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## Selected Medicinal Plants from Morocco: Biological Activities, Electrochemical Properties and Secondary Metabolites Screening

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*To my parents*

*To Wisam and Naima*

*To my aunts and awesome friends*

*In testimony of my deep affection to you*

*Know that this work is the fruit of your support*

*I am very grateful to you. Your pride to my regard today is for me the best of rewards*

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**Walid Belmaghraoui, Aimad Mazkour, Hicham Harhar, Mourad Harir, Souad El Hajjaji, (2018)** "Investigation of corrosion inhibition of C38 steel in 5.5 M $H_3PO_4$ solution using *Ziziphus lotus* oil extract: an application model", *Anti-Corrosion Methods and Materials*, Vol : 66 (1), https://doi.org/10.1108/ACMM-02-2018-1901

**Belmaghraoui, W., El Madani, N., Manni, A., Harir, M., Filali-Maltouf, A., El Hajjaji, S., El Fatni O.K. (2018).** Total phenolic and flavonoid content, antioxidant and antibacterial activity of *Ziziphus lotus* from Morocco, *Pharmacologyonline*, Vol: 3, 176-183.

**Belmaghraoui, W., Manni, A., Filali-Maltouf, A., H., Harir, M., El Fatni O.K. and El Hajjaji, S. (2019).** Phenolic compounds quantification, antioxidant and antibacterial activities of different parts of *Urtica dioica* and *Chenopodium murale*, *Research journal of pharmacy and technology*, 12 (1).

# LIST OF COMMUNICATIONS

**Belmaghraoui W., Manni A., El Madani N., Harir M., Charouf Z., El Hajjaji S. (2016),** "Determination of the total phenolic, flavonoids compounds, the antioxidant and the antibacterial effect of Moroccan *Ziziphus Lotus*". SNDD international confress 2016 – Rabat, 19-20 Mai 2016.

**Belmaghraoui W., El Madani N. , Manni A., Filali-maltouf A., Charouf Z., El Hajjaji S. (2015),** "Evalutation of the therapeutic effect of the kingdom's wild plants : *Urtica Dioica L.*", JIJC04 – El Jadida, 19-20 November 2015.

**Belmaghraoui W., El Hajjaji S., Schmitt-Kopplin P., El Monfalouti H., Charrouf Z., Harir M. (2015),** "High-field FTICR mass spectrometry for metabolites screening of *Urtica Dioica (L.)*: an analytical approach", IOCD International Symposium – Marrakech, 7 April to 11 April 2015.

**Belmaghraoui W., El Hajjaji S. (2015),** "Utilization of *Urtica Dioica L.* Exracts as a corrosion inhibitor", 4th Edition of the FSR Doctorial 2015 - Rabat, 19, 20, 21 February 2015.

**Belmaghraoui, W. (2014),** "12th edition of the week of science: Innovation for the Economic and Social Development" – Rabat, 28 April to 2 May 2014.

# SUMMARY

The biodiversity of Morocco has recently been recognized as of global interest. This rich biodiversity provides services to mankind, especially through the aromatic and medicinal plants traditionally used. The four plants used in this study, namely, *Urtica dioica*, *Chenopodium murale*, *Mentha rutondifolia* and *Ziziphus lotus*, are well known to Moroccans and are widely consumed for their aromatic, medicinal and nutritional properties. An integral part of this project, aims to study some biological and chemical aspects of these species. Thus, biological activities, anti-corrosive properties and secondary metbaolites composition were investigated. The studied extracts exhibited for most cases good antibacterial and antioxidant properties. *Mentha rutondifolia* seeds extract for instance, has an $IC_{50}$ value of 5.98 µg/mL, which is comparable, if not better, than synthetic antioxidants. Whatsmore, the corrosion inhibition of these extracts reached an efficiency of about 72 % for the polarization study and ~91% efficiency for EIS measurements at 3g/L. Accordingly, interesting classes of compounds were in parallel identified using ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The results helped to improve the knowledge of these species, and provided a solid basis for their development, as well as for the appropriate pharmacological exploitation.

*Keywords: Corrosion inhibition, Urtica dioica, Chenopodium murale, Mentha rotundigolia, Ziziphus lotus.*

# RÉSUMÉ

La biodiversité du Maroc a récemment été reconnue comme d'intérêt mondial. Cette riche biodiversité fournit des services à l'homme, notamment par le biais des plantes aromatiques et médicinales, utilisées traditionnellement. Les quatres plantes etudier, à savoir, Urtica dioica, Chenopodium murale, Mentha rutondifolia et Ziziphus lotus, sont bien connue des Marocains et largement consommée pour leurs propriétés aromatiques, médicinales et alimentaires.

Le présent travail a pour objectif d'étudier certains aspects de la biologie et la chimie de ces espèces. Ainsi, les activités biologiques, anti-corrosive et la composition métabolomique ont été étudiés.

Les extraits présentaient dans la plupart des cas de bonnes propriétés antibactériennes et antioxydantes. L'extrait de graines de Mentha rutondifolia, par exemple, avait une IC50 de 5,98 µg /mL, comparable aux antioxydants synthétiques. De plus, l'inhibition de la corrosion de ces extraits a atteint une efficacité ~ 72% pour l'étude de polarisation et ~ 91% pour les mesures EIS à 3 g / L. Quelques classes intéressantes de composés ont été identifiées à l'aide de la spectrométrie de masse à résonance cyclotronique ionique (FT-ICR-MS). Les résultats concourent à améliorer la connaissance de ces espèces, et fournissent une base solide pour leur mise en valeur, ainsi que pour l'exploitation pharmacologique appropriées.

*Mots-clés: Inhibiteur de corrosion, Urtica dioica, Chenopodium murale, Mentha rotundigolia, Ziziphus lotus.*

# RÉSUMÉ DÉTAILLÉ

La biodiversité du Maroc a récemment été reconnue comme d'intérêt mondial. Cette riche biodiversité fournit des services à l'homme, notamment par le biais des plantes aromatiques et médicinales, utilisées traditionnellement. Les quatre plantes étudier, à savoir, *Urtica dioica, Chenopodium murale, Mentha rutondifolia* et *Ziziphus lotus*, sont bien connue des Marocains et largement consommée pour leurs propriétés aromatiques, médicinales et alimentaires.

Le présent travail a pour objectif d'étudier certains aspects de la biologie et la chimie de ces espèces. Ainsi, les activités biologiques, anti-corrosives et la composition métabolomique ont été étudiés.

Les extraits présentaient dans la plupart des cas de bonnes propriétés antibactériennes et antioxydantes. L'extrait de *Ziziphus lotus* était le meilleur antibactérien testé, avec une meilleure efficacité contre la souche *Rhizobium Sp.*, une valeur de 16 mm a été enregistrée au test de diffusion par disque, et 3.2 µg/mL comme concentration minimale d'inhibition. L'extrait de graines de *Mentha rutondifolia*, par exemple, avait une IC50 de 5,98 µg /mL, comparable aux antioxydants synthétiques.

Pour les essais d'inhibition contre la corrosion, l'huile extraite par hexane du *Ziziphus lotus* a démontré une efficacité de 61% en polarization, en utilisant le C38 dans un milieu 5.5 M d'acide phosphorique. Cette efficacité a été largement supérieur dans un milieu moins corrosif comme l'acide hydrochloridrique 1M, en atteignant une efficacité de ~ 98%.

De plus, l'inhibition de la corrosion des extraits obtenus des plantes *Urtica dioica, Chenopodium murale* et *Mentha rutondifolia* ont atteint une efficacité ~ 72% pour l'étude de polarisation et ~ 91% pour les mesures EIS à 3 g / L. Les extraits obtenus par 80% éthanol ont démontré une performance meilleure que celle des extraits obtenus par le méthanol.

Quelques classes intéressantes de composés ont été identifiées à l'aide de la spectrométrie de masse à résonance cyclotronique ionique (FT-ICR-MS). Les résultats concourent à améliorer la

connaissance de ces espèces, et fournissent une base solide pour leur mise en valeur, ainsi que pour l'exploitation pharmacologique appropriée. Cette méthode, grâce à sa précision, a révélé que plus de 50% des composés contenu dans les extraits étudiés sont des composés inconnus.

Il a été démontré à travers cette méthode que *Mentha rutondifolia* est la plante la plus abondante en métabolites secondaires, plus précisément en flavonoïdes. *Urtica dioica* était plus abondante en Stéroïdes et leurs dérivés, alors que *Chenopodium murale*, la moins abondante, contenait des flavonoïdes et des iso flavonoïdes.

# LIST OF ABREVIATIONS

DPPH: 2,2-diphényl-1-picrylhydrazyle

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid

$IC_{50}$: The half-maximal inhibitory concentration is a measure of the potency of a substance in inhibiting a specific biological or biochemical function.

MIC: Minimal inhibitory concentration

TPC: Total phenolics content

TFC: Total flavonoids content

TTC / CTC: Total condensed tannins

UD: *Urtica Dioica*

CM: *Chenopodium Murale*

MR: *Mentha rutondifolia*

ZL: *Ziziphus Lotus*

FT-ICR-MS: Fourier transform ion cyclotron resonance mass spectrometry

NMR: Nuclear magnetic resonance

QE: Quercetin equivalent

GAE: Gallic acid equivalent

CE: Catechine equivalent

AA: Antioxidant activity

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# General Introduction

In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells (Fang et al. 2002; Peng et al. 2014; Li et al. 2015). Normally, antioxidant system occurring in human body can scavenge these radicals, which would keep the balance between oxidation and anti-oxidation. Nonetheless, the exposure of cigarette smoking, alcohol, radiation, or environmental toxins induces the production of excessive ROS and RNS, which disrupt the balance between oxidation and anti-oxidation and result in some chronic and degenerative diseases (Li et al. 2015; Wang et al. 2016; Zhou et al. 2016). The increment of intake of exogenous antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen and reducing agents (Baiano and Del Nobile 2016). The exogenous antioxidants are mainly derived from food and medicinal plants, such as fruits, vegetables, cereals, mushrooms, beverages, flowers, spices and traditional medicinal herbs (Cai et al. 2004; Fu et al. 2010; Fu et al. 2011).

Besides, the industries processing agricultural by-products are also potentially important sources of natural antioxidants (Deng et al. 2012). These natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) (Baiano and Del Nobile 2016; Manach et al. 2004). The nature of the chemical function of the major compound (phenol, alcohol, aldehyde, ketone ...) plays a preponderant role in the efficiency of their biological activities. Generally, these natural antioxidants, especially polyphenols, exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer (Manach et al. 2004; Peng et al. 2014).

Considering their important health effects, the efficient extraction methods of natural antioxidants, appropriate assessment of antioxidant activity as well as their main resources from food and medicinal plants are drawing great attention in food science and nutrition. Moreover, to further assess the antioxidant capacities of extracts from natural products, especially those frequently

consumed by people, different evaluation assays have been developed, e.g., free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2'-azino-bis(3-éthylbenzothiazoline-6-sulphonique) (ABTS). These assays have been used for ranking the antioxidant plants and recommending best antioxidant foods for consumption.

Morocco has a rich flora that is little valued. Spaces that traditionally harbor rich and diverse vegetation tend to decline. Among this rarefied vegetation, we find the medieval aromatic plants traditionally used by our ancestors for the flavoring of food, the culinary arts and medicinal virtues. These plants are undervalued and understudied from a scientific point of view. This led us to study antioxidant, antimicrobial and electrochemical activities, as well as the composition of extracts of forgotten aromatic plants from Morocco. This subject seemed even more interesting as the flora of Morocco is extremely rich in aromatic plants rich antioxidants.

# CHAPTER I

# Bibliographical review

# I. Aromatic and medicinal plants

## I.1. Definition

Medicinal plants are vegetal drugs as described by the Pharmacopoeia, of which at least an organ has medicinal properties. It is not common for the plant to be used completely; most often, it is one or more parts that may have different uses. Since ancient times, men have been treated with plants. Several theorists have begun to explain the action of plants on the body (Chevallie 2001). The great ancient civilizations resorted to aromatic and medicinal plants for their medicinal, fragrant and ritual uses. Medicinal plants have always been associated with traditional and cultural knowledge. According to 2003 World Health Organization (WHO) statistics, 80% of the world's population uses traditional medicines to meet primary health care needs (Bhar and Balouk 2011). It grows in the world more than 20000 species of plants, for culinary uses, medicinal or cosmetic, of which 50% is used in pharmaceutical industry (Bermness 2005).

## I.2. Aromatic and medicinal plants in the world

Recognition of the clinical, pharmaceutical and economical value of herbal medicines continues to grow, although this varies greatly between countries. Each country defines in different ways simple medicinal plants and derived products. Thus, countries have adopted several approaches for plants licensing, preparation, manufacturing and marketing to ensure their safety, quality and effectiveness. The growth of the pharmaceutical industry and the continued development of new, more effective synthetic and biological medical products have not reduced the importance of the use of medicinal plants. On the contrary, population growth in the developing world and growing interest in industrialized nations have significantly increased the demand for aromitc and medicinal plants and their derivatives. Regulations concerning the evaluation of the quality, safety and efficacy of medicinal plants in certain countries (United States, Japan, China, European Union, etc.) and the activities of WHO with a view to supporting the development of standard guidelines has helped to strengthen recognition of the role that medicinal plants play in health care. The use of herbal remedies has recently experienced an unprecedented craze. People are increasingly looking for "natural" medicines and it seems that cosmetics and herbal products are being used more and more today.

Accordingly, world trade in aromatic and medicinal plants is estimated at nearly 0.5 million tonnes at a value of ~1.2 billion Euros. Five European countries are among the 12 largest importers in the world of medicinal plants: Germany, Spain, France, Italy and the United Kingdom. Europe as a whole plays a leading role in the international trade of these factories, with a quarter of the world's annual imports coming back to it. In recent years, Europe has imported annually an average of 120000 tonnes of medicinal plants from more than 120 different countries. Three European countries also rank among the top 12 plants exporters, Germany, Bulgaria and Poland. In Europe, at least 2000 medicinal and aromatic plant taxa are used on a commercial basis. Two-thirds of these taxa are native to Europe. Amongst the European plant species traded, at least 90% are still harvested in the wild. The overall volume of plant material harvested each year in the wild would be in the order of 30000 tonnes. In the European Union, the total area of medicinal and aromatic plants cultivated is about 70000 hectares.

