and the sampling sites; ii) characterize the used parts, use forms, and use mode for each plant; and iii) investigate the efficacy of plants against treated diseases and side effects of each species (toxicity) among the interviewed participants. In this study, we targeted the populations in seven cities from three regions:

Taounate and Taza from the Northern region, Meknes and Fez from the plain of Saiss (central region), and Immouzzer, Sefrou, and Ifrane from the Middle Atlas region.

2. Material and methods

2.1. Study areas

Fig. 1 displays the sampling sites, including Taounate and Taza from the Northern region, Meknes and Fez from the plain of Saiss (central region), and Immouzzer, Sefrou, and Ifrane from the Middle Atlas region. These regions are characterized by different climatic and topographic contexts. The Middle Atlas is a hilly region defined by a semiarid climate. The average annual rainfall is estimated at 364 mm, while the average temperature is estimated at 5.3–35 °C annually (Linares *et al.* 2013). The winters are severely cold and snowy, while the summers are moderately hot. The Saiss Plain is located in central Morocco and covers an area estimated at 40.075 km^2, which represents 5.7% of the country's area. The average annual temperature is estimated at 7–45 °C, while the average rainfall is estimated at 490 mm (500 mm in Fez and 600 mm in Meknes) (El Garouani *et al.* 2021). The winters are cold and rainy, while the summer is severely hot during July and August. The Northern region is dominated by the Mediterranean climate due to its location in the South of the Mediterranean basin. This zone is characterized by a rainy winters, while summers are hot and dry. The average annual temperature is estimated at 17 °C, while the average annual rainfall is estimated at 700 mm.

The recorded climatic conditions and topographical characteristics in the studied zones support an important vegetal diversity. Further many important medicinal plants, including *Pistacia atlantica*, *Thymus algeriensis*, and *Thymus zygis* from the Middle Atlas (Hachi *et al.* 2016), *Origanum vulgare* and *Teucrium fruticans* from Saiss Plain, and *Carum carvi* L., *Coriandrum sativum* L., and *Pistacia lentiscus* L. from the North of Morocco (El-Hilaly *et al.* 2003).

Data were collected from seven cities, including Taza and Taounate in the north of Morocco, Azrou, Ifrane, Immouzar, and Sefrou in the Middle Atlas, Fez and Meknes on the Saiss plain, and we selected this number of cities, characterized by different climatic conditions and geographical locations, to provide accurate and representative data on the use of medicinal plants in the entire country of Morocco.



Figure 36: Samples sites from North, Plain of Saiss, and Middle Atlas (Morocco)

2.2. Data collection and interviews

The ethnomedical investigation was conducted for one year, from 2019 to 2020. This period was selected to cover the entire sampling zone. In order to carry out the goals of this survey, a questionnaire was created and disseminated around the sampled sites. We aimed to collect the maximum amount of data on the uses selected for each planned site. Therefore, the questionnaire was divided into six principal sections: i) biographical details of targeted populations (surname, first name, sex, age, education, family...), ii) information about the study plants (knowledge and source of information), iii) therapeutic uses of each medicinal plant, and iv) usage forms, modes, and parts for each species; v) effects of the used plants on the health of participants (cure, toxicity, and side effects), and vi) storage conditions for each species.

According to (Daget and Godron 1982), the ethnomedical surveys were carried out using a random and stratified sampling approach, allowing for the use of a representative sample.. The most thorough inventory can be obtained using this technique (Lahsissene *et al.* 2009). Then, the questionnaire was distributed among the populations of the sampling sites. Using the Plant List website, all species' scientific names were reviewed and revised (Rivera *et al.* 2014). Additionally, the WHO's taxonomy of world illnesses was applied (Staub *et al.* 2015).

2.3. Statistic tests

Recorded data for each parameter was organized in Microsoft Excel before analysis. In addition, we calculated the percentages for each parameter. We calculated the percentage for five age categories (number of participants for each age category ⁄ Total number of participants), educational levels (primary, college, high school, university, and illiterate), family situations (married, single, and divorced), and health disorders among participants. Similarly, we calculated the percentages for therapeutic uses, used parts, usage modes, and therapeutic effects for each medicinal plant. Then, we tested for the normality of the data via the Shapiro-Wilk test before starting the statistical analysis. A paired T-test was used to compare parameters with two groups of data, including knowledge of the plants, use form (dried or fresh), and use dose (precise or inaccurate). Parameters with three or four groups (source of information, preparation modes, and use form) were compared with an ANOVA one way test. Parameters with up to five groups were compared with the multiple range test.

To investigate the effect of sampling site (n=7) on the age of participants (n=5), sources of information (n=4), therapeutic uses (n=12), used parts (n=5), preparation modes (n=3), and forms of use (n=4) we used Correspondence Analysis Test (multivariate statistics). The sampling sites were considered as factors, while the other parameters were considered as dependent variables. In Correspondence Analysis plots we considered axes with eight values greater than 1 and superior percentage of inertia. All statistics were done in IBM SPSS 25 software.

3. Results and discussions

3.1. Demographic features of Participants

In total, 600 informants in the research area were questioned; the results are displayed in Fig.37. The ages of the questioned participants from 20 to 60 years old; though, the percentage of each age category was variable. The category of age between 51 and 60 years old was the most recorded among participants, with 26%. Further, 22.67% and 22.33% of participants have the age categories 31–40 and 41–50 years old, respectively. The ages between 20 and 30 and up to

60 years were the least recorded among the interviewed participants, with 13% and 16%, respectively. Further, the age of the investigated populations among sampled sites. Ifrane, the 51-60 years old was the most dominant category, while age of >60 years was dominant in Sefrou and Meknes. In Immouzer, the age category 20-30 was the most recorded among the explored participants. The participants of Fez were dominated by those aged between 31 and 40 years old. In Taza and Taounate, the age category of 41-50 years old was the most principal among interviewed participants (Fig. 37).

In terms of gender, men dominated the participants with 57.33%, while women represented only 42.67% of the participants. Additionally, only 10.83% of participants were divorced, compared to 32.25% of participants who were single. In contrast, the majority of participants (67.67%) were married. On the other hand, the percentages of education levels were different among participants. Further, 35.33% of participants were illiterate, followed by college level with 29.33%. Primary level was present in 23.5% of participants, followed by 12.33% with high school diplomas. Similarly, the education levels of populations were variable among sampled sites. Participants with secondary level and Illiterates were the most observed in Ifrane, Sefrou, Taounate, and Immouzer. The university level corresponds to Taza and Taounate (Fig.38).

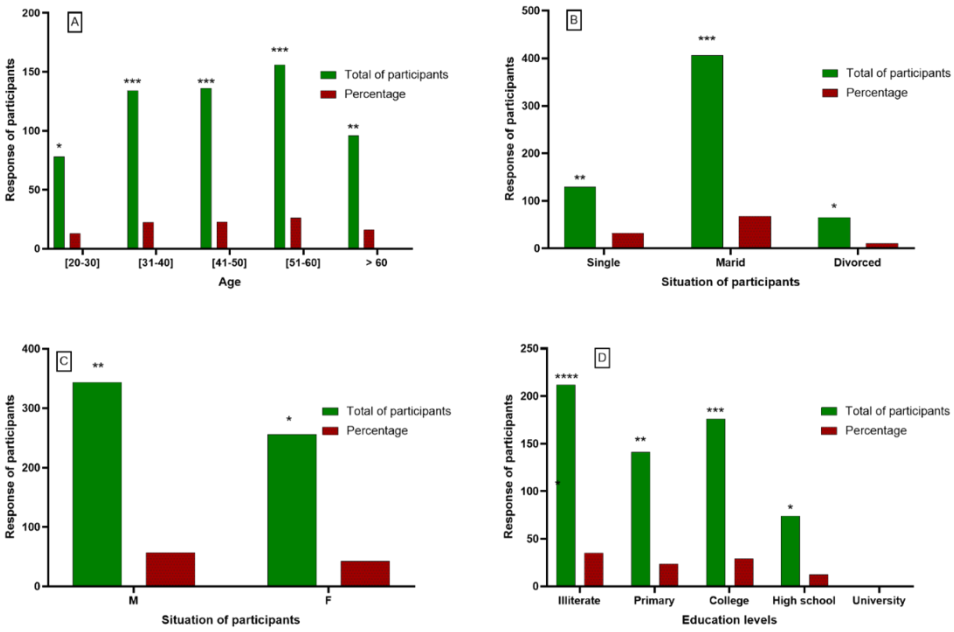


Figure 37 : Demographic features

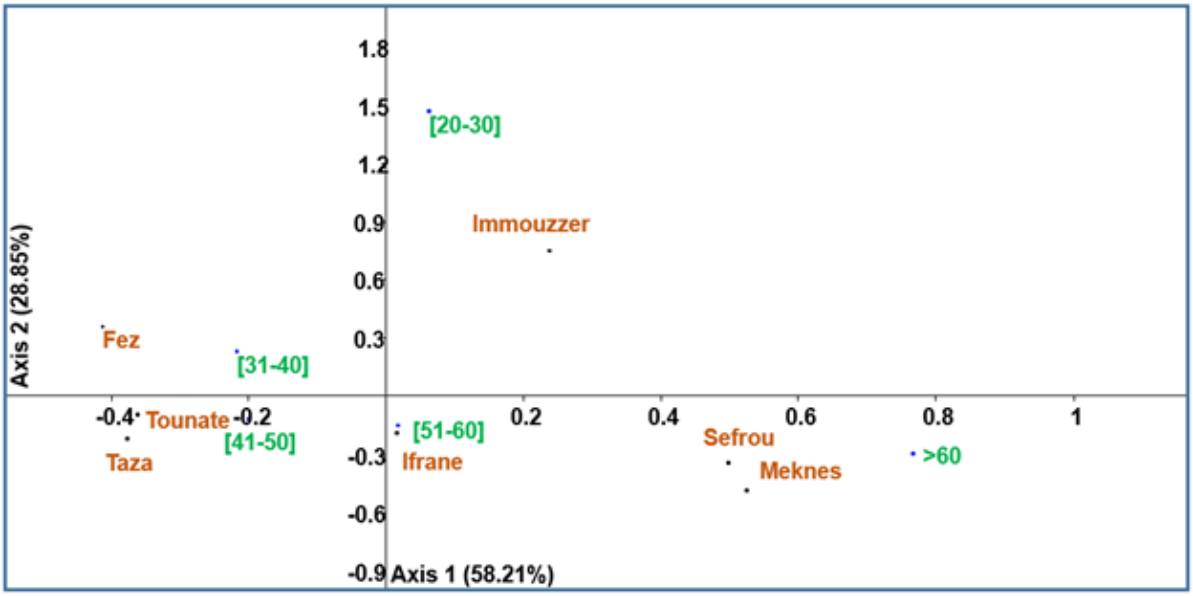


Figure 38: Variation of age categories among interviewed populations depending on sampling sites

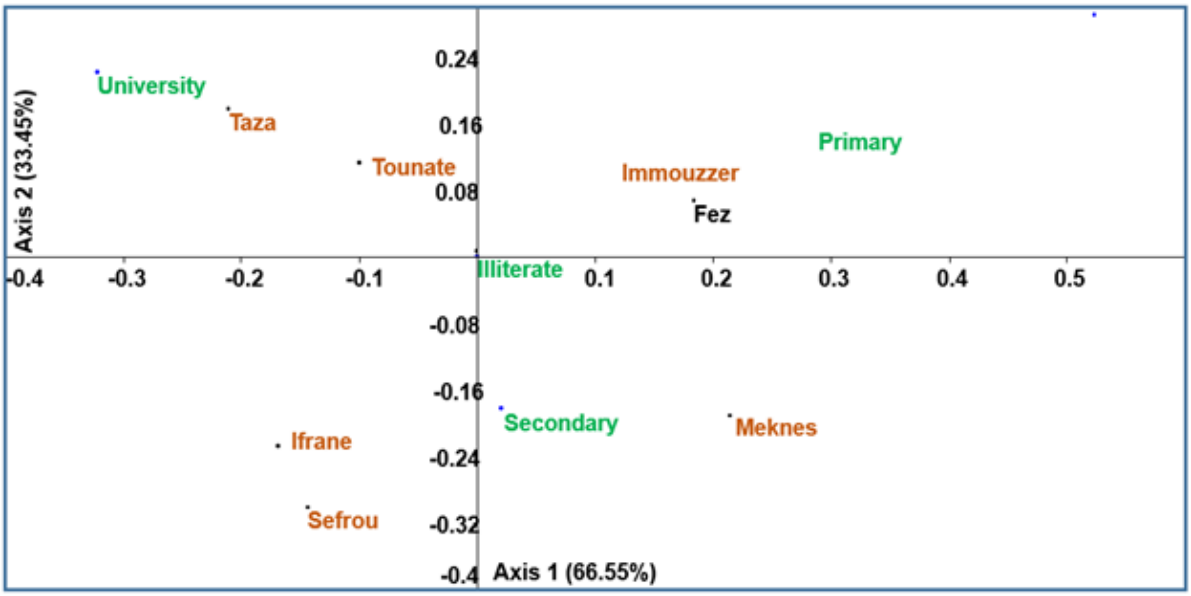


Figure 39: Variation of education levels among interviewed populations depending on sampling sites

Numerous ethnomedical studies on various aspects of medicinal plants, such as Middle Atlas antidiabetic herbs, have been carried out in the same regions (Hachi *et al.* 2016), economic evaluation of medicinal plants in Taounate province (El-Hilaly *et al.* 2003), and medicinal plants used to treat acute digestive system problems in the region of Fez-Meknes (Es-Safi *et al.* 2020). These studies have addressed the demography and status of these populations. Es-Safi *et al.* (2020) mentioned that the age of populations in Fez-Meknes is variable between 20 and up to 60 years old, with the dominance of the 20–40 and 40–60 categories at 44.75% and 37.35%, respectively. In terms of education levels, illiterates and primary school students were dominant with 36.11% and 25.62%, while the gender was dominated by women with 54.63%

compared to 45.37% for men. In another study, the therapeutic plants utilized by diabetes patients in the Fez-Meknes region were researched by Mechchate *et al.* (2020), who also noted the range in patient ages from 25 to >65 years old with the dominance of 50–65 years at 53.10%. The majority of patients were married (88.10%), while the education level was dominated by illiterate elementary students (65.70% and 25.80%, respectively). Najem *et al.* (2020) investigated the ethnobotanical treatments of oral disorders among 58 herbalists in the Central Middle Atlas, dominated by men (87.40%) and recorded the variation of ages from <30 to >60 years old, with the dominance of 50–60 and over 60 years with 30.37% and 24.36%, respectively. In terms of education level, participants with secondary education were dominant (42%), followed by primary education (28%. In the northern zone of Morocco, Bencheikh *et al.* (2021) investigated medicinal plants used conventionally to treat kidney diseases among 12 health herbalists and 476 non-specialists. The results revealed the variation of age from less than 25 years to more than 65 years, with dominance of 46–65 (53%) and 25–45 years (27%), while gender was dominated by women (58%) compared to men (42%). The education level was dominated by the illiterate (59%) and secondary education (17%). The analysis of all these previous studies showed that the demographic characteristics of participants varied depending on the sampling sites and studied topic, which is in agreement with our results. However, our study investigated the variation of demographic features at a large scale (600 participants) in seven sites belonging to three regions of Morocco, which is more representative compared to small-scale investigations in the bibliography.

3.2. Well-being of participants

Fig.40 shows the health and illnesses of the people that were interviewed at the studied sites. Only 29.66% of the 600 participants were healthy, while 69.44% were suffering from at least one disease. The unhealthy participants were affected by four diseases at various percentages. Further, high blood pressure dominated all diseases with 21.83% of cases, followed by diabetes in 20.17% of participants. Digestive problems were recorded in 19.5% of participants, while cardiovascular issues were recorded in a small number of participants, estimated at 8.83%. On the other hand, the recorded diseases had a different distribution at each site that had been sampled (Fig.41). In Taza, stomach issues were most frequently reported, but in Immouzer, participants' high blood pressure levels were most frequently noted. In Taounate, Ifrane, and Meknes, diabetes and cardiovascular disorders were most frequently reported. The healthy individuals, on the other hand, were not associated with any particular site because they dominated all places.

Numerous investigations have looked into disorders that affect people who utilize medicinal plants in different zones of Morocco (Chaachouay *et al.* 2019, 2020; Bencheikh *et al.* 2021; Mohamed *et al.* 2022; Benkhnigue *et al.* 2023). These studies reported the presence of various diseases among participants, including anemia, cancer, dermatological diseases, liver diseases, insomnia, asthenia, and oral and gum infections. However, these diseases were distributed in different regions of Morocco. For example, among 600 participants (users of medicinal herbs) in the Tarfaya Province, Idm'hand *et al.* (2020) found that the prevalence of gastrointestinal ailments (31.96%) and genito-urinary problems (12.19%) was different from our findings. In this study, we showed that among participants, diabetes was the most often reported illness, while the other diseases were distributed differently depending on the sampled site. In the same sense, El-Hilaly *et al.* (2003) revealed stomach issues as the most dominant illness in the Taounate region (North Morocco), which are also treated with medicinal herbs. In El Jadida (Northwest Morocco), digestive issues were the most commonly reported diseases among participants and were treated mostly by traditional medicine (Briguiche and Zidane 2016). In the Middle Atlas, Chaachouay *et al.* (2022) reported the use of medicinal plants to treat various diseases such as diarrhea, anthelminthic infections, and indigestion. These confirm the variation of illness from one site to the next, as we recorded in our study. This spatial variation could be governed by the health infrastructure or alimentary practices in each area (Wilson *et al.* 2017; Przybyłowicz and Danielewicz 2022). For example, Vitale *et al.* (2020) demonstrated that pasta consumption and related dietary habits influence cardiovascular risk factors in people with type 2 diabetes. Equally, the age of populations could interfere with the type of disease (Forni *et al.* 2019; Hou *et al.* 2019).

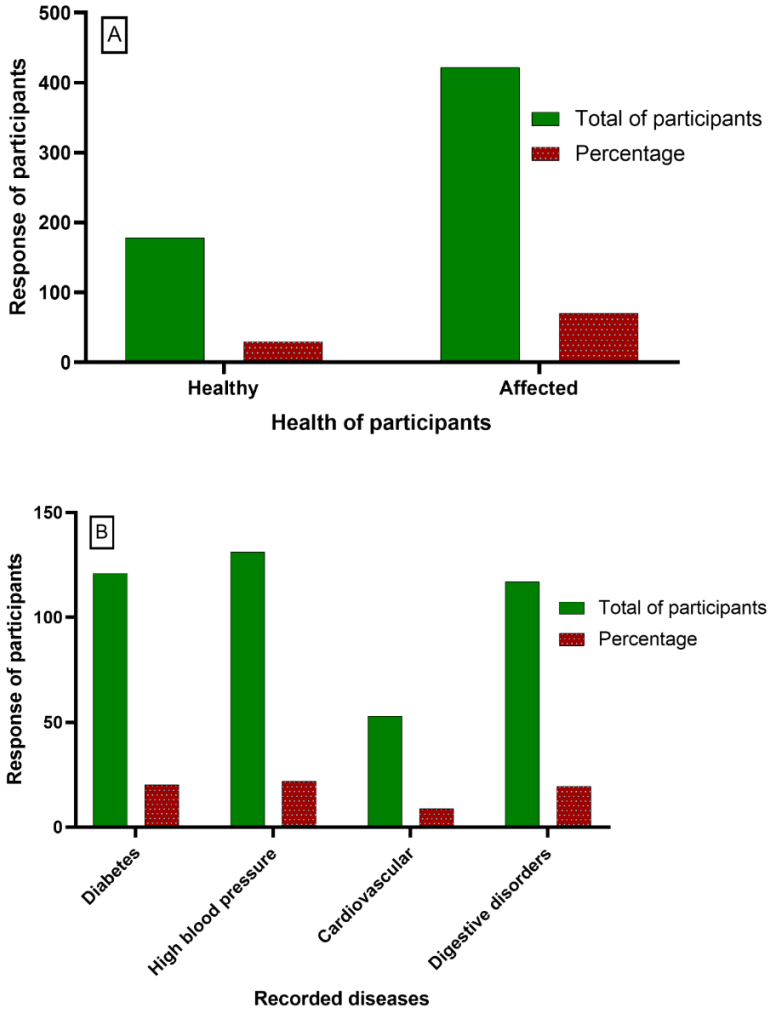


Figure 40: Health status (A) and recorded diseases (B) among interviewed participants

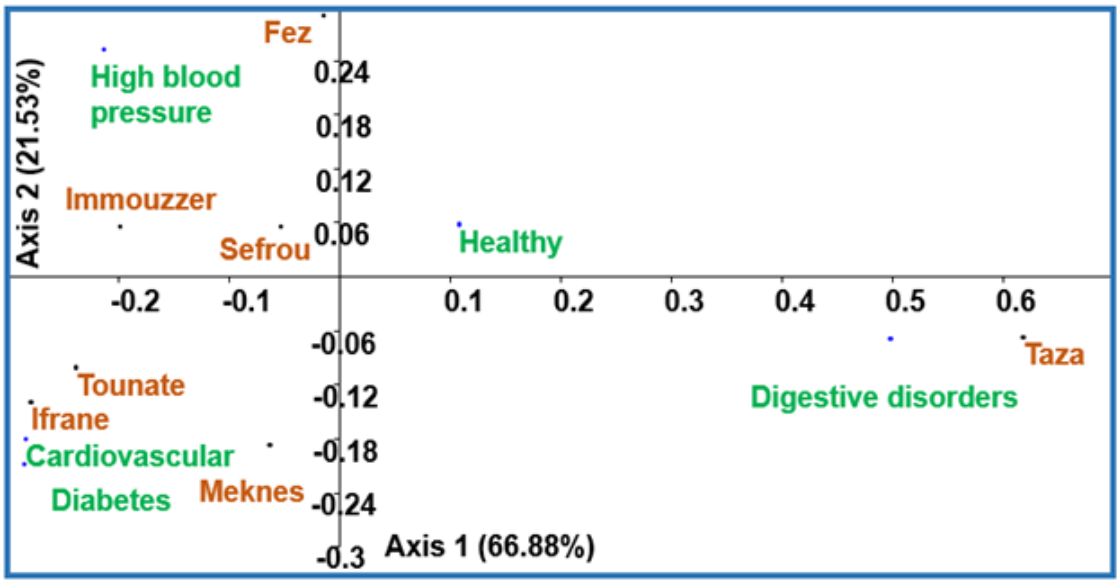


Figure 41: Geographical distribution of recorded diseases among populations of sampled sites analyzed with FCA

3.3. Source of information on medicinal benefits of plants

Fig.42 displays the knowledge of studied medicinal plants among investigated populations. The majority of participants are aware of the studied medicinal plants. Further, 100% of the participants knew *Pimpinella anisum* and *Coriandrum sativum* compared to 85% of *Carum carvi*. In contrast, only 14.44% of participants know *Carum carvi*. The sources of information about the studied plants are presented in Fig. 7. The participants mentioned different sources of information about each medicinal plant. In *Pimpinella anisum*, the most important sources of information were herbalists (55.5%), other sources (30.5%), and the media (14.5%). Similarly, herbalists were the principal source of *Carum carvi* (52.5%), followed by other sources (21.16%) and the media (12.16%). The herbalists were the principal source of information about *Coriandrum sativum* with 60.84%, followed by others with 27% and the media with 12.16%. However, these sources vary depending on the sampling site.

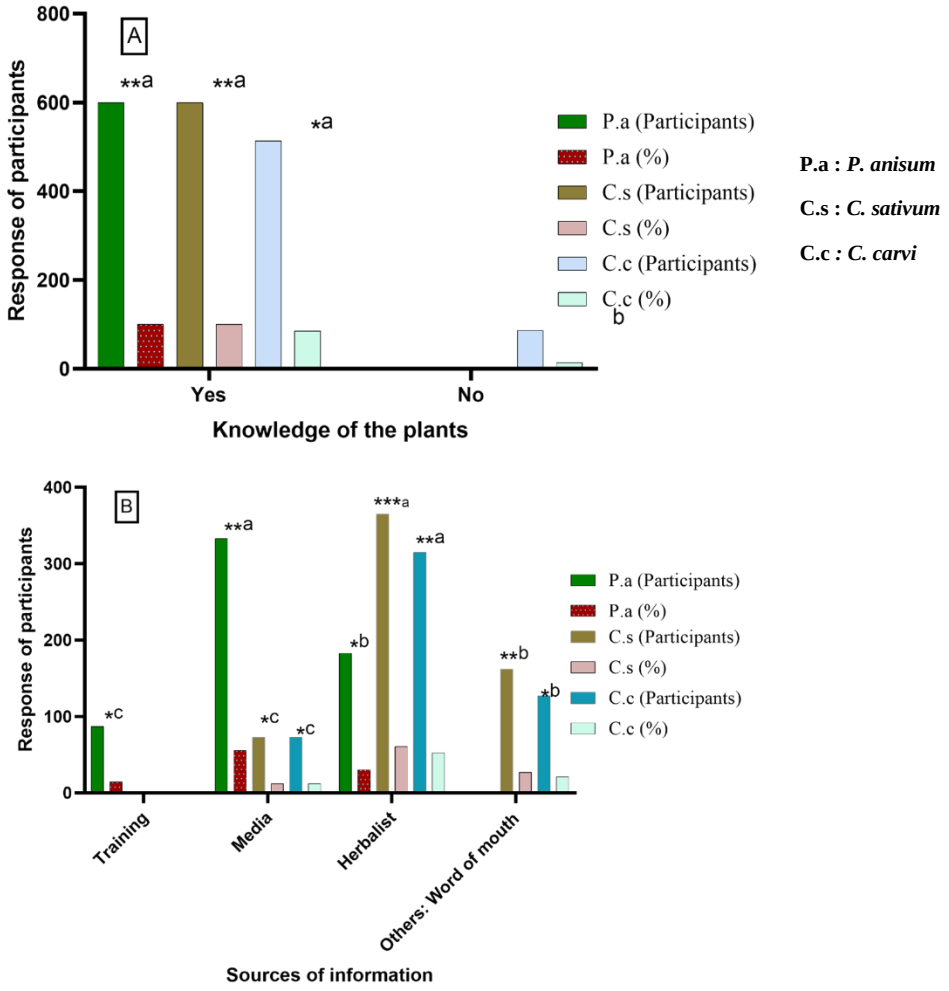


Figure 42: Knowledge of the plants (A) and Sources of information (B)

The source of information is the channel through which the therapeutic practices of traditional medicine are transmitted from one generation to another among populations (Bencheikh *et al.* 2021; Eshete and Molla 2021). In Morocco, many studies have addressed the transmission of traditional knowledge about medicinal plants. For example, Es-Safi *et al.* (2020) investigated the source of information on medicinal plants used to treat acute digestive system problems in the Fez-Meknes region. In the results, many sources were mentioned with various percentages; the most important were other people's experiences at 48.5%, followed by traditional health practitioners at 45.5%, then virtues explored by themselves at 3%, the internet at 2.4%, and finally books (0.6%). In another study, Mechchate *et al.* (2020) reported various sources of information, counting other experiences (84.8%), herbalists (6.2%), the internet (5.3%), exploring by themselves (2.0%), and books (1.7%). Similar results were reported by Benkhnigue *et al.* (2014) in the Al Haouz-Rhamna region on plants used to treat diabetes.

Concerning the studied plants, Soussi *et al.* (2023) investigated the source of information about the therapeutic uses of *Pimpinella anisum* in different areas of Morocco. The recorded results showed that the principal sources of information were media, counting TV, radio, and social networks with 68.24%, followed by educational training with 15.29%, while families and between friends' discussions, books, represented only 16.47%. Further, *Carum carvi* was reported as one of the plants used by Afghan refugees in the Northwest of Pakistan, and the know-how was related to the dietary habits of the participants (Shah *et al.* 2023). However, the *Carum carvi* is widely known in traditional medicine in Asia, counting India, Iran, Pakistan, and Afghanistan (Amiri and Joharchi 2016; Singh *et al.* 2016), Europe, such as Ukraine and Italy (Leporatti and Ghedira 2009; Pieroni and Sõukand 2017), and Africa counting Morocco (Najem *et al.* 2019) and Tunisia (Leporatti and Ghedira 2009). Concerning coriander (*Coriandrum sativum* L.), information on the therapeutic roles of this annual has been available since ancient times (Khan *et al.* 2014; Sobhani *et al.* 2022). It is used by a wide range of populations to treat different diseases, including intestinal disorders as mentioned in Colombia (Paniagua-Zambrana *et al.* 2020), diseases of the gastrointestinal system as reported in India (Sivasankari *et al.* 2014), and diabetes in Morocco (Skalli *et al.* 2019).