## I.3. Artomatic and medicinal plants in Morocco

Morocco is a traditional producer of aromatic and medicinal plants, one of the world's leading suppliers of rosemary, verbena, rose, coriander, pennyroyal, etc., and an exclusive supplier of several essential oils such as mugwort, wild chamomile and annual tansy. By its geographical contrasts, Morocco offers a varied range of bioclimates allowing the installation of a rich flora (more than 4200 species) and a diversity of phylogenetic resources in medicinal plants (600 species). Besides this promising natural context, Morocco has an ancestral expertise: the medication using plants, their use for the aromatisation and the conservation of the food, as well as for the extraction of the aromatic principles intended for the perfumery family or market. The production of these plants activates both spontaneous plants and dried plants for herbal and food spices. More than twenty species are used for the production of essential oils or other aromatic extracts intended mainly for the perfume and cosmetics industry as well as for the preparation of hygiene products and the formulation of flavors. Plants related activities in Morocco are thus rich and diversified, which constitutes an important asset for the establishment and development of the sector. Several products are known as typically Moroccan products. This means that medicinal plants operating profession in Morocco, despite its weaknesses, has managed to introduce several new products to the international market. Seasonal harvesting of high value plants has traditionally provided a source of income for families in rural areas. Men and women in rural areas have

considerable knowledge and practical skills gained through long years of life with plants and herbs in the wild (Zrira 2017).

## II. Phytochemical study of secondary metabolites

Plants are of a great importance for the survival of humans and the ecosystems. They contain a large proportion of compounds involved in the enzymatic or biochemical reactions taking place in the organism. Two groups of metabolites can be distinguished: primary metabolites and secondary metabolites (Heldt et al. 2011).

- Primary metabolites are organic molecules found in all the cells of the plant's organism to ensure its survival. These compounds are classified into four main groups, carbohydrates, proteins, lipids and nucleic acids (Heldt et al. 2011).

- Secondary metabolites are molecules with a limited distribution in the organism of the plant. They are necessary for its defense against external aggressions (Heldt et al. 2011).

However, they are not always necessary for the survival of the plant. The products of the secondary metabolism, which are emitted in very small quantities, are of great variety (more than 200000 defined structures). These compounds mark in an original way, a genus, a family or a species of plant and sometimes allow establishing a chemical taxonomy.

Secondary metabolites are the subject of several research, they are used in food, cosmetic and pharmaceutical fields (Heldt et al. 2011). They are widely used in therapy as vasculo-protective, anti-inflammatory, enzymatic inhibitors, antioxidants and anti-free radicals. The pharmaceutical industry still uses a great number of plant origin medicines, and research still finds new active molecules or substances in plants, destined for the hemisynthesis of active compounds. We have long used traditional herbal remedies without knowing what their beneficial actions were due to.

At the beginning of the $20^{th}$ century, syntheses of analogous compounds (secondary metabolites) began; and in order to increase their pharmacological efficiencies, studies of the biological structures and activities derived from the prenylated derivatives of these metabolites were performed. Prenylation consisted of the fixation of a lateral chain (Pentenyl, geranyl and farnesyl) on an acceptor molecule. Secondary metabolites are a group of natural products to explore for their

antioxidant, anti-microbial and anti-inflammatory properties, carcinogens or mutagens (Genovese et al. 2012).

They are very heterogeneous compounds in their either composition or structure. For a long time, these compounds were considered secondary and metabolically inactive, they therefore attracted little interest. Nowadays, this perception has changed, due to extensive research which has largely shown that these compounds are not inert and efficiently contribute to the biosynthesis of various metabolites in the organism. In plants, they are subject to significant quantitative and qualitative variations, which testifies to an incontestable biochemical dynamic. They are involved in a wide range of life processes (Heldt et al. 2011). Their mode of action and their physiological significance are not yet sufficiently clear, which lead to the increasing number of studies of these compounds and their functions. Secondary metabolites are found in all parts of plants, but they are distributed differently according to their roles. This distribution varies from one plant to another. Among the main families of secondary metabolites found in plants are:

- Phenolic compounds involved in plant-plant interactions (allelopathy, inhibition of germination and growth). Among these compounds, polyphenols, lignins, stilbenes, flavonoids, phenylpropanoids, anthocyanins and tannins.

- The alkaloids contain a nitrogen atom in their structure. Among these, some release hydrocyanic acid when the plants are damaged. They are synthesized from amino acids. These include nicotine, atropine, codeine, lupinin.

- Mucilages: These are complex polymers of fructose, glucuronic acid and manuronic acid. The mucilages are colloidal mixtures, which swell with water.

- Gums and resins: These are substances produced by the plant because of an injury.

- Essential oils: These are concentrated and hydrophobic liquids containing aromatic compounds (odoriferous), aromas of a plant, these species are highly volatile and not miscible with water.

- Latex: These are substances secreted or manufactured by laticiferous cells (true or anastomosed) and which have the particularity of solidifying in contact with air.

## II.1. Classification of secondary metabolites

### II.1.1. Alkaloids

Nitrogen compounds are divided into two groups: amino acids (Fig 1) and alkaloids. Amino acids (which do not belong to secondary metabolites) are what constitutes proteins and other peptides. Most of the compounds of these two groups are water-soluble.

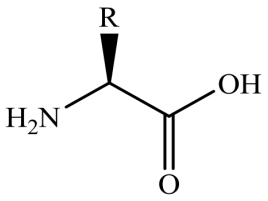



**Fig 1.** Amino acids general structure.

Alkaloids are organic substances of vegetables that mostly contain basic nitrogen atoms. Although many of them are toxic (such as strychnine or aconitine), some are used in medicine for their analgesic properties (such as morphine, codeine), in the context of sedation protocols (Atropine) often hypnotics, or as antimalarial agents (Quinine, chloroquinine) or anticancer agents (Taxol, Cinblastine, Vincristine). Morphine was the first alkaloid isolated from opium (1805). Then strychnine was discovered (1818). Other known alkaloids are: Colchicine, atropine, tubocurarine, theine, cocaine, mescaline, lysergic acid and aconitine (Matsuura and Fett-Neto 2017).

Accordingly, pyrazoles form a group of alkaloids containing two nitrogen atoms in the aromatic nucleus, these are not of natural origin. Alkaloids are natural heterocyclic organic compounds with a nitrogen atom as a heteroatom. Their molecular structures are complex, more or less basic and endowed with pronounced physiological properties even at low doses (Zenk and Juenger 2007). They are one of the groups of secondary metabolites containing more than 10000 to 12000 different structures (Stöckigt et al. 2002). The structures of two imino-sugars having antibiotic properties belonging to the alkaloid family are shown below in Fig 2 (c, d).

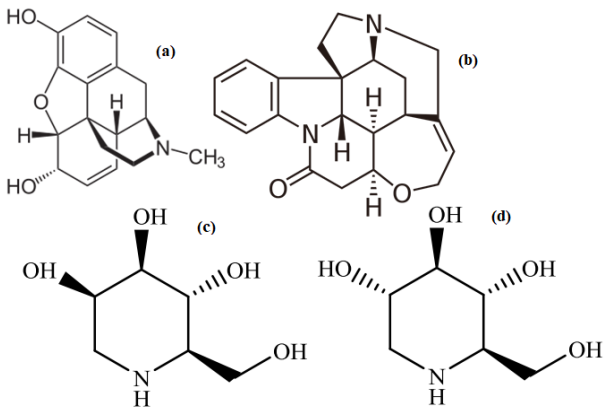



**Fig 2.** Chemical structure of Morphine (a), Strychnine (b), L-Deoxymannojirimycin (c) and L-Deoxyjirimycin (d).

### II.1.2. Terpenes

Terpenes form a class of hydrocarbons (Fig 3), produced by many plants, especially conifers. These are major components of the resin and Turpentine produced from resin. Terpenes are derivatives of isoprene $C_5H_8$ (Fig.3) and have the basic formula of multiples of the latter $(C_5H_8)_n$. Isoprene can be considered as one of the favorite building elements of nature (Nuutinen 2018).

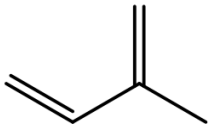


**Fig 3**. Chemical structure of Isoprene.

Their carbon skeleton consists of interconnected isoprene units. It is what is called the isoprene rule. These skeletons can be arranged in a linear manner or forming cycles. Depending on the number n (integer) of units, we can distinguish for, n=2: the Monoterpenes ($C_{10}$), n=3: sesquiterpenes ($C_{15}$), n=4: diterpenes ($C_{20}$), n=5: Sesterpenes ($C_{25}$), n=6: triterpenes ($C_{30}$). For instance, carotene is a tetraterpene ($C_{40}H_{64}$). It plays the role of pigment in photosynthesis vegetation. Materials as diverse as rubber, vitamin A1 or cholesterol are constructed essentially of "bricks" of isoprene. Among the most important terpenes are: α-pinene, β-pinene, δ-3-Carene,

limonene, carotene, etc. (Nuutinen 2018). On the other hand, carotenoids which contain oxygen atoms are not terpenes, but technically they're terpenoids (lutein). Two of the basic properties of terpenes are their odoriferous characteristics (geranium) and their sensitivity to light.

### II.1.3. Sterols

These are derivatives of phytosterols. These compounds are naturally present in the lipid fraction of plants. They are not synthesized by man and animal, they can only come from alimentation. Several studies have shown that phytosterols and phytostanols reduce the absorption of cholesterol in the small intestine. Cholesterol (Fig 4) is the most common example of sterols. Their general structure is composed of 4 cycles with a hydroxyl group at the third position of the A-ring (Piironen et al. 2000).

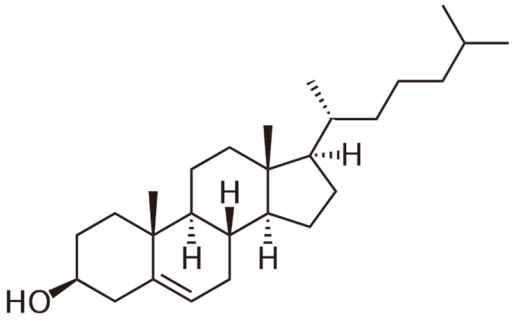



**Fig 4.** Chemical structure of cholesterol.

### II.1.4. Steroids

Steroids are a group of lipids derived from triterpenoids (lipids with 30 carbon atoms), mostly squalene (Fig 5). They are characterized by a hydrophobic cyclopentanophenanthrenic nucleus (cortisol) partially or totally hydrogenated. Usually, the $C_{10}$, $C_{13}$ carbons are linked to a methyl group - $CH_3$ and the $C_{17}$ carbon group to an alkyl group (Takafuta and Fujimura 2017). By extension, steroids also include lipids whose cyclopentanophenanthrenic ring has been modified by cleavage of a bond and the addition or deletion of a carbon. In medicine, the term "steroid" refers to steroid hormones. In a sporting context, "steroid" is usually used to refer to anabolic steroids.

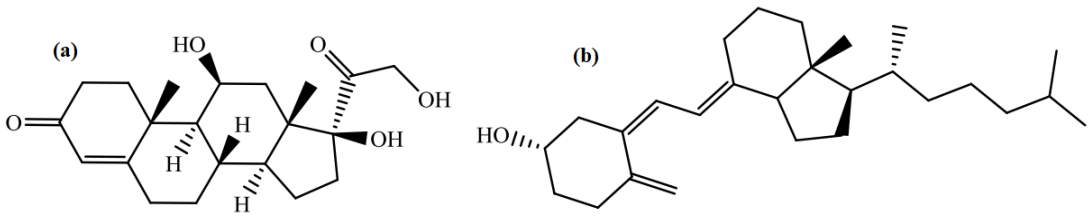



**Fig 5.** Chemical structure of Cortisol (a) and Cholecalsiferol (b).

### II.1.5. Saponins

The name saponin derives from the Latin word "sapo", which means soap, because these compounds foamed once stirred with water. They consist of non-polar aglycones linked to one or more sugars. This combination of polar and non-polar structural elements polar groups explains their foaming behavior in aqueous solution. As a definition, we could say that a saponin is a steroid or triterpene glycoside. Basically, we distinguish steroid saponins and triterpenic saponins both derived biosynthetically from squalene oxide (Manach et al. 2004). Soybean saponin is an example (Fig 6).

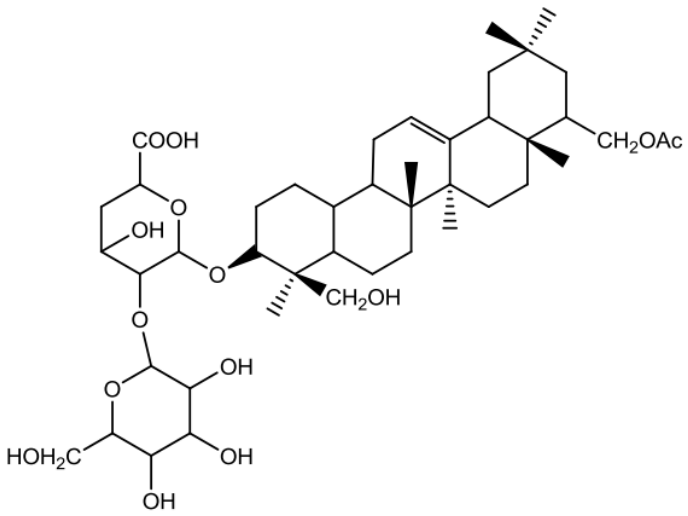



**Fig 6.** Chemical structure of Soja saponin.

Fig 7 illustrates a classification of some secondary metabolites in relation to a few phenolic compounds.