3.4. Curative uses of medicinal plants

Therapeutic uses of studied medicinal plants are presented in Table 1. In total, the investigated plants were used in the treatment of eight health troubles, two food purposes, and one cosmetic role. However, these roles were variable depending on the plant and sampling area. *Pimpinella anisum* is used to treat three diseases with different percentages. Further, 34.85% of

participants used it to treat dysmenorrhea, followed by anti-constipation uses for 33.02% of participants, 21.31% used it in antitussive roles, and only 10.82% used it as a promoter of milk flow (food purpose). *Carum carvi* was used by 44.51% of participants to treat bloating and cramps intestine, 43.97% to treat digestive troubles, and 11.51% to favor lactation and regulate menstruation. In contrast, *Coriandrum sativum* was used by 42.5% of participants to treat both troubles digestifs and spasms intestine, followed by antidiarrhea roles for 38.25% of participants, while alimentary roles were limited to 19.25% of participants.

Table 14 : Principal therapeutic uses of studied medicinal plants

THERAPEUTIC USES	ANTI-CONSTIPATION	DYSMENORRHEA	Promotes milk flow	Antitussive	Degestive disorders	lactation	Bloating and intestinal cramps	Antidiarrheal	Digestive disorders and intestinal spasms	Food
Anis	33.02%***	34.85%***	10.82%*	21.31%**	0	0	0	0	0	0
carvi	0	0	0	0	43.97%**	11.51%*	44.51%**	0	0	0
Coriandre	0	0	0	0	0	0	0	38.25%**	42.5%****	19.25%*

In terms of sampling site, *Pimpinella anisum* is used principally to treat dysmenorrhea in Meknes, as a promoter of milk flow in Taza, as an anti-constipation agent in Ifrane and Immouzer, and as an antitussive in Taounate and Sefrou. In Ifrane, *Carum carvi* is mostly used to treat bloating and intertinal cramps; in Meknes and Taza, it is used to treat digestive disorders, in Fez and Immouzzer, it is used in cosmetic and other roles, and in Taounate, *Carum carvi* is used to promote lactation and regulate menstruation. *Coriandrum sativum* is used to treat digestive disorders and intestinal spasms in three sites (Meknes, Ifrane, Immouzzer, and Sefrou); in Taounate, it is used as an antidiarrheal agent; and in Taza and Fez, it is used for cosmetic, food, and other purposes (Fig.43).

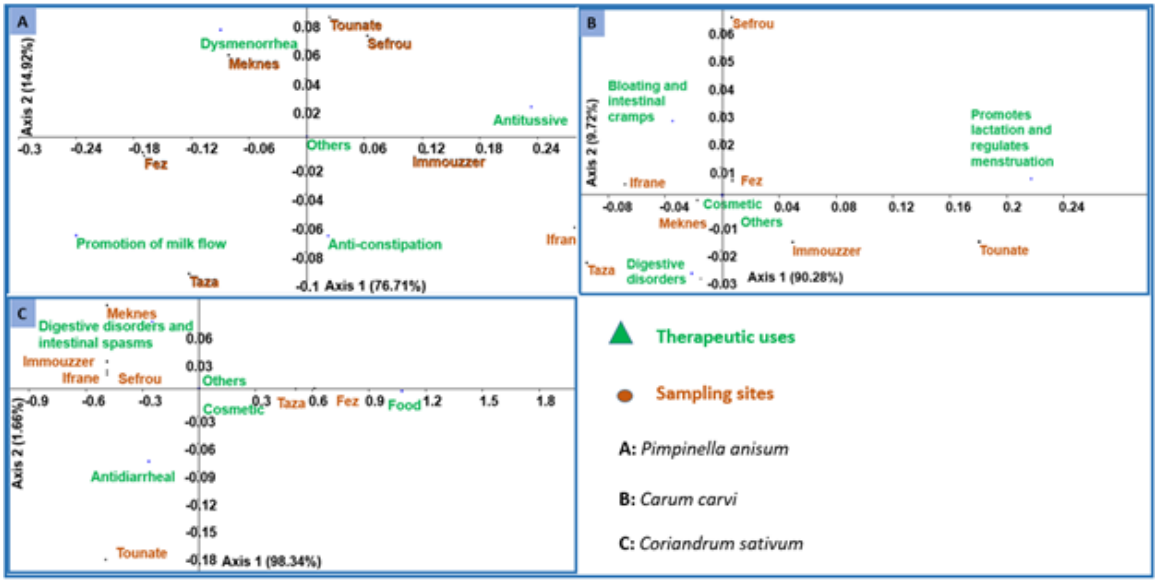


Figure 43: Therapeutic uses of medicinal plants depending on sampling sites

Carum carvi, coriander (*Coriandrum sativum* L.), and *Pimpinella anisum* are among the most popular species used worldwide. Their therapeutic uses vary depending on the geographical area, availability, health status, and knowledge of the population. In Rabat (Northwest Morocco), *Coriandrum sativum* L. is used traditionally to treat diabetes (Skalli *et al.* 2019), while in Agadir (southwest Morocco), it is reported that the plant is used in food receipts (Ouhaddou *et al.* 2014). Kothalawala *et al.* (2020) reported the use of *Coriandrum sativum* L. in traditional Sri Lankan concoctions to improve immunomodulatory activity. Chew *et al.* (2022) mentioned the benefits of *Coriandrum sativum* in food ingredients and its capacity to manage atopic dermatitis A. *Carum carvi* L. or caraway, is a common household plant grown around the world (Miraj and Kiani 2016). Caraway fruits are used as flavoring agents in foods and beverages and have various traditional uses in ethnomedicine. *Carum carvi* is reported to be useful for digestive problems including heartburn, bloating, gas, loss of appetite, and mild spasms of the stomach and intestines (Saghir *et al.* 2012; Lim 2013). The oil of *Carum carvi* is also used to help people cough up phlegm, improve control of urination, kill bacteria in the body, and relieve constipation. Women use the oil of caraway to start menstruation and relieve menstrual cramps; nursing mothers use it to increase the flow of breast milk (Munshi *et al.* 1990; SOFI *et al.* 2009). Caraway is used in mouthwashes and skin rubs to improve local blood flow (Miraj and Kiani 2016). Caraway was also used to improve lactation in nursing mothers (Sachan *et al.* 2016). The essential oil is used as a constituent in mouthwashes and bath additives, in perfumery, for scenting soap, and as a parasiticide (Hill 1952; Chiej 1984). It was commonly used as a flavoring in ice-cream, candy, meat, cheese, condiments, soft drinks, and alcoholic beverages (Morton and Zallinger 1976). Therapeutic uses of *Pimpinella anisum* are

reported in different scientific reports from all continents. In Asia, counting Iran, India, and China, , *Pimpinella anisum* is used as an analgesic in migraine and also as a carminative, aromatic, disinfectant, and diuretic in traditional medicine (Shojaii and Abdollahi Fard 2012; Kadan *et al.* 2013; Sun *et al.* 2019). In Europe (i.e., Italy, Turkey, and Greece), *Pimpinella anisum* is used as a digestive, stomachic, carminative, antitussive, expectorant, anti-spasmodic, galactogogue, diuretic, and diaphoretic agent (ARSLAN *et al.* 2004; Iannarelli *et al.* 2017; Anastasopoulou *et al.* 2020; Boztaş and Bayram 2020). Similarly, the species and its derivatives are used in African traditional medicine, such as in Morocco, Algeria, and Tunisia, to treat various diseases, counting the prevention purposes during the COVID-19 pandemic, galactagogue activity, and antidepressant-like effects (Elyebdri *et al.* 2017; Alami *et al.* 2020; Soussi *et al.* 2023).

All the cited bibliographies and our results confirm the utility of these plants against a wide range of diseases. These biological properties are due to the richness of these plants with chemical compounds (Wei *et al.* 2019; Aly *et al.* 2023; AlBalawi *et al.* 2023). For example, Matasyoh *et al.* (2009) identified 24 components in the essential oils from the leaves of *Coriandrum sativum* L., and the oil was dominated by aldehydes and alcohols (56.1% and 46.3%, respectively). Sixteen compounds were identified in *C. carvi* essential oils, out of which, cuminaldehyde was the principal component with 22.08%, followed by γ-terpinene with 17.86%), γ-terpinene-7-al with 15.41%, and *p*-cymene with 7.99% (Razzaghi-Abyaneh *et al.* 2009). Similar results were recorded in the seeds, fruits, and leaves of *Pimpinella anisum* (Figueredo *et al.* 2020; Anastasopoulou *et al.* 2020; Boumahdi *et al.* 2021). These compounds are responsible for the management of treated diseases.

3.5. Used fragments of medicinal plants

Different parts and usage forms were recorded for each species. Seeds are the only part used for anise and *Carum carvi* at 100%. In contrast, the used parts of *Coriandrum sativum* were divided equally between the whole plant (50%) and the seeds (50%). On the other hand, all plants were used in dried forms compared to fresh materials. Equally, all investigated medicinal plants were used in infusion preparation by participants in all sampling sites. *Pimpinella anisum* is used principally in the form of tisane by 77.83% of participants, followed by powder form by 22.17% of participants. In *Carum carvi*, all participants used only the Tisane form (100%). Further, 36.5% of participants used *Coriandrum sativum* in the tea form, compared to 63.5% of participants in the powder form. Distilled materials and essential oils were absent among the interviewed participants for all plants.

Table 15 : Used fragments of studied medicinal plants among interviewed participants

Parts	Species	Whole plant	Seed	Fruit	Root	Stem
	Pimpinella anisum	0	100*	0	0	0
	Carum carvi	0	100*	0	0	0
	Coriandrum sativum	50%*	50%*	0	0	0
Form		Fresh	Dried			
	Pimpinella anisum	0	100*	0	0	0
	Carum carvi	0	100*	0	0	0
	Coriandrum sativum	0	100*	0	0	0
Preparation		Infusion	Decoction	Others		
	Pimpinella anisum	100*	0	0	0	0
	Carum carvi	100*	0	0	0	0
	Coriandrum sativum	100*	0	0	0	0
Mode		Tisane	Powder	Distilled	essential oil	
	Pimpinella anisum	77.83**	22.17*	0	0	0
	Carum carvi	100*	0	0	0	0
	Coriandrum sativum	36.5*	63.5**	0	0	0

Many studies reported the use of studied species in traditional medicine; however, these investigations reported the use of different parts depending on the targeted disease. (Soussi *et al.* 2023) reported the use of seeds (dried powder and tisane) from *Pimpinella anisum* in traditional medicine from various regions of Morocco. In other studies, Jouad *et al.* (2001) reported the use of the fruits of *Pimpinella anisum* to treat diabetes in the Fez-Boulemane region (central Morocco). (Jouad *et al.* 2001; El-Hilaly *et al.* 2003) reported the use of leaves and seeds of *Coriandrum sativum* L. to treat intestinal pains, kidney stones, and insomnia in the North of Morocco and fruits to treat diabetes in central Morocco. In Iran, local populations use the leaves of *Coriandrum sativum* L. (decoction) to manage gasteralgia and sore throats (Ghorbani 2005). Fruits of *C. carvi* L. were reported among the medicinal plants used in the treatment of diabetes in the Central Zone of Morocco (Jouad *et al.* 2001).

3.6. Used dose and effects

The results of the dose used and their effects on each medicinal plant are presented in Table 16. The majority of participants use imprecise doses of medicinal preparations from all plants. In *Pimpinella anisum*, 89.67% of participants used imprecise spoonfuls to treat health disorders, compared to 10.33% who used accurate quantities. Further, 83.17% of participants use imprecise spoonfuls to treat health disorders and for food purposes with *Coriandrum sativum* compared to 16.83% of participants who use it in inaccurate mode. Further, 79.55% of participants used inaccurate doses of *Carum carvi* preparations, compared to accurate doses for 19.45% of participants.

All medicinal plants were used by the totality of participants (100%) only when they were needed (after the occurrence of diseases). All participants (100%) mentioned the amelioration of their health status (cure of the treated disease) after the use of the studied medicinal plants. All participants mentioned the absence of side effects after the use of these medicinal plants in the treatment of health disorders. Similarly, no toxicity was recorded after the use of the plants at all sampling sites. To avoid the toxicity and side effects of used plants, all participants (100%) store the plants' materials at ambient temperature and away from light. Equally, the participants avoid mixing the investigated medicinal plants with other supplementary materials.

Table 16 : Posology, effects (cure, side, and toxicity), and storage conditions of studied medicinal plants

	Pimpinella anisum	*Carum carvi*	*Coriandrum sativum*
Storage conditions	Protected in ambient temperature and far from light	Protected in ambient temperature and far from the light	Protected in ambient temperature and far from the light
Posology	Only when needed	Only when needed	Only when needed
Precautions for use	No mixing with other plants or products	No mixing with other plants or products	No mixing with other plants or products
Cases of toxicity	No case	No case	No case
Side effects	No case	No case	No case
Results	Cure of treated disease with 100%	Cure of treated disease with 100%	Cure of treated disease with 100%

This is the first study to evaluate the cure, side effects, toxicity, and precautions for the use of medicinal plants in Morocco. All participants mentioned the efficacy of all plants in the treatment of the mentioned diseases and the absence of side effects or toxicity. Soussi *et al.* (2023) conducted deep ethnobotany research on the therapeutic uses of *Pimpinella anisum* among local populations in Morocco. Similarly, laboratory studies have tested the toxicity of *P. anisum* (Sihoglu Tepe and Tepe 2015). de Mello *et al.* (2007) have investigated the potential toxic effect of *P. anisum* formulation on female Wistar rats during pregnancy and lactation (44 days), on male Wistar rats, and on New Zealand rabbits (males and females) for 30 days. According to the results, *P. anisum* showed no toxic effect when administered to rats and rabbits at a dose equivalent to 10 times the human dose. Despite the absence of toxicity among the participants who use *Carum carvi*, (Agrahari 2014) demonstrated that the seed of *Carum carvi* contains >90% fumigant toxicity against adult male and female *Blatella germanica*. In

another study, Kumar and Singh (2006) investigated the toxicity of three plants, counting *Carum carvi, Syzygium aromaticum*, and *Ferula asafetida*, as well as their active components against the snail *Lymnaea acuminate*. In the results, the toxicity of *S. aromaticum* flower-bud powder (96 h LC50: 51.98 mg/l) was more pronounced than that of root latex powder of *F. asafoetida* (96 h LC50: 82.71 mg/l) and seed powder of *C. carvi* (96 h LC50: 140.58 mg/l). Further, ethanol extract was more toxic than other organic extracts. The ethanol extract of *S. aromaticum* (24 h LC50: 83.53 mg/l) was more effective than that of *F. asafoetida* (24 h LC50: 132.31 mg/l) and *C. carvi* (24 h LC50: 130.61 mg/l) in killing the test species.

4. Conclusion

This survey presents new ethnobotanical data on the therapeutic uses of three medicinal plants, *Pimpinella anisum, Carum carvi,* and *Coriandrum sativum,* in traditional medicine in three geographical zones from Morocco and the south of the Mediterranean. We investigated the use of these plants in folk medicine, food, and cosmetics among populations in the north, center, and Saiss plains of Morocco. Equally, we searched for the effect of demographic features, geographical location, and health status of the interviewed populations on the responses of participants. The most relevant conclusions were that populations in the three zones were aged from 20 to 60 years and used all these plants to treat a wide range of diseases, including digestive disorders, dysmenorrhea, lactation, and regulation of menstruation. Equally, these plants are used for food and cosmetic purposes, such as the promotion of milk flow. The use of each plant varies depending on the sampling site. The principal sources of information about the therapeutic benefits of the plants were the media, including radio, TV, and current social networks. Populations use the seeds or whole plants alone in dried form to treat their illnesses. The usage forms were principally tisane and powder, which were prepared in the mode of infusion. To avoid toxicity or side effects, local populations use these plants only when needed, with storage in obscurity and at ambient temperature. The treatment with the mentioned plants was 100% effective against the treated diseases, but the doses were not precise. The findings demonstrated the significance of *Pimpinella anisum, Carum carvi,* and *Coriandrum sativum* in Moroccan traditional medicine. To turn intuition into knowledge, however, and scientifically confirm the treatments found by exact scientific procedures, it is crucial to expand this kind of research to many regions of the nation. It is thought that this scientific assessment will increase the economic value of natural resources and, as a result, rural and poor communities' incomes.

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OPTIMISATION DE L'EXTRACTION DES COMPOSES PHENOLIQUES DES PLANTES ETUDIEES

Optimisation simultanée des composés phénoliques et des capacités antioxydantes d'extraits marocains de *Pimpinella anisum* à l'aide d'une méthodologie de conception de mélange

(A fait l'objet d'un article publié dans le journal Processes)

Synthèse d'étude

Cette étude consiste à définir le mélange des solvants optimale pour l'extraction des composés polyphénoliques de *Pimpinella anisum*, en se basant sur la conception « Augmented Simplex-Centroid ». Le choix du mélange idéal repose sur une concentration élevée en polyphénols et flavonoïdes, ainsi qu'une activité antioxydante maximisée.

Les graines d'anis ont été collectées dans la région d'El Hajeb au cours de l'automne 2021 (33°41'22" N, 5°21'13" W). Les échantillons ont été ramenés au laboratoire et séchés jusqu'à un poids constant à température ambiante puis, ils sont broyés en poudre.

Les extraits ont été préparés en mélangeant 50 mg de plante avec 1 ml de solvant d'extraction à savoir : Éthanol, méthanol, eau, dichlorométhane, chloroforme, acétone, acétate d'éthyle, hexane, butanol, acétonitrile. Les extraits réalisés avec de l'eau, du méthanol et de l'éthanol ont affiché les concentrations les plus élevées de TPC, atteignant respectivement 13,2 ± 0,36, 11,29 ± 0,11 et 7,5 ± 0,16 mg GAE/g. Il ressort que l'eau s'est avérée être le solvant le plus efficace pour extraire une grande quantité de composés phénoliques. À l'opposé, l'extrait de dichlorométhane a présenté la concentration la plus basse de TPC, enregistrant des valeurs de 2,1 ± 0,1 mg GAE/g. en se basant sur ces résultats les 3 solvants : éthanol, méthanol et eau ont été retenu pour le reste du travail.

Notre étude s'est basée sur l'utilisation du plan de mélange pour optimiser le processus d'extraction. La formule optimale du solvant a été déterminée à l'aide de courbes de niveau et de surface basées sur des courbes d'iso-réponse. La teneur en composés phénoliques et en flavonoïdes totaux a été quantifiée. L'activité antioxydante des extraits optimisés a été évaluée à l'aide du test DPPH.

L'analyse des résultats a révélé que le mélange binaire des deux solvants d'extraction (eau et méthanol) à volumes égaux a montré d'importantes valeurs de TPC. Le mélange avec des proportions équivalentes des trois solvants était la formule la plus extractible et possédait les valeurs TPC et TFC les plus élevées. La combinaison du mélange ternaire avec des volumes

égaux de trois solvants (eau-éthanol-méthanol, v/v/v), s'avère la plus appropriée pour une extraction optimale.

L'analyse des courbes de niveau et de surface ainsi que le diagramme de désirabilité simultanée suggèrent que pour atteindre une extraction maximale et en tenant compte de toutes les réponses, le mélange ternaire optimal s'avère être celui composé de (48 :22 :29 v/v/v) d'eau, d'éthanol et de méthanol. Les meilleures réponses étaient : 18,55 mg GAE/g, 7,16 mg QE/g et 0,56 mg/ml pour le TPC, le TFC et le $DPPH_{IC50}$, respectivement.

Les résultats indiquent que la proportion des solvants extracteurs influence les contenus bioactifs des extraits optimisés. Par conséquent, il s'avère que l'utilisation du plan de mélange est un outil efficace pour déterminer le meilleur mélange d'extraction afin d'obtenir des extraits dotés d'un fort potentiel antioxydant.

Chap I: Simultaneous optimization of phenolic compounds and anti-oxidant abilities of Moroccan *Pimpinella anisum* extracts using mixture design methodology

Abstract

Pimpinella anisum (anise) is a dense vegetal matrix with considerable amounts of bioactive com-ponents known for its pharmacological properties. The optimization of extraction constitutes an important key to improving efficacy and avoiding wasting time. Within this frame, the present study was designed to select the most appropriate extractor solvent mixture to extract phenolic and flavonoids using Mixture Design Methodology. The concerned responses were the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant ability examined by 2,2-diphenyl-l-picrylhydrazyl (DPPH) assay. Before mixture design optimization, a screening of solvents was conducted on ten polar and non-polar solvents to choose the best solvents that give a maximum of total phenolic compounds. This first step has shown that water, ethanol, and methanol were the best-used solvents. Later, an augmented centroid design investigated the solvent system's optimization. The results of simultaneous optimization have shown that the ternary mixture containing 44% of water, 22% of ethanol, and 34% of methanol was the most appropriate for simultaneous maximization of TPC, TFC, and antioxidant activity with 18.55 mg GAE/g, 7.16 mg QE/g and 0.56 mg/ml, respectively. Our results have shown that using mixture design as an optimization technique was an excellent way to choose the most suitable mixture to extract bioactive compounds, which may represent a promising way of multi-purpose extraction, especially in the pharmaceutical and food sectors.

Keywords: *Pimpinella anisum*; optimization; total phenolic content; total flavonoid content; anti-oxidant activity.

1. Introduction

Medicinal plants have occupied a significant role in human food since early. They are used for many purposes as raw matter in culinary and therapeutic systems, either in traditional or modern medicine [1]. The dense chemical composition of medicinal plants was the main factor for their uses in folk and modern medicine. The therapeutic abilities of medicinal drugs depend on numerous factors, as well as the part of the plant, the nature of the extract, isolated components, and purification technique [2–4]. Thus, classical techniques of extraction have continually been used in traditional therapy. However, recent studies focus on optimizing the extraction as the most efficacious tool for extracting a high amount of bioactive compounds

from plant matrix [5,6]. In this way, several researchers have focused their interest on studying various factors such as extraction method, the effect of extraction time, temperature, the form of a material matrix, and the nature of the solvent [7–9]. Actually, there is no magic recipe or mixture of solvents to elevate the yield of extractable bioactive compounds. Therefore, different ways were investigated to find the optimal operating conditions that can lead to the best high number of bioactive compounds. Among these techniques, the designs of experiments, which are reliable and precise chemometric techniques based on the mathematical modeling of a response in terms of the dependent factors [10,11].

Numerous mathematical models were developed to elevate the amount of extractable bioactive compounds, including response surface methodology (RSM) [12,13] and mixture design [14,15]. These models are an alternative approach for recovering a high amount of bioactive components, and allow more than one response to be optimized simultaneously [16]. The optimization of phenolic compounds amount seems to be related to several proprieties such as antioxidant activity. The modeling of the extraction process using response surface methodology is widely used to choose the right amounts of different solvents to recover the maximum yield of pharmacologically active ingredients from a matrix plant [17]. This technique has been used in various fields, including experiment studies in medicinal plants [18]. Indeed, it has been proved that the optimized extract exhibits high antioxidant potency. These findings were confirmed by the chemical profile of the extract, indicating the presence of different pharmacological active molecules with high antioxidant potential [13,16,19].

Pimpinella anisum (*P. anisum*) is a member of *Umbelliferae* family known for its traditional uses in the folk medicine of several civilizations [20]. Mounting evidence confirmed the ability of *P. anisum* to exert numerous pharmacological properties, including antibacterial [21], antifungal [22], insecticidal [23], anticonvulsant [24], analgesic [24], antidepressant and anxiolytic activities [20,25]. Furthermore, these pharmacological properties are associated with phytochemical content, such as polyphenolic compounds. Indeed, it has been confirmed that *P. anisum* contains numerous phytochemical constituents, including flavonoids and polyphenolic components [26].

Experimental designs are among the most common avenue adopted to optimize the extraction of phenolic compounds from different vegetal matrices [27–29]. In addition, other methods gained increased interest, including microwave and ultrasonic-assisted extract thanks to their simplicity, rapidity, and low cost [27]. These methods play a pivotal role in examining the possible interaction of different parameters, providing the most appropriate combinations to

minimize the time and number of experiments [13,27,30]. Within this frame, the current study is designed to identify the most suitable solvent mixture for polyphenolic compounds extraction and antioxidant ability from *P. anisum* using an augmented simplex-centroid design. Before proceeding with the solvent system optimization, a screening was carried out on ten pure solvents among the most used in the extraction of phenolic compounds to select those which give the best total phenol content. The solvents chosen from this screening were used as constituents of the mixture of the system of solvents. The appropriate mixture was selected based on the optimized high total phenolic-flavonoid content and antioxidant power. To the best of our Knowledge, It is the first time that such a work has been done on the species *P. anisum.*

2. Materials and Methods

2.1. Chemicals and materials

Seeds of anise were collected from El Hajeb region during the autumn season of 2021 (33°41’22’’ N, 5°21’13’’ W). Ethanol, methanol, dichloromethane, chloroform, acetone, ethyl acetate, hexane, butanol, acetonitrile, water, DPPH, Folin-ciocalteu reagent, gallic acid, quercetin were the chemicals used in different experiments and were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Samples preparation

The plant was identified by the Botanist of the National Agency of Medicinal and Aromatic Plants (NAMAP/ANPMA)-Taounate, Morocco. The samples were dried to constant weight in a room in which the temperature was fixed at 25 °C and then powdered in a lab grinder machine.

2.3. Ultrasonic-assisted extraction

Initially, the extraction was performed by ten pure polar and non-polar solvents, including water, ethanol, methanol, dichloromethane, chloroform, acetone, ethyl acetate, hexane, butanol, and acetonitrile. 50 mg of powdered seeds were mixed with 1 mL of each solvent. The mixtures were sonicated for 30 min in a P30 H ultrasonic cleaner bath with a power of 100W and a frequency of 37 kHz (Elmasonic, Germany), and centrifuged at 10000 rpm/10 min in a Hettich ROTOFIX 32A centrifuge (Hettich, Tuttlingen, Germany). The mixture was filtered and stored at 4 °C until experimental analysis, as previously described by Ousaaid et al. [31]. Afterward, an extraction under the same conditions was carried out by a solvent

system formulated from the three most effective solvents. The solvent fractions of each experiment are generated from the chosen mixture design.

2.4. Total Phenolic content quantification

The phenolic content was quantified using the colorimetric methods previously described by Singleton et al. [32]. In brief, 25µL of extract and 450µL of Folin-Ciocalteu reagent (0.5N) were mixed. Then, the mixture was treated with 450µL of sodium carbonate and incubated in the dark for 2 hours. The absorption was read at 760 nm, and the results were expressed as mg GAE/g using a calibration curve of gallic.

2.5. Total flavonoid content quantification

The total flavonoid content was determined by the colorimetric method according to the method described by Laaroussi et al. [33]. The protocol involves mixing 100µL of extract, 150µL of $Alcl_3$ reagent (10%), and 200µL of NaOH (1%). The obtained mixture was incubated in the dark condition for one hour, and then, the absorbance was read at 510 nm.

The results are expressed as mg of quercetin equivalent per gram (QE/g) of seeds anise using a calibration curve of quercetin.

2.6. Antioxidant activity

The antioxidant activity of extracts was evaluated using the DPPH test [34]. In brief, the extract and DPPH solution were mixed with the following quantities, 25µL and 875µL, respectively. The mixture vortexed and incubated for one hour in the dark condition. The absorbance of the mixture was measured at 517 nm. The percentage inhibition (PI) of the DPPH radical by the samples was calculated according to the formula:

$$PI(\%) = \left(\frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\right) \times 100 \qquad (1)$$

The antiradical activity was expressed as $DPPH_{IC50}$ (mg/mL), which is the exact dose required concentration of extract able $DPPH_{IC50}$ value corresponds to a higher antioxidant activity.

2.7. Mixture design

The current study used a mixture design methodology to optimize the extraction process. The test is based on combining different independent factors to create a set of equations that donate theoretical values [13]. The solvent system components are presented in table 3. According to this table, each of the three components can have a value between 0 and 1, and the sum of the three proportions will always be 1. Besides, a total of 12 experiments were

performed to examine the impact of solvent proportions on total phenolic and flavonoid content and antioxidant activity, as described in figure 44.

Table 17 : Identification of solvent system factors

Components	Coded variables	Level -	Level +
Water	X1	0	1
Ethanol	X2	0	1
Methanol	X3	0	1
Sum of proportions		1	

N° of experiment	X1	X2	X3
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.33	0.33	0.33
8	0.33	0.33	0.33
9	0.33	0.33	0.33
10	0.66	0.17	0.17
11	0.17	0.66	0.17
12	0.17	0.17	0.66

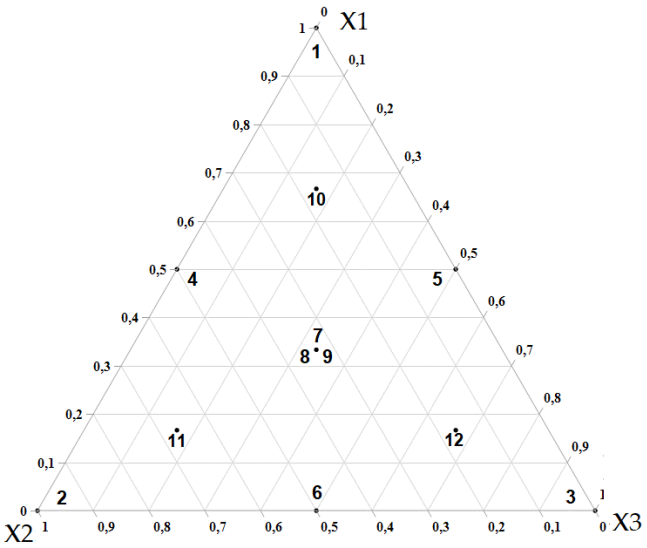


Figure 44: Illustration representing the augmented simplex-centroid design.

As shown by the equation below, the specific cubic model was employed to express the interaction between the components of the analyzed mixtures as well as to predict the maximal recovery of bioactive chemicals and antioxidant activity:

$$Y = \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_{12} X_1 X_2 + \alpha_{13} X_1 X_3 + \alpha_{23} X_2 X_3 + \alpha_{123} X_1 X_2 X_3 + \varepsilon \quad (2)$$

Y represents the experimental response expressed by mg GAE/g, mg QE/g, and mg/ml for TPC, TFC, and $DPPH_{IC50}$, respectively. α_1, α_2, α_3, are linear regression coefficients, α_{12}, α_{23}, and α_{23}, are binary regression coefficients, α_{123}: is the ternary regression coefficient, while ε: is the regression error term. The findings were expressed as mean, and ANOVA was used to analyze the results.

2.8. Statistical analysis

Optimization tools were used as an excellent appliance to predict the appropriate combination of extractor solvents. In this context, simplex centroid design was used to assess different interactions among extractor solvents on TPC, TFC, and $DPPH_{IC50}$.

The validation of the postulated models was carried out using the F-test for ANOVA. The $F_{R/r}$ (the ratio between the mean square due to the regression (MS_R) and the mean square due to the residuals (MS_r)) was calculated and then compared to the theoretical F for the same degree of freedom. The lack of fit test was used was used to refine the model fit by comparing the ratio between the mean square lack of fit (MS_{LOF}) and the mean square pure error (MS_{PE}) [35]. High values of $F_{LOF/PE}$ indicate a lack of fit to the model. The coefficient of determination R^2, R^2 adjusted and R^2 predicted were used to verify the quality of the postulated models, while Student's test was used to express the significance of the model's coefficients. The experimental design conception and the statistical and graphical analysis were performed using JMP software V.14 (SAS Institute Inc., Cary, NC, USA) and Design Expert V.11 (Stat-Ease, inc., Minneapolis, USA).

The optimum solvent formula was determined using contour and surface plots based on iso-response curves, which resulted in a compromise of responses. The intensity of the red color indicates the response elevation, while the blue color indicates the opposite. In addition, the desirability function was carried out to determine accurately the desired response based on the optimum conditions. A number of 0% is given when the system produces an undesirable response, while a score of 100% represents the highest possible desired response [36]. Finally, the comparison of means was performed using the ANOVA F-test while Pearson's test was used to evaluate the correlation between the antioxidant capacity and TPC and TFC. All experiments were performed in triplicate and the results were expressed as the mean ± SD.

3. Resultats et Discussion

3.1. Solvents screening

Ten solvents were tested to select the most appropriate in maximizing the extractable efficacy on TPC. The obtained results of quantifying phenolic content in terms of used solvents are presented in Figure 45.

The observation of the graph revealed that water was the most suitable solvent to extract the highest quantity of phenolic content with 13.2 ± 0.36, followed by methanol and ethanol with 11.29 ± 0.11 and 7.5 ± 0.16 mg GAE/g, respectively. Acetone and ethyl acetate gave moderate values of TPC with 6.4 ± 0.62 and 5.9 ± 0.79, respectively. In contrast, dichloromethane extract

showed the lowest amount of TPC with only 2.1 ± 0.1 mg GAE/g. In addition, the results obtained for the three solvents water, ethanol and methanol were statistically different from the other seven solvents (p-value<0.05). The obtained findings were in good concordance with those evoked previously by several researchers for the genius *Rosa canina* L indicating that high amounts of TPC and TFC were obtained in the aqueous extract compared to ethanolic and methanolic extracts [31,37]. In the same way, Cavalcanti et al. have found that water was the most extractable solvent of TPC with 3.82±0.07 mg GAE/g against 0.84±0.01 mg GAE/g and 0.35±0.02 mg GAE/g obtained by ethanol and acetone, respectively [19]. However, our results did not agree with those provided by Mohsen et *al.*, which indicated that ethanol was the most effective solvent for TPC recovering from corn tassel, followed by methanol and then water [38].

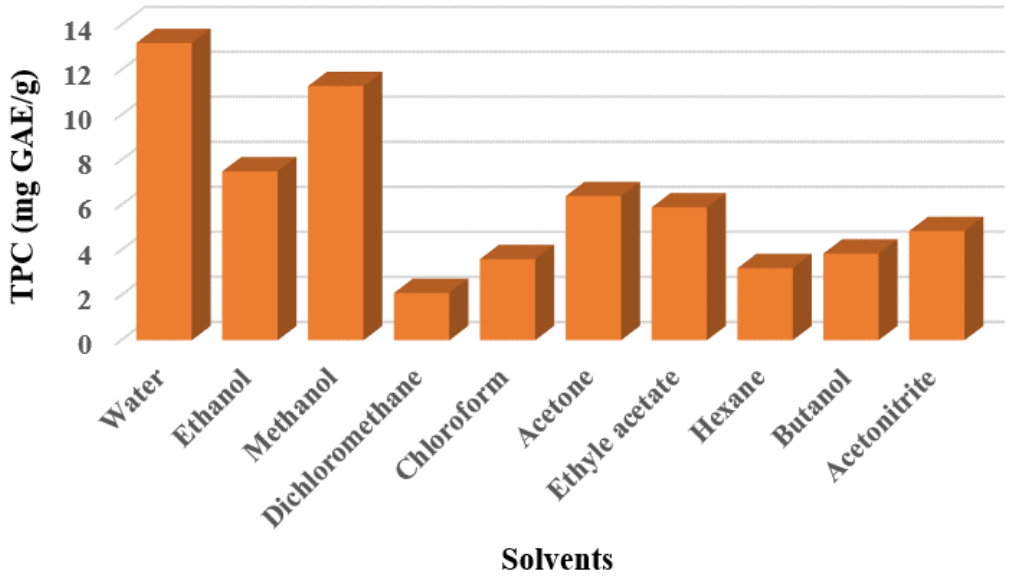


Figure 45: Total phenolic compounds extracted from P. anisum seeds using different solvents.

3.2. Solvent Mixture

The simplex-centroid design, with different combinations of the solvents and the recorded response of each experiment on TPC, TFC, and $DPPH_{IC50}$ are listed in Table 18. The experiments were carried out after randomization, and every response averages three replicate.

Table 18 : Experimental conditions of mixture design. actual and predicted values and residuals for each response

Experiment number [a]	**Solvent's proportions**			**TPC (mg GAE/g)**			**TFC mg QE/g**			**$DPPH_{IC50}$ (mg/ml)**		
	Water	Ethanol	Methanol	Actual [b]	Predicted	Residual	Actual [b]	Predicted	Residual	Actual [b]	Predicted	Residual
1	1	0	0	14.14±0.23	13.9	0.24	4.12±0.72	4.12	0	0.69±0.08	0.69	0
2	0	1	0	5.62±0.56	5.66	-0.04	2.05±0.26	2.03	0.02	1.46±0.06	1.47	-0.01
3	0	0	1	11.29±0.11	11	0.29	3.54±0.41	3.51	0.03	0.77±0.13	0.77	0
4	0.5	0.5	0	11.84±0.62	11.64	0.2	3.84±0.93	3.82	0.02	0.72±0.05	0.74	-0.02
5	0.5	0	0.5	16.52±0.17	15.99	0.53	4.92±0.12	4.89	0.03	0.68±0.05	0.68	0
6	0	0.5	0.5	10.6±0.35	10.35	0.25	2.95±0. 38	2.9	0.05	0.97±0.18	0.99	-0.02
7	0.33	0.33	0.33	18.45±0.28	17.99	0.46	7.15±0.56	7.17	-0.02	0.6±0.06	0.61	-0.01
8	0.33	0.33	0.33	18.15±0.09	17.99	0.16	7.38±0.67	7.17	0.21	0.59±0.03	0.61	-0.02
9	0.33	0.33	0.33	18.86±0.85	17.99	0.87	7.13±0.18	7.17	-0.04	0.57±0.21	0.61	-0.04
10	0.66	0.17	0.17	15.72±0.43	16.92	-1.2	5.99±0.87	6.04	-0.05	0.6±0.07	0.58	0.02
11	0.17	0.66	0.17	11.91±0.57	12.29	-0.38	4.56±0.43	4.67	-0.11	1±0.19	0.94	0.06
12	0.17	0.1	0.6	14.16±0.92	15.53	-1.37	5.39±0.25	5.53	-0.14	0.71±0.05	0.69	0.02

[a] Experiments were carried out after randomization.

[b] Each response is the average of three replicates with standard error.

The analysis of results revealed that the mixture with equivalent proportions of the three solvents was the most extractable formulation and possessed the highest TPC, TFC, and $DPPH_{IC50}$ values (Table 18). Furthermore, the binary mixture of both extractor solvents (water and methanol) with equal volumes showed important TPC values. While pure ethanol was the weakest extractor solvent for TPC yielded, as previously mentioned in the screening of pure solvent extractor efficacy. In fact, no solvent can extract all kinds of bioactive compounds because of their variable solubility and polarity [39].

The efficacy of a mixture of three solvents water, ethanol, and methanol in extracting the highest content of TPC and TFC is demonstrated by the highest antioxidant ability of the optimized extracts. Based on our findings, the mixture of three solvents was paramount in elevating bioactive components recovery due to the complex composition of plant matrix containing molecules with different polarities [31,40].

This antioxidant activity could be explained by the presence of polyphenols and flavonoids, which have strong antioxidant power in plants [30]. In a study carried out by Bekara et *al.*, the results showed that the aqueous extract of *P. anisum* possesses an important antiradical power [41].

3.2.1. Statistical validation of postulated models

The analysis of variance was carried out to study the interaction of different constituents of the mixture, as displayed in Table 19. The results show that the regression's main effect was significant for all studied responses with low *p-values* (0.0011, <0.0001, and <0.0001 for TPC, TFC, and $DPPH_{IC50}$, respectively). In addition, the three postulated models do not show a lack of fit since their *p-values* were greater than 0.05, and their calculated $F_{LOF/PE}$ was lower than the theoretical $F_{(0.05;\ 3;2)}$ equal to 19.16 at 95% of confidence.

Table 19 : The analysis of variance (ANOVA) of studied fitted models.

	TPC					**TFC**				**$DPPH_{IC50}$**			
Model	**DF**	**SS**	**MS**	**F**	**p-value**	**SS**	**MS**	**F**	**p-value**	**SS**	**MS**	**F**	**p-value**
R	6	163.1	27.2	27.3	0.0011*	33.1	5.52	326	<0.0001*	0.711	0.119	81	<0.0001*
R	5	4.970	0.99			0.08	0.016			0.007	0.001		
Lof	3	4.71	1.57	12.37	0.0700	0.05	0.015	0.80	0.5987	0.0068	0.0023	9.76	0.09
Pe	2	0.25	0.12			0.04	0.019			0.0005	0.0002		
total	11	168.11				33.23				0.72			
R^2	0.97					0.99				0.98			
R^2_{adj}	0.93					0.99				0.97			
R^2_{pred}	0.89					0.97				0.81			

DF: degrees of freedom; SS: sum of squares; MS: mean square; R: regression; r: residual; Lof: lack of fit; Pe: pure error; adj: adjusted; pred: predicted; *: statistically significant;

The quality of the studied model was confirmed by the coefficient of determination (R^2). The latter was equal to 0.97, 0.99, and 0.98 for TPC, TFC, and $DPPH_{IC50}$, respectively. The R^2 adjusted and predicted are also sufficient to testify the prediction quality of the chosen models. Notably, an excellent correlation was observed between the experimental and predicted results. The graph in Figure 46 confirms this good agreement by showing a linear curve for the actual values in terms of the predicted ones.

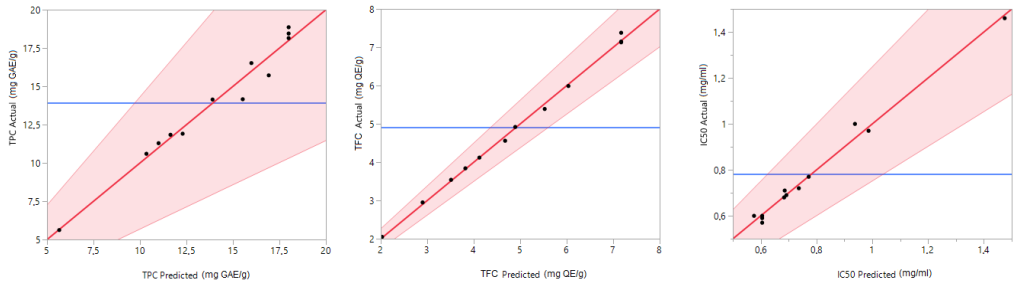


Figure 46: Curves of observed values versus predicted effects and fitted models

Table 20 summarizes the estimated regression coefficients of the special cubic model. The regression equations with significant coefficients were used to determine the relationships between all studied parameters and the obtained responses for TPC, TFC, and $DPPH_{IC50}$).

Table 20 : Estimated regression coefficients of the special cubic model.

Term	**Coeff.**	**TPC**		**TFC**		**$DPPH_{IC50}$**	
		Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|
Water	α_1	13.90	<.0001*	4.12	<.0001*	0.69	<.0001*
Ethanol	α_2	5.66	0.0020*	2.03	<.0001*	1.47	<.0001*
Methanol	α_3	11.00	<.0001*	3.51	<.0001*	0.77	<.0001*
Water*Ethanol	α_{12}	7.44	0.19	2.98	0.0053*	-1.39	0.0007*
Water*Methanol	α_{13}	14.16	0.0330*	4.30	0.0010*	-0.19	0.35
Ethanol*Methanol	α_{23}	8.07	0.16	0.52	0.45	-0.55	0.0321*
Water*Ethanol*Methanol	α_{123}	121.82	0.0057*	83.25	<.0001*	-3.73	0.0141*

*Statistically significant at P<0.05

The significant extractor solvent coefficients for TPC are α_1, α_2, and α_3, for pure extractor solvents, followed by the coefficient of the binary formulation between water and methanol (α_{13}). The following equation represents the mathematical model adopted for TPC:

$$Y = 13.90X_1 + 5.65X_2 + 11\ X_3 + 14.16X_{13} + 121.82X_{123} + \varepsilon \quad (3)$$

Concerning TFC, the significant terms are all the terms of the adapted mathematical model (α_1, α_2, α_3, α_{12}, α_{13}, and α_{123}), except the coefficient that corresponds to the binary combination between ethanol and methanol (α_{23}). The following equation is used to represent the retained mathematical model for TFC:

$$Y = 4.12X_1 + 2.03X_2 + 3.51\ X_3 + 2.98X_{12} + 4.30X_{13} + 83.25X_{123} + \varepsilon \quad (4)$$

Regarding $DPPH_{IC50}$, all terms are significant except the coefficient corresponding to the binary term α_{13}. $DPPH_{IC50}$ response was determined using the following mathematical model:

$$Y = 0.69X_1 + 1.47X_2 + 0.77X_3 - 1.39X_{12} - 0.55X_{23} - 3.73X_{123} + \varepsilon \quad (5)$$

3.2.2. Solvent system optimization

3.2.2.1. Total polyphenolic content

Figure 4 presents the contour and surface plots realized for total polyphenolic content obtained using a different mixture of water, ethanol, and methanol.

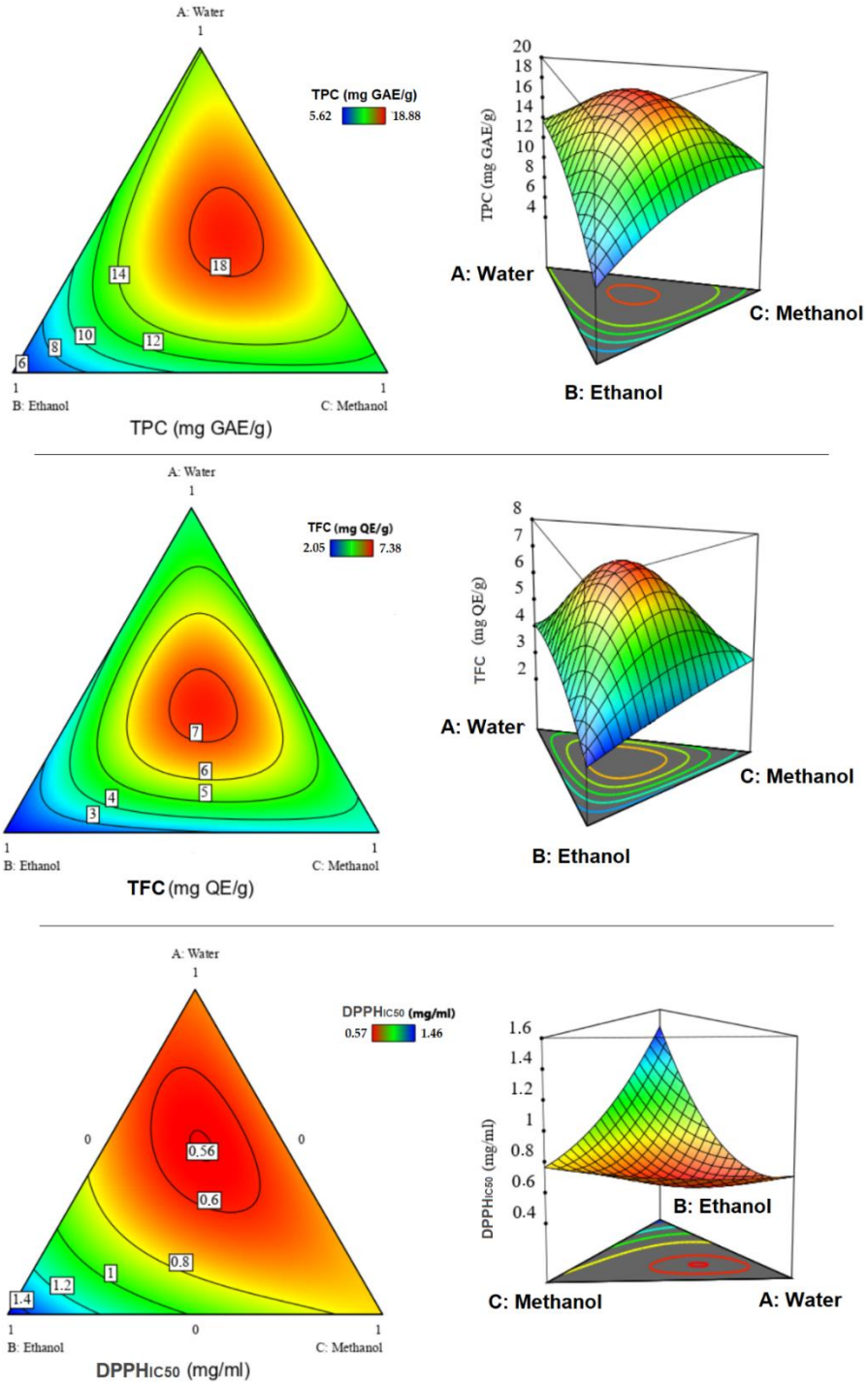


Figure 47: Contour and surface plots, which indicate the optimal compromise area leading to the best value of TPC (A), TFC (B) and $DPPH_{IC50}$ (C).

The TPC of 12 different extracts of the samples ranged from 5.62 to 18.86 mg GAE/g. The illustrations in the contour and surface plots (Figure 47A) show that a mixture of water, methanol, and ethanol is needed to reach a value of around 18 mg GAE/g. Furthermore, the desirability plot (Figure 48A) shows that the maximum value of the TPC that can be achieved

is equal to18.5 mg GAE/g. This value is possible with a desirability of 99% by ensuring the following operating conditions (40:23:37 v/v/v) water: ethanol: methanol.

This obtained TPC was higher than previous studies that used only simple solvents, as in the case of Wang et *al.*, who obtained only 7.50 ±0.11 mg GAE/g DW using methanol, and 10.20±0.09 mg GAE/g DW using aqueous extracts [42]. The only acceptable reason to explain the higher quantity of polyphenol content in this study is that the combined effects (synergy) of the used solvents counting water, ethanol, and methanol, are superior to simple methods of dissolving polyphenols. Similar results were revealed in other plants, such as *Coffea arabica* L. and *Spondias mombin* L. in which mixture design recorded superior results for polyphenols as compared to simple methods [43, 44]. In another study, Herrera-Pool et al. have found that the highest recovery of TPC from *Capsicum Chinense* was obtained using methanol 50% by ultrasound-assisted extraction [45]. With the same Target of maximizing the total phenolic compounds recovery, Šaponjac et al. found that the most appropriate combination to maximize the recovery of phenolic compounds from carrot required mixing 20% of water, 49% acetone, and 31% ethanol [46]. Besides, a solvent system consisted of (53:35:12 v/v/v) methanol: ethanol: acetone ternary mixture was found to be the most adequate to recover a high amount of TPC from Moroccan *Lavendula stoechas* [47].

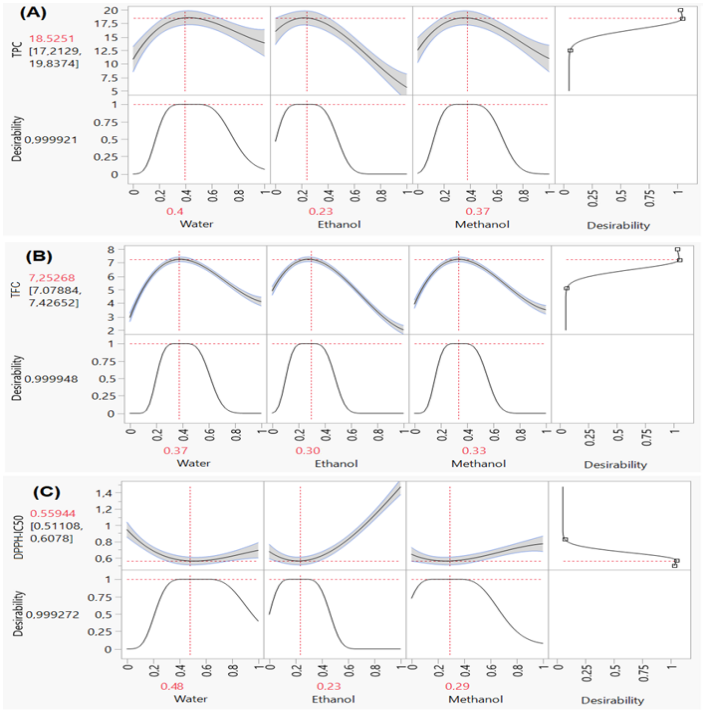


Figure 48 : Desirability plots used to identify the optimum value of (A) TPC; (B) TFC; (C) DPPHIC50 separately.

3.2.2.2. Total flavonoid content

Thanks to Figure 47B, which displays the contour and surface plots of the response TFC, we can see that a content of 7 mg QE/g was registered for the ternary combination with equal proportions of three studied solvents containing water-ethanol-methanol (v/v/v). In addition, the desirability function indicates that the precise optimal amount of TFC was equal to 7.25 mg QE/g with a compromise percentage of 99% (Figure 48B).

These results were significantly superior to those recorded by Balanescu et *al.,* who found 5.55 ± 0.42 mg QE/g TFC using the Ultrasound-Assisted Extraction method [48]. The effect of mixture solvent on TFC was previously reported for extra virgin olive oil and *Pimpinella barbata (dc.)* Boiss. *Jundishapur*. [49,50]. Moreover, in the study conducted by Fadil et *al.,* a solvent system containing (40:27:33 v/v/v) ethyl acetate: methanol: ethanol was the best-recommended mixture to optimize total flavonoids content from *Lavandula stoechas* [47].

3.2.2.3. Antioxidant activity

The efficacy of recovering phytochemicals with high antioxidant potential constitutes an important step to elevate the beneficial properties of the optimized extracts. Within this background, the main objective of the current study was to determine the efficacy of the simplex centroid mixture design in finding the most effective mixture to extract the highest amounts of bioactive ingredients with antioxidant potential. The analysis results of the contour and surface plots (Figure 47C) revealed that we could reach a value of $DPPH_{IC50}$ around 0.56 mg/ml by ensuring the ternary combination with water, ethanol, and methanol. Moreover, the desirability test in (Figure 48C) showed that the ternary mixture with the following proportion (48:22:29 v/v/v) water:ethanol:methanol gives the best antioxidant ability. In the same way, Aazza et al. have reported that the solvent mixture containing 25% ethanol and 75% methanol was the most appropriate solution to improve the antioxidant ability of *Cannabis sativa* Waste [30]. While the binary mixture 75:25 of water: acetone was better for the DPPH scavenging activity of *Allium sativum L*. [19]. In a recent study, Fadil et al. demonstrated that the ternary mixture (50:36:14 v/v/v) methanol: ethanol: acetone was the optimal solvent system for better optimizing the antioxidant power of Moroccan *Lavandula stoechas* [47].

3.2.2.4. Simultaneous optimization of the three responses

Depending on the priority of the responses under study, the simultaneous optimization of all responses using the desirability function could provide various solutions. In our case, the response $DPPH_{IC50}$ was the most important. Thus, simultaneous optimization involves finding

the best compromise to improve all responses with the priority of the response $DPPH_{IC50}$. According to the desirability plot (Figure 48), the maximum responses for TPC, TFC, and antioxidant activities by DPPH assays were obtained by the ternary solvents mixture (44:22:34 v/v/v) water: methanol: ethanol. With this solvent system, we can obtain a value of 18.55 mg GAE/g, 7.16 mg QE/g, and 0.56 mg/ml for TPC, TFC, and $DPPH_{IC50}$, respectively. The mixture contour plot of all the responses, which is created by the three solvents water, methanol, and ethanol (Figure 49), makes the effects of the simultaneous optimization more obvious. The position of these three responses in figure 49 suggests a possible correlation between them. Indeed, it has been previously demonstrated that a statistical correlation exists between the amount of total phenols and flavonoids content and the antioxidant activity [47]. In this way, a correlation test was performed to check this hypothesis. As a result, and since the plant extracts with higher levels of total phenolics exhibit low values of $DPPH_{IC50}$, a significant negative correlation was observed for the correlation between $DPPH_{IC50}$ with TPC (r= -0.89; *p-value*= 0.0001) and TFC(r= -0.78; *p-value*= 0.028).