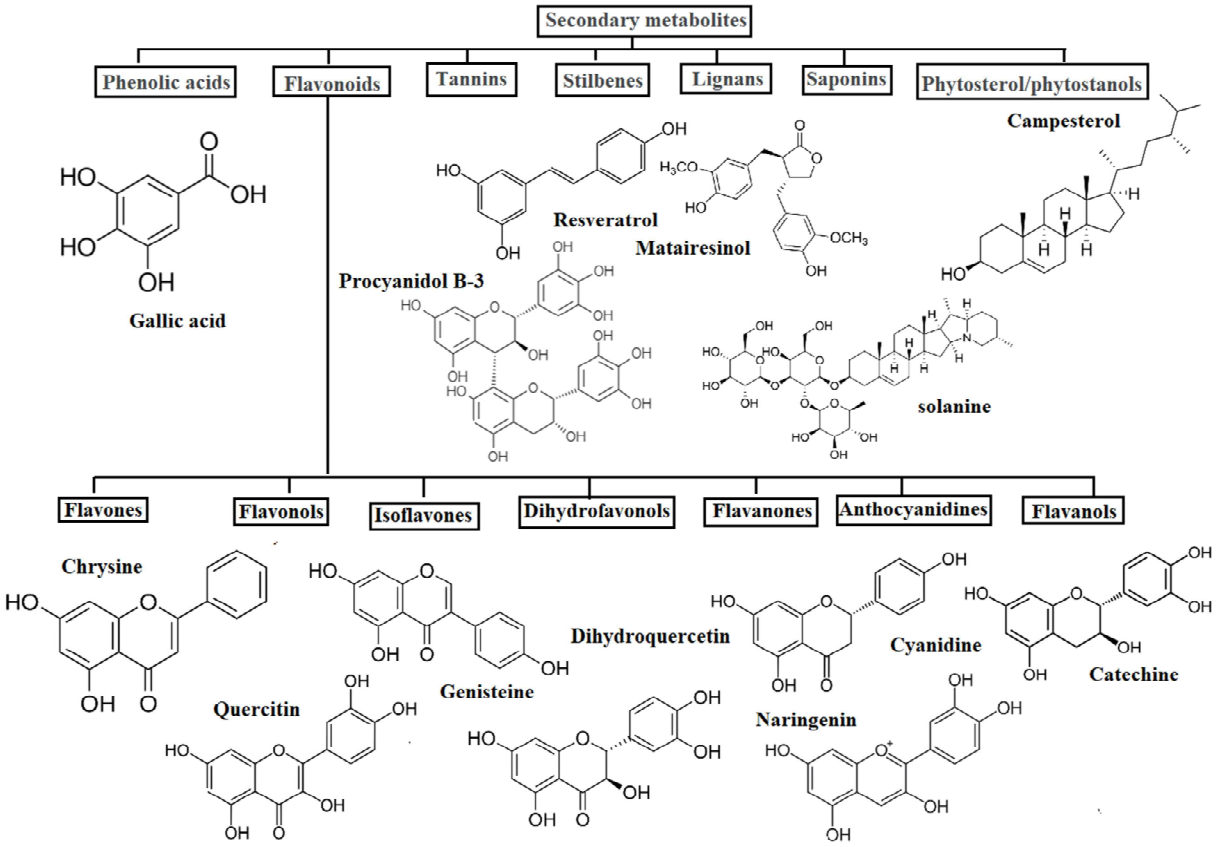



**Fig 7.** Classification of some known secondary metabolites (Muanda 2010).

## II.1.6. Phenolic compounds

The term "polyphenols" is frequently used to describe all phenolic compounds of plants. In fact, it should be reserved for only molecules with several phenol functions. This would then exclude the monophenols, which are abundant and important in plants. Therefore, the general designation "phenolic compounds" concerns both the mono, di and polyphenols whose molecules contain respectively one, two or more phenolic functions (Macheix et al. 2006). The polyphenols have several phenolic groups, with or without other functional groups (i.e., OH, COOH, etc.). They are probably the most prevalent natural compounds in nature and as such are elements of animal food. These compounds exhibit a wide variety of structures, divided into non-flavonoids and flavonoids. The first is represented by phenolic acids, which include benzoic acids (gallic acid). The second class consists of a phenolic nucleus with an unsaturated lateral chain $C_3$ (caffeic acid). The last

class is based on a $C_6$-$C_3$-$C_6$ skeleton (catechin) (Fig 8); it contains several groups that are distinguished by the degree of oxidation of the $C_3$ central heterocycle. All classes of phenolic compounds have a large number of different structures, depending on the number and position of the hydroxyl groups on the skeleton base. These structures can also be variously substituted (glycosylated, esterified, acylated, etc.).

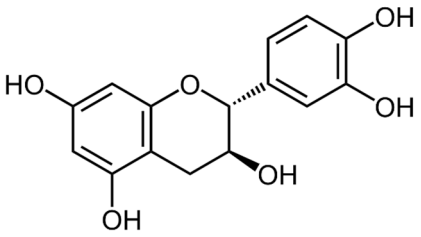


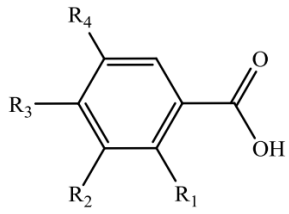

**Fig 8.** Chemical structure of Catechin.

### II.1.6.1. Non flavonoids

This group comprises several compounds among which are distinguished the phenolic acids, stilbenes, lignans, coumarins and xanthones.

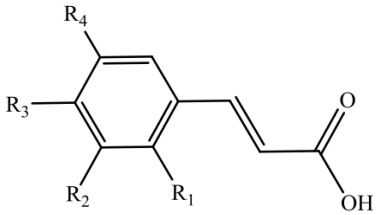
#### a) Phenolic acids

There are two main classes of phenolic acids namely benzoic acid (Fig 9) and cinnamic acid (Fig 10) derivatives. The concentration of the hydroxybenzoic acid is generally very low in edible plants. These derivatives are quite rare in the human diet, whereas those of hydroxycinnamic acids are very abundant (Macheix et al. 2006).




| Name of compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Benzoic acid | H | H | H | H |
| Salicylic acid | OH | H | H | H |
| p-hydroxybenzoic acid | H | H | OH | H |
| Gallic acid | H | OH | OH | OH |
| Protocatechuic acid | H | OH | OH | H |

**Fig 9.** Chemical structure of Benzoic acid and its derivatives.




| Name of compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Cinnamic acid | H | H | H | H |
| o-Coumaric acid | OH | H | H | H |
| m-Coumaric acid | H | OH | H | H |
| p-Coumaric acid | H | H | OH | H |
| Caffeic acid | H | OH | OH | H |

**Fig 10.** Chemical structure of Cinnamic acid and its derivatives.

### b) Stilbenes

Stilben is found in small quantities in the human diet, among these compounds resveratrol, which is an anti-cancer agent present in some medicinal plants, for instance the trans-resveratrol (Fig 11) (Macheix et al. 2006).

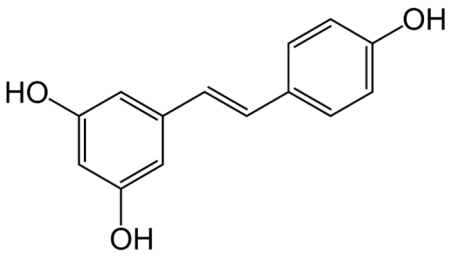



**Fig 11.** Chemical structure of Trans-resveratrol.

### c) Lignans and lignins

Monolignols are derivatives of cinnamic acid; they serve as precursors for compounds of phenylpropanoid types such as lignans and lignins. The lignans correspond to a structural representation of $(C_6\text{-}C_3)_2$ type; the unit $(C_6\text{-}C_3)$ is considered as propylbenzene. The plants produce them by oxidative dimerization of two units of coniferous alcohol. When this dimerization involves an oxidative bond by the $C_8$ of the propenyl side chains of two bonded coniferous alcohol units, forming the $(C_8\text{-}C_8)$ bond, the metabolites are called lignan. The term neolignan is used to define all other link types. When there is no direct bond (C-C) between $(C_6\text{-}C_3)$ units but linked by an ether oxygen atom, the compound is called oxyneolignan. There are other types of lignans such as sesquineolignans (having three units $(C_6\text{-}C_3)$) and dineolignans (containing four units of $(C_6\text{-}C_3)$) (Stalikas 2007). Lignans are mainly found in oil seeds (Macheix et al. 2006).

Accordingly, the lignans matairesinol (Fig 12), secoisolariciresinol and others were detected in red wine obtained from the grapes of vines belonging to the *Vitaceae* family**.** But neoligame biphenyls are isolated from *Magnolia officinalis* (Nurmi et al. 2003). And oxyneolignans, from *Bursera tonkinensis* of the family *Burcaceraceae* (Fukuyama et al. 1996).

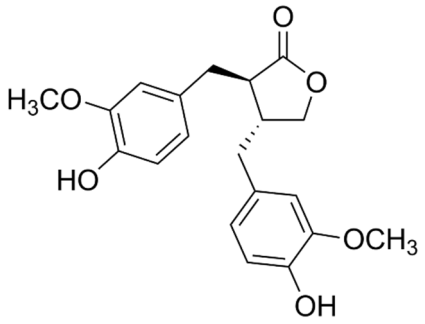



**Fig 12.** Chemical structure of Matairesinol.

Lignins constitute an important class of natural products in the Plant and would be formed by oxidative polymerization of monolignols (monomers) which are p-coumaric, coniferic and sinapic alcohols (Jutiviboonsuk et al. 2005).

## d) Coumarins

Coumarins are oxygenated heterocycles having the basic structure of benzo-2-pyrene. They were isolated for the first time by Vogel in 1820 in the Coumarouna odorata. Today, nearly 1000 coumarin compounds are isolated in more than 800 species of plants and in microorganisms. In plants, they are found in *Apiaceae, Asteraceae, Fabaceae, Rosaceae, Rubiaceae, Rutaceae* and *Solanaceae*. From a structural point of view, they are classified as simple coumarins with substituents on the benzene ring, furanocoumarins, pyranocoumarins, coumarins substituted in position 3 and / or 4 (Fig 13). The last group would be that of the dimers (Sakagami et al. 2005).

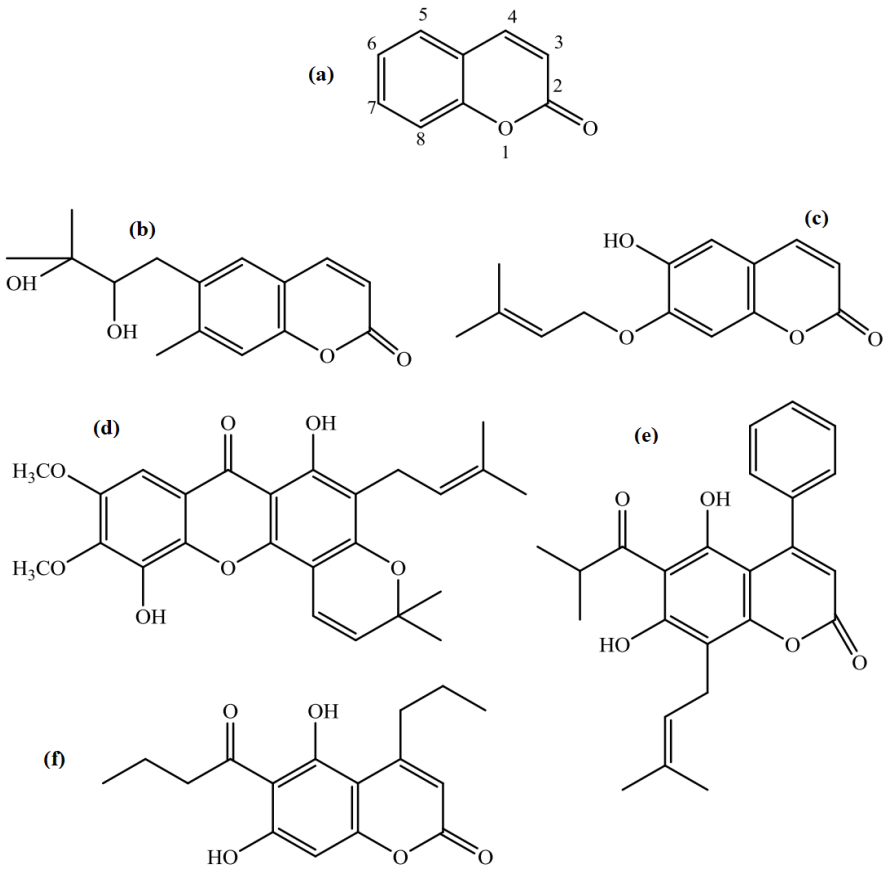



**Fig 13.** Chemical structure of Benzo-2-pyrone (a), Peucedanol (b), Prenyletine (c), Syphonine (d), Mensuol (e) and Mammea B/AC (f).

### e) Xanthones

It is a family consisting of polyphenolic compounds generally isolated in the upper plants and in the microorganisms responding to a basic structure ($C_6$-$C_1$-$C_6$). Some examples of these compounds are shown in Fig 14: gaboxanthone, xanthone and globuliferin (Fig 14) (Gutierrez-Orozco and Failla 2013).

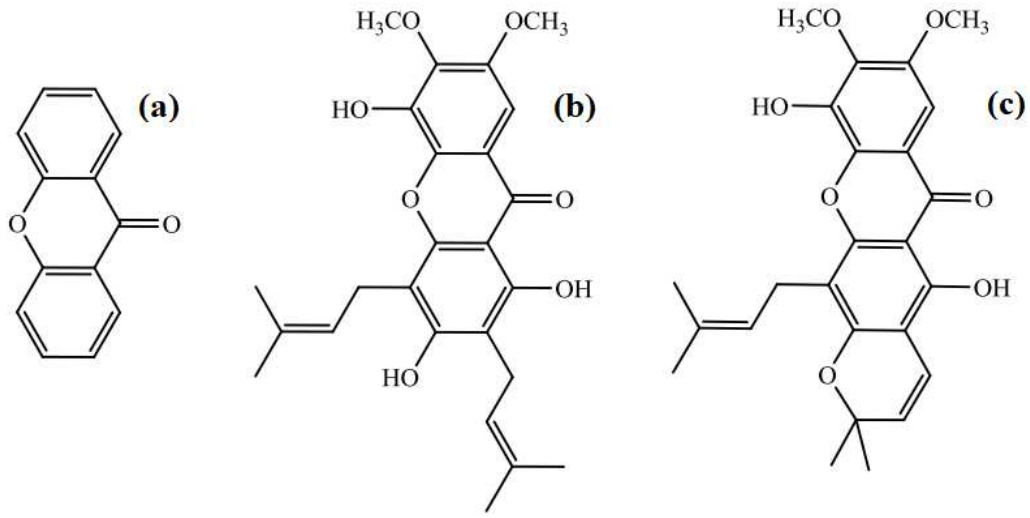



**Fig 14.** Chemical structure of Xanthone (a), Globulferin (b), Gaboxanthone (c).

### II.1.6.2. Flavonoids

Flavonoids represent a class of secondary metabolites spread in the vegetable kingdom. They are virtually universal pigments of plants and are partly responsible for the coloring of flowers, fruits and sometimes leaves. It is found in the vacuole of the cells in the form of heterosides or as constituents of chromoplasts (Guignard 1996). The term flavonoid comprises a very broad range of natural polyphenolic compounds. There are nearly 6500 flavonoids divided into 12 classes (Stöckigt et al. 2002). Their numbers are constantly increasing. By definition, flavonoids are compounds which have in common the structure of diphenylpropane ($C_6$-$C_3$-$C_6$); the three carbons serving as junction between the two benzene rings denoted A and B form generally an oxygen containing heterocycle (Fig 15) (de Rijke et al. 2006).

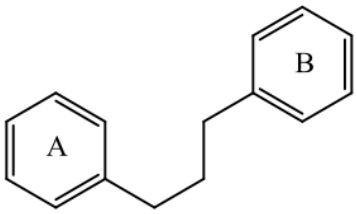



**Fig 15.** General structure of flavonoids.

Their main function seems to be the coloring of plants (along with chlorophyll, carotenoids and betalaines) (Gàbor et al. 1988). Different structures of flavonoids are distinguished among which are flavones, flavonols, flavanones, flavanonols, flavanes, flavan-3-oles, flavylium, chalcones, aurones, isoflavones, isoflavonols, Isoflavanes, pterocarpans, coumaronochromones, 3-arylcoumarins, coumestanes and rotenoids. De Rijke et al. (2006) have classified the flavonoids into 6 families which are flavonols, flavones, flavans, isoflavones, anthocyanins and flavanols (Fig 16). Within these six families, two types of structures have been identified, that of flavonoids in the strict sense, the structure of which carries the aromatic nucleus B in position 2 on the chain $C_3$, and that of the isoflavonoids whose aromatic ring B is in position 3 on the chain $C_3$.