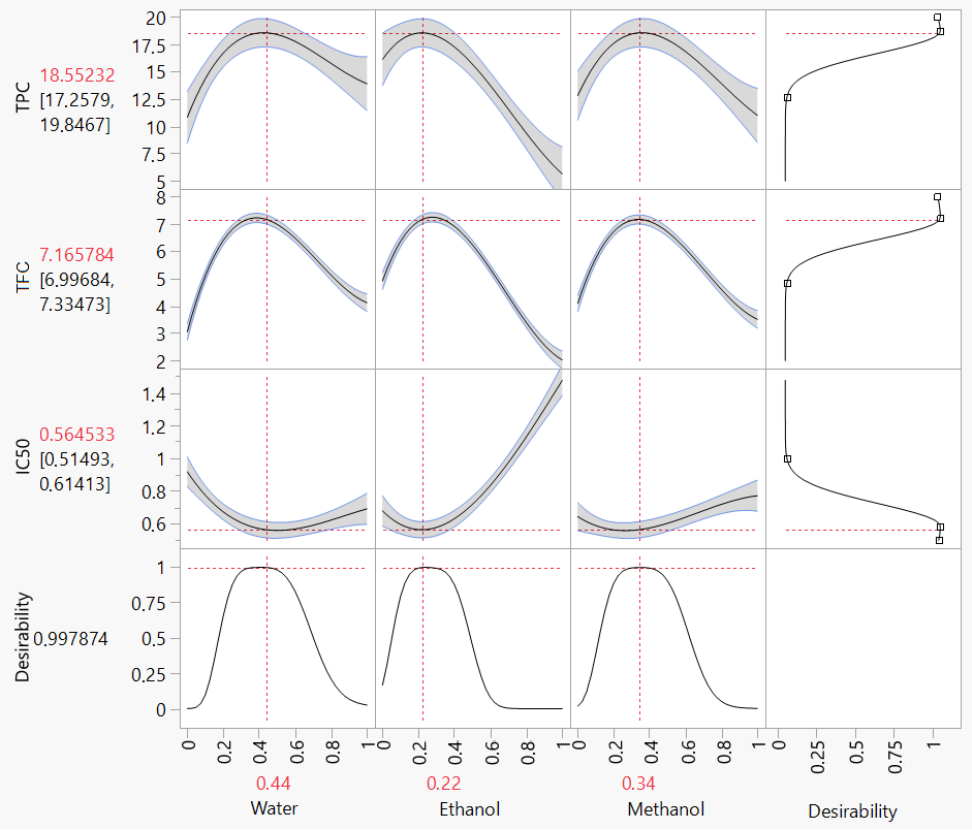


Figure 49: Desirability plots of simultaneous optimization demonstrating the maximum value of TPC, TFC and $DPPH_{IC50}$.

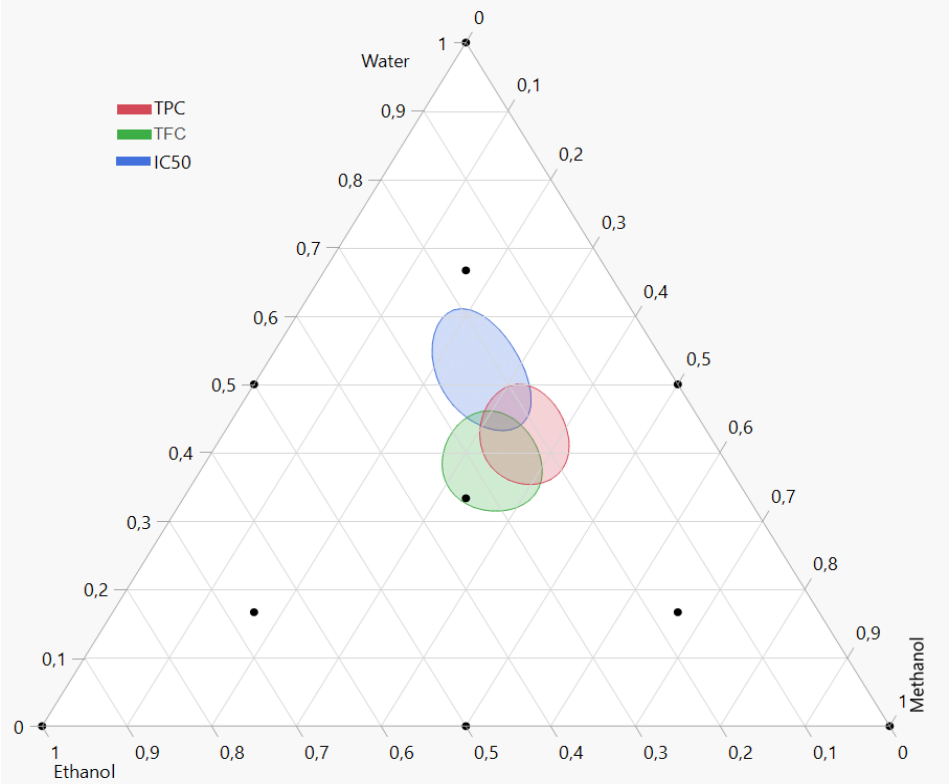


Figure 50:Mixture contour plot, which illustrates the simultaneous optimization of the three responses based on the values given by desirability functions.

3.2.2.5. Experimental validation of the optimal conditions

The current study's final step was validating the generated special cubic models. The suitability of the prediction model was evaluated by conducting another experiment analysis under the appropriate conditions and comparing predicted and experimental values. The chosen test point coordinates were the proportions of the optimal solvent system obtained for simultaneous optimization. Table 21 shows that the experimental and predicted values are in good concordance, demonstrating that the postulated models' choice has been judicious.

Table 21 : Predicted and experimental values for the test point realized by the optimal found mixtures.

		TPC mg GAE/g		TFC mg QE/g		$DPPH_{IC50}$ (mg/ml)	
Comp. of mixture	Propor. of Solvents (%)	Predicted Value [a]	Experimental value [b]	Predicted Value [a]	Experimental value [b]	Predicted Value [a]	Experimental value [b]
Water	44						
Ethanol	22	18.55±1.29	19.01±0.23	7.16±0.17	7.02±0.12	0.56±0.05	0.56±0.01
Methanol	34						

[a] The predicted value is given with the standard deviation of the response calculated from the model.

[b] The observed value is the average of three replicates with standard error.

4. Conclusion

In the current study, the optimization of the extraction of bioactive compounds from anise was performed using a simplex-centroid design. A solvent system containing water, ethanol and methanol was optimized to improve the three responses, TPC, TFC and $DPPH_{IC50}$. The validated special cubic models allowed the optimization of all responses simultaneously. Thus, the findings indicated that the ternary mixture of water:ethanol:methanol (44:22:33 *v*/*v*/*v*) was the most appropriate for simultaneous maximization of TPC, TFC and antioxidant activity. The optimized responses were 18.55 mg GAE/g, 7.16 mg QE/g and 0.56 mg/mL for TPC, TFC and $DPPH_{IC50}$, respectively. These conclusions could be a key development in managing the extraction of phenolic compounds for specific applications, such as their application as a substitute for traditional antioxidants utilized mostly in the food industry to enhance nutrition quality. In addition, our work has demonstrated that mixture designs are a valuable and effective tool for organizing and refining experimental parameters to obtain the best findings with the fewest experiments possible.

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Analyse comparative des composés phénoliques et des capacités antioxydantes des extraits de *Carum carvi* L. du Maroc pour l'optimisation à l'aide d'une méthodologie de conception de mélange.

(A fait l'objet d'un article soumis pour publication)

Synthèse d'étude

Carum carvi L., ou carvi, de la famille des Apiacées, est une espèce végétale bisannuelle aux tiges fines et cannelées. Carvi est composé d'huile essentielle riche en D-carvone, ainsi que d'huiles grasses et de polysaccharides. Le carvi possède plusieurs propriétés pharmacologiques, notamment des propriétés antispasmodiques, emménagogue, expectorante, galactagogue, stimulante, stomacale et tonique. Ces propriétés biologiques sont étroitement liées aux molécules actives contenues dans les dérivés de la plante. Cependant, les approches d'extraction influencent la quantité de molécules extraites, ce qui a un effet significatif sur la valeur commerciale et les applications des extraits de la plante. Les composés bioactifs, tels que les polyphénols et les flavonoïdes font partie des molécules les plus importantes enregistrées chez les plantes médicinales et aromatiques. Ces molécules se caractérisent par diverses propriétés biologiques, telles que l'activité antioxydante. Cependant, l'extraction constitue la première étape de toute étude des composants chimiques des végétaux et joue un rôle important et crucial. L'efficacité des méthodes d'extraction conventionnelles et non conventionnelles dépend fortement des paramètres d'entrée, de la nature de la matrice végétale, de la chimie des composés bioactifs et des solvants utilisés. La conception expérimentale étant la voie la plus couramment adoptée pour optimiser l'extraction des composés phénoliques à partir de différentes matrices végétales. Cette technique joue un rôle central dans l'examen de l'interaction possible de différents paramètres étudiés, en fournissant les combinaisons les plus appropriées pour minimiser la durée, le coût et le nombre d'expériences.

Par la présente étude, nous avons essayé de sélectionner le meilleur mélange de solvants et ceci via la technique de conception de mélange pour extraire les produits chimiques polyphénoliques et flavonoïdes et évaluer leurs propriétés antioxydantes à partir des extraits de *Carum carvi* L. pour ce faire les graines de carvi ont été récoltées dans la région de Hajeb en 2021. Les échantillons ont été réduits en poudre via un broyeur de laboratoire et séchés jusqu'à obtenir un poids constant à température ambiante. Les extraits ont été réalisés, en combinant 50 mg de matière végétale avec 1 ml de solvant d'extraction, dont l'eau, méthanol, éthanol, hexane, dichlorométhane, acétone, acétate d'éthyle, hexane, butanol et l'acétonitrile. Ensuite,

nous avons mesuré à la fois la teneur totale en flavonoïdes et en phénols, tandis que le test DPPH a été utilisé pour évaluer l'activité antioxydante. D'après les résultats obtenus les solvants polaires dont l'eau, méthanol et éthanol ont présenté les teneurs les plus élevées en polyphénols totaux, ce qui nous a permis de faire une optimisation à partir de ce mélange ternaire.

Une méthodologie mixte a été utilisée dans la présente étude pour améliorer la procédure d'extraction. Des tracés de contour et de surface basés sur des courbes d'iso-réponse ont été utilisés pour estimer la formule de solvant idéale, ce qui a donné un compromis de réponses.

Les résultats obtenus diffèrent en fonction des solvants d'extraction utilisés et des paramètres d'extraction. Le mélange comprenant de l'eau, de l'éthanol et du méthanol présente les taux de polyphénols totaux plus élevés, allant jusqu'à 27,23 mg GAE/g. En revanche, le solvant contenant 100 % d'éthanol présente des valeurs TPC plus faibles avec 13,76 mg GAE/g. De même, les meilleures réponses étant, 8.02 mg QE/g et 0,48 mg/ml pour, le TFC et le $DPPH_{IC50}$ respectivement avec le mélange ternaire comprenant l'eau, l'éthanol et méthanol à des proportions égales.

Les coefficients de détermination R^2 sont tous égaux à 0,99, ce qui démontre un accord suffisant entre les valeurs expérimentales et prédites du modèle adapté. La désirabilité montre que ces meilleures valeurs pouvant être atteintes avec une désirabilité de 99%.

Nos résultats montrent que l'optimisation des solvants d'extraction des composés bioactifs du *Carum carvi,* entraînent une augmentation de la teneur totale en phénols et flavonoïdes, ainsi que l'activité antioxydante. En outre les contenus bioactifs des extraits optimisés sont influencés par la proportion de solvant de l'extracteur. La combinaison ternaire de l'eau, l'éthanol et le méthanol semblent être la meilleure pour le rendement de l'extraction. Par conséquent, il semble logique d'utiliser un modèle expérimental afin de maximiser l'efficacité de la récupération et d'éviter toute perte de temps, ceci via l'utilisation du plan d'expériences qui constitue un outil très utile pour estimer le mélange d'extraction approprié afin de produire divers extraits à fort potentiel antioxydant.

Chap II Comparative analysis of Phenolic Compounds and Antioxidant Abilities in the extracts of Extraction of *Carum carvi* from Morocco and Use of Mixture Design Methodology for Optimization

Abstract

Carum carvi is a dense vegetal matrix with considerable amounts of bioactive components known for its pharmacological properties. The optimization of extraction constitutes an important key to improving efficacy and avoiding wasting time. Within this framework, the present study was designed to select the most appropriate extractor solvent mixture to extract phenolic and flavonoids using Mixture Design Methodology. The concerned responses were the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant ability examined by 2,2-diphenyl-l-picrylhydrazyl (DPPH) assay. Before mixture design optimization, a screening of solvents was conducted on ten polar and nonpolar solvents to choose the best solvents that give a maximum of total phenolic compounds. This first step has shown that water, ethanol and methanol were the best-used solvents. Later, an augmented centroid design investigated the solvent system's optimization. The results of simultaneous optimization have shown that the ternary mixture containing 33% of water, 33% of ethanol and 33% of methanol was the most appropriate for simultaneous maximization of TPC, TFC and antioxidant activity. Our results have shown that using mixture design as an optimization technique was an excellent way to choose the most suitable mixture to extract bioactive compounds, which may represent a promising method of multi-purpose extraction, especially in the pharmaceutical and food sectors.

Keywords: *Carum carvi*; optimization; total phenolic content; total flavonoid content; antioxidant activity.

1. Introduction

Bioactive compounds, including flavonoids, phenols, anthocyanins, ascorbic acid, amides, alkaloids, tannins, saponins and glycosides, from different part of medicinal species play an important role in human health, due to their biological activity (Alamgir and Alamgir 2018). Polyphenols are natural compounds that are widely found in plants and their importance continues to grow, in particular because of their impact on organoleptic and health benefits (Pinto *et al.*, 2021). Their role as natural antioxidants is useful in the prevention and treatment of cancer, inflammation and cardiovascular diseases (Cheynier, 2012). Indeed, in the extraction of active ingredients with high added value from plants, especially polyphenols, the extraction

method applied is a very important step in the isolation, as well as in the identification, of phenolic compounds. As a result, many authors have studied the influence of different extraction methods on the extraction yields of phenolic compounds in plant sources (Babbar *et al.,* 2014, Alara *et al.,* 2021). Among others things, the solubility of the phenolic compounds is affected by the polarity of the solvent used. Further, the determination of phenolic compounds from plant materials is influenced by the extraction technique, the extraction time and the temperature. Therefore, it is difficult to develop an extraction process suitable for the extraction of all the phenolic compounds of plants (Garcia-Salas *et al.,* 2010, Lama-Muñoz and Contreras 2022).

Despite several disadvantages, liquid-liquid and solid-liquid extraction are still the most commonly used extraction procedures for phenolic acids and flavonoids (Sudha *et al.,* 2012; Putnik *et al.,* 2017). For many years, the conventional techniques have been widely accepted, mainly because of their ease of use, efficiency, and wide-ranging applicability (Sudha *et al.,* 2012; Putnik *et al.,* 2017). Maceration and decoction techniques are very useful methods for weakening the cell wall in vegetal tissues, but in different ways. Experimental designs are among the most common avenues adopted to optimize phenolic compounds' extraction from different vegetal matrices (Vázquez-Espinosa *et al.,* 2019; Bodoira *et al.,* 2017, 2019). In addition, other methods have gained increased interest, including microwave and ultrasonic-assisted extraction, thanks to their simplicity, rapidity and low cost (Vázquez-Espinosa *et al.,* 2019). These methods play a pivotal role in examining the possible interaction of different parameters, providing the most appropriate combinations to minimize the time and number of experiments (Vázquez-Espinosa *et al.,* 2019; Aazza, 2021).

Caraway [*Carum carvi*] [black zeera] belongs to Apiaceaefamily (MArdani *et al.,* 2016; Dar and Zeerak,2010) is a biennial plant grown in western Asia, Europe and Northern Africa (Keshavarz *et al.,* 2013) It is also known as Persian cumin. It is a biennial, aromatic, umbelliferous plant. The plant grows 20–30 cm (Kamaleeswari and Nalini, 2006; Sadiq *et al.,* 2010). The main flower stem is small white or pink flowers in umbels. Caraway fruits are crescent-shaped achenes, around 2 mm long. Caraway is useful for digestive problems including heartburn, bloating, gas, loss of appetite, and mild spasms of the stomach and intestines (Saghir *et al.,* 2012; Lim, 2013). Caraway oil is also used to help people cough up phlegm, improve control of urination, kill bacteria in the body, and relieve constipation. The seeds of Carum carvi [caraway] are considered as antispasmodic, carminative, astringent and used in the treatment of stomatitis stimulant, diarrhea, dyspepsia, hysteria, flatulent indigestion,

colic, dyspeptic headache and improve liver function, for gastrointestinal problems and obesity, anticonvulsant, Reno-protective effect, anti-hyperglycemic and antimicrobial effects (Kazemipoor *et al.*, 2014, Choudhary *et al.*, 2014).

In this study, we aimed to optimize the extraction of polyphenolic compounds including total polyphenolic and flavonoid content, as well as the antioxidant activities in *Carum carvi* extracts. In this way, the first step in this work consists of screening ten pure solvents among the most commonly used in the extraction of phenolic compounds to select those with the most suitable extractive properties. The screened solvents include nonpolar ones such as hexane, solvents with low polarity such as dichloromethane, solvents with important polarity such as acetone and acetonitrile and solvents with high polarity such as water, ethanol and methanol. Then, the solvents chosen from this screening were used as constituents of the mixture of the system of solvents. The appropriate mixture was selected based on the optimized high total phenolic-flavonoid content and antioxidant power. To the best of our knowledge, this is the first time that such work has been done on the species *Carum carvi*.

2. Materials and Methods

2.1. Chemicals and Materials

Seeds of *Carum carvi* were collected from El Hajeb region during the autumn season of 2021 (33°41′22″ N, 5°21′13″ W). Ethanol, methanol, dichloromethane, chloroform, acetone, ethyl acetate, hexane, butanol, acetonitrile, water, DPPH, Folin-Ciocalteu reagent, gallic acid and quercetin were the chemicals used in different experiments and were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA).

2.2. Samples Preparation

The plant was identified by the botanist of the National Agency of Medicinal and Aromatic Plants (NAMAP) in Taounate, Morocco. The samples were dried to constant weight in a room where the temperature was fixed at 25 °C and then powdered in a lab grinder machine.

2.3. Ultrasonic-Assisted Extraction

Initially, the extraction was performed by ten pure polar and nonpolar solvents, including water, ethanol, methanol, dichloromethane, chloroform, acetone, ethyl acetate, hexane, butanol and acetonitrile. 50 mg of powdered seeds were mixed with 1 mL of each solvent. The mixtures were sonicated for 30 min in a P30 H ultrasonic cleaner bath with a power of 100 W and a frequency of 37 kHz (Elmasonic, Singen, Germany) and centrifuged at 10,000 rpm/10 min in a Hettich ROTOFIX 32A centrifuge (Hettich, Tuttlingen, Germany). The mixture was filtered

and stored at 4 °C until experimental analysis, as previously described by Ousaaid *et al.*, (2020). Afterward, an extraction under the same conditions was carried out by a solvent system formulated from the three most effective solvents. The solvent fractions of each experiment are generated from the chosen mixture design.

2.4. Total Phenolic Content Quantification

The phenolic content was quantified using the colorimetric methods previously described by Singleton et al. (1999). In brief, 25 µL of extract and 450 µL of Folin-Ciocalteu reagent (0.5N) were mixed. Then, the mixture was treated with 450 µL of sodium carbonate and incubated in the dark for 2 hours. The absorption was read at 760 nm and the results were expressed as mg GAE/g using a gallic calibration curve.

2.5. Total Flavonoid Content Quantification

The total flavonoid content was determined by the colorimetric method according to the method described by Laaroussi et al. (2020). The protocol involves mixing 100 µL of extract, 150 µL of $Alcl_3$ reagent (10%) and 200 µL of NaOH (1%). The obtained mixture was incubated in the dark for one hour and the absorbance was read at 510 nm. The results are expressed as mg of quercetin equivalent per gram (QE/g) of anise seeds anise using a quercetin calibration curve.

2.6. Antioxidant Activity

The antioxidant activity of extracts was evaluated using the DPPH test (Miguel *et al.*, 2014). In brief, the extract and DPPH solution were mixed with the following quantities, 25 µL and 875 µL, respectively. The mixture was vortexed and incubated for one hour in the dark condition. The absorbance of the mixture was measured at 517 nm. The percentage inhibition (PI) of the DPPH radical by the samples was calculated according to the formula:

$$PI(\%)=(control\ absorbance-sample\ absorbance:control\ absorbance\ )\times 100$$

The antiradical activity was expressed as $DPPH_{IC50}$ (mg/mL), which is the exact concentration dose required of extractable $DPPH_{IC50}$ whose value corresponds to a higher antioxidant activity.

2.7. Mixture Design

The current study used a mixture design methodology to optimize the extraction process. The test is based on combining different independent factors to create a set of equations that donate theoretical values [13]. The solvent system components are presented in Table 1. According to this table, each of the three components of the mixture can have a value between 0 and 100%

and the sum of the three proportions will always be equal to 100%. Besides, 12 experiments were performed to examine the impact of solvent proportions on total phenolic and flavonoid content and antioxidant activity, as described in Figure 51.

N° of experiment	X1	X2	X3
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.33	0.33	0.33
8	0.33	0.33	0.33
9	0.33	0.33	0.33
10	0.66	0.17	0.17
11	0.17	0.66	0.17
12	0.17	0.17	0.66

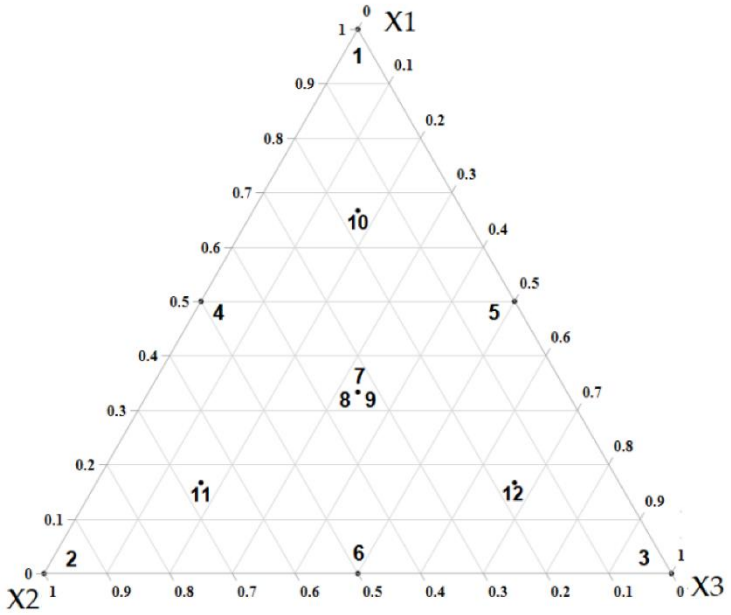


Figure 51: Illustration representing the augmented simplex-centroid design for three components.

Identification of solvent system factors			
Components	Coded variables	Level -	Level +
Water	X1	0	1
Ethanol	X2	0	1
Methanol	X3	0	1
Sum of proportions		1	

As shown by the equation below, the specific cubic model was employed to express the interaction between the components of the analyzed mixtures as well as to predict the maximal recovery of bioactive chemicals and antioxidant activity:

$$Y=\alpha 1X1+\alpha 2X2+\alpha 3X3+\alpha 12X1X2+\alpha 13X1X3+\alpha 23X2X3+\alpha 123X1X2X3+\varepsilon \quad (2)$$

Y represents the experimental response expressed by mg GAE/g, mg QE/g and mg/mL for TPC, TFC and DPPHIC50, respectively. α1, α2, α3 are linear regression coefficients, α12, α23 and α23 are binary regression coefficients, α123: is the ternary regression coefficient, while ε: is the regression error term. The findings were expressed as mean.

2.8. Statistical Analysis

Optimization tools were used as an excellent appliance to predict the appropriate combination of extractor solvents. In this context, simplex centroid design was used to assess different interactions among extractor solvents on TPC, TFC and $DPPH_{IC50}$. The validation of the postulated models was carried out using the F-test for ANOVA. The $F_{R/r}$ (the ratio between the mean square due to the regression (MS_R) and the mean square due to the residuals (MS_r)) was calculated and then compared to the theoretical F for the same degree of freedom. The lack of fit test was used to refine the model fit by comparing the ratio between the mean square lack of fit (MS_{LOF}) and the mean square pure error (MS_{PE}) (Fadil et *al.,* 2018). High values of $F_{LOF/PE}$ indicate a lack of fit to the model. The coefficient of determination R^2, R^2 adjusted and R^2 predicted were used to verify the quality of the postulated models, while Student's test was used to express the significance of the model's coefficients. The experimental design conception and the statistical and graphical analysis were performed using JMP software V.14 (SAS Institute Inc., Cary, NC, USA) and Design Expert V.11 (Stat-Ease, inc., Minneapolis, MN, USA).

The optimum solvent formula was determined using contour and surface plots based on iso-response curves, which resulted in a compromise of responses. The intensity of the red color indicates the response elevation, while the blue color indicates the opposite. In addition, the desirability function was carried out to accurately determine the desired response based on the optimum conditions. A number of 0% is given when the system produces an undesirable response, while a score of 100% represents the highest possible desired response (Izadiyan *et al.,* 2016). Finally, the comparison of means was performed using the ANOVA F-test, while Pearson's test was used to evaluate the correlation between the antioxidant capacity and TPC and TFC. All experiments were performed in triplicate and the results were expressed as the mean ± SD.

3. Results

3.1. Solvents Screening

Ten solvents were tested to select the most appropriate in maximizing the extractable efficacy on TPC. The results obtained when quantifying phenolic content in terms of used solvents are presented in figure 52

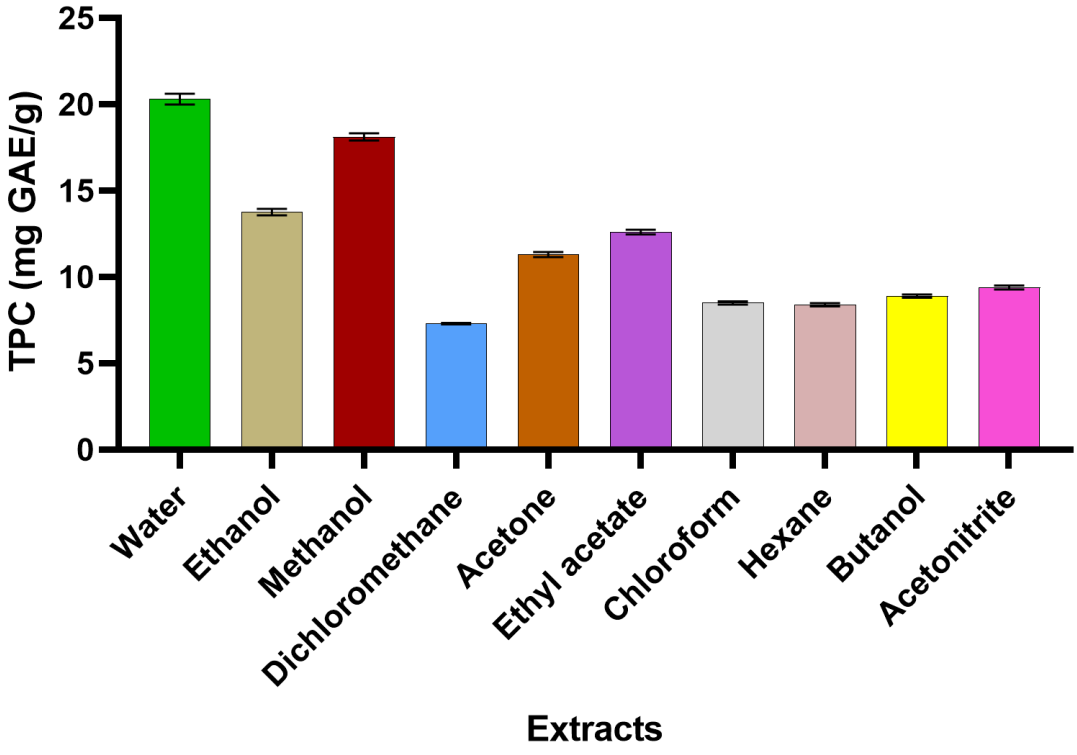


Figure 52: Total phenolic compounds extracted from *Carum carvi* seeds using different solvents.

Based on the graph analysis, it was shown that water had the highest phenolic content extraction rate (20.3 ± 0.32 mg GAE/g), followed by methanol and ethanol (18.12 ± 0.21 and 13.76 ± 0.18 mg GAE/g, respectively). Moderate TPC values were obtained from acetone and ethyl acetate, with 11.3 ± 0.62 and 12.6 ± 0.79, respectively. Conversely, the extract of dichloromethane exhibited the least quantity of TPC, measuring just 7.1 ± 0.14 mg GAE/g. Additionally, there was a statistically significant difference (p-value < 0.05) between the results obtained for the three solvents, water, ethanol, and methanol, and the other seven solvents.

3.2.Solvent Mixture

The simplex-centroid design, with different combinations of the solvents and the recorded response of each experiment on TPC, TFC and $DPPH_{IC50}$, are listed in Table 19. In Carvi (*Carum carvi*), the obtained data showed different results depending on the used extraction solvents and extracted parameters. The mixture comprising water (33.33%), ethanol (33.33%), and methanol (33.33%) had the highest total polyphenol levels, ranging from 26,87 to 27,23 mg GAE/g. On the other hand, the solvent containing 100% ethanol with 13,76 mg GAE/g had the lowest TPC values.