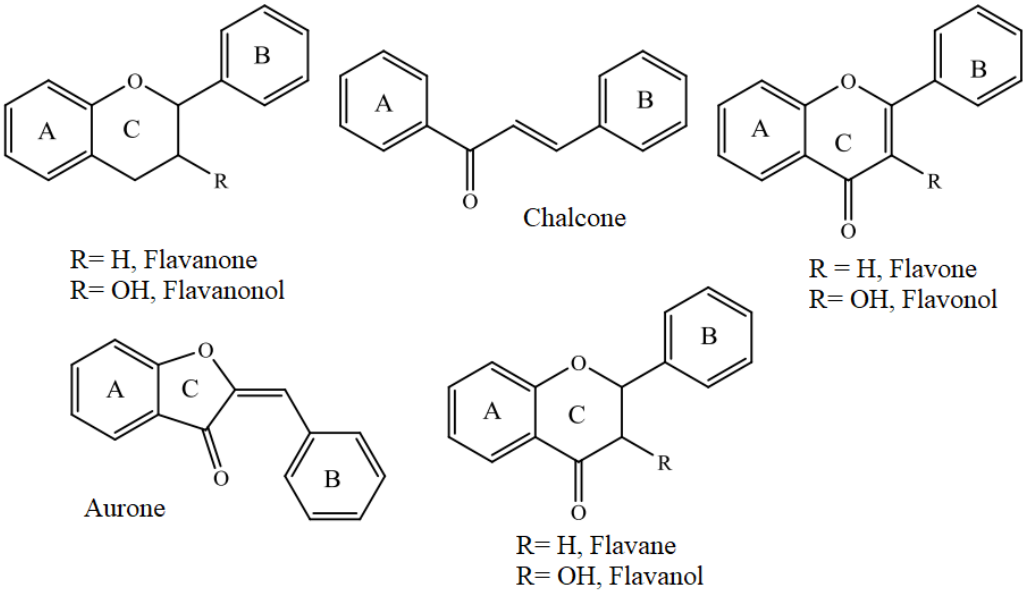



**Fig 16.** Chemical structure of some types of flavonoids.

### a) Flavonoids in the strict sense

In this group we can distinguish chalcones, aurones, flavones, flavanes, flavanones, flavanols, flavonols and flavanonols.

### b) Chalcones and derivatives

The chalcones are flavanoids which do not contain a C heterocycle. They are prenylated more often on the ring A while the ring B remains little to not substituted. This prenylation may be cyclic of the pyrano or furan type. Some of them have a linear O-prenylation (Fig 17) (Yerragunta et al. 2013).

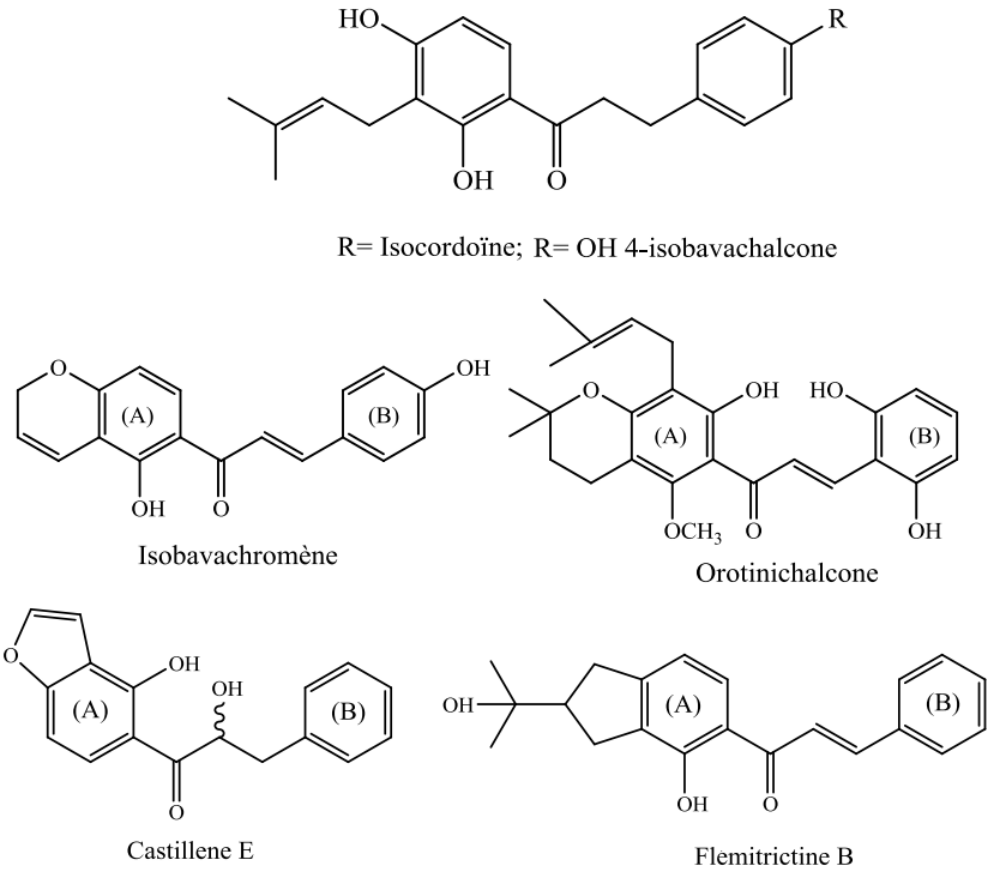



**Fig 17.** Chemical structure of some chalcones.

### c) Aurones and derivatives

Aurons are structural isomers of flavones (Fig 18). They have a close but different structure from most other flavonoids. These molecules are derived from chalcone. Indeed, in the case of the aurones, the chalcone closes forming a 5-atoms ring, whereas it forms a 6-atom ring for the other flavonoids (Haudecoeur and Boumendjel 2012).

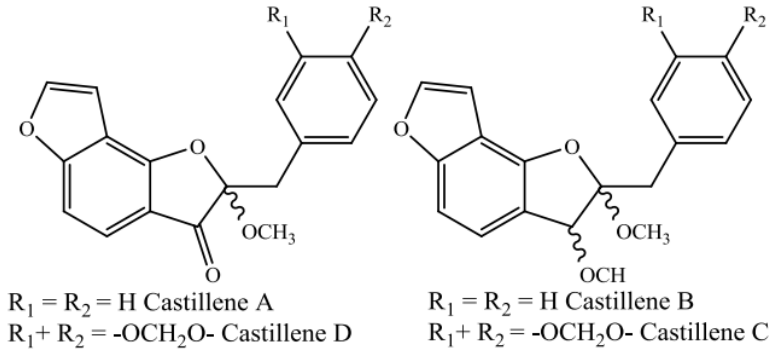



**Fig 18.** Chemical structure of some compounds from the aurone family.

### d) Flavones and flavanones

Flavones, like all flavonoids, have a $C_6$-$C_3$-$C_6$ structure with a $C_3$ like appearance of a heterocycle bearing a carbonyl group and an unsaturation. The flavanones have a structure similar to that of the flavones but do not possess and unsaturation at the level of the heterocycle (Fig 19) (Martens and Mithöfer 2005). Flavonols and flavanonols correspond to the hydroxylated derivatives of flavones and flavanones. Several compounds are listed at this group level. In addition to their almost frequent prenylation, C-glycosyl flavonoids or O-glycosyl flavonoids are occasionally found. Note that flavones and flavonols also occur in glycosylated form, this is the case of vicemin-2, 5-hydroxy-6', 6'-dimethylpyrano (2', 3', 7', 8) flavone, apigenin-7-O-glucoside, luteolin-7-O-glucoside. Quercetin, rutin and hyperseroid are almost exclusively prenylated flavones of type "DMP" (dimethylpyran) or of the "furano" type.

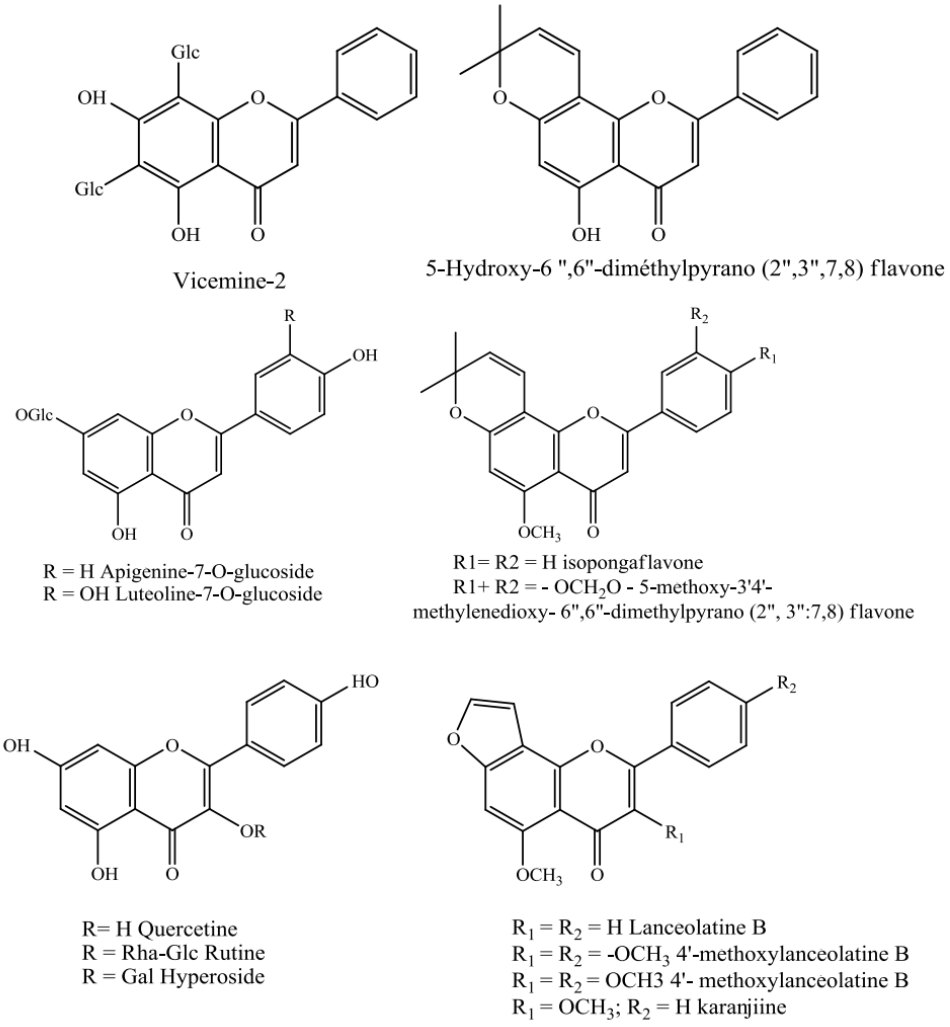
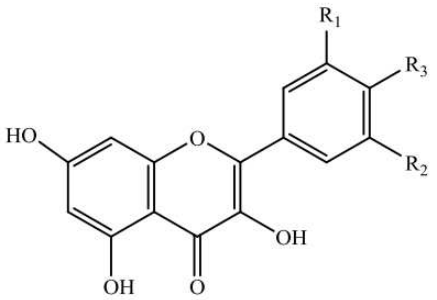



**Fig 19.** Chemical structure of some flavones and flavonones.

The general structure of flavonols is shown below (Fig 20), flavonols (hydroxy-3 flavone) are the most widely spread. They are colorless, and characterized by the presence of a carbonyl group in the 4-position and a hydroxyl group in the 3-position. Flavonols which possess in addition hydroxides in 6 or 8, color some flowers in yellow primrose (Guignard 1996). Among the most replicated flavonols are kaempferol, queretol, myricetol and isorhametol. The chemical structures of some flavonols are listed in Fig 20.




$R_1 = R_2 = R_3 = H$ : general structure
$R_1 = R_2 = H$ ; $R_3 = OH$ : kaempferol
$R_1 = OH$; $R_2 = H$ ; $R_3 = OH$ : Quercetrine
$R_1 = OH$; $R_2 = OH$ ; $R_3 = OH$ : Myricetine
$R_1 = OCH_3$; $R_2 = H$ ; $R_3 = OH$ : Isorhamnetine

**Fig 20.** Chemical structures of some flavonols.

Flavanones and derived flavononols are the flavonoids responsible for the bitter taste of certain grapefruits, lemons, oranges (naringin, hesperidin) (Alais et al. 2003). There are also simple flavanols such as taxifolins, glycosylates such as astilbines, fustine-o-glucoside, glcfustine-3-O-glucide and those of one or more prenylations. In the latter group, we distinguish several categories of prenylations:

- Linear O-prenylation is the case of isoderricidine, as well as a C linear prenylation.
- Single prenylatin of the DMP type on the nucleus A, a double prenylation (C-and O-) of the nucleus A.
- Double C-prenylation of the nucleus A.
- Simple furan-type prenylation of nucleus A.
- The kernel B is unsubstituted or a bit substituted.

The structures shown below (Fig 21,22) are representative of some subclasses of flavonoids in the strict sense.