In *Carum carvi*, the highest values of Total Flavonoid Content were recorded in the extracts realized with water (33.33%), ethanol (33.33%), and methanol (33.33%) with values ranging

from 5,98 to 6,13 mg QE/g. In the second rank, the medium values were recorded in the combinations Water, Ethanol, and Methanol (66.66%, 16.66% and 16.66% respectively ranging from 5.14 to 5.75 mg QE/g. In contrast, the lowest values were recorded in Ethanol (100%) with 2.6 and 50% ethanol, 50% methanol with 2.98 mg QE/g.

The analysis of recorded results showed a variation of $DPPH_{IC50}$ depending on used extraction solvents and their fractions. The highest $DPPH_{IC50}$ was recorded in solvent combining 33.33% water, 33.33% ethanol, and 33.33%.

Table 22 : Experimental conditions of mixture design, actual and predicted values and residuals for each response.

Water	Ethanol	Methanol	TPC	TFC	$DPPH_{IC50}$
1	0	0	20,3	3,59	0,69
0	1	0	13,76	2,6	1,32
0	0	1	18,12	3,42	0,69
0,5	0,5	0	18,85	3,54	0,71
0,5	0	0,5	22,18	4,19	0,693
0	0,5	0,5	15,09	2,98	0,96
0,33	0,33	0,33	27,08	7,61	0,43
0,33	0,33	0,33	26,87	7,89	0,48
0,33	0,33	0,33	27,23	8,02	0,39
0,66	0,16	0,16	23,56	5,75	0,53
0,16	0,66	0,16	20,63	5,14	0,87
0,16	0,16	0,66	22,85	5,43	0,63

3.3.Statistical Validation of Postulated Models

According to the analysis of variance table 22, we can conclude that the main effect of the regression is significant since the probability of the significance of the risk p-value is less than 0.05 (<0.0001 , <0.0001 and <0.0002). Furthermore, the three models do not present a lack of fit, because p-value>0.05 (0.06, 0.59 and 0.56).

Table 23 : The analysis of variance (ANOVA) of studied fitted models

		TPC					*TFC*					*DPPH$_{IC50}$*			
model	DF	SS	MS	F	p-value	DF	SS	MS	F	p-value	DF	SS	MS	F	p-value
R	6	219,32	36,55	124,72	<,0001*	6	41,96	6,99	180,03	<,0001*	6	0,72	0,12	62,585	0,0002*
R	5	1,46	0,29			5	0,19	0,03			5	0,01	0,001		
LOF	3	1,40	0,46	14,27	0,0662	3	0,10	0,03	0,80	0,5943	3	0,005	0,001	0,911	0,5612
Pe	2	0,06	0,03			2	0,08	0,04			2	0,004	0,002		
total	11	220,79				11	42,16				11	0,73			
R^2			0.99					0.99					0.99		

The determination coefficients R^2 are all equal to 0.99. These values demonstrate sufficient agreement between the experimental and predicted values of the adapted model. The graph (figure 53) shows that the curves of observed values versus predicted values have the appearance of a straight line.

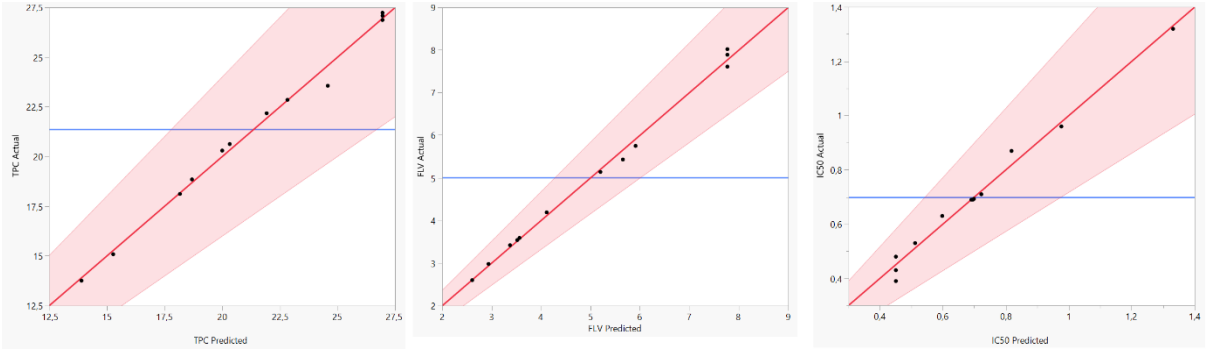


Figure 53: Curves of observed values versus predicted values.

3.3.1. Effects of factors and models

Table 23 summarizes the estimated regression coefficients of the special cubic model. The regression equations with significant coefficients were used to determine the relationships between all studied parameters and the obtained responses for TPC, TFC and DPPH$_{IC50}$).

Effects of model coefficients that relate response to factors

The statistically significant coefficients for the TPC response are:

The linear terms α1, α2 and α3;

Binary terms α12 α13

The ternary term α123

The mathematical model retained is presented by the equation:

Y=〖19.99X〗_1 + 〖13.89X〗_2 + 〖18.16X〗_3+ 6.95X_1 X_2+11.37X_1 X_3+213.03X_1

The statistically significant coefficients for the FLV response are:

The linear terms α1, α2 and α3;

Binary terms α13

The ternary term α123

The mathematical model retained is presented by the equation ():

Y=〖3.56X〗_1 + 〖2.6X〗_2 + 〖3.37X〗_3+ 2.58X_1 X_3+21.39X_1 X_2 X_3+ε

The statistically significant coefficients for the IC50 response are:

The linear terms α1, α2 and α3;

Binary terms α12

The ternary term α123

Y=〖0.69X〗_1 + 〖1.33X〗_2 + 〖0.69X〗_3- 1.15X_1 X_2-8.45 X_1 X_2 X_3+ε

Table 24 : Estimated regression coefficients of the special cubic model.

Term	Coeff.	TCP			FLV			$DPPH_{IC50}$		
		Estimate	t-student	*p-value*	Estimate	t-student	*p-value*	Estimate	t-student	*p-value*
Water	α_1	19,99974	38,25	<,0001*	3,56129	18,71	<,0001*	0,69045	16,2	<,0001*
Ethanol	α_2	13,89974	26,58	<,0001*	2,60492	13,68	<,0001*	1,33163	31,4	<,0001*
Methanol	α_3	18,16610	34,74	<,0001*	3,37038	17,71	<,0001*	0,69499	16,4	<,0001*
Water*Ethanol	α_{12}	6,958981	2,64	0,0458*	1,73243	1,81	0,130	-1,15583	-5,42	0,0029*
Water*Methanol	α_{13}	11,37170	4,32	0,0076*	2,58334	2,70	0,0430*	0,02289	0,11	0,9187
Ethanol*Methanol	α_{23}	-3,028291	-1,15	0,3021	-0,20938	-0,22	0,835	-0,14674	-0,69	0,5222
Water*Ethanol*Methanol	α_{123}	213,0336	14,87	<,0001*	111,549	21,39	<,0001*	-8,45352	-7,28	0,0008*

Coefficient statisticaly significant at 0.05

3.3.2. Solvent System Optimization

The objective of this work is to achieve the best value for the five responses using the iso response profile and the desirability function. From the 2D and 3D mixing graph, we can see that we can achieve a higher TPC value of around 27 mg GAE/g, an FLV value of around 7mgQE/g and an IC50 value. less than 0.45 mg/ml, ensuring a mixture consisting of the three

components of the water and ethanol and methanol mixture since the optimal zone of the two responses exists in the center of the triangle.

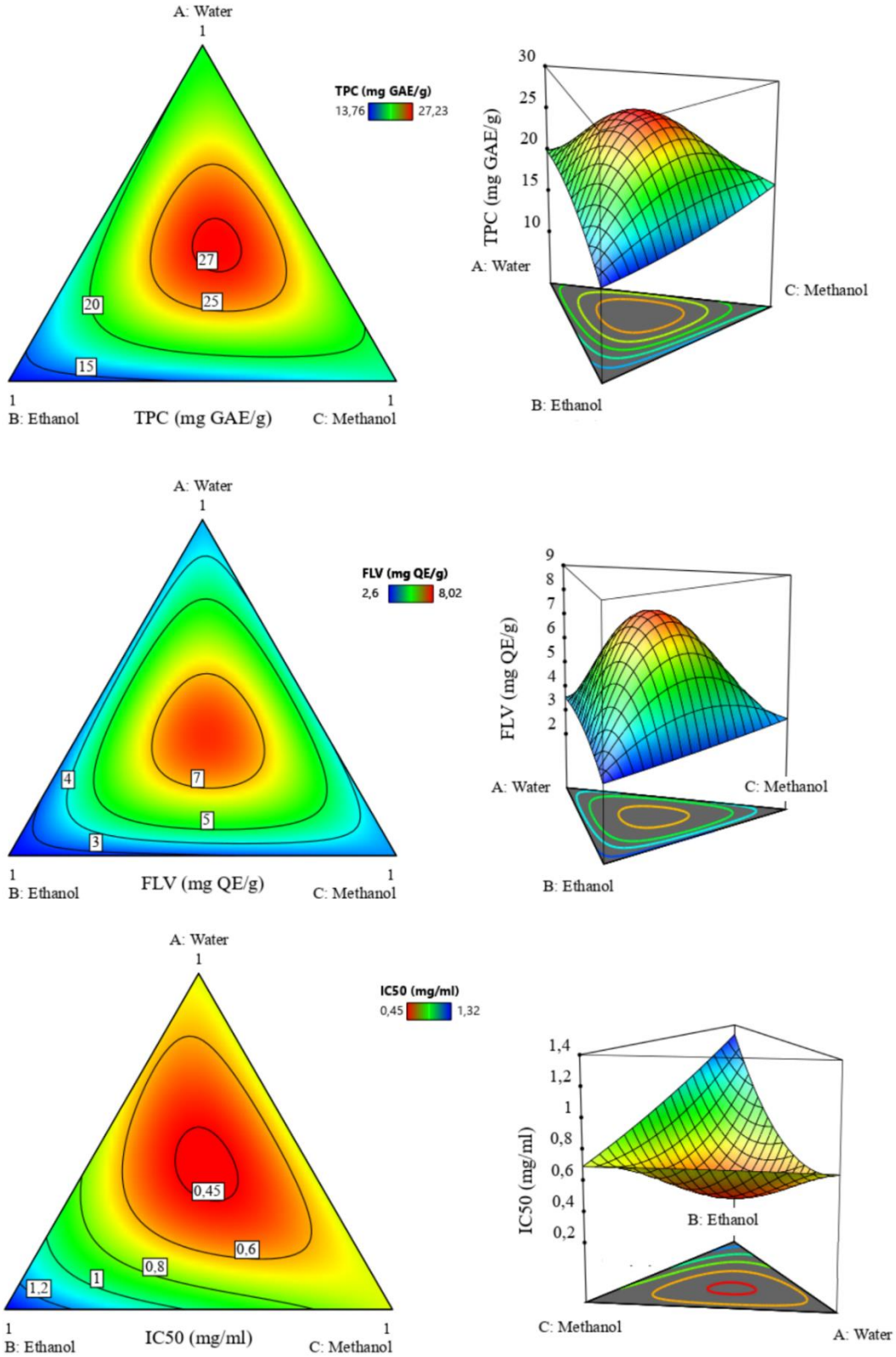


Figure 54: Contour and surface plots, which indicate the optimal compromise area leading to the best value of TPC (A), TFC (B) and DPPHIC50 (C).

3.3.3. Desirability study

In order to know the maximum value of the two responses that we can achieve and the exact values of the constituents, we will use the desirability function (Figure 55). The desirability graph shows that we can have a simultaneous adjustment of the two responses since they show correlation trends. Thus this graph shows that the best values that could be achieved with a desirability of 99% are equal to 27.29 mgGAE/g, 7.75 mg QE/g and 0.43 mg/ml for the TPC and IC50 response, respectively. These values are accessible by ensuring the following mixture: 38% water, 28% ethanol, and 34% Methanol.

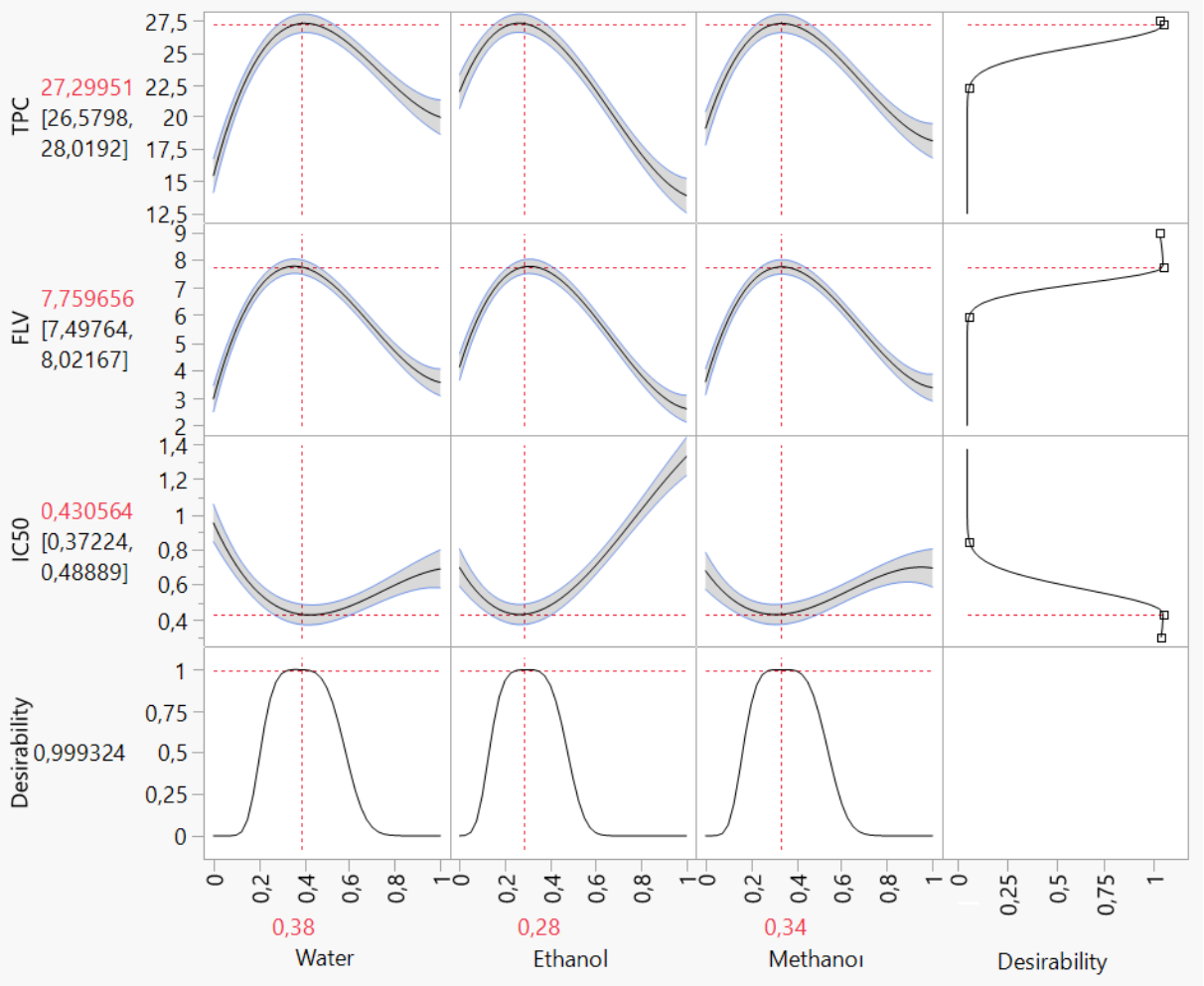


Figure 55: Desirability plots used to identify the optimum value of TPC (A), TFC (B) and DPPHIC50 (C), separately.

4. Discussion

Caraway (*Carum carvi* L.) is an aromatic, biennial umbelliferous plant belonging to the Apiaceae family. This plant has been served traditionally for a long time for various medical prescriptions such as digestive unrest including gas, loss of appetite, bloating, heartburn, as well as mild spasms of the stomach and intestines (Hajlaoui et *al.*, 2021, Hashem *et al.*, 2023).

These biological activities are closely related to the chemical compounds of the plant (Joshi and Soulimani 2020). The EO and extracts of Carum carvi are composed of many bioactive molecules mainly used in the food and cosmetic industries, because of their perfume and antioxidant potential (Sherafatmanesh *et al.*, 2020, Ghannay *et al.*, 2022). Therefore, we investigated the optimized extraction solvent to isolate the polyphenols and flavonoids, as well as the antioxidant properties from seed extracts of the plant.

The obtained data showed that the mixture comprising water (33.33%), ethanol (33.33%), and methanol (33.33%) had the highest total polyphenol levels, while the solvent containing 100% ethanol had the lowest TPC values. In a comparative study, Thippeswamy *et al.,* (2013) investigated the amount of TPC in different extracts of Carum carvi. To simplify the extraction of a variety of polyphenols, the polyphenolic components from defatted C. carvi powder were extracted progressively with water, 50% ethanol, and a 1:1 combination of 70% aqueous methanol and 70% aqueous acetone. According to the findings, there were 8.76, 12.63, and 50.20mg of polyphenols per gram of defatted powder, respectively. Therefore, we were able to extract many phenolic acids and flavonols from *C. carvi* using the solvent systems mentioned above. On the other hand, Mehwish Wajidi *et al.,* (2019), investigated the total phenolic compounds in the extracts and fractions from *Carum carvi*. The recorded values were significantly variable depending on the used solvents. In the extracts, the highest value of TPC was recorded in the aqueous extract with 112.5 ±0.36 mg/g Gallic Acid, followed by Ethyl acetate with 91.6 ± 0.29 mg/g Gallic Acid, and ethanol with 57.5 ± 0.21 mg/g Gallic Acid. In contrast, the lowest value of TPC was recorded in the hexane with 29.5 ±0.49 mg/g Gallic Acid.

In terms of TFC, the obtained data showed that the mixture comprising water (33.33%), ethanol (33.33%), and methanol (33.33%) had the highest levels, while the solvent containing 100% ethanol had the lowest TFC values. In comparison, Mehwish Wajidi *et al.,* (2019), investigated the total flavonoid compounds in the extracts and fractions from Carum carvi. The recorded values were significantly variable depending on the used solvents. In the extracts, the highest value of TFC was recorded in the aqueous extract with 85.4 ± 0.12 mg/g Quercetin, followed by ethanol with 81.1± 0.16 mg/g Quercetin, and Ethyl acetate with 49.6 ± 0.93 mg/g Quercetin. In contrast, the lowest value of TFC was recorded in the hexane with 3.08 ± 0.02 mg/g Quercetin. In another study, Mišan et *al.,* (2011) compared the total flavonoid content in five medicinal plants including *Carum carvi*. The recorded results showed a significant variation in TFC values depending on the used plant. In the extract of Carum carvi, the TFC was estimated

at 1.78 ± 0.070%, compared to 1.97 ± 0.037% in Mint leaves and 2.05 ± 0.060% in Vitalplant. Mehwish Wajidi *et al.*, (2019) investigated the Total Flavonoid content in of crude and fractions of *Carum carvi*, including extract of ethanolic (EE), hexane fraction (HF), ethyl acetate fraction (EAF), and aqueous fraction (AQF). The highest flavonoid contents were observed 85.4±0.124, 81.1± 0.16, 49.6 ± 0.93, and 3.08 mg/g Querecitin for aqueous, ethanol, ethyl acetate and hexane respectively. In *Coriandrum sativum*, Rajeshwari *et al.*, (2012) investigated the effect of solvents and their fractions, including in water and hexane (He), ethyl acetate (Ea), benzene (Be), n-butanol (nBu), and water (Aq). In the leaves, the highest amount of TFC was recorded in the Ethyl acetate 4.82±1.1 mg/100g RE, while in the other extracts the values were statistically similar. In the seeds, the highest value of TFC was recorded in Ethyl acetate with 3.40±1.1 mg/100g RE.

The analysis of recorded results showed a variation of $DPPH_{IC50}$ depending on used extraction solvents and their fractions. The lowest anti-oxidant activity was recorded in the ethanol (100%) with 1,32mg/mL in *Carum carvi*. The solvent combining 33.33% water, 33.33% ethanol, and 33.33% methanol showed the most important value of $DPPH_{IC50}$. In other studies, Thippeswamy et *al* (2013) investigated the antioxidant properties of phenolic extract from seeds using water, 50% alcohol and 1:1 mixture of methanol (70%) and acetone (70%). The extract was tested in the concentration range of 0.1–2 μg/ml and the activity were observed in a dose dependent manner. At a concentration of 0.1 μg/ml, the scavenging activity was 13.7%, whereas at 2 μg/ml, the scavenging activity was 84.6%. The IC50 value of C. carvi phenolic extract was found to be 2.7 μg/ml. Atrooz, (2013) tested the antioxidant activity of seed extract from *Cuminum cyminum* L. and *Carum carvi* L. Both cumin and caraway seed extracts were able to neutralize free radicals over a period of 60 min in the DPPH assay, with most of the neutralization occurring quickly within the first 30 seconds. The steady state was reached within 10 min, and it appeared that the acetonic extracts of both seeds had less antioxidant activity than the methalonic extracts. The methanolic and acetonic extracts of cumin showed slightly higher neutralization ability than the respective extracts from caraway within three minutes of assay initiation (57.0 and 52.4% for cumin and 44.7 and 39.5% for caraway. Importantly, the neutralization level stayed relatively the same over 60 min. Mišan et *al.*, (2011) evaluated the antioxidant activity of ethanolic extracts from four medicinal plants. The obtained results showed a significant variation depending on used plant. In extracts from Caraway fruit, the value of $DPPH_{IC50}$ scavenging activity was estimated at 2.06 ± 0.137 mg extract/mL, while the highest value was recorded in Parsley fruit with 4.65 ± 0.820mg/mL.

There are many techniques to recover antioxidants from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction. However, extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Turkmen et *al.*, 2006). Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols (Dai and Mumper 2010). The maximum total phenolic content was obtained from barley flour by extraction using a mixture of ethanol and acetone (Bonoli et *al.*, 2004). For extracting flavonoids from tea, aqueous ethanol performed better than aqueous methanol and aqueous acetone (Wang and Helliwell 2001). Extracts with the greatest antioxidant activity were obtained in mate tea and black tea by using 50% aqueous ethanol and 50% aqueous acetone, respectively (Turkmen et *al.*, 2006). These are suggested to be the best explanation of our results. Therefore, the highest values recorded in the optimized extract is suggested to be governed by the combination of the characteristics of each solvent (Monteiro et *al.*, 2020, Lezoul et *al.*, 2020).

5. Conclusion

In the current study, the optimization of the extraction of bioactive compounds from *Carum carvi* was performed using a simplex-centroid design. A solvent system containing water, ethanol and methanol was optimized to improve the three responses, TPC, TFC, and $DPPH_{IC50}$. The validated special cubic models allowed the optimization of all responses simultaneously. Thus, the findings indicated that the ternary mixture of water: ethanol: methanol (33%:33%:33%) was the most appropriate for simultaneous maximization of TPC, TFC, and antioxidant activity. These conclusions could be a key development in managing the extraction of phenolic compounds for specific applications, such as their application as a substitute for traditional antioxidants utilized mostly in the food industry to enhance nutrition quality. In addition, our work has demonstrated that mixture designs are a valuable and effective tool for organizing and refining experimental parameters to obtain the best findings with the fewest experiments possible.

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Optimisation simultanée des composés phénoliques et des capacités antioxydantes des extraits de *Coriandrum sativum* à l'aide d'une méthodologie de conception de mélange

(A fait l'objet d'un article soumis pour publication)

Synthèse d'étude

La conception expérimentale représente la voie la plus couramment adoptée pour optimiser l'extraction des composés phénoliques à partir des différentes matrices végétales.

Cette partie d'étude porte sur une optimisation de l'extraction des composés chimiques, ainsi qu'une évaluation de l'activité antioxydante de la plante *Coriandrum sativum*. Elle consiste à utiliser une conception de mélange pour déterminer le meilleur mélange de solvants afin d'extraire les produits polyphénoliques et évaluer leur capacité antioxydante. Sur la base de la teneur totale optimale en polyphénols et flavonoïdes ainsi, que le potentiel antioxydant, le bon mélange sera choisi.

Pour ce faire, des graines de coriandre ont été récoltées dans la région d'El Hajeb, ramenées au laboratoire. Broyées jusqu'à obtenir une poudre de poids constant à température ambiante.

Les extraits ont été réalisés, en combinant 50mg de matière végétale avec 1 ml de solvant d'extraction, dont l'eau, méthanol, éthanol, hexane, dichlorométhane, acétone, acétate d'éthyle, hexane, butanol et l'acétonitrile. Ensuite, nous avons mesuré à la fois la teneur totale en flavonoïdes et en phénols, tandis que le test DPPH a été utilisé pour évaluer l'activité antioxydante. D'après les résultats obtenus les solvants polaires dont l'eau, méthanol et éthanol ont présenté les teneurs les plus élevées en polyphénols totaux, ce qui nous a permis de faire une optimisation à partir de ce mélange ternaire.

Une méthodologie mixte a été utilisée dans la présente étude pour améliorer la procédure d'extraction. Des tracés de contour et de surface basés sur des courbes d'iso-réponse ont été utilisés pour estimer la formule de solvant idéale, ce qui a produit un compromis de réponses.

Les données obtenues ont montré des résultats différents en fonction des solvants d'extraction utilisés et des paramètres d'extraction.

En termes de polyphénols totaux, des flavonoïdes et $DPPH_{IC50}$ les valeurs les plus élevées ont été enregistrées dans le mélange ternaire contenant de l'eau (1/3), de l'éthanol (1/3) et du méthanol (1/3) avec 13,88 mg GAE/g, 6,13mg QE/g et 0.38 mg/mL respectivement.

Des résultats obtenus, il ressort que les contenus bioactifs des extraits optimisés sont influencés par la proportion de solvant de l'extracteur. L'eau, le méthanol et l'éthanol combinés pour former une combinaison ternaire donnant les valeurs TPC les plus élevées. Par conséquent, il parait logique d'utiliser un modèle expérimental afin de maximiser l'efficacité de la récupération et d'éviter toute perte de temps. Dans ce cas, il semble que le plan expérimental constitue un outil utile pour estimer le mélange d'extraction approprié afin de produire divers extraits à fort potentiel antioxydant.

Chap III. Combination Design Methodology for the Concurrent Optimization of Phenolic Compounds and Antioxidant Capabilities in the extracts of Moroccan *Coriandrum sativum*

Abstract

Coriandrum sativum is a dense vegetal matrix with considerable amounts of bioactive components known for its pharmacological properties. The optimization of extraction constitutes an important key to improving efficacy and avoiding wasting time. Within this framework, the present study was designed to select the most appropriate extractor solvent mixture to extract phenolic and flavonoids using Mixture Design Methodology. The concerned responses were the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant ability examined by 2,2-diphenyl-l-picrylhydrazyl (DPPH) assay. Before mixture design optimization, a screening of solvents was conducted on ten polar and nonpolar solvents to choose the best solvents that give a maximum of total phenolic compounds. This first step has shown that water, ethanol and methanol were the best-used solvents. Later, an augmented centroid design investigated the solvent system's optimization. The results of simultaneous optimization have shown that the ternary mixture containing 33% of water, 33% of ethanol and 33% of methanol was the most appropriate for simultaneous maximization of TPC, TFC and antioxidant activity. Our results have shown that using mixture design as an optimization technique was an excellent way to choose the most suitable mixture to extract bioactive compounds, which may represent a promising method of multi-purpose extraction, especially in the pharmaceutical and food sectors.

1. Introduction

Bioactive compounds, including flavonoids, phenols, anthocyanins, ascorbic acid, amides, alkaloids, tannins, saponins and glycosides, from different part of medicinal species play an important role in human health, due to their biological activity [1-3]. Polyphenols are natural compounds that are widely found in plants and their importance continues to grow, in particular because of their impact on organoleptic and health benefits [1,2]. Their role as natural antioxidants is useful in the prevention and treatment of cancer, inflammation and cardiovascular diseases [2]. Indeed, in the extraction of active ingredients with high added value from plants, especially polyphenols, the extraction method applied is a very important step in the isolation, as well as in the identification, of phenolic compounds. As a result, many authors have studied the influence of different extraction methods on the extraction yields of

phenolic compounds in plant sources [3]. Among others things, the solubility of the phenolic compounds is affected by the polarity of the solvent used. Further, the determination of phenolic compounds from plant materials is influenced by the extraction technique, the extraction time and the temperature. Therefore, it is difficult to develop an extraction process suitable for the extraction of all the phenolic compounds of plants [3,4,5].