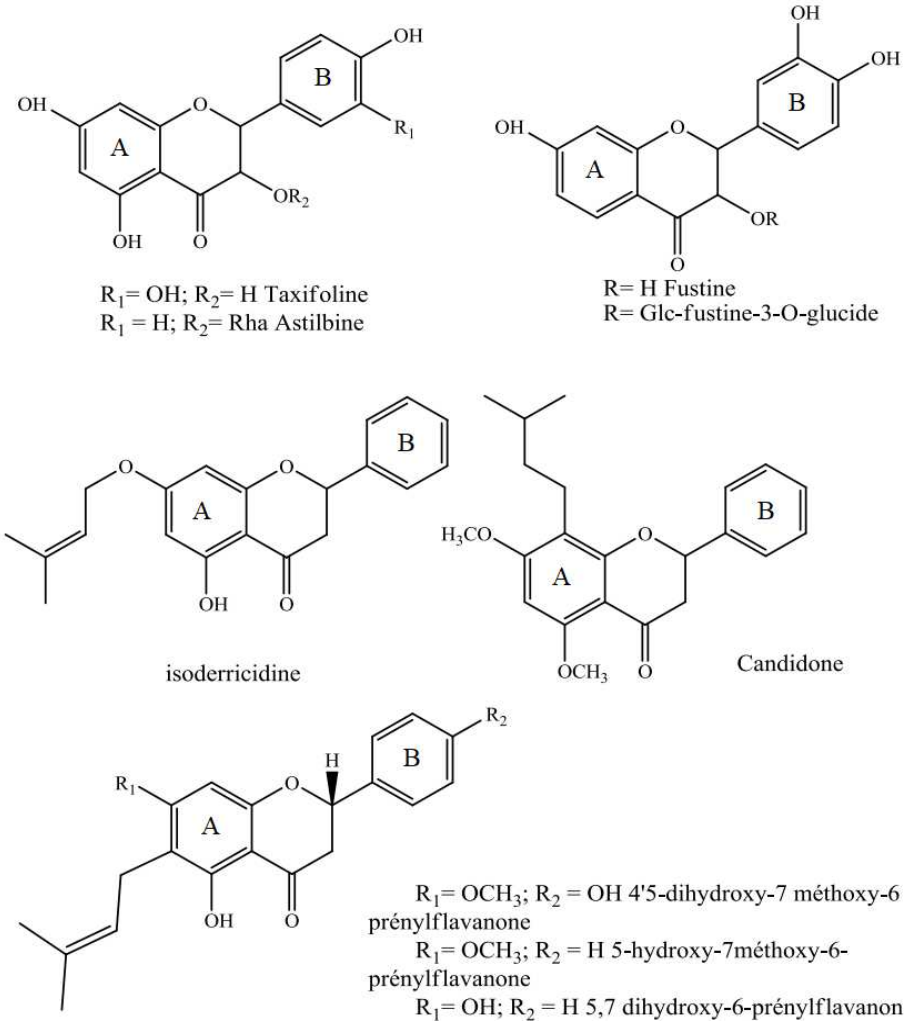



**Fig 21.** Chemical structure of some flavanols.

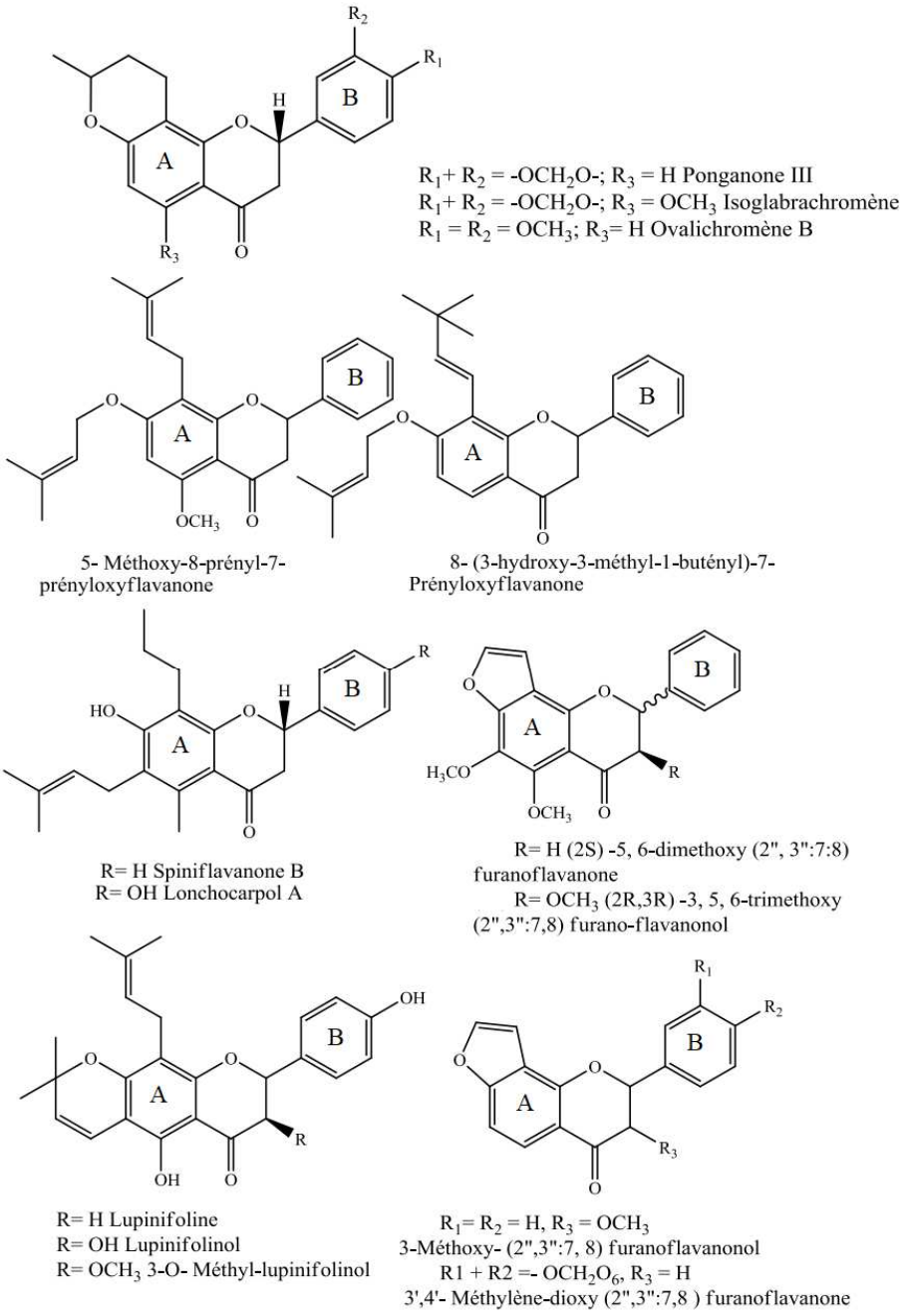



**Fig 22.** Chemical structure of some flavanols.

Flavanes are saturated and non-carbonylated derivatives of flavones, all of which have a cyclized prelylation of the DMP or furan type, they are characterized by the presence of a double prenylation of the ring A (cyclized and linear), and of an epoxy bridge connecting the carbons “4 and 6” of the nucleus B. Their molecular structures are shown in Fig 23.

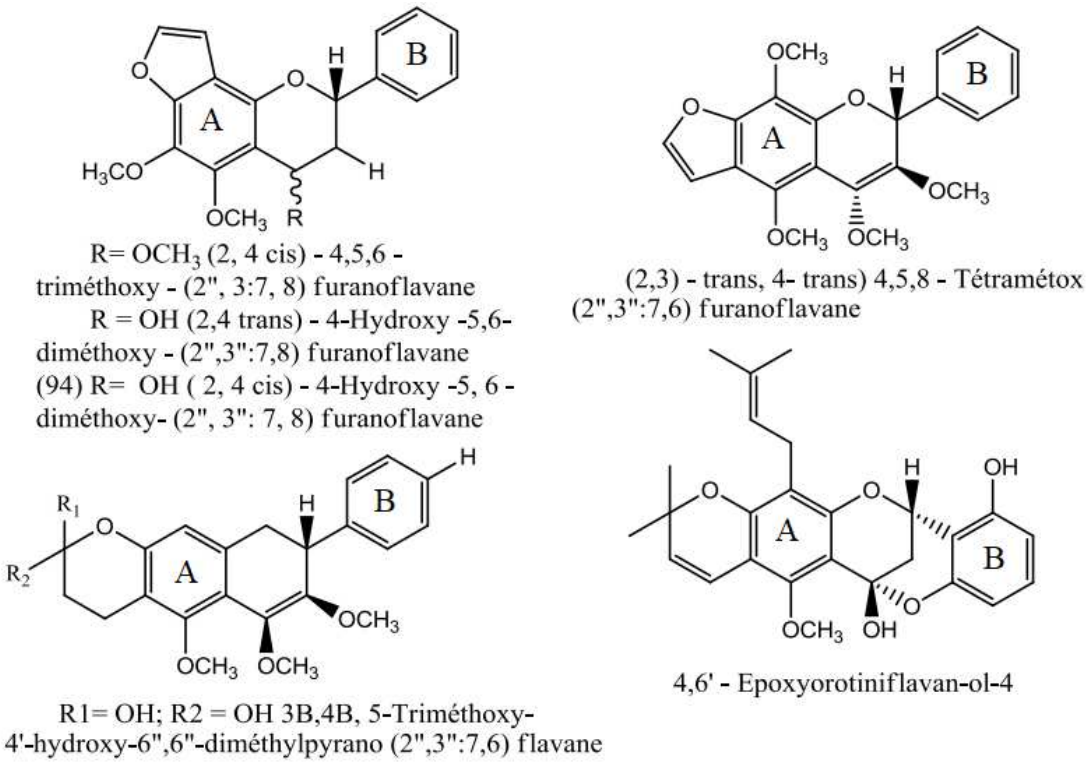



**Fig 23.** Chemical structure of some flavanes.

### e) Isoflavonoids

The isoflavonoids constitute a large and very diverse subclass of flavonoids. They are derived from a 1,2-diphenylpropane structure. In spite of their sporadic characterization in the *Dicotyledon* class, they are almost specific to the *Fabaceae* family. This specificity is probably due to the presence of the enzyme responsible for the rearrangement of 2-phenylchromone (flavanone) to 3-phenylchromone (isoflavone) in this family. They can be classified into a dozen structural categories: 3-arylcoumarins, coumaronochromones, coumestanes, isoflavanes, isoflavenes,

isoflavones, rotenoids, and pterocarpans (Fig 24). These categories differ from one another by the degree of oxidation and the existence or not of additional heterocycles (Dewick 2017).

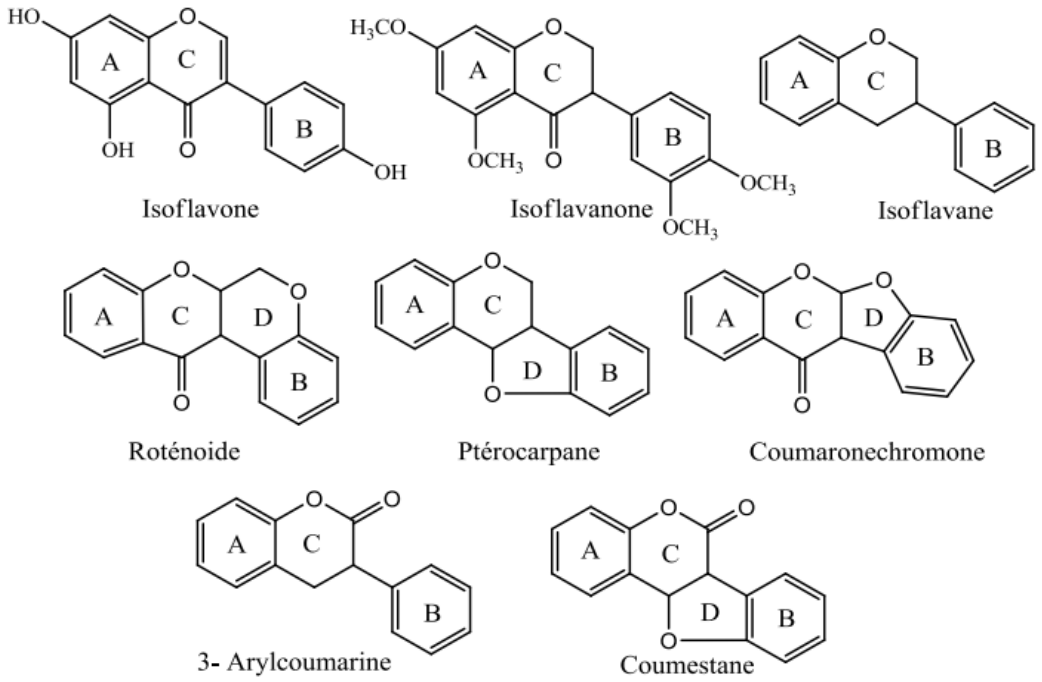



**Fig 24.** Different subclasses of isoflavonoids.

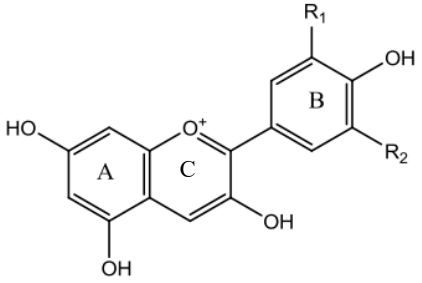
In each category, there is also the frequent presence of prenylated derivatives. The term "prenyl" is used in the broad sense meaning the prenyl substituent and or isopentenyl, furan and dimethylpyrano derivatives or the geranyl or other substituent. From all the isoflavonoids listed in the plant area, the most widely represented is that of non-glycosylated isoflavones. Glycosylated isoflavones however exist, but are rarer (O-glycosylated and C-glycosylated). Some isoflavonoids have an additional cycle resulting from the cyclization of 2'-hydroxy derivative. Pterocarpans and their derivatives as well as coumaronochromones are included. Others have a coumarin like structure induced by the oxidation of an isoflavene. This is the case, for example, of coumestans and their derivatives. A final group of isoflavonoids possesses not only a ring but also an additional carbon. This is the case of the rotenoids, which result from an oxidative cyclization of 2'-methoxyisoflavone. The rotenoids are encountered mainly in the *Fabaceae* but are also found sporadically in other families in particular the genera: *Boerhaavia* and *Mirabilis* (*Nyctaginaceae*)

(Santhos et al. 1998; Wang et al. 2002; Wangensteen et al. 2005). The rotenoids are the most important class and are exclusively prenylated whereas isoflavones, isoflavanes, pterocarpans and 3-arylcoumarins are poorly represented. The structures below represent the Different subclasses of isoflavonoids.

### II.1.6.3. Anthocyanins

Anthocyanins (from the Greek anthos, flower and kuanos, purple blue) groups the anthocyanidols and their glycosylated derivatives (Guignard 1996). Anthocyanins are flavonoids carrying a charge on the oxygen of the heterocycles C. The basic structure of the anthocyanins is characterized by a "flavon" nucleus generally glucosylated at the $C_3$ position (Ribereau 1968). Anthocyanins are distinguished by their degree of hydroxylation and methylation, by nature, the number and position of the links bound to the molecule. The aglycone or anthocyanidin constitutes the chromophoric group of the pigment (Fig 25).