Despite several disadvantages, liquid-liquid and solid-liquid extraction are still the most commonly used extraction procedures for phenolic acids and flavonoids. For many years, the conventional techniques have been widely accepted, mainly because of their ease of use, efficiency, and wide-ranging applicability [6]. Maceration and decoction techniques are very useful methods for weakening the cell wall in vegetal tissues, but in different ways. Experimental designs are among the most common avenues adopted to optimize phenolic compounds' extraction from different vegetal matrices [27,28,29]. In addition, other methods have gained increased interest, including microwave and ultrasonic-assisted extraction, thanks to their simplicity, rapidity and low cost [27]. These methods play a pivotal role in examining the possible interaction of different parameters, providing the most appropriate combinations to minimize the time and number of experiments [13,27,30]. Within this framework, the current study is designed to identify the most suitable solvent mixture for polyphenolic compound extraction and antioxidant ability from C. *sativum* using an augmented simplex-centroid design.

Coriandrum sativum L. (*Coriandrum diversifolium* Gilib., *Coriandrum majus* Gouan, *Coriandrum testiculatum* Lour., *Selinum Coriandrum* (Vest) E. H. L. Krause) is a culinary herb that belongs to the family Apiaceae (Umbelliferae). It is referred to as Kusthumbari (Sanskrit), Dhania (Hindi), Kottamalli (Tamil), Kothimeera (Telugu), Kottambari (Kannada) and many other names [12]. At present, coriander has been cultivated globally in countries such as the USA, the UK, Argentina, India, France, Italy, Morocco, Myanmar, Mexico, Netherlands, Pakistan, Turkey, Spain, and Romania [13]. Coriander is easily identifiable due to its unique morphology. This erect annual herb has a predominant taproot system and thin, soft branching stems, with a height of 20–70 cm [2]. The pale/dark green leaves are glabrous, lanceolate, lobed, and variable in shape. The white or light pink flowers are first borne in peripheral umbellets of small umbels. The peripheral flowers are protandrous and the central flowers are staminiferous or sometimes sterile [12]. The seeds of the plants appear almost ovate and globular. It is a dry schizocarp with multiple longitudinal ridges on the surface and two mericarps [14].

In this study, we aimed to optimize the extraction of polyphenolic compounds including total polyphenolic and flavonoid content, as well as the antioxidant activities in *Coriandrum Sativum* extracts. In this way, the first step in this work consists of screening ten pure solvents among the most commonly used in the extraction of phenolic compounds to select those with the most suitable extractive properties. The screened solvents include nonpolar ones such as hexane, solvents with low polarity such as dichloromethane, solvents with important polarity such as acetone and acetonitrile and solvents with high polarity such as water, ethanol and methanol. Then, the solvents chosen from this screening were used as constituents of the mixture of the system of solvents. The appropriate mixture was selected based on the optimized high total phenolic-flavonoid content and antioxidant power. To the best of our knowledge, this is the first time that such work has been done on the species *Coriandrum Sativum*.

2. Materials and Methods

2.1. Chemicals and Materials

Seeds of *Coriandrum Sativum* were collected from El Hajeb region during the autumn season of 2021 (33°41′22″ N, 5°21′13″ W). Ethanol, methanol, dichloromethane, chloroform, acetone, ethyl acetate, hexane, butanol, acetonitrile, water, DPPH, Folin-Ciocalteu reagent, gallic acid and quercetin were the chemicals used in different experiments and were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA).

2.2.Samples Preparation

The plant was identified by the botanist of the National Agency of Medicinal and Aromatic Plants (NAMAP) in Taounate, Morocco. The samples were dried to constant weight in a room where the temperature was fixed at 25 °C and then powdered in a lab grinder machine.

2.3.Ultrasonic-Assisted Extraction

Initially, the extraction was performed by ten pure polar and nonpolar solvents, including water, ethanol, methanol, dichloromethane, chloroform, acetone, ethyl acetate, hexane, butanol and acetonitrile. 50 mg of powdered seeds were mixed with 1 mL of each solvent. The mixtures were sonicated for 30 min in a P30 H ultrasonic cleaner bath with a power of 100 W and a frequency of 37 kHz (Elmasonic, Singen, Germany) and centrifuged at 10,000 rpm/10 min in a Hettich ROTOFIX 32A centrifuge (Hettich, Tuttlingen, Germany). The mixture was filtered and stored at 4 °C until experimental analysis, as previously described by Ousaaid et al. [33]. Afterward, an extraction under the same conditions was carried out by a solvent system

formulated from the three most effective solvents. The solvent fractions of each experiment are generated from the chosen mixture design.

2.4.Total Phenolic Content Quantification

The phenolic content was quantified using the colorimetric methods previously described by Singleton et al. [34]. In brief, 25 µL of extract and 450 µL of Folin-Ciocalteu reagent (0.5N) were mixed. Then, the mixture was treated with 450 µL of sodium carbonate and incubated in the dark for 2 hours. The absorption was read at 760 nm and the results were expressed as mg GAE/g using a gallic calibration curve.

2.5.Total Flavonoid Content Quantification

The total flavonoid content was determined by the colorimetric method according to the method described by Laaroussi et al. [35]. The protocol involves mixing 100 µL of extract, 150 µL of $Alcl_3$ reagent (10%) and 200 µL of NaOH (1%). The obtained mixture was incubated in the dark for one hour and the absorbance was read at 510 nm. The results are expressed as mg of quercetin equivalent per gram (QE/g) of anise seeds anise using a quercetin calibration curve.

2.6.Antioxidant Activity

The antioxidant activity of extracts was evaluated using the DPPH test [36]. In brief, the extract and DPPH solution were mixed with the following quantities, 25 µL and 875 µL, respectively. The mixture was vortexed and incubated for one hour in the dark condition. The absorbance of the mixture was measured at 517 nm. The percentage inhibition (PI) of the DPPH radical by the samples was calculated according to the formula:

$$PI(\%)=(control\ absorbance-sample\ absorbance:control\ absorbance\ )\times 100$$

The antiradical activity was expressed as $DPPH_{IC50}$ (mg/mL), which is the exact concentration dose required of extractable $DPPH_{IC50}$ whose value corresponds to a higher antioxidant activity.

2.7.Mixture Design

The current study used a mixture design methodology to optimize the extraction process. The test is based on combining different independent factors to create a set of equations that donate theoretical values [13]. The solvent system components are presented in Table 1. According to this table, each of the three components of the mixture can have a value between 0 and 100% and the sum of the three proportions will always be equal to 100%. Besides, 12 experiments

were performed to examine the impact of solvent proportions on total phenolic and flavonoid content and antioxidant activity, as described in Figure 56.

N° of experiment	X1	X2	X3
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.33	0.33	0.33
8	0.33	0.33	0.33
9	0.33	0.33	0.33
10	0.66	0.17	0.17
11	0.17	0.66	0.17
12	0.17	0.17	0.66

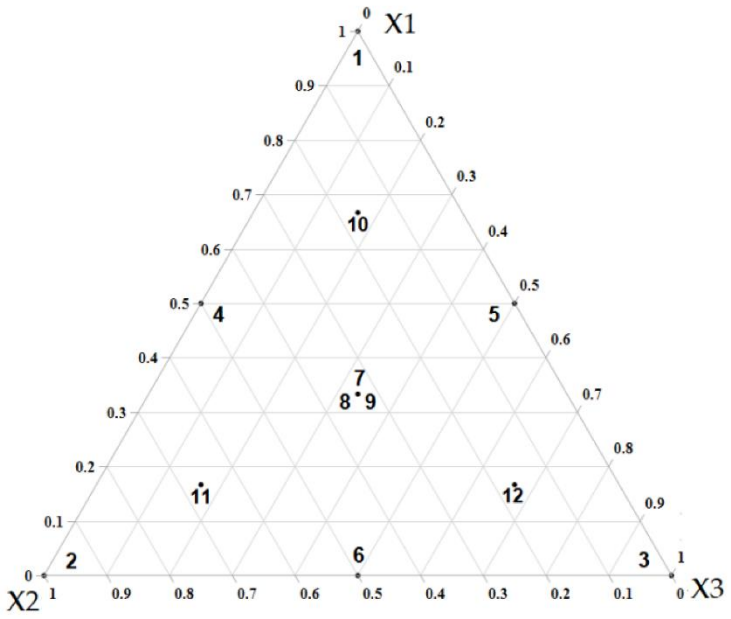


Figure 56: Illustration representing the augmented simplex-centroid design for three components.

Table 25 : Identification of solvent system factors.

Components	Level − (%)	Level + (%)	Coded Variables	Level −	Level +
Water	0	100	X1	0	1
Ethanol	0	100	X2	0	1
Methanol	0	100	X3	0	1
Sum of proportions		100 (%)		1	

As shown by the equation below, the specific cubic model was employed to express the interaction between the components of the analyzed mixtures as well as to predict the maximal recovery of bioactive chemicals and antioxidant activity:

$$Y=\alpha 1X1+\alpha 2X2+\alpha 3X3+\alpha 12X1X2+\alpha 13X1X3+\alpha 23X2X3+\alpha 123X1X2X3+\varepsilon \quad (2)$$

Y represents the experimental response expressed by mg GAE/g, mg QE/g and mg/mL for TPC, TFC and $DPPH_{IC50}$, respectively. α1, α2, α3 are linear regression coefficients, α12, α23 and α23 are binary regression coefficients, α123: is the ternary regression coefficient, while ε: is the regression error term. The findings were expressed as mean.

2.8.Statistical Analysis

Optimization tools were used as an excellent appliance to predict the appropriate combination of extractor solvents. In this context, simplex centroid design was used to assess different interactions among extractor solvents on TPC, TFC and $DPPH_{IC50}$.

The validation of the postulated models was carried out using the F-test for ANOVA. The $F_{R/r}$ (the ratio between the mean square due to the regression (MS_R) and the mean square due to the residuals (MS_r)) was calculated and then compared to the theoretical F for the same degree of freedom. The lack of fit test was used to refine the model fit by comparing the ratio between the mean square lack of fit (MS_{LOF}) and the mean square pure error (MS_{PE}) [37]. High values of $F_{LOF/PE}$ indicate a lack of fit to the model. The coefficient of determination R^2, R^2 adjusted and R^2 predicted were used to verify the quality of the postulated models, while Student's test was used to express the significance of the model's coefficients. The experimental design conception and the statistical and graphical analysis were performed using JMP software V.14 (SAS Institute Inc., Cary, NC, USA) and Design Expert V.11 (Stat-Ease, inc., Minneapolis, MN, USA).

The optimum solvent formula was determined using contour and surface plots based on iso-response curves, which resulted in a compromise of responses. The intensity of the red color indicates the response elevation, while the blue color indicates the opposite. In addition, the desirability function was carried out to accurately determine the desired response based on the optimum conditions. A number of 0% is given when the system produces an undesirable response, while a score of 100% represents the highest possible desired response [38]. Finally, the comparison of means was performed using the ANOVA F-test, while Pearson's test was used to evaluate the correlation between the antioxidant capacity and TPC and TFC. All experiments were performed in triplicate and the results were expressed as the mean ± SD.

3. Results

3.1.Solvents Screening

Ten solvents were tested to select the most appropriate in maximizing the extractable efficacy on TPC. The results obtained when quantifying phenolic content in terms of used solvents are presented in (figure 57).

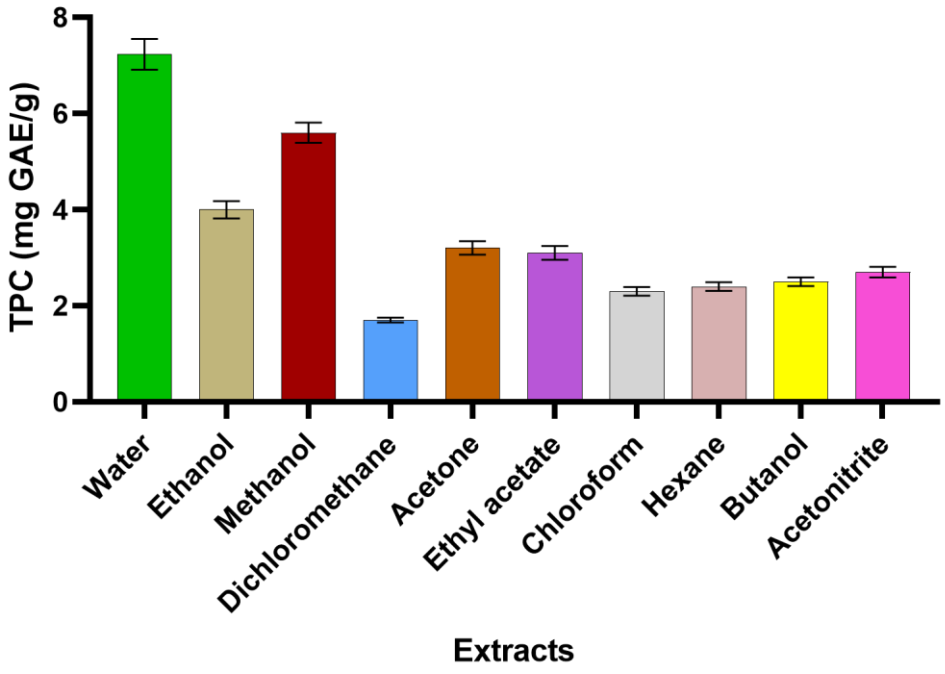


Figure 57: Total phenolic compounds extracted from *Coriandrum Sativum* seeds using different solvents.

According to the graph, water had the highest phenolic content (7.23 ± 0.32 mg GAE/g) of any solvent. Methanol and ethanol came in second and third, with 5.6 ± 0.21 and 4.00 ± 0.18 mgGAE/g, respectively. Moderate values of TPC were obtained from acetone and ethyl acetate, with 3.2 ± 0.62 and 3.1 ± 0.79mgGAE/g, respectively. Conversely, the extract of dichloromethane exhibited the least quantity of TPC, measuring just 2.1 ± 0.1 mg GAE/g. Additionally, there was a statistically significant difference (p-value < 0.05) between the results obtained for the three solvents, water, ethanol, and methanol, and the other seven solvents.

3.2.Solvent Mixture

The simplex-centroid design, with different combinations of the solvents and the recorded response of each experiment on TPC, TFC and $DPPH_{IC50}$, are listed in Table 26. The experiments were conducted after randomization and every response averages three replicates. The analysis of results revealed that the mixture with equivalent proportions of the three solvents was the most extractable formulation and possessed the highest TPC, TFC and $DPPH_{IC50}$ values (Table 26). In *Coriandrum sativum*, the solvent combining 33.33% water, 33.33% ethanol, and 33.33% methanol showed the highest values of TFC raging between 6,02 and 6,13 mg QE/g. In contrast, the lowest values of TFC were recorded in 100% ethanol with 1,68 mgQE/g. The maximum responses for anti-oxidant activity was obtained with the ternary solvents mixture.

Table 26 : Experimental conditions of mixture design, actual and predicted values and residuals for each response.

WATER	ETHANOL	METHANOL	TPC	TFC	$DPPH_{IC50}$
1	0	0	7,23	2,35	0,7
0	1	0	4	1,68	1,45
0	0	1	5,9	2,15	0,91
0,5	0,5	0	6,02	2,64	0,83
0,5	0	0,5	9,6	3,89	0,62
0	0,5	0,5	4,98	2,05	1,1
0,33	0,33	0,33	13,5	6,13	0,38
0,33	0,33	0,33	13,73	5,98	0,38
0,33	0,33	0,33	13,88	6,02	0,32
0,66	0,16	0,16	10,02	4,84	0,45
0,16	0,66	0,16	7,69	3,54	0,79
0,16	0,16	0,66	9,76	4,02	0,51

3.3.Statistical Validation of Postulated Models

The analysis of variance was carried out to study the interaction of different constituents of the mixture, as displayed in Table 24. According to the analysis of variance in the table 27, we can conclude that the main effect of the regression is significant since the probability of the significance of the risk p-value is less than 0.05 (<0.0002 , <0.0001 and <0.0001). Furthermore, the three models do not present a lack of fit, because p-value>0.05 (0.052, 0.07 and 0.28).

Table 27 : The analysis of variance (ANOVA) of studied fitted models.

	TPC					***TFC***					***$DPPH_{IC50}$***				
Model	DF	SS	MS	F	p-value	DF	SS	MS	F	p-value	DF	SS	MS	F	p-value
R	6	130,59	21,76	52,34	0,0002*	6	29,80	4,96	107,31	<,0001*	6	1,23	0,205	84,892	<,0001*
R	5	2,07	0,41			5	0,23	0,046			5	0,01	0,002		
LOF	3	2,005	0,66	18,25	0,0524	3	0,21	0,073	12,111	0,0772	3	0,009	0,003	2,697	0,2820
PE	2	0,073	0,036			2	0,01	0,006			2	0,002	0,001		
Total	11	132,67				11	30,03				11	1,245			
R^2	0.98					0.99					0.99				

The determination coefficients R^2 are all equal to 0.99. These values demonstrate sufficient agreement between the experimental and predicted values of the adapted model. The graph in figure 58 confirms this good agreement by showing a linear curve for the actual values in terms of the predicted ones.

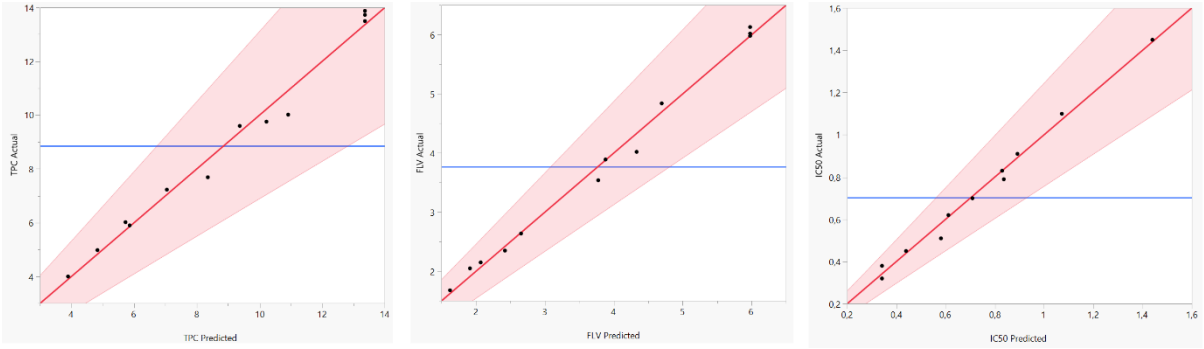


Figure 58: Curves of observed values versus predicted values.

3.3.1. Effects of factors and models

Table 27 summarizes the estimated regression coefficients of the special cubic model. The regression equations with significant coefficients were used to determine the relationships between all studied parameters and the obtained responses for TPC, TFC and $DPPH_{IC50}$).

The significant extractor solvent coefficients for TPC are α1, α2 and α3, for pure extractor solvents, followed by the coefficient of the binary formulation between water and methanol (α13) and then the ternary term (α123). The following equation represents the mathematical model adopted for TPC:

The statistically significant coefficients for the TPC response are:

The linear terms α1, α2 and α3;

Binary terms α13

The ternary term α123

The mathematical model retained is presented by the equation ():

Y= 〖7.04X〗_1 + 〖3.89X〗_2 + 〖5.85X〗_3+11.68X_1 X_3+172.15X_1 X_2 X_3+ε

The statistically significant coefficients for the FLV response are:

The linear terms α1, α2 and α3;

Binary terms α13

The ternary term α123

The mathematical model retained is presented by the equation ():

Y= 〖2.42X〗_1 + 〖1.62X〗_2 + 〖2.06X〗_3+6.53X_1 X_3+78.35X_1 X_2 X_3+ε

The statistically significant coefficients for the IC50 response are:

The linear terms α1, α2 and α3;

The binary terms α12 and α13

The ternary term α123

Y= 〖0.70X〗_1 + 〖1.44X〗_2 + 〖0.89X〗_3- 0.98X_1

Table 28 : Estimated regression coefficients of the special cubic model.

Term	Coeff.	TCP			FLV			$DPPH_{IC50}$		
		Estimate	t-student	*p-value*	Estimate	t-student	*p-value*	Estimate	t-student	*p-value*
Water	α_1	7,0434909	11,31	<,0001 *	2,4205	11,65	<,0001 *	0,7094	14,93	<,0001 *
Ethanol	α_2	3,8916727	6,25	0,0015 *	1,6241	7,82	0,0005 *	1,4403	30,31	<,0001 *
Methanol	α_3	5,8580364	9,41	0,0002 *	2,0687	9,96	0,0002 *	0,8921	18,77	<,0001 *
Water*Ethanol	α_{12}	1,0303273	0,33	0,7558	2,5293	2,42	0,0603	-0,9803	-4,10	0,0094 *
Water*Methanol	α_{13}	11,683055	3,73	0,0136 *	6,5384	6,25	0,0015 *	-0,7567	-3,16	0,0250 *
Ethanol*Methanol	α_{23}	-0,180582	-0,06	0,9563	0,2657	0,25	0,8096	-0,3749	-1,57	0,178
Water*Ethanol*Methanol	α_{123}	172,1592	10,09	0,0002 *	78,350	13,77	<,0001 *	-11,808	-9,07	0,0003 *

Coefficient statisticaly significant at 0.05

3.3.2. Solvent System Optimization

Figure 59 presents the contour and surface plots realized for total polyphenolic content obtained using a different mixture of water, ethanol and methanol. From the 2D and 3D mixing graph, we can see that we can achieve a higher TPC value of around 13 mg GAE/g, an FLV value of around 6 mg QE/g and an $DPPH_{IC50}$ value. less than 0.32 mg/ml, ensuring a mixture consisting of the three components of the water and ethanol and methanol mixture since the optimal zone of the two responses exists in the center of the triangle.

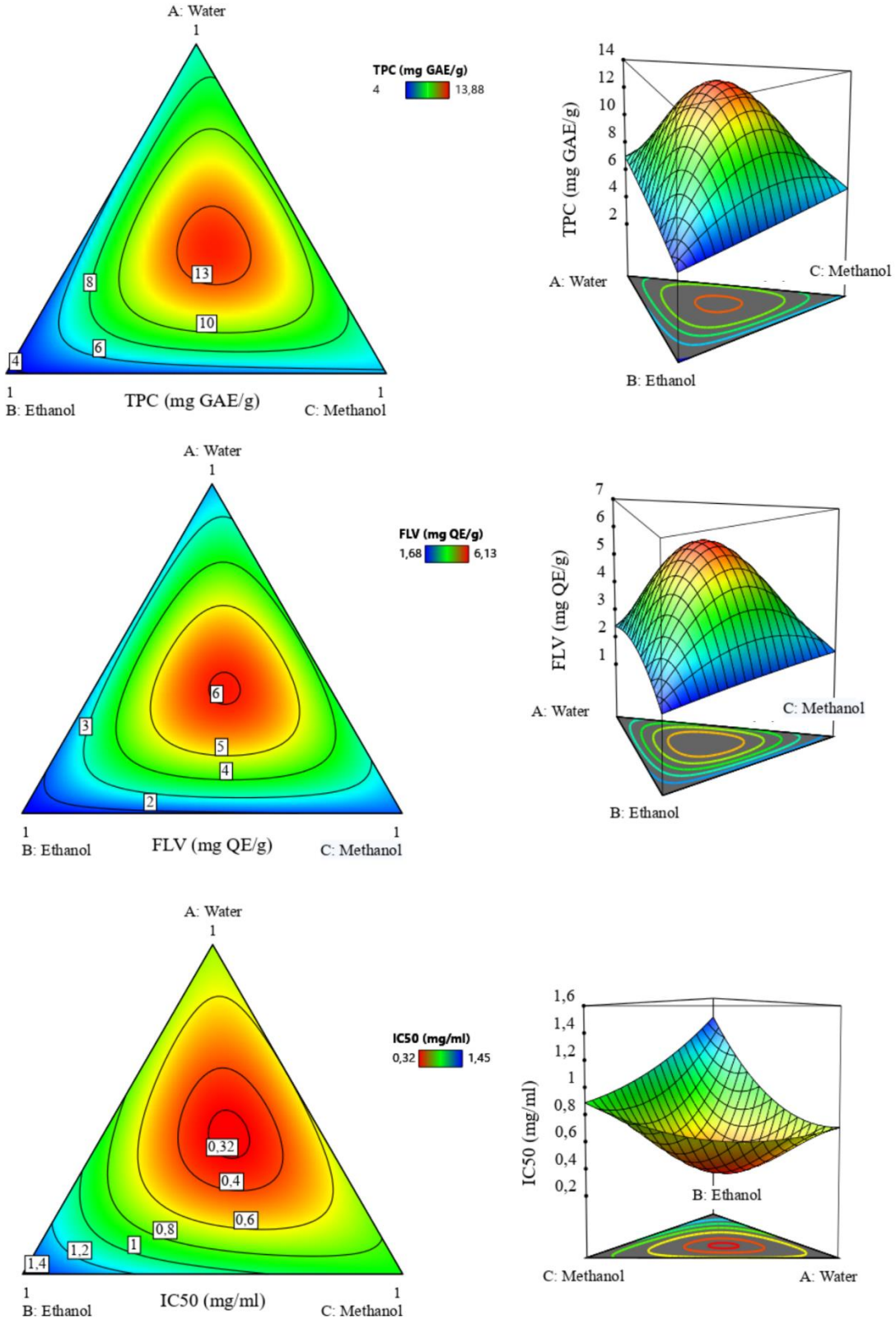


Figure 59: Contour and surface plots, which indicate the optimal compromise area leading to the best value of TPC (A), TFC (B) and $DPPH_{IC50}$ (C).

3.3.3. Desirability study

In order to know the maximum value of the two responses that we can achieve and the exact values of the constituents, we will use the desirability function (Figure 60). The desirability graph shows that we can have a simultaneous adjustment of the two responses since they show correlation trends. Thus this graph shows that the best values that could be achieved with a desirability of 99% are equal to 13.59 mgGAE/g, 6.03 mg QE/g and 0.3 mg/mL for the TPC, TFC and $DPPH_{IC50}$ response, respectively. These values are accessible by ensuring the following mixture: 40% water, 25% ethanol, and 35% Methanol.

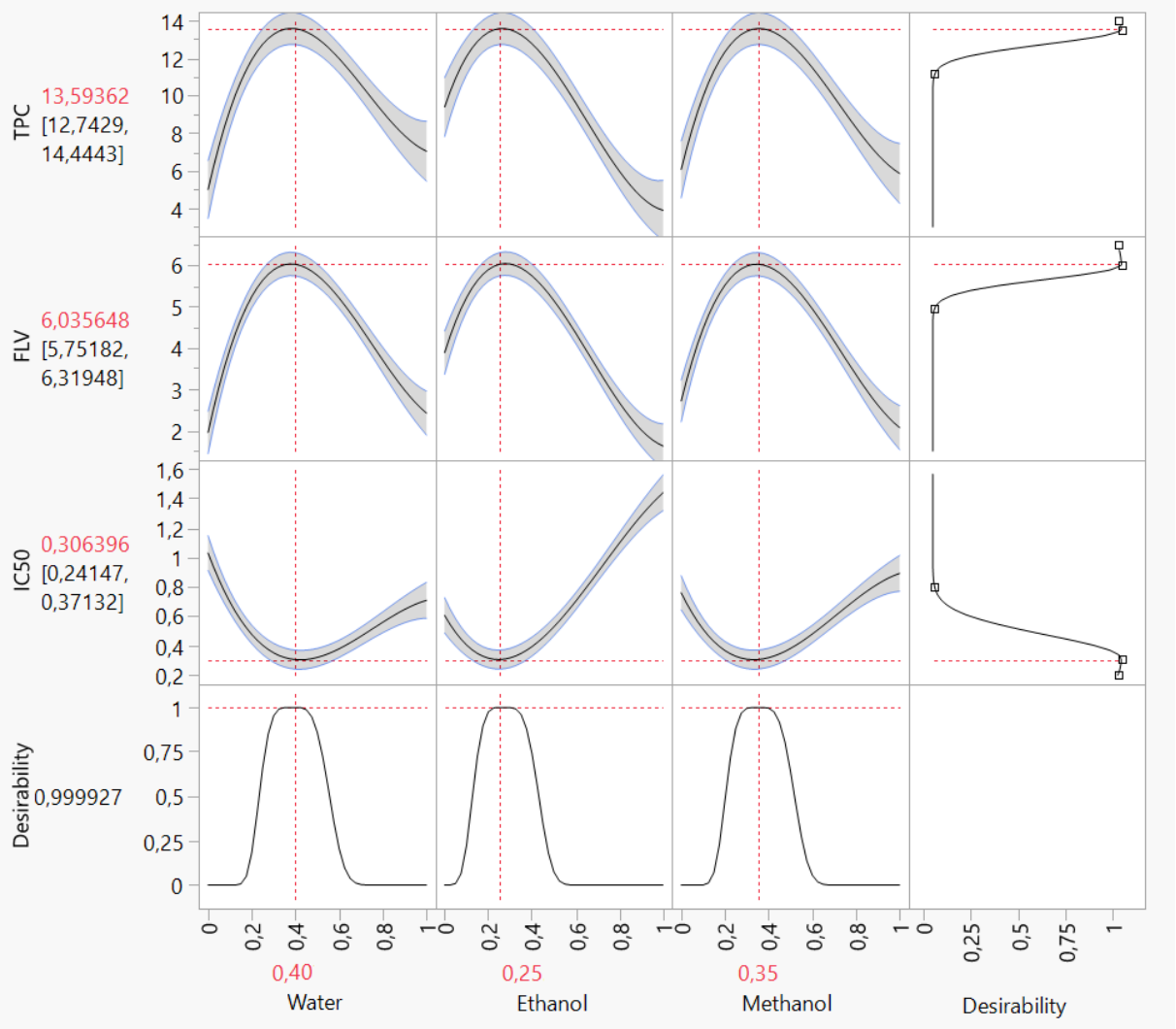


Figure 60 : Desirability plots used to identify the optimum value of TPC (A), TFC (B) and $DPPH_{IC50}$ (C), separately.

4. Discussion

In Coriander (*Coriandrum sativum* L.), the highest values of TPC were recorded in the mixture containing water (33.33%), ethanol (33.33%), and methanol (33.33%), while the lowest values of TPC were recorded in the solvent containing 100% ethanol. These confirm the effect of

extraction solvent on the amount of TPC. Zeković et *al.,* 2014 [39] tested the Total polyphenol in the water extracts of Coriander with by response surface methodology. The obtained results showed that values for total phenols content (TP) varied from 0.5119 to 2.6297 g GAE/100 g CSS. In addition to the effect of extraction solvent, the temperature was the most significant factor influencing the response since TP did not vary significantly on fixed level of temperatures. TP obtained with SWE at 200 °C was significantly higher comparing to previously reported results of TP obtained with maceration for 24 h using ethanol, methylenechloride, ethyl acetate, butanol and water, which varied from 0.09 g GAE/100 g CSS obtained with methylenechloride to 1.89 g GAE/100 g CSS obtained with ethyl acetate [40]. Even though, modern extraction techniques such as ultrasound-assisted (UAE) and microwave-assisted extraction (MAE) can provide higher yields and improve process, TP obtained with these techniques were 0.0418 and 0.0821 g GAE/100 g CSS [41], which once again confirmed higher yields obtained using SWE. Tunisian coriander methanolic extract had a polyphenol content of 1.04 mg GAE/g DW [42]. Sriti et al. (2011) [43] reported that total polyphenol contents found in coriander fruit methanolic extracts were 15.16 mg GAE/g DW for Canadian variety and 12.10 mg G GAE/g DW for Tunisian one. Wangensteen *et al.,* [40] reported that the extraction of total polyphenols with ethyl acetate from coriander fruit samples from Norway contained 1.9 g GAE/100 g DW. On the other hand, Ali Al-Mamary [44] found that polyphenol contents of coriander cultivated in Yemen were 701.21 ± 6.07 mg/100 g DW. The difference in the extraction yield could be the result of using different extraction solvents in other works and methanol in ours. The importance of the solvent type used in the extraction has already been mentioned [45]. These authors showed significant variability on phenolic contents in the same extract when using solvents with different polarities.

It is well known that an important function of flavonoids and phenolic acids was their action in plant defense mechanisms [46]. Indeed, flavonoids have many biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release, potent nitric oxide radical scavenging activity and exhibiting antibacterial, antiviral, anti-inflammatory and antiallergenic effects [47].

The Tunisian coriander variety extract showed the highest capacity to neutralize this radical. Moreover, the obtained IC50 values were 27.00 ± 6.57 µg/mL, 36.00 ± 3.22 µg/mL and 32.00 ± 2.87 µg/mL in Tunisian, Syrian and Egyptian varieties, respectively [48]. The IC50 of BHT was 18.00 ± 1.61 µg/mL. In this study, DPPH radical scavenging activity of the tested samples was in the order BHT > Tunisian variety > Egyptian variety > Syrian variety. On the other

hand, the ethanolic extracts from both leaves and seeds showed a concentration-dependent DPPH scavenging activity with IC50 values of 389 ± 5 and 510 ± 12 μg/mL, respectively [11]. Moreover, the DPPH radical scavenging activity was strongly ($P < 0.001$) affected by the coriander varieties. Sriti et al. [43] have showed a lower free radical scavenging activity (IC50 of 32 ± 0.78 μg/mL) in comparison with our result (27.00 ± 6.57 μg/mL). In addition, the ethyl acetate extract from coriander leaves showed the most potent activity (IC50 value of 147 ± 3 μg/mL) and indicate that compounds with strongest radical-scavenging capacity are of medium polarity [11]. This antiradical activity could be due to the phenolic compounds. In fact, it has been found that antioxidant molecules such as polyphenols, flavonoids, and tannins reduce and discolor DPPH due to their hydrogen donating ability [49]. Comparing obtained results with results of previously conducted studies on antioxidant activity of CS extracts obtained by SFE technique revealed the similar activity of SFE extracts. Obtained extracts (experimental conditions: pressure in range of 115.5–279.5 bar, temperature in range of 37.85–57.85 ◦C and CO2 flow rate of 0.08 kg/h) in concentration of 2.5 mg/mL expressed antioxidant activity in the range of 32.0–57.4% of DPPH scavenging [50]. On the other hand, extracts obtained using ultrasound-assisted (UAE) extraction showed antioxidant activity in concentration range of 0.02479–0.04183 mg/mL (for 70% ethanolic extracts), while aqueous extracts obtained by the same extraction technique exhibited activity in concentration range of 0.07499–0.1007 mg/mL [51]. Extracts obtained during the optimization of microwave-assisted extraction (MAE) expressed activity against DPPH radical in concentration range of 0.0302–0.0665 mg/mL [51], while the subcritical water extracts (SWE) exhibited activity in concentration range of 0.01706–0.06336 mg/mL [39]. Comparing the activity obtained in this study with activity of CS extracts obtained by other extraction techniques, it could be concluded that SFE extracts exhibited the lowest activity toward DPPH radical. Reason for such difference in activity against DPPH radicals may lie in fact that UAE, MAE and SCW techniques provide extracts with high content of polyphenolic compounds unlike the SFE technique which mostly isolate volatile oil components and other nonpolar compounds such are fatty acids.

5. Conclusion

In the current study, the optimization of the extraction of bioactive compounds from *Coriandrum sativum* was performed using a simplex-centroid design. A solvent system containing water, ethanol and methanol was optimized to improve the three responses, TPC, TFC and $DPPH_{IC50}$. The validated special cubic models allowed the optimization of all responses simultaneously. Thus, the findings indicated that the ternary mixture of water:

ethanol: methanol (33:33:33 *v*/*v*/*v*) was the most appropriate for simultaneous maximization of TPC, TFC and antioxidant activity. These conclusions could be a key development in managing the extraction of phenolic compounds for specific applications, such as their application as a substitute for traditional antioxidants utilized mostly in the food industry to enhance nutrition quality. In addition, our work has demonstrated that mixture designs are a valuable and effective tool for organizing and refining experimental parameters to obtain the best findings with the fewest experiments possible.

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MICROBIOLOGIE DES PLANTES ETUDIEES

Analyse comparative des propriétés phytochimiques, des activités antioxydantes et antimicrobiennes des extraits de *pimpinella anisum*, *carum carvi* et *coriandrum sativum*

(A fait l'objet d'un article soumis pour publication)

Synthèse d'étude

Dans cette étude, nous avons examiné les composés chimiques et les activités biologiques de trois plantes médicinales et aromatiques : *Pimpinella anisum*, *Carum carvi* et *Coriandrum sativum*. Pour les composés chimiques, nous avons évalué les teneurs totales en polyphénols et flavonoïdes dans quatre extraits, notamment le méthanol (ME), l'éthanol (EE), l'extrait aqueux (AE) et l'extrait optimisé (OE).

Quant aux activités biologiques, nous avons évalué les effets antioxydants et antimicrobiens des extraits des trois plantes étudiées. Les activités antimicrobiennes ont été testées contre des bactéries Gram positives et Gram négatives (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa et Escherichia coli)* ainsi que des champignons (*Candida tropicalis et Saccharomyces*). De plus, nous avons déterminé la concentration minimale inhibitrice (CMI) et la concentration minimale bactéricide (CMB) pour évaluer le niveau d'activité antimicrobienne dans les extraits des plantes étudiées. L'activité antioxydante a été testée via le dosage de l'assay 2,2-diphényl-1-picrylhydrazyle (DPPH).

Les résultats obtenus ont montré des teneurs importantes de polyphénols totaux et de flavonoïdes dans les extraits des trois plantes étudiées. De même, les activités antioxydantes et antimicrobiennes des extraits étudiés étaient significativement importantes. Cependant, les valeurs obtenues étaient statistiquement variables en fonction de la plante utilisée, du type d'extrait et du microorganisme testé.

Le mélange ternaire : eau, éthanol et méthanol avec mêmes proportions était la combinaison la plus adaptée pour extraire la plus grande quantité de contenu phénolique pour les trois plantes étudiées avec 18.86± 0,85 mg GAE/g ; 13.88± 0,36 mg GAE/g and 27.23± 0,75 mg GAE/g, pour *Pimpinella anisum*, *Coriandrum sativum* et *Carum carvi* respectivement. De même, pour les flavonoïdes les trois solvants mélangés à même proportion ont présenté la valeur la plus élevée de flavonoïdes totaux (7.38 ± 0,72 mg QE/g) pour l'anis vert. Le carvi (7.89± 0,65 mg QE/g) et (5.98± 0,43 mg QE/g) pour la coriandre. L'extrait éthanolique pur étant le solvant qui a présenté la teneur la plus faible en polyphénols totaux et flavonoïdes.

L 'activité antioxydante la plus importante a été enregistrée dans le mélange ternaire contenant (33.33% eau, 33.33% éthanol et 33.33% méthanol). Avec des valeurs de (0.59 ± 0,06 mg/ml) pour *Pimpinella anisum*, (0.38± 0,01 mg/ml) concernant *Coriandrum sativum* et (0.48 ± 0,03 mg/ml) pour *Carum carvi.*

Les résultats obtenus ont montré que les effets inhibiteurs des extraits étudiés différent significativement en fonction de la plante utilisée, des microorganismes testés et du type de solvant. Pour la plante de l'anis, l'analyse des valeurs de CMI a montré que l'activité antimicrobienne la plus élevée a été enregistrée dans l'extrait de méthanol contre *Pseudomonas aeruginosa* ATCC 27,853 (CMI=0,195) et dans l'OE contre *Staphylococcus aureus* ATCC 25,923, *Bacillus subtilis*, *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 27,853 et *Candida tropicalis* (CMI=0,195). La valeur de CMB la plus basse a été enregistrée dans l'extrait méthanolique, aqueux et l'extrait optimisé contre B. subtilis, S. aureus et E. coli (CMB=1,563).

Pour l'extrait de la plante *Carum carvi*, l'activité antimicrobienne la plus élevée a été enregistrée dans l'extrait de méthanol contre *Staphylococcus aureus*, dans l'extrait aqueux contre *Escherichia coli et Pseudomonas aeruginosa*, et dans l'extrait optimisé contre *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* et *Pseudomonas aeruginosa* (CMI=0,781). Les valeurs les plus basses de CMB ont été obtenues dans l'extrait optimisé contre Escherichia coli et Pseudomonas aeruginosa (CMB=3,25). Dans *Coriandrum sativum*, l'activité antimicrobienne la plus élevée a été enregistrée dans l'extrait aqueux contre *S. aureus* et *B. subtilis*, dans l'extrait d'OE contre *Staphylococcus aureus, Bacillus subtilis, Escherichia coli et Pseudomonas aeruginosa* avec une valeur de CMI estimée à 0,391. Les valeurs les plus importantes de CMB ont été enregistrées dans l'extrait optimisé contre *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* et *Pseudomonas aeruginosa* (CMB=1,563).

Dans l'ensemble des résultats obtenus, il ressort que les extraits des trois plantes étudiées (*Pimpinella anisum, Carum carvi* et *Coriandrum sativum)* sont riches en composés chimiques qui sont responsables des activités biologiques observées.

Comparative analysis of Phytochemical properties, antioxidant, and antimicrobial activities of extracts of *Pimpinella anisum*, *Carum carvi*, and *Coriandrum sativum*

Abstract

This study aimed to investigate the chemical compounds and biological activities of three medicinal plants *Pimpinella anisum* (PA), *Carum carvi* (CC), and *Coriandrum sativum* (CS). We evaluated Total polyphenol and flavonoid contents, DPPH, and antimicrobial activities in four extracts including Methanol (ME), Ethanol (EE), Aqueous (AE), and optimized extract (OE). The antimicrobial activities were tested against bacteria and Fungi, and we noted the minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC). The obtained results showed that the amount of chemicals and biological activities are significantly variable depending on the used plant, extraction solvent, and tested microorganism. The recorded results showed that the amount of TPC and TFC was variable depending on used extract solvents among tested plants. The highest TPC, TFC and DPPH in all studied plants was recorded in the extracts realized with water (33.33%), Ethanol (33.33%), and Methanol (33.33%). In *Pimpinella anisum*, the optimized extract showed the highest antimicrobial activities against *S. aureus* ATCC 25,923, *B. subtilis*, *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853, and one fungi *C. tropicalis.* In *Carum carvi*, the optimized extract, the lowest MIC was recorded against *S. aureus* ATCC 25,923, *B. subtilis*, *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853 with a value estimated at 0.781. In *Coriandrum sativum*, the extracts showed different antimicrobial effects depending on tested microorganisms and the type of extract. In the optimized extract, the lowest MIC was recorded against *S. aureus* ATCC 25,923, *B. subtilis*, *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853. These results demonstrated the efficacy of optimized extract against tested microorganisms. However, more research is needed to test the safety of these plants.

Keywords: Chemical compounds, antioxidant, inhibitory effect, extracts, medicinal plants.

1. Introduction

The use of herbal plants for the treatment of various human ailments has a long history in many civilizations (Ssenku et *al.*, 2022). According to some estimates, 75% of people worldwide rely on indigenous medicine. The Global Health Organization (WHO, 2019) also notes that a significant section of the inhabitants in underdeveloped countries continue to rely on medicinal

plants as their primary sources of healthcare. Because of a variety of factors, including expensive pricing and the inaccessibility of contemporary synthetic pharmaceuticals, people of rural regions chose herbal plants as their major source of health care (WHO, 2019). Few or no side effects of herbal medicines are reported as compared to allopathic medics (Faizi *et al.*, 2017). Plants have a wide range of bioactive substances such as alkaloids, flavonoids, lignins, tannins, terpenoids, polyphenols, vitamins, and other secondary metabolites, making them potential antioxidant and antibacterial agents (Azizah *et al.,* 2021; Agbor *et al.,* 2016).

The bioactive compounds in plants act significantly on parasitic and pathogenic organisms (Myburgh, 2014; Blokhina *et al.,* 2002; Halliwell, 2007). The polyphenols, flavonoids and other associated secondary metabolites significantly reduce the damaging effect of reactive oxygen species (ROS) which are resulted from oxidative stress and cause many human illnesses such as cardiac, degenerative disorders, diabetes, and cancer (Nijveldt *et al.,* 2001). Although various antibiotics have been formulated for the treatment of infectious diseases and it was expected that infectious diseases will be eradicated after the discovery of antibiotics (Serrano *et al.,* 2009). But the fight against infectious diseases is progressively getting weaker because of antibiotic resistance (Gill and Tuteja, 2010; Jajic *et al.,* 2015). In this context, the evaluation of medicinal plants for phytochemicals, antioxidant, and antibacterial activity is necessary for finding new possible therapeutic compounds (Halliwell, 2007). Indeed, one fourth of all synthetic drugs used today were made up of any plant-based compound as the active ingredient (Karlsen *et al.*, 1954).

The herbal medicines have advantages of no or minimal side effects as compared to synthetic antibiotics (Hostett, 1998). Identifications and isolation of target bioactive compounds had been a challenge during the entire era of modern pharmacognosy. One major bottle neck has been the selection of appropriate solvent system for crude extract preparation and selection of vehicles for bioassays (Lampinen, 2005). Appropriation of solvent system thus, is highly selective and needs considerable attention for better exploration of chemical diversity of plant extract under examination. One plausible approach is to use a panel of most commonly used solvents as a variable during extract preparation and bioassays (Emma *et al.*, 2001; Fazly Bazzaz *et al.,* 2016).

Traditional medicines (TM) are commonly utilized in India, Pakistan, China, Korea, Tailand, Japan and Sri Lanka. More than 75% of Pakistanis use TM for all or most of their medical needs (Shinwari and Shinwari, 2011). Approximately 600 plant species are utilized medicinally in Pakistan (Hamayun *et al.,* 2006). In Azad Kashmir, there are many plant species which are being used for the treatment of various human diseases. Traditional medicine is heavily used

in this region to satisfy people's daily health care requirements. For example, A. *millefolium*, B. *ciliata*, and A. *vera* are the most popular plants used by the local inhabitants and traditional healers for the treatment of different diseases. A. *vera*, belongs to family Xanthorrhoeaceae, is an herb found all over the world. It is found that it has noticeable pharmacological activities such as antioxidant (Baradaran *et al.*, 2013; Ray *et al.*, 2016; Kang *et al.*, 2014), antibacterial (Fani and Kohanteb, 2012; Nejatzadeh-Barandozi, 2013), anti-aging and anti-inflammatory (Vijayalakshmi *et al.*, 2012; Bhattacharjee *et al.*, 2014). Since long time it is used as an herbal drug containing more than 75 bioactive components (Radha and Laxmipriya, 2014). *A. vera* holds health supporting effect and is a major source of several phytochemicals like amino acids, salicylic acids, minerals, polysaccharides, anthraquinones, saponins, vitamins and may more (Surjushe *et al.*, 2008; Chatterjee *et al.*, 2013).

In this study, we investigated the chemical compounds and biological activities in three medicinal plants *Pimpinella anisum*, *Carum carvi*, and *Coriandrum sativum*. In terms of chemical compounds, we evaluated the Total polyphenol and flavonoid contents in four extracts including Methanol (ME), Ethanol (EE), Aqueous (AE), and OE. In terms of biological activities, we evaluated the antioxidant and anti-microbial effects in the extracts of studied plants. The antimicrobial activities were tested against Gram (+) and Gram (-) bacteria (*S, aureus, B, subtilis, P, aeruginosa, and E, Coli) and Fungi (C, tropicalis and Saccharomyces*). Further, we noted the minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) to evaluate the level of microbial activity in the extracts of studied plants. The antioxidant activity was tested via 2,2-diphenyl-l-picrylhydrazyl (DPPH) assay.

2. Materials and methods

2.1. Plant materials

Seeds of *Pimpinella anisum*, *carum carvi* and *coriandrum sativum* were collected from El Hajeb region during the autumn season of 2021 (33°41'22'' N, 5°21'13'' W).

The samples were dried to constant weight in a room in which the temperature was fixed at 25 °C and then powdered in a lab grinder machine.

2.2.Extraction

2.2.1. Solvent Extraction

The extracts of the different plants were prepared in triplicate by adding 1 ml of the solvent (pure solvents and mixtures) to 50 mg of powder and subjected to sonication for 30 min in an ultrasonic bath at ambient temperature, so the extract is recovered after centrifugation for 10

minutes at 10,000 rpm and stored in the dark at 4 °C. The first step of extraction consists of a screening using solvents with different polarities water, ethanol, methanol, acetone, ethyl acetate, dichloromethane, chloroform, hexane, petroleum ether, di-ethyl ether, butanol in order to choose the best solvent to use for the next step. The second step consists of extraction of the pure selected solvent and their mixtures.

2.2.2. Total phenolic content (TPC)

The total phenolic content (TPC) was determined spectrophotometrically, according to the colorimetric method using the Folin-Ciocalteu reagent [1] with modification. A volume of 50 µL of the sample was mixed with 450 µL of a solution of the Folin-Ciocalteu reagent diluted 10 times. After 5 minutes of incubation at room temperature, 450 µL of a solution of Na_2CO_3 (75 g L^{-1}) was added. All samples were subsequently incubated in the dark for 2 h at room temperature and their absorbance was read at 760 nm in a spectrophotometer. The concentration of the calibration curve ($y = 2.8388x + 0.0556$ $R^2 = 0.9994$) ranged from 0.062 to 1 mg mL^{-1} in an ethanolic solution of gallic acid. The experiment was carried out in triplicate and the results are expressed in mg of gallic acid equivalent (GAE) g^{-1} of the dry plant.

2.2.3. Total flavonoid compounds (TFC)

The aluminum chloride colorimetric technique was used to measure the TFC of studied extracts (with some adjustments) [33]. Further, 0.5 mL of each extract was mixed with 5% NaNO2 (0.3 ml), and after 5 min, we added 0.3 ml of AlCl3 (10%) and 1 ml of 1M NaOH to the mixture. The tubes were adjusted to 5 ml via distilled water. Then, the absorbance of the extract was quantified at 415 nm and quercetin was used to prepare the standard calibration curve (y= 3,8762x - 0.044, R2= 0.9993). Obtained results were presented as milligrams of quercetin corresponding to a gram of dried sample (mg Qu/g dw) and three replicates were used for each experiment.

2.2.4. Antioxidant activity:

- ***Free radical scavenging activity: DPPH***

DPPH (2,2-diphenyl-1-picrylhydrazyl) was produced as described by [4]. 25 µl of different concentrations of samples or standards were added to a 60 µM ethanolic solution of DPPH (1 mL). The absorbance measurements were read at 517 nm, after 60 min of incubation time at room temperature. The absorption of a blank sample containing the same amount of methanol and DPPH solution served as a negative control. The experiment was performed in triplicate and the percentage inhibition of the free radical scavenging activity of each extract was calculated as follows:

% inhibition = [(Abs $_{control}$ - Abs $_{sample}$ / Abs $_{control}$) × 100]

The percent inhibition was plotted against the content of the sample or standard and the IC50 was determined (concentration of the extract or standard capable of trapping 50% of the free radical of DPPH)

2.2.5. Antimicrobial Activity

- ***Inoculum preparation***

The extracts from the study plants were tested against a series of pathogenic microorganism obtained from the culture collection of the laboratory of Functional Ecology and Engineering Environment, Faculty of Sciences and Technologies (FST), Fez. These strains were stored in 15% (v/v) glycerol at -80°C. Each bacterium was added to fresh Mueller – Hinton broth and incubated overnight at 37°C. subsequently, bacterial suspensions were prepared in sterile distilled water and adjusted to 0.5Mc Farland standard (108CFU/mL), the antifungal efficacy of the extracts was assessed against two yeast strains responsible for human respiratory, gastrointestinal and urinary infections namely candida tropicalis (EF 14) and saccharomyces cerevisiae.

These two fungal strains were sourced from the culture collection the laboratory of Functional Ecology and Engineering Environment, Faculty of Sciences and Technologies (FST), Fez. They were cultivated in yeast Extract - Peptone - glycerol (IPG) medium compressing 1% yeast extract, 2% peptone and 2% glucose. Subsequently, the cultures were diluted with distilled water 6×108CFU/mL. inoculum suspension of C. tropicalis and S. cerevisiae remained viable for processing for up to one week.

- ***Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)***

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were evaluated by the broth microdilution assay. In 96 well microplates, 50µL of sterile distilled water was added to each microplate will. Then 50µL of each extract was added to the first well plate and mixed to determine the serial dilutions. Additionally, 50mL of MHB and 50mL of bacterial /fugal suspension were added to each well.

The wells containing the bacterial/fungal suspension without extract and those containing Gentamicin/Amphotericin (30µg/mL).

The extracts with no microbial inoculation served us negative and positive controls, respectively.

Moreover, wills containing the extract with no microbial inoculation were established us blanks. The plates were incubated at 37°C for 24h for bacteria and at 30°C for 24h for fungi.

Next, 40µL of TTC (2, 3, 5 – triphenyltetrazolium chloride) was added to each will and incubated at 37°C for 30min. The MIC values were determined at the lowest concentrations of extracts at which bacterial growth was not observed. At the same time, the MBC values were determined us the lowest concentrations of extract that did not produce a bacterial colony following plating of 100µL from each well on tryptone soy yeast extract agar.

2.3.Statistics

All experiments selecting the solvent proportion were performed in triplicate and the results were reported as a medium ± standard error and IC_{50} values have been determined. Statistical comparisons were made with a one-way ANOVA followed by a Tukey multiple comparison test. The significance level was set at $p \leq 0.05$. Principal component Analysis was used to analyze the principal compounds (Polyphenols and Flavonoids) characterizing the fractions of studied plants. The statistical calculation was performed using the free version of STATISTICA version 10 software (StatSoft, INC., 2011).

3. Results and discussion

3.1. Total polyphenols

The results of Total polyphenols in the extracts of studied plants are presented in Table 26. The analysis of recorded results showed a variation of total polyphenols depending on used extraction solvents, and used plant. Based on the Table analysis concerning *Pimpinella anisum*, it was shown that ternary combination of water, ethanol and ethanol with equal proportions had the highest phenolic content extraction ratio (18.86 ± 0.36), followed by methanol – ethanol (50%;50%) (16.52 ± 0.11mg GAE/g). Conversely, the extract of ethanol exhibited the least quantity of TPC, measuring just 5.62 ± 0.1 mg GAE/g.

In Coriander (*Coriandrum sativum* L.), the obtained data showed different results depending on used extraction solvents and extracted parameter. In terms of total polyphenols, the highest values were recorded in the mixture containing water (33.33%), ethanol (33.33%), and methanol (33.33%) with 13,73 to 13,88 mg GAE/g. In contrast, the lowest values of TPC were recorded in the pure solvent containing ethanol.

In Carvi (*Carum carvi*), the obtained data showed different results depending on the used extraction solvents and extracted parameters. The mixture comprising water (33.33%), ethanol (33.33%), and methanol (33.33%) had the highest total polyphenol levels, ranging from 26,87

to 27,23 mg GAE/g. On the other hand, the solvent containing 100% ethanol with 13,76mgGAE/g had the lowest TPC values.

Table 29 : Comparison of Total Polyphenols among the extracts of studied plants *(Carum carvi, Coriandrum sativum*, and *Pimpinella anisum)*

	Extracts			*Carum carvi*	*Coriandrum sativum*	*Pimpinella anisum*
	Water	Ethanol	Methanol	TPC	TPC	TPC
1	1	0	0	20,3	7,23	14.14
2	0	1	0	13,76	4	5.62
3	0	0	1	18,12	5,9	11.29
4	0,5	0,5	0	18,85	6,02	11.84
5	0,5	0	0,5	22,18	9,6	16.52
6	0	0,5	0,5	15,09	4,98	10.6
7	0,33	0,33	0,33	27,08	13,5	18.45
8	0,33	0,33	0,33	26,87	13,73	18.15
9	0,33	0,33	0,33	27,23	13,88	18.86
9	0,66	0,16	0,16	23,56	10,02	15.72
10	0,16	0,66	0,16	20,63	7,69	11.91
11	0,16	0,16	0,66	22,85	9,76	14.6

In *Pimpinella anisum*, the results showed that the aqueous extract had higher concentrations of TPC than the ethanolic and methanolic extracts using purrs solvents, the ternary combination showed the most value of TPC. Bettaieb Rebey et *al.,* (2017) compared TPC of Tunisian (TAS) and Egyptian anise (EAS) (*Pimpinella anisum* L.) seeds. According to the obtained results, the phenolic content of aniseeds ranged noticeably from 31.22 to 1.82 for TAS and 17.43 to 1.03 for EAS. The total phenolic contents that were extracted from TAS were considerably more than those from EAS. Ethyl acetate yielded the highest total phenolic content in both accessions, with ethanol, dichloromethane, and hexane fractions following suit. The ethyl acetate extract from Indian aniseeds has a higher total phenolic content than other solvent extracts, according to Shobha *et al.,* (2013). This outcome is consistent with the study by Scholz and Rimpler, (1989), which demonstrated that methanol is the best solvent for phenolic compound extraction and that ethyl acetate is frequently used as an extraction solvent with a significant selectivity in the extraction of high-molecular-weight polyphenols and low-molecular-weight phenolic compounds. Gülçin *et al.,* (2003) found, in contrast to these findings, that the Turkish aniseed ethanol extract contained the largest level of total phenolic components (77.5 mg GAE/g DW) when compared to the water extract (30 mg GAE/g DW).