These molecules belonging to the flavonoid family are capable of absorbing visible light, and are also pigments that color the plants in blue, red, purple, pink or orange (Harborne 1967). Their presence in plants is therefore detectable with the naked eye. At the origin of the color of the flowers, the fruits and the red or blue bays, they are generally localized in the vacuoles of epidermal cells, which are pockets filled with water (Richardson et al. 1984). Even if the colorization of flowers and fruits is their most well known role, anthocytes are also found in roots, stems, leaves and seeds. In autumn, the characteristic colors of the leaves of trees is due to anthocyanins and carotenes, which are no longer masked by chlorophyll.




| Anthocyanidine | R1 | R2 |
|---|---|---|
| Malvidine | O-$CH_3$ | O-$CH_3$ |
| Péonidine | O-$CH_3$ | H |
| Delphinidine | OH | OH |
| Petunidine | O-$CH_3$ | OH |
| Cyanidine | OH | H |

**Fig 25.** Chemical structure of some Anthocyanins.

### II.1.6.4. Tannins

The term tannin derives from the tanning capacity of the animal skin by transforming it into leather by said compound. Tannins are a group of high molecular weight polyphenols. Tannins are highly hydroxylated molecules and can form insoluble complexes when combined with carbohydrates, proteins and digestive enzymes, thus reducing the digestibility of food. They can be linked to cellulose and to numerous mineral elements (Alkurd et al. 2010). Two types can be distinguished: hydrolyzable and condensed tannins.

#### a) Hydrolysable tannins

These tannins are dimers of gallic acid condensed on a glycosyl derivative, include gallic acid and the condensation products of its dimer, hexahydroxydiphenic acid (Fig 26). These tannins undergo acid and basic hydrolysis, they hydrolyse under enzymatic action as well as hot water (Conrad et al. 1998).

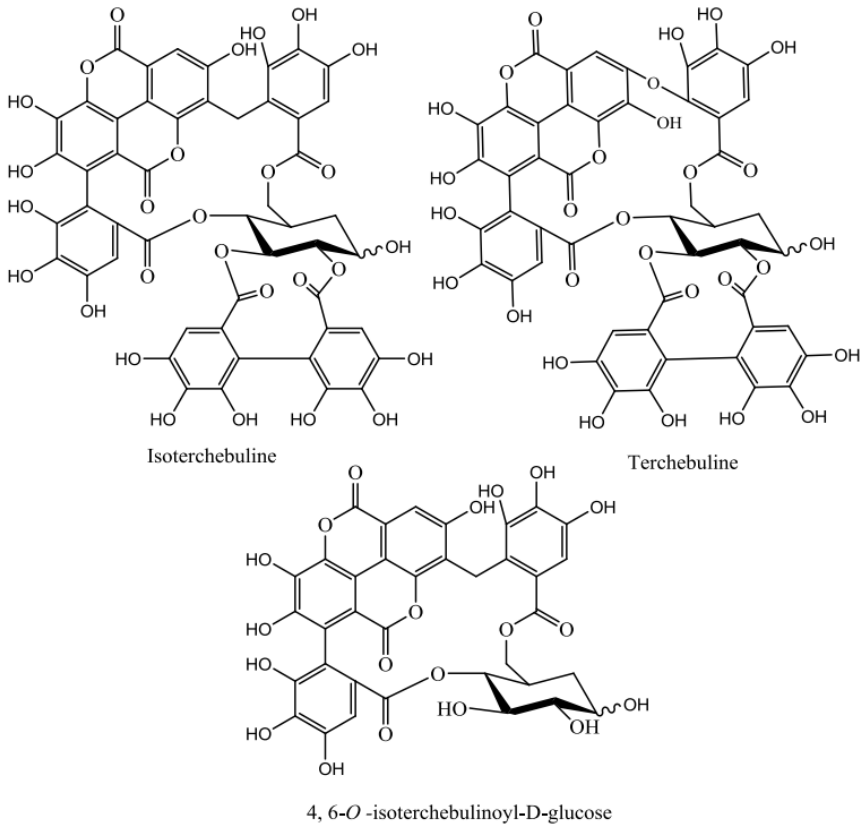



**Fig 26.** Chemical structure of some Hydrolysable tannins.

### b) Condensed tannins

Also known as proanthocyanidins or procyanidins, condensed tannins are polyphenols of high molecular mass weight. They are the result of the auto-oxidative or enzymatic polymerization of the flavan-3,4-diol units predominantly linked by the $C_4$-$C_8$ bonds (Sometimes $C_4$-$C_6$) of the adjacent units, and are thus named pro anthocyanidins type B. When condensation occurs between adjacent units via the $C_4$-$C_8$ bond and an additional ether bond between $C_2$ and $C_7$, proanthocyanidins are said to be types A. In Fig 27, the structure model of a type B tannin is shown.

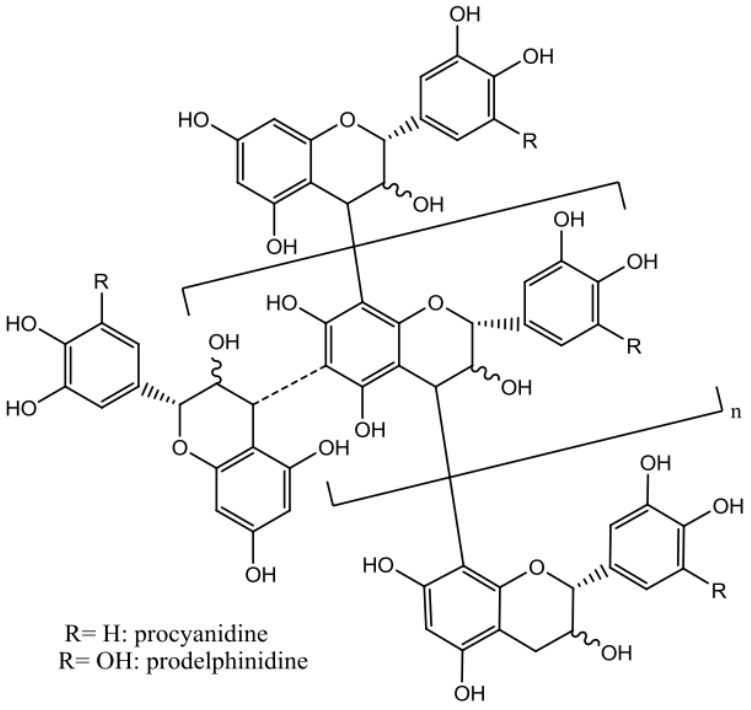



**Fig 27.** Chemical structure of type B Tannins.

If R = H or OH, the structure represents respectively procyanidin or prodelphinidin (Fig 28). The dashed 4-6 bond is a binding alternative interflavanic. The presence of a terminal unit in such structure is noted (Schofield et al. 2001).

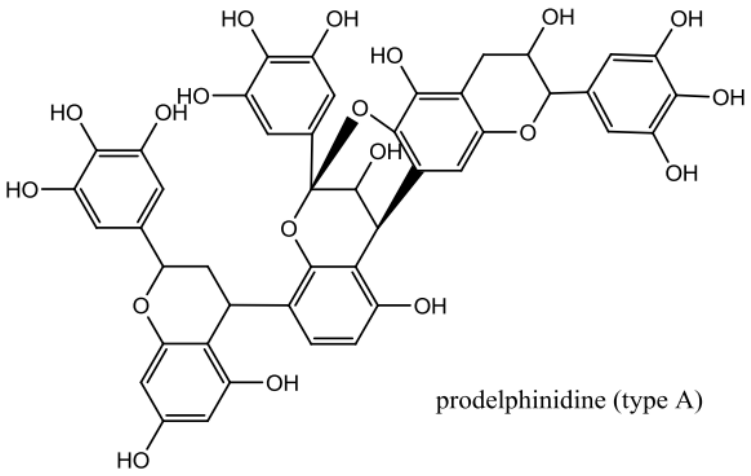



**Fig 28.** Chemical structure of Prodelphinidine.

## II.2. Role and interest of phenolic compounds

### II.2.1. In plants

Phenolic compounds may be involved in certain aspects including ***(i)*** plant physiology (i.e., lignification, growth regulation, molecular interactions with certain symbiotic or parasite microorganisms, etc.), ***(ii)*** interactions of plants with their biological and physical environment (i.e., relationships with bacteria, fungi, Insects, UV resistance); either directly in nature or during storage after harvesting of certain plants, ***(iii)*** quality criteria (color, astringency, bitterness, nutritional qualities ...) that guide the choices of humans in their consumption of (fruits, vegetables, tubers, etc.) and ***(iv)*** the products derived from transformation mainly variations in plant characteristics during technological treatments (preparation of fruit juices, fermented beverages, etc.) during which frequently appear enzymatic brownings which alter the quality of the finished product (Macheix et al. 2006).

### II.2.2 In humans

The role of phenolic compounds is widely shown in the protection against certain diseases because of their possible interaction with many enzymes and their antioxidant properties (Macheix et al. 2006). Specifically, flavonoids are attributed various properties: veinotonic, anti-tumor,

antiradical, anti-inflammatory, analgesic, antiallergic, antispasmodic, antibacterial, hepatoprotective, estrogenic and / or anti-estrogenic. They are also known to modulate the activity of several enzymes or cellular receptors. Flavonoids promote vascular relaxation and prevent agglutination of blood platelets. Therefore, they reduce blood clotting and make it more fluid. They limit the oxidation of blood lipids and contribute to the control of atheroma plaques. They are also anxiolytic, protect our arteries against atherosclerosis, and reduces thrombosis (clots in the arteries). Examples of some phenolic compounds and their biological activities are summarized in Tab 1 below.

**Tab 1.** Biological properties of some polyphenols on human organism.

| Polyphenols | Biological activities | Reference |
|---|---|---|
| Phenolic acids | Antibacterial, anti-ulcerative, antiparasitic antifungals, antioxidant | (Sannomiya et al. 2005; Barros et al. 2008; Gurbuz et al. 2009) |
| Coumarines | Vascular protective, anti inflammatory, parasitic analgesic and anti-edematous | (Ito et al. 2005; Win et al. 2008; Hirata et al. 2009; Melagraki et al. 2009; Kalkhambkar et al. 2007; Smyth et al. 2009) |
| Flavonoids | Antitumor, antiparasitic, vaso, dilatory, antibacterial, anticarcinogens, anti-inflammatories, analgesics, hypotensives, antiviral, diuretic, osteogenic, antioxidants, anti-atherogenic, antithrombotic, antiallergic | (Wollgast and Anklam 2000; Hirata et al. 2009; Tripoli et al. 2007; F. Li et al. 2008; Rao et al. 2009; Vafeiadou et al. 2009; Sutradhar et al. 2008; Choi et al. 2009; Maurya et al. 2009; Raimundo and Shadel 2009; Sohn et al. 2004) |
| Anthocyanes | Anti-capillary-venous protectors, antioxidant | (Bruneton 1993) |
| Proanthocyanidines | Stabilizing effects on collagen, antioxidants, antitumorals, antifungal and anti-inflammatory | (Masquelier et al. 1979) |
| Gallic and Catechic Tannins | Antioxidants | (Okamura et al. 1993; Kubata et al. 2005) |
| Lignans | Anti-inflammatories, analgesics | (Kim et al. 2009) |
| Saponins | Antitumor, anticarcinogenic | (Nebeling 2002) |
| Phytosterols | Protection agent against the colon cancer dependent hormone | (Nebeling 2002) |

### II.2.3. In the regeneration of polluted soils

This process was ignored until recently and consists of the biotransformation of organic matter in the soil where syringyl lignin (one of the millions of phenolic compounds) plays an essential role, as well as a large number of other phenolic compounds. This biotransformation is only the beginning of a long process related to soil transformation, which is the regulation of soil life, by controlling the availability of nutrients. It directly influences resistance to erosion, stimulates and protects at the same time different phases of animal, bacterial and fungal life which are the main culprits of pedogenesis (Lemieux and Germain 2002). Therefore, the soil remains stable and fertile. The biotransformation of organic tissues is responsible for preserving biodiversity and the physical structure of the soil. These biological characteristics govern the availability of nitrogen and phosphorus.

## II.3. Antioxidants

### II.3.1. Generalities on antioxidants

Oxygen is the source of life for aerobic organisms. But oxygen can be also a source of aggression for these bodies (Ekoumou 2002). Indeed, highly derivatives oxygen reagents may occur during enzymatic reactions or under the effect of UV radiations, ionizing radiation and transition metals (Ekoumou 2002). The forms of the oxygen causing these disorders are: oxygen singlet $O_2$, hydrogen peroxide $H_2O_2$, ROOH alkyl peroxides, $O_2^{\cdot -}$ superoxide radical, $HO^{\cdot}$ hydroxyl radicals, peroxides $ROO^{\cdot -}$ and alkoxyls RO (Cavin 1999). The consequences for the organism are displayed on DNA, lipids and proteins (Ahamet 2003).

### II.3.2. Mechanisms of action of free radicals

Free radicals can be considered as leftover of cellular metabolism. They are atoms and molecules with high energy. They are produced in all cells of the organism quite normally and in small quantity in the mitochondria. These are oxygen, hydroxide and hydrogen peroxide, which are released during the biochemical reactions. Before being neutralized they cause lesions on all the elements that they encounter (Franceschini 1994).

In genral, the body knows how to protect itself against them, thanks to the antioxidant enzymes present in our cells. These enzymes are aided in their anti-free radical action by vitamin E, C, provitamin A, zinc and selenium. If these defense systems are overcome or inadequate, free radicals have tendencies to be harmful. They attack the cell membranes whose unsaturated fatty acids are denatured (their structure is modified); they also attack proteins, collagen microfibrils, hyaluronic acid, the nucleic acids of the chromosomes and the DNA itself is transformed causing the appearance of a series of abnormalities leading to risks of cancerization. When the Free radicals damage the unsaturated fatty acids, we speak of lipidoperoxidation of the membranes cells. This then triggers a chain reaction on the various fatty acids in the vicinity until they are neutralized. These results in lesions of the cell membrane, which can lead to disruptions of variable intensity, possibly leading to cell death. They have a similar effect on mitochondria, cellular enzymes, chromosomes, collagen and hyaluronic acid. Over the course of our lives, we are subject to millions of circumstances favoring the production of these free radicals, particularly harmful to the skin (Franceschini 1994).

In Fig 29, ROS generation is initiated during respiration. This generation is facilitated by the involvement of various physiological and environmental factors (UV, radiation, ozone, cigarette, pollution, etc.). In the organization, the production of an assortment of ROS from molecular $O_2$ and L-arginine is realized through various enzymes such as MPO (myloperoxidase), NADPH Oxidase, SOD (superoxide dismutase) and NOS (nitrite oxide synthase). The presence of these compounds in the body generate several phenomena at the cellular level, namely, DNA repair proteins damage, caspase and lipid peroxidation. Note that the damage of DNA is followed by mutation and activation of NF-kB (Nuclear factor-kappa B). All these phenomena give rise to a wide range of diseases.

Secondary metabolites inhibit generations of free radicals by trapping not only the mother, the daughter but also the products by inducing an increase in the SOD, CAT, GST and GSH, resulting in an obstruction of the formation of various diseases (Mandal et al. 2011). It is undeniable that polyphenols have important properties on various biological systems. These effects may have therapeutic applications. However, all these potentialities are not yet realized even if many works support the possible use of these compounds in therapeutics. In addition, derivatives of rutin are actually used in therapeutics to improve capillary resistance. Flavonoids are the usual constituents

of vasculoprotective and veinotonic vectors used in phlebology. The use of plants containing flavonoids, alone or in combination, is steadily rising because of growing consumer demand for products of natural origin, and due to interest in both medicinal and nutritional plants containing this class of compounds of natural origin, having properties justifying their use in the prophylaxis of cardiovascular diseases, alzheimer's, cancers, etc.

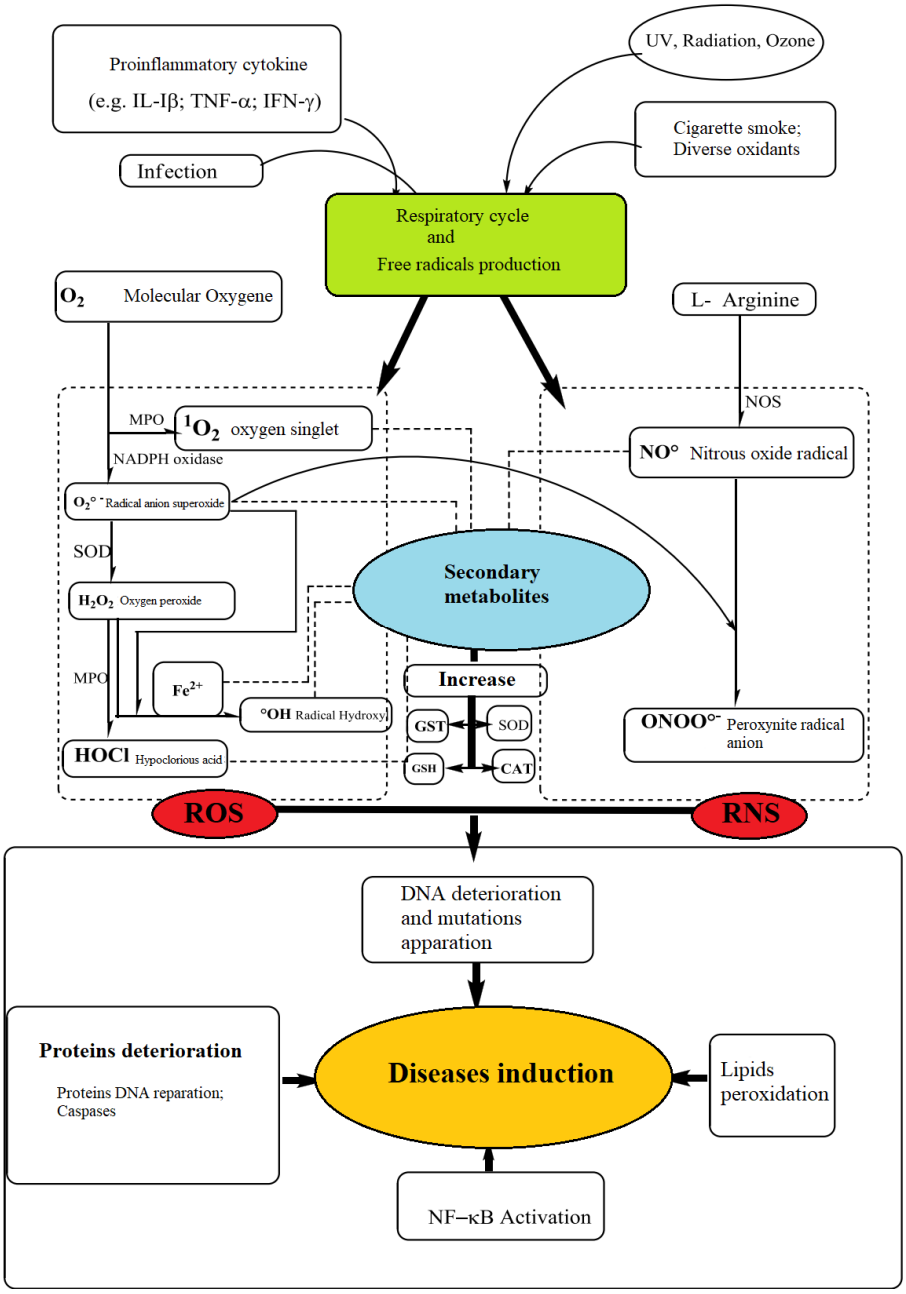



**Fig 29.** Interaction between secondary metabolites and disease prevention.

### II.3.3. The main sources of antioxidants

Certain therapeutic classes such as NSAIDs (non-inflammatory Steroids), antihyperlipoproteinemics, β-blockers and antihypertensive agents are known for their antioxidant properties (Ahamet 2003). The simplest free radical scavenger is ethyl alcohol, transfer agent of

hydrogen, which leads to a biologically compatible compound, acetaldehyde, which is bio-oxidized by the enzymatic chain with production of energy.

$$CH_3CH_2OH + 2\,R\cdot \rightarrow CH_3CHO + 2RH \quad (R1)$$

Certain medicines (eg. Probucol) lower cholesterol level in the blood, N-acetylcysteine acts in the regeneration of glutathione in penetrating the cells. The properties of glutathione have been recognized in phospholipid studies of leaves of certain plants. Indeed, thiols are much more active than hydrocarbons, alcohols or phenols as free radical scavenging agents (Le Perchec and Malik 1994).

$$GSH + OH\cdot \rightarrow GS + H_2O \quad (R2)$$

$$2\,GS \rightarrow GS - GS \quad (R3)$$

The protective capacity of glutathione is considered superior to that of an antioxidant and as potent as α-tocopherol. In vitro, it was observed that glutathione introduces a period of induction to oxygen uptake by hemoglobin and delays the oxidation of the unsaturated hydrocarbon fraction of the lecithins (unsaturated esters of fatty acid phospholipids) and aniline (Mieyal 1978). Thus, vitamin C contains an enediol form, which produces the diketonic form by successive transfers of its two H-atoms. The enediol form is regenerated by the intervention of superoxide dismutase enzyme in the presence of a catalase. Vitamin C is found in vegetables, cabbage, sweet pepper, parsley, citrus fruits and kiwi. It plays an important role in the regeneration of vitamin E (Igor 2003). Vitamin E seems to fix the hydroxyl radical with the formation of a ring-opening molecule. It is found in vegetable oils (cold pressed peanuts, soybean, thistle, sunflower, olive, almonds, seeds, milk, eggs, green leafy vegetables) (Igor 2003).

Accordingly, among the active photo-protectors, β-carotene appears to be an effective scavenger. The polyisene composition gives it an ability to trap oxygen by the formation of a dioxetane (addition of an olefin and an oxygen molecule) or by production of hydroperoxides (insertion of oxygen into all C-H bonds conjugated to a double bond) which may in turn be reduced. It is found in green vegetables, salad, carrots, apricot, melon, spinach, papaya (Igor 2003). Furthermore, antioxidants are present in all parts of higher plants. They are phenolic compounds (flavonoids, xanthones, coumarins, carotenoids, phenolic acid derivatives, tannins, anthocyanins, etc.).

### II.4. Economic interests of phenolic compounds

The use of plants by man is confused with the history of mankind, for food and for domestic animals (progressively), protection (that of its shelters and for its clothing), energy (fire, fossil energy) and the fight against disease through the synthesis of drugs and a traditional pharmacopoeia. Among all the chemical constituents of plants, the phenolic compounds occupy only a modest quantitative place (with the exception of lignin). They have multiple properties sought by mankind for a long time, first empirically and then by a more reasoned approach that benefited from the progress of science, particularly in the analytical and biotechnological fields (Macheix et al. 2006). Thus, the economic implications of the results obtained with respect to phenolic compounds are considerable. They concern the direct use of numerous agriculture products (direct marketing of fruit, vegetables and cereals). They exist also in several sectors of biotechnology (agro-food industries, pharmaceuticals and cosmetics).

## III. Corrosion Inhibition

### III.1. Corrosion and protection of materials

Corrosion can be defined as the chemical degradation of a material and the alteration of its physical properties (especially mechanical) under the influence of its surrounding environment. It is a natural phenomenon that tends to change metals and alloys to their original state of oxide, sulphide, carbonate or any other salt, more stable in the environment. Corrosion can affect many structures, especially those made of metallic materials (Cwalina 2014). Indeed, metallic materials and more particularly steels, which constitute the basic materials in the construction of many structures, are highly exposed to corrosion when in contact with wet atmospheres, immersed in fresh or salt water, implanted in soils or in the presence of more or less aggressive solutions. The corrosion processes in these environments depend on a large number of factors (the nature and composition of the material, the environment itself, its chemical characteristics, its temperature, etc.) which intervene not individually but in a more or less complex way with each other. In terms of protection against corrosion, it is possible to act on the material itself (judicious choice of the material, adapted forms, constraints according to the applications), on the surface of the material (coating, painting, any type of surface treatment) or the environment with which the material is in contact using corrosion inhibitors. The use of inhibitors to prevent the process of metal deterioration remains an

unavoidable and widespread application (Left et al. 2013). Synthetic inhibitors are effective in protecting against the corrosion of metals, but they are highly toxic to humans and the environment. These compounds are also expensive and non-biodegradable. As a result, the use of oils, plant extracts and biomolecules as corrosion inhibitors has become a key niche in applied electrochemical research. Agro-resources and their co-products constitute a deposit of biodegradable natural compounds that can be extracted from plant products and co-products by simple and cost-effective procedures (Raja and Sethuraman 2008).

## III.2. Corrosion inhibitors

According to the definition given by the National Association of Corrosion Engineering (NACE) "a corrosion inhibitor is a substance which, when added at low concentrations in a corrosive medium, decreases the reaction rate of oxidation of the metal in its environment " (Dariva and Galio 2014). The reduction of the corrosion rate is mainly achieved either by limiting the chemical or electrochemical reactions or by modifying the aggressiveness of the electrolyte. Thus, protection by a corrosion inhibitor on the surface of the material may be a permanent protection, or a temporary protection, during a period where the material is particularly sensitive to the corrosion (storage, cleaning, pickling, etc.) or when subjected to very severe machining such as drilling, tapping, and threading. An inhibitor can be combined with other protective means such as the additional protection of an alloy with high corrosion resistance, the addition of a surface coating such as paint, grease and oil, etc. A corrosion inhibitor must lower the rate of corrosion of the metal while maintaining the physicochemical characteristics of the latter. It must not only be stable in the presence of the other constituents of the medium, but also not affect the stability of the compounds contained in this medium. An inhibitor is definitely recognized as such if it is stable at the use temperature and effective at low concentration. It must be nontoxic, cheap and available (Raja and Sethuraman 2008). There are several possibilities for classification of inhibitors, which differ from each other in various ways (Landolt 1993):

- From the nature of the products (organic or mineral inhibitors);
- From their mechanism of electrochemical action (cathodic, anodic or mixed inhibitors);

- From their interface mechanisms and their principles of action (adsorption on the surface of the metal and / or formation of a protective film);
- From the application field.

### III.2.1. Classification by inhibitor's nature

#### a) Mineral inhibitors

The mineral molecules are used most often in a medium close to neutrality, even in alkaline medium and more rarely in acidic medium. The products dissociate in solution and it is often their products of dissolution which ensure the phenomena of inhibition (anions and cations) (Dariva and Galio 2014). The inhibitory cations are essentially $Ca^{2+}$ and $Zn^{2+}$ and those, which form insoluble salts with certain anions such as hydroxyl ($OH^-$). The main inhibiting anions are the oxo-anions of the type $XO_4^{n-}$, chromates, molybdates, phosphates, silicates (Rozenfel′d 1981). The number of mineral molecules currently used as a corrosion inhibitor is limited, because most of the effective products are harmful to the environment. However, new organic complexes of chromium III and other cations ($Zn^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Sr^{2+}$, $Al^{2+}$, $Zr^{2+}$, $Fe^{2+}$, etc.) that are effective against corrosion and non-toxic have been developed.

#### b) Organic inhibitors

Organic molecules are increasingly used for the development of corrosion inhibitors. Their use is currently preferred to that of inorganic inhibitors for essentially ecotoxic reasons (Dariva and Galio 2014). Organic inhibitors generally consist of by-products of the petroleum industry. They have at least one active center capable of exchanging electrons with the metal, such as nitrogen, oxygen, phosphorus or sulfur. The usual functional groups, allowing their fixation on the metal are:

- The amino radical ($-NH_2$),
- The mercapto radical (-SH),
- The hydroxyl radical (-OH),
- The carboxyl radical (-COOH).

Inhibitors that contain sulfur are more effective than those that contain nitrogen, because sulfur is a better electron donor than nitrogen. The main feature of these inhibitors is their high efficiency,

even at low concentrations. The inhibitory action of these organic compounds, which is generally independent of the anodic and cathodic corrosion processes, is related to the formation (adsorption) of a barrier more or less continuous, but of finite thickness, which prevents the access of the solution to the metal.

**c) Classification by action mecanism**

There are many modes of action for corrosion inhibitors. The same compound will often have a mechanism of action that is specific to the corrosion system (metal/solution). However, and regardless the exact mechanism by which each inhibitor acts under the conditions in which it is placed, there are a number of basic considerations valid for all inhibitors.

- **Mechanisms of electrochemical action**

This classification of inhibitors takes into account the electrochemical nature of the liquid phase corrosion, which involves at least two reactions:

- An anodic reaction of dissolution of the metal (oxidation reaction):

$$M \rightarrow M^{n+} + ne^- \quad (R4)$$

Example:

$$Fe \rightarrow Fe^{2+} + 2e^- \quad (R5)$$

- A cathodic reaction for reducing an oxidant of the solution:

$$H^+ + e^- \rightarrow \frac{1}{2} H_2 \text{ (non-aerated acidic medium)} \quad (R6)$$

Or: $$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \text{ (aerated acidic medium)} \quad (R7)$$

The role of the inhibitor will necessarily be to reduce the speed of one of the two reactions and in some cases both. If the inhibitor slows down the oxidation reaction by blocking the anodic, it is called an anodic inhibitor (Dariva and Galio 2014). Similarly, if the inhibitor slows down the reduction reaction by blocking the cathodic sites (the site of the reduction of dissolved oxygen in an aerated environment or the site of the reduction of the proton $H^+$ in an acidic medium), it is

called a cathodic inhibitor. Mixed inhibitors act both to decrease the rate of the anodic reaction and that of the cathodic reaction (Dariva and Galio 2014). Anodic inhibitors are inorganic substances such as orthophosphates, silicates, chromates, etc. Their mode of action is to increase the value of the corrosion potential of the material in order to bring it to a value for which there is formation of a passive protective film on the anode.