According to Bagdassarian *et al.*, (2013), Bulgarian aniseed methanolic extract had a total phenolic concentration of 46.17 mg/g DW. These modifications may be attributed to differences in pedoclimatic circumstances.

In Coriander (*Coriandrum sativum* L.), the highest values of TPC were recorded in the mixture containing water (33.33%), ethanol (33.33%), and methanol (33.33%), while the lowest values of TPC were recorded in the solvent containing ethanol. These confirm the effect of extraction solvent on the amount of TPC. Zeković *et al.,* (2014) tested the Total polyphenol in the water extracts of Coriander with by response surface methodology. The obtained results showed that values for total phenols content (TP) varied from 0.5119 to 2.6297 g GAE/100 g CSS. In addition to the effect of extraction solvent, the temperature was the most significant factor influencing the response since TP did not vary significantly on fixed level of temperatures. TP obtained with SWE at 200 °C was significantly higher comparing to previously reported results of TP obtained with maceration for 24 h using ethanol, methylenechloride, ethyl acetate, butanol and water, which varied from 0.09 g GAE/100 g CSS obtained with methylenechloride to 1.89 g GAE/100 g CSS obtained with ethyl acetate (Wangensteen *et al.*, 2004). Even though, modern extraction techniques such as ultrasound-assisted (UAE) and microwave-assisted extraction (MAE) can provide higher yields and improve process, TP obtained with these techniques were 0.0418 and 0.0821 g GAE/100 g CSS (Gallo *et al.*, 2010), which once again confirmed higher yields obtained using SWE.

In Carvi (*Carum carvi*), the obtained data showed that the mixture comprising water (33.33%), ethanol (33.33%), and methanol (33.33%) had the highest total polyphenol levels, while the solvent containing 100% ethanol had the lowest TPC values. In a comparative study, Thippeswamy *et al.*, (2013) investigated the amount of TPC in different extracts of *Carum carvi*. To simplify the extraction of a variety of polyphenols, the polyphenolic components from defatted *C. carvi* powder were extracted progressively with water, 50% ethanol, and a 1:1 combination of 70% aqueous methanol and 70% aqueous acetone. According to the findings, there were 8.76, 12.63, and 50.20 mg of polyphenols per gram of defatted powder, respectively. Therefore, we were able to extract many phenolic acids and flavonols from C. carvi using the solvent systems mentioned above.

3.2.Total flavonoids

The results of Total Flavonoid Content in the extracts of studied plants are presented in Table 30. The analysis of recorded results showed a variation of total flavonoid content depending on used extraction solvents, their fractions, and used plant.

The highest TFC in *Pimpinella anisum* was recorded in the extracts realized with water (33.33%), ethanol (33.33%), and methanol (33.33%) with values ranging from 7,15 to 7.38 mg QE/g. in contrast, the lowest values of TFC were recorded in Ethanol (100%) with 2,05 mg QE/g and the combination of Ethanol and Methanol (50:50) with 2.95 mg QE/g. In *Coriandrum sativum*, the solvent combining 33.33% water, 33.33% ethanol, and 33.33% methanol showed the highest values of TFC raging between 6,02 and 6,13 mg QE/g. In contrast, the lowest values of TFC were recorded in 100% ethanol with 1,68 mg QE/g. In *Carum carvi*, the highest values of Total Flavonoid Content were recorded in the extracts realized with water (33.33%), ethanol (33.33%), and methanol (33.33%) with values ranging from 5,98 to 6,13 mg QE/g. In the second rank, the medium values were recorded in the combinations Water (0,666667, 0,166667, and 0,166667), Ethanol (0,166667, 0,666667, and 0,166667), and Methanol (0,166667, 0,166667, and 0,666667) ranging from 5.14 to 5.75 mg QE/g. In contrast, the lowest values were recorded in Ethanol (100%) with 2.6 and 50% ethanol, 50% methanol with 2.98 mg QE/g.

Table 30 : Comparison of Total Flavonoid Content among the extracts of studied plants (*Carum carvi*, *Coriandrum sativum*, and *Pimpinella anisum*)

Extracts			*Carum carvi*	*Coriandrum sativum*	*Pimpinella anisum*
Water	Ethanol	Methanol	TFC	TFC	TFC
1	0	0	3,59	2,35	4,12
0	1	0	2,6	1,68	2,05
0	0	1	3,42	2,15	3,54
0,5	0,5	0	3,54	2,64	3,84
0,5	0	0,5	4,19	3,89	4,92
0	0,5	0,5	2,98	2,05	2,95
0,33	0,33	0,33	7,61	6,13	7,15
0,33	0,33	0,33	7,89	5,98	7,38
0,33	0,33	0,33	8,02	6,02	7,13
0,66	0,16	0,16	5,75	4,84	5,99
0,16	0,66	0,16	5,14	3,54	4,56
0,16	0,16	0,66	5,43	4,02	5,39

The highest TFC in *Pimpinella anisum*, *Coriandrum sativum* and *Carum carvi* was recorded in the extracts realized with water (33.33%), ethanol (33.33%), and methanol (33.33%). These is different compared with literature.

Mehwish Wajidi *et al.,* (2019) investigated the Total Flavonoid content in of crude and fractions of *Carum carvi*, including extract of ethanolic (EE), hexane fraction (HF), ethyl acetate fraction (EAF), and aqueous fraction (AQF). The highest flavonoid contents were observed 85.4±0.124, 81.1± 0.16, 49.6 ± 0.93, and 3.08 mg/g Querecitin for aqueous, ethanol, ethyl acetate and hexane respectively. In *Coriandrum sativum*, Rajeshwari *et al.,* (2012) investigated the effect of solvents and their fractions, including in water and hexane (He), ethyl acetate (Ea), benzene (Be), n-butanol (nBu), and water (Aq). In the leaves, the highest amount of TFC was recorded in the Ethyl acetate 4.82±1.1 mg/100g RE, while in the other extracts the values were statistically similar. In the seeds, the highest value of TFC was recorded in Ethyl acetate with 3.40±1.1 mg/100g RE.

Bettaieb Rebey *et al.,* (2017) compared TFC of Tunisian (TAS) and Egyptian anise (EAS) (*Pimpinella anisum* L.) seeds. The range of total flavonoid content in aniseeds for TAS was 2.76 to 48.52 mg CE/g DW, while for EAS it was 1.88 to 31.08 mg CE/g DW. The total flavonoid concentrations of the two accessions differed significantly. The amount of total flavonoids extracted from TAS was greater than that of EAS. In terms of flavonoid solubility, the solvent classification based on extraction efficiency was comparable to the one used for polyphenols, which are soluble in ethyl acetate, ethanol, dichloromethane, and hexane in that order. Additionally, Shobha *et al.,* (2013) demonstrated the effectiveness of ethyl acetate as a solvent for flavonoid extraction from aniseeds.

3.3.Antioxidant activity

The results of antioxidant activity in the extracts of studied plants are presented in Table 31. The analysis of recorded results showed a variation of $DPPH_{IC50}$ depending on used extraction solvents, and used plant. In all studied plants, the highest $DPPH_{IC50}$ was recorded in the ternary combination with equal proportions with 0.43mg/ml in *Carum carvi*, 0.38mg/mL in *Coriandrum sativum*, and 0.6mg/mL in *Pimpinella anisum.* The solvent with 100% showed the lowest values of $DPPH_{IC50}$ for all studied plants.

Table 31 : Comparison of Antioxidant activity (DPPHIC50) among the extracts of studied plants (*Carum carvi*, *Coriandrum sativum*, and *Pimpinella anisum*)

Extracts			*Carum carvi*	*Coriandrum sativum*	*Pimpinella anisum*
Water	Ethanol	Methanol	$DPPH_{IC50}$	$DPPH_{IC50}$	$DPPH_{IC50}$
1	0	0	0,69	0,7	0,69
0	1	0	1,32	1,45	1,46
0	0	1	0,69	0,91	0,77
0,5	0,5	0	0,71	0,83	0,72
0,5	0	0,5	0,69	0,62	0,68
0	0,5	0,5	0,96	1,1	0,97
0,33	0,33	0,33	0,43	0,38	0,6
0,33	0,33	0,33	0,48	0,38	0,59
0,33	0,33	0,33	0,39	0,32	0,57
0,66	0,16	0,16	0,53	0,45	0,6
0,16	0,66	0,16	0,87	0,79	1
0,16	0,16	0,66	0,63	0,51	0,71

The DPPH method with an organic radical, 1,1-diphenyl-2-picrylhydrazyl is applied to determine the free radical scavenging activity and is expressed by IC50 (Gulcin and Alwasel 2023), which is interpreted as the amount of antioxidants needed to reduce the initial DPPH concentration to 50%. The antioxidant activity of a substance is called high when its IC50 value is lower (Jug *et al.*, 2021).

Coriandrum sativum L. has an advantageous flower antioxidant profile including radical free radical scavenging activity, phospholipid peroxide inhibition, chelation activity, hydroxyl radical and peroxidid peroxidation scavenging. (Nhut *et al.*, 2020) investigated the antioxidant activity in ethanolic and aqueous extracts of *Coriandrum sativum* sampled from Vietnam. The obtained results showed that the lowest IC50 was recorded in the ethanolic extracts with 78.309 µg/mL compared to 108,233 µg/mL in the aqueous extract. Besides, ABTS root removal is determined by observing color change at 734nm wavelength. The similarity with the DPPH assay is shown in EE at 54,489 µg/mL and aqueous extract at 97,368 µg/mL. The results showed that extracted from *Coriandrum sativum* L with ethanolic extract for high antioxidant activity. In another study, Msaada *et al.*, (2017) investigated the antioxidant activity in methanolic extracts from three fruit varieties of coriander (*Coriandrum sativum* L.). recorded results showed that fruit methanolic extracts exhibited remarkable DPPH radical scavenging activity with IC50 values ranged from 27.00 ± 6.57 to 36.00 ± 3.22 µg/mL. EC50 values of

reducing power activity varied significantly ($P < 0.05$) from 54.20 ± 6.22 to 122.01 ± 13.25 μg/mL. The IC50 values of β-carotene bleaching assay were between 160.00 ± 18.63 and 240.00 ± 26.35 μg/mL. Therefore, coriander fruit might constitute a rich and novel source of natural antioxidants and may be suggested as a new potential source of natural antioxidants and could be used as a food additive. Tang *et al.* (2013) investigated the Antioxidant activity in different parts of *Coriandrum sativum* and their correlation with polyphenols. The results showed that among C. *sativum* root extracts, the ethyl acetate extract had the highest FRAP value of 0.129 ± 0.007 mmol/g (Table 1). As for the leaf and stem, highest FRAP value was seen in the dichloromethane extract (0.136 ± 0.008 mmol/g). The leaf and stem aqueous extract had the lowest IC50 value of 1335.0 ± 37.7 μg/ml (Table 1). As for the root, the ethyl acetate extract which had highest FRAP value, also displayed highest DPPH radical scavenging activity (IC50 = 2348.3 ± 184.1 μg/ml) among root extracts. The relationship between phenolic content with FRAP and DPPH scavenging activity of plant extracts were evaluated by Pearson correlation analyses. A strong and statistically significant positive correlation was identified between the phenolic content and FRAP values of the root ($r = 0.982$, $P < 0.01$) while a weak positive correlation was seen between its TPC and DPPH scavenging activity ($r = 0.663$, $P < 0.01$). As for the leaf and stem, significant positive correlation was observed between its TPC and DPPH scavenging activity ($r = 0.906$, $P < 0.01$).

In terms of *Carum carvi*, Thippeswamy *et al.*, (2013) investigated the antioxidant properties of phenolic extract from seeds using water, 50% alcohol and 1:1 mixture of methanol (70%) and acetone (70%). The extract was tested in the concentration range of 0.1–2 μg/ml and the activity were observed in a dose dependent manner. At a concentration of 0.1 μg/ml, the scavenging activity was 13.7%, whereas at 2 μg/ml, the scavenging activity was 84.6%. The IC_{50} value of *C. carvi* phenolic extract was found to be 2.7 μg/ml. Atrooz, (2013) tested the antioxidant activity of seed extract from *Cuminum cyminum* L. and *Carum carvi* L. Both cumin and caraway seed extracts were able to neutralize free radicals over a period of 60 min in the DPPH assay, with most of the neutralization occurring quickly within the first 30 seconds. The steady state was reached within 10 min, and it appeared that the acetonic extracts of both seeds had less antioxidant activity than the methalonic extracts. The methanolic and acetonic extracts of cumin showed slightly higher neutralization ability than the respective extracts from caraway within three minutes of assay initiation (57.0 and 52.4% for cumin and 44.7 and 39.5% for caraway. Importantly, the neutralization level stayed relatively the same over 60 min. Mišan et *al.*, (2011) evaluated the antioxidant activity of ethanolic extracts from four medicinal plants.

The obtained results showed a significant variation depending on used plant. In extracts from Caraway fruit, the value of IC50 DPPH scavenging activity was estimated at 2.06 ± 0.137 mg extract/mL, while the highest value was recorded in Parsley fruit with 4.65 ± 0.820 mg extract/mL.

Gülçın *et al.,* (2003) conducted a screening of antioxidant activities of water and ethanol extracts from anise (*Pimpinella anisum* L.) seed. Total antioxidant activity of PAS extracts was determined by the thiocyanate method. Both PAS extracts exhibited effective antioxidant activity at all concentrations. The antioxidant activity of both PAS extracts increased with increasing concentration. The different concentration of water and ethanol extracts (10, 20 and 50 μg/ml) of PAS showed higher antioxidant activities than that of 20 μg/ml concentration of α-tocopherol. The percentage of inhibition in linoleic acid system was 88.3, 94.1, 97.6, 56.5, 77.5, and 91.2%, respectively, and greater than that of 20 μg/ml of α-tocopherol (36.1%). In another study, Mohammad Al-Ismail *et al.*, (2004) evaluated the Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. In results, the water extracts showed higher antioxidant activity than the corresponding alcohol extracts. The antioxidant activity of both water and alcohol extracts decreased in the order chamomile flowers > gtill seeds > gtnise seeds. The extracts also showed good free radical-scavenging activity, indicating that they act as hydrogen donors.

There are many techniques to recover antioxidants from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction. However, extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Turkmen *et al.,* 2006). Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols (Dai and Mumper 2010). The maximum total phenolic content was obtained from barley flour by extraction using a mixture of ethanol and acetone (Bonoli *et al.,* 2004). For extracting flavonoids from tea, aqueous ethanol performed better than aqueous methanol and aqueous acetone (Wang and Helliwell 2001). Extracts with the greatest antioxidant activity were obtained in mate tea and

black tea by using 50% aqueous ethanol and 50% aqueous acetone, respectively (Turkmen *et al.,* 2006). These are suggested to be the best explanation of our results. Therefore, the highest values recorded in the optimized extract is suggested to be governed by the combination of the characteristics of each solvent (Monteiro *et al.,* 2020).

3.4.Anti-microbial activity

The results of antimicrobial activities in extracts of *Pimpinella anisum*, *Carum carvi*, and *Coriandrum sativum* are presented in Table 32. The obtained results showed that the inhibitory effects of the extracts differed significantly, depending on the used plant, tested microorganisms, and the type of solvent.

In *Pimpinella anisum*, the extracts showed different antimicrobial effects. The lowest MIC values (0.195) were recorded in the aqueous extract against B. subtilis, *E. Coli* ATCC 25,922, and *P. aeruginosa* ATCC 27,853, Methanolic extract against *B. subtilis* and *P. aeruginosa* ATCC 27,853, and optimized extract against four bacteria *S. aureus* ATCC 25,923, B. subtilis, *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853, and one fungi *C. tropicalis*. These values are statistically inferior when compared to the values demonstrated by the commercial antibiotic Gentamicin and Amphotericin. On the other hand, the lowest MBC value was recorded in the aqueous, methanol, and optimized extracts against *S. aureus* ATCC 25,923, B. subtilis, *E. Coli* ATCC 25,922, and P. aeruginosa ATCC 27,853 with a value estimated at 1.563. However, the recorded inhibitory effects were statistically inferior as compared to the antibiotics Gentamicin and Amphotericin.

In *Carum carvi*, the extracts showed different antimicrobial effects depending on tested microorganisms and the type of extract. In the optimized extract, the lowest MIC was recorded against *S. aureus* ATCC 25,923, *B. subtilis, E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853 with a value estimated at 0.781. A similar value was recorded in the aqueous extract against *S. aureus* ATCC 25,923 and *B. subtilis*, and Methanol extract against *S. aureus* ATCC 25. However, the recorded inhibitory activities were statistically inferior as compared to the antibiotics Gentamicin and Amphotericin. In terms of MBC, the lowest value was recorded in the optimized extract against *S. aureus* ATCC 25,923, *B. subtilis, E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853. A similar value was recorded in the aqueous extract against *S. aureus* ATCC 25,923 and B. subtilis. However, the recorded inhibitory activities were statistically inferior as compared to the antibiotics Gentamicin and Amphotericin.

In *Coriandrum sativum*, the extracts showed different antimicrobial effects depending on tested microorganisms and the type of extract. In the optimized extract, the lowest MIC was recorded

against *S. aureus* ATCC 25,923, *B. subtilis*, *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853 with a value estimated at 0.391. A similar value was recorded in the aqueous extract against *S. aureus* ATCC 25,923 and *B. subtilis*. The MIC values in the mentioned extracts were similar to those demonstrated by the antibiotics Gentamicin and Amphotericin against the same bacteria. In terms of MBC, the lowest value was recorded in the optimized extract against *S. aureus* ATCC 25,923, *B. subtilis*, *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853 with a value estimated at 1.356. Similar results were recorded in the aqueous extract against *E. Coli* ATCC 25,922, *P. aeruginosa* ATCC 27,853. The recorded inhibitory effect was inferior compared to the antimicrobial activity demonstrated by Gentamicin and Amphotericin against the same bacteria.

Table 32 : Comparison of antimicrobial activity among the extracts of studied plants (*Pimpinella anisum*, *Coriandrum sativum*, and *Carum carvi*)

Pimpinella anisum			**AE**		**ME**		**EE**		**OE**		**Gentamicin/Amphotericin**	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC/MFC
Bacteria	Gram-positive	*S. aureus* ATCC 25,923	0.391	1.563	0.391	1.563	3.125	3.125	0.195	1.563	0.32	0.64
		B. subtilis	0.195	1.563	0.195	1.563	1.563	3.125	0.195	1.563	0.35	0.69
	Gram-negative	*E. Coli* ATCC 25,922	0.195	1.563	0.371	1.563	1.563	3.125	0.195	1.563	0.37	0.7
		P. aeruginosa ATCC 27,853	0.195	1.563	0.195	1.563	1.563	3.125	0.195	1.563	0.35	0.69
Fungi		*C. tropicalis*	0.781	6.25	0.391	3.125	3.125	6.25	0.195	3.125	2.08	1.04
		Saccharomyces	0.781	6.25	0.781	3.125	12.5	25	0.391	3.125	2.12	2.65

Carum carvi			**AE**		**ME**		**EE**		**OE**		**Gentamicin/Amphotericin**	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC/MFC
Bacteria	Gram-positive	*S. aureus*	1.563	6.25	0.781	12.5	6.25	25	0.781	3.25	0.32	0.64
		B. subtilis	1.563	6.25	1.563	12.5	6.25	12.5	0.781	3.25	0.35	0.69
	Gram-negative	*E. Coli*	0.781	3.25	1.563	12.5	6.25	12.5	0.781	3.25	0.37	0.7
		P. aeruginosa	0.781	3.25	1.563	12.5	6.25	12.5	0.781	3.25	0.35	0.69
Fungi		*C. tropicalis*	3.125	12.5	3.125	12.5	12.5	25	1.563	6.25	2.08	1.04
		Saccharomyces	3.125	12.5	6.25	25	25	-	3.125	12.5	2.12	2.65
Coriandrum sativum			**AE**		**ME**		**EE**		**OE**		**Gentamicin/Amphotericin**	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC/MFC
Bacteria	Gram-positive	*S. aureus*	0.391	3.125	0.781	3.125	3.125	6.25	0.391	1.563	0.32	0.64
		B. subtilis	0.391	3.125	0.781	3.125	3.125	6.25	0.391	1.563	0.35	0.69
	Gram-negative	*E. Coli*	0.781	1.563	0.781	3.125	3.125	6.25	0.391	1.563	0.37	0.7
		P. aeruginosa	0.781	1.563	0.781	3.125	3.125	6.25	0.391	1.563	0.35	0.69
Fungi		*C. tropicalis*	1.563	12.5	1.563	6.25	6.25	12.5	0.781	6.25	2.08	1.04
		Saccharomyces	1.563	12.5	3.125	12.5	25	-	0.781	6.25	2.12	2.65

The extracts of studied plants showed a significant microbial activity, which is ion agreement with literature. For example, Gülçın et *al.,* (2003) investigated the antimicrobial activities in the extracts of anise (*Pimpinella anisum* L.) seeds with Disc diffusion methods. Further, nine different microbial and one fungi species were used to screen the possible antimicrobial activities of the extracts. All tested extracts showed strong antibacterial activity against this bacterium (Staphylococcus aureus). Most of the bacterial species and the fungi species were inhibited antimicrobial activity. However, the antimicrobial activity of water extract was not detected against Pseudomonas aeruginosa and Escherichia coli. The inhibition zone against tested microorganisms varied between 7 and 11 mm. In another study, Amer et *al.,* (2019) investigated the antibacterial properties from the extracts of anise (*Pimpinella anisum* L.) seeds sampled from Egypt. The ethanolic extract of seeds showed significant inhibition against Staphylococcus aureus (18.3±1.5 mm) and Bacillus cereus (21.0±1.2 mm). On the other hand, Begnami et *al.,* (2010) testes the antimicrobial potential of *Coriandrum sativum* against different Candida species in vitro. The EO and its fractions showed antimicrobial activity against all the species of *Candida* tested, except for Candida tropicalis CBS 94. Sasi Kumar *et*

al., (2014) tested the antimicrobial activity in the extracts of solvent extracts of *Coriandrum sativum* L. roots. The ethanol extract of roots was found to be exhibit Staphylococcus aureus, Bacillus cereus, Salmonella typhi, Klebsiella and Candida. The extract and fractionates of fresh roots of Coriandrum sativum showed a significant and remarkable activity against Staphylococcus aureus, Bacillus cereus, Salmonella typhi, Klebsiella, when compared to standard.

4. Conclusion

This study presents new data on the bioactive compounds and biological activities (antioxidant and anti-microbial) of three Moroccan medicinal plants *Pimpinella anisum*, *Carum carvi*, and *Coriandrum sativum.* Equally, this study showed the effect of extraction solvent and their combinations on the amount of extracted compounds, and their biological properties. The obtained results showed that the amount of TPC, TFC and $DPPH_{IC50}$ were significantly variable depending on used combination of solvents and tested plant. In *Pimpinella anisum*, *Coriandrum sativum* and *Carum carvi* the highest values were recorded in the mixture containing water (33.33%), ethanol (33.33%), and methanol (33.33%). In parallel, the tested extracts of all studied planta showed significant antimicrobial activities against all tested microorganisms. Despite the importance of this study for traditional medicine and phytochemical investigations in Morocco and the Mediterranean basin, more research is needed to clarify how climatic conditions, soil constituents, and extraction solvents influence the biosynthesis and then the yield of bioactive compounds in medicinal plants.

5. References

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CONCLUSION GENERALE

Notre travail de recherche est centré sur l'étude de trois plantes médicinales et aromatiques marocaines qui sont *Pimpinella anisum, Coriandrum sativum et Carum carvi* essentiellement connues par leur utilisation par des populations locales en médecine traditionnelle. Ainsi, des études ethno-pharmacologiques réalisées dans différentes régions du Maroc nous ont permis de mettre en évidence un grand nombre d'informations sur l'importance thérapeutique de ces trois espèces étudiées. Effets qui semblent liés à la composition chimique des dérivés des plantes dont le rendement en composés phénoliques est relativement important.

L'optimisation de l'extraction des dérivés des plantes étudiées par la méthodologie de conception de mélange nous a permis d'identifier la combinaison pondérale des différents solvants utilisés pour avoir le Meilleur rendement en composés chimiques. Cette partie d'étude semble d'un grand intérêt dans le choix du ou des solvants pour extraire le maximum des composés chimiques des plantes. Elle nous permettra de minimiser le temps, le coût et l'utilisation aléatoire de différents solvants et aboutir ainsi, à un rendement important en composes chimiques bioactifs.

En outre, par une étude phytochimique, nous avons essayé de comprendre la composition chimique des plantes, notamment des composés naturels produits par les plantes appelés métabolites secondaires. Ces dernières nous seront d'une grande importance pour expliquer les effets bénéfiques déclarés par les populations enquêtées, suite à l'utilisation de ces plantes étudiées et aussi, d'éviter tout effet toxique relatifs à ces plantes.

Les différents tests microbiologiques menées dans notre travail, nous ont permis de montrer l'efficacité remarquable des différents extraits dans l'inhibition des microorganismes étudiés, (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* et *Escherichia coli*) ainsi que des champignons (*Candida tropicalis* et Saccharomyces).

La concentration minimale inhibitrice et la concentration bactéricide ont été évaluées par la méthode de la microdilution en plaques à puits.

Les résultats des essais antimicrobiens des extraits des trois plantes étudiées ont révélé un potentiel promoteur pour le développement de nouveaux agents thérapeutiques. Pour *Pimpinella anisum,* l'extrait aqueux (AE) a montré une efficacité notable contre *B.Subtilis* et *E.Coli* (MIC=0.195,CMB=1.563), tandis que l'extrait optimisé a démontré une activité puissante contre *C.tropicalis* (MIC= 0.195 , MBC=3.125).

L'extrait aqueux du *Carum carvi* a montré une inhibition significative contre *E.Coli* (MIC=0.781, MBC=3.25) et l'extrait optimisé contre *S.Aureus* (MIC=1.563, MBC=3.25).

Concernant *Coriandrum sativum* l'extrait optimisé (OE) s'est révélé efficace contre *E. Coli* (MIC=0.391, MBC= 1.563), et *C. Tropicalis* (MIC=0.781, MBC=6.25). Notamment, l'extrait optimisé a présenté une activité antimicrobienne supérieure à celle des antibiotiques standards (Gentamicine/Amphotéricine), mettant en évidence le potentiel antimicrobien très important des plantes étudiées.

Ces tests antimicrobiens des extraits étudiés joueront un rôle important dans le traitement et la prévention des infections, contribuant ainsi à améliorer la santé des populations locales. Cependant, certaines plantes médicinales traditionnelles il y en a celles qui peuvent être toxiques à de fortes doses ou après administration prolongée, en même temps qu'elles sont une source de molécules naturelles actives douées de propriétés pharmacologiques. A cet effet, il semble indispensable d'étudier les effets toxiques des extraits.

Notre étude montre que les différents extraits des plantes étudiées peuvent donc, être exploités pour d'autres activités biologiques, parmi lesquelles l'action antimicrobienne et le piégeage des radicaux libres, en raison de leur richesse en composés phénoliques. Pour la suite, il serait intéressant d'isoler et de caractériser les composés phénoliques des extraits étudiés et d'évaluer les activités biologiques in vitro et in vivo de chacun de ces composés pris séparément.

En perspectives, les résultats obtenus lors de cette étude, nous encouragent à approfondir nos recherches pour :

- Identification et caractériser les composés chimiques des extraits étudiés. Les molécules actives identifiées pourront par la suite être réévaluées et éventuellement modulées par rapport à leurs réponses biologiques et toxicologiques.
- Evaluer leurs effets "*in vivo*", sur un modèle animal, afin de mieux comprendre le profil de sécurité de la plante,
- Exploration davantage la recherche qui conduira à un effet pharmacologique plus sûr et plus efficace dans le traitement de certaines maladies,
- Développer des produits biologiques à base de plantes, doués d'activité antioxydante et/ou antimicrobienne.

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