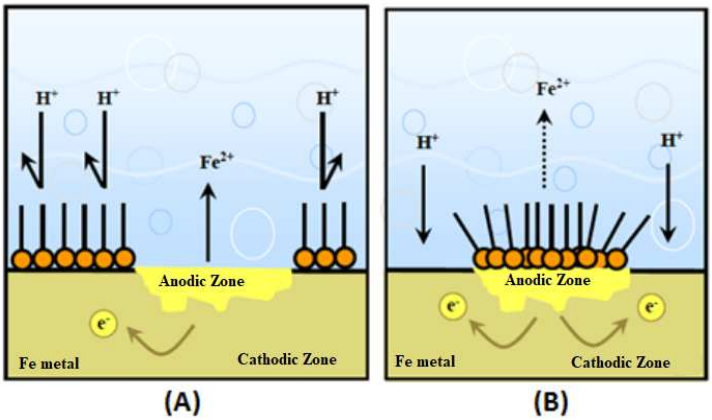



**Fig 30.** Formation of cathodic (A) anodic (B) and barrier layers interfering with electrochemical reactions, in the case of inhibition in an acid medium.

Indeed, the ratio of the surfaces (anodic and cathodic) is important in this case. The anode corrodes more rapidly as the anode surface is smaller than the cathode surface. In other words, if the protective film is altered by scratching or dissolution, and if the amount of inhibitor is insufficient to restore the film, the exposed portion corrodes deeply. Thus, in localized corrosion, pitting corrosion is a particularly insidious form: the attack is limited to holes, very localized and can progress very rapidly in depth while keeping the rest of the surface unscathed. The action of the cathodic inhibitors results in a decrease in the speed of the cathodic reaction and thus in a displacement of the corrosion potential towards less noble values. These are usually cations that can migrate to the cathode surface, where they precipitate as basic salts or hydroxides, forming adherent and compact films. These inhibitors are "safer" than anodic inhibitors, because they are not likely to promote localized corrosion, even in case of underdosing.

- **Mechanisms of interfacial action**

This other mode of classification of the inhibitors differentiates them from their mode of fixation on the metallic surface. Adsorption inhibitors or "interface" inhibitors and so-called "interphase"

inhibitors. The former are rather observed in acidic medium and act by forming single or two dimensional films of molecules by adsorption on the surface of the metal, whereas the latter are specific for neutral or alkaline medium and form three dimensional films between the corroded substrate and the molecules of the metal inhibitor. Interphase inhibitors are thus not only adsorbed at the metal/oxide and oxide/electrolyte interfaces, but are also incorporated into the barrier layers (forming complexes for example); thus these interphase inhibiting molecules lead to homogeneous and dense networks thus having a low porosity and a good stability. This surface therefore tends to capture nearby atoms and molecules. There are two types of bonds between the adsorbed species and the metal surface: electrostatic bonding and chemical bonding, thus two distinct types of adsorption: physisorption and chemisorption. The mode of organization of the inhibitory molecules on the surface.

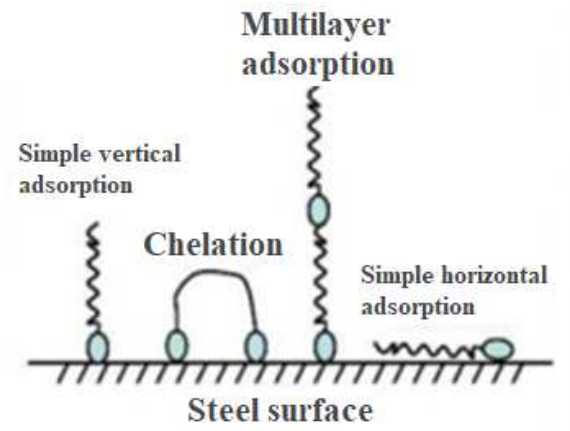



**Fig 31.** Illustation of the differents modes of adsorption of organic molecules on steel surface.

- **Physical adsorption**

Physical adsorption retains the identity of the adsorbed molecules. Three types of bonds are distinguished here ***(i)*** Van der Waals bonds (always present), ***(ii)*** polar bonds (dependent of surface charges and inhibitor) and ***(iii)*** hydrogen bonds (between a hydrogen bond donor and a hydrogen bond acceptor, only N, O, P, S carrying free doublets (Khaled and Al-Qahtani 2009). Physical adsorption is due to the electrostatic attraction between the inhibitory ions or the dipoles and the electrically charged surface of the metal. Thus, the electrostatic forces adsorption are generally low. Furthermore, inhibitory species adsorbed on the metal, due to electrostatic forces, can also be

easily desorbed. A main function of electrostatic adsorption is that the ions are not in direct physical contact with the metal. A layer of water molecules separates the metal from the ions. The physical adsorption process has low activation energy and is relatively temperature independent (Mansfeld 1985).

- **Chemisorption**

Chemisorption is a more common mechanism than the physisorption mechanism and leads to greater efficacy of the inhibitor (Hackerman and Makrides 1954). Chemisorption results from sharing electrons between the metal surface and the inhibition molecule (an active center such as the N, S, P, O atoms) and thus very strong covalent chemical bonds are formed. The inhibitor has the behavior of an electron donor vis-a-vis the metal atom of the surface. In addition, chemical adsorption is accompanied by a profound change in the distribution of the electronic charges of the adsorbed molecules. Chemisorption is often an irreversible mechanism (Left et al. 2013).

### III.2.2. Green inhibitors

Organic inhibitors generally have heteroatoms. O, N, and S are found to have higher basicity and electron density and thus act as corrosion inhibitor. O, N, and S are the active centers for the process of adsorption on the metal surface. The inhibition efficiency should follow the sequence O < N < S < P. The use of organic compounds containing oxygen, sulphur, and especially nitrogen to reduce corrosion attack on steel has been reported in detail. The existing data show that the most organic inhibitors adsorbed on the metal surface by displacing water molecules on the surface and forming a compact barrier (Rani and Basu 2012).

The environmental toxicity of organic corrosion inhibitors has prompted the search for green corrosion inhibitors as they are biodegradable, do not contain heavy metals or other toxic compounds. In addition to being environmentally friendly and ecologically acceptable, plants products are inexpensive, readily available and renewable. Investigations of corrosion inhibiting abilities of tannins, alkaloids, organic, amino acids, and organic dyes of plant origin are of great interest. Although substantial research has been devoted to corrosion inhibition by plant extracts, reports on the detailed mechanisms of the adsorption process and identification of the active ingredient are still scarce (Rani and Basu 2012).

Bendahou et al. (2006) evaluated the effect of natural rosemary oil as non toxic inhibitor on the corrosion of steel in $H_3PO_4$ medium at various temperatures. The oil was initially hydrodistilled and used as inhibitor in various corrosion tests with gravimetric and electrochemical techniques being used to characterise the corrosion mechanisms. Chromatographic analysis by gas chromatography showed that the oil was rich in 1,8-cineole. Furthermore, Bendahou et al. (2006) proved good agreement between the various methods explored for corrosion inhibition analysis. The polarisation measurements showed that rosemary oil acted essentially as a cathodic inhibitor. The efficiency of the oil increased with the concentration (to attain 73% at 10g/L) but decreased with the rise of temperature in the 25-75°C range. In another study, Bothi and Sethuraman (2008) invesitigated the corrosion inhibitive effect of the extract of black pepper on mild steel in 1M $H_2SO_4$ medium by conventional weight loss (33–50°C), electrochemical, tafel polarization, impedance and scanning electron microscope (SEM). Results of weight loss study revealed that black pepper extract acts as a good inhibitor even at high temperatures. The inhibition is through adsorption, which is found to follow Temkin adsorption isotherm. Tafel polarization method revealed the mixed mode inhibition of black pepper extract. Analysis of impedance data has been made with equivalent circuit with constant phase angle element for calculation of double layer capacitance value. SEM analysis provide the confirmatory evidence for the protection of mild steel by the green inhibitor.

Accordingly, El-Etre et al. (2005) tested the aqueous extract of the leaves of henna (*Lawsonia*) as a corrosion inhibitor of C-steel, nickel and zinc in acidic, neutral and alkaline solutions, using polarization technique. El-Etre et al. (2005) showed that the extract acted as a good corrosion inhibitor for the three tested electrodes in all tested medium. The inhibition efficiency increased as expected with the concentration of the extract. The inhibitive action of the extract was also discussed in view of adsorption of the complex *Lawsonia* molecules onto the metal surface. El-Etre et al. (2005) showed that this adsorption followed Langmuir adsorption isotherm in all tested systems. In other studies, electrochemical and gravimetric assays for the corrosion of dental amalgam were carried out in electrolytes similar to artificial saliva with and without *Anise* extracts. The electrochemical study showed that the anise extract act as an anodic-type inhibitor. The results obtained proved that the extract of the anise plant could serve as an effective inhibitor for the corrosion of amalgam in saliva medium. The inhibition efficiency was found to increase with extract concentration until 0.33g/L with an efficiency of more than 90% (Saufi et al. 2014).

## IV. Ethanobotanical synthesis of the studied species

### IV.1. *Urtica Dioica*

#### IV.1.1. Description

*Urtica dioica* is originally from the colder regions of northern Europe and Asia. Today, this herbaceous shrub grows all over the world. Stinging nettle grows well in nitrogen-rich soil, blooms between May and September of every year, and reaches nearly 3 feet high. The stem is erect and green; the leaves are opposite, and cordate at the base, oblong or ovate finely toothed, dark green above and paler beneath. The flowers are in reddish-brown to greenish-white colour. The small, green, dioecious flowers occur as racemes in the axils of the upper leaves. Usually, the plant has either male or female flowers, in separate inflorescences, hence the specifc name of the plant *dioica*, which means “two houses” (Wagner et al. 1989).

**Tab 2.** *Urtica Dioica* Taxonomic Hierarchy (Le Moal and Truffa-Bachi 1988).

| **Kingdom** | **Plantae** |
|---|---|
| **Subkingdom** | *Viridiplantae* |
| **Infrakingdom** | *streptophyta* |
| **Superdivision** | *embryophyta* |
| **Division** | *tracheophyta* |
| **Subdivision** | *Spermatophytina* |
| **Class** | *Magnoliopsida* |
| **Superorder** | *Rosanae* |
| **Order** | *Rosales* |
| **Family** | *Urticaceae* |
| **Genus** | *Urtica* |
| **Species** | *Urtica dioica* |
| **Arabic name** | **الحريقة** |

### IV.1.2. Traditional uses

*Urtica dioica* have a long history of use in the household home remedies and nutritious diet. The powdered leaf extract used as antihemorrhagic agent to reduce excessive menstrual flow and nose bleedings. This plant was used for the treatment of arthritis, anemia, hay fever and used as diuretics, astringents and blood builders in folk medicine. Traditionally, a tea made from the leaves of *Urtica dioica* has been used as a cleansing tonic and blood purifer. Externally, this plant is used to treat skin complaints, gout, sciatica, neuralgia, haemorrhoids, hair problems, etc. (Bombardelli and Morazzoni 1997). For medicinal purposes, the plant is harvested between May and June of every year as it is coming into flower and dried for later use. The root has a benefcial effect upon enlarged prostate glands and it is used for the treatment of rheumatic gout, nettle rash and chickenpox, externally is applied to bruises. The plant has been widely used by herbalists around the world for centuries. In the first century, Greek physicians Pedanius Dioscorides and Galen reported that the leaf of *Urtica dioica* had diuretic and laxative properties and was useful for treatment of asthma, pleurisy and spleen illnesses. The nettle leaves are used as a nutritious supplement and as a weight loss aid (Ji et al. 2009). Nowadays, in Germany this plant is sold as herbal drug for prostate diseases and as a diuretic.

### IV.1.3. Ethnopharmacology

In preclinical animal trials *Urtica dioica* along with *Nigella sativa* reduced carbon tetrachloride induced elevated levels of serum potassium and calcium levels and decreased the levels of red blood cells, weight of blood cells, packed cell volume and haemoglobin levels (Kanter et al. 2003). Türkdoğan et al. (2003) and Kanter et al. (2003), also reported the hepatoprotective effects of *N. sativa* and *U. dioica* in carbon tetrachloride induced liver fibrosis and cirrhosis model. In another study, the affects of ethanol-water (80%-20%) extract of *U. dioica* and butylated hydroxyanisole were investigated, for phase I and phase II enzymes, antioxidant enzymes, lactate dehydrogenase, lipid peroxidation and sulfydryl groups in the liver of Swiss albino mice. It was found that the extract was affective in inducing GST, DTD, SOD and CAT activity (Ozen and Korkmaz 2003). The aqueous extract of *U. dioica* is characterized for the specifc cardiac and vascular affects using isolated Langendorff perfused rat heart, and it produced a vasoconstriction of the aorta, which is

due to the activation of alpha1-adrenergic receptors. However, aqueous extract of *U. dioica* also induced a strong bradycardia through non-cholinergic and non-adrenergic pathways that might compensate for its vascular effect and account for the hypotensive action of *U. dioica* (Legssyer and Ziyyat 2002). The aqueous extract of *U. dioica* was also studied for its antioxidant, antimicrobial, antiulcer and analgesic properties, and the study resulted with the conclusion that the plant exhibits antioxidant properties, antimicrobial activity, antiulcer activity and an analgesic effect (Gülçin and Küfrevioğlu 2004). It has been also reported that the *U. dioica* roots extract had an hypotensive effect by decreasing the vascular pressure (Legssyer and Ziyyat 2002).

## IV.2. *Chenopodium Murale*

### IV.2.1. Description

This plant is a common annual herbaceous weed in 57 countries. It originated in Eurasia and now occurs over a wide range of latitudes (New Zealand to Sweden) and altitudes (the coasts of Lebanon to the Bolivian Andes). It is found in cropland and wastelands, especially those with rich fertile soils. Its species name suggest the plant "grows on walls" (Holm 1997). *Chenopodium murale* is a stout, succulent somewhat aromatic annual herb; root a branched taproot; stems branched, erect except lower branches somewhat decumbent, slightly striated, smooth, 30 to 80 cm tall; leaves alternate, mealy when young, then dark green and shiny, ovate usually pointed at tip, 2 to 8 cm long, 1 to 6 cm wide, coarsely toothed with teeth directed forward, lower leaves on rather long petioles, upper ones shortly petioled, leafy almost to the top; inflorescence a duster of flowers in loose cymose panicles, terminal and axillary; flowers perfect, small, greenish, sepals 5, conspicuously keeled, united below, not completely covering the fruit; stamens 5, stigmas 2; fruit an utrick; seed lens-shaped, 1.2 to 1.5 mm long, 1 to 13 mm wide, finely granular, yellowish-brown to dull black with distinctly keeled margin giving the appearance of a pie-plate rim. The somewhat unpleasant odor, toothed leaves, conspicuously keeled sepals and pie-plate margins of the seeds are the distinguishing characteristics of this species (Holm 1997).

**Tab 3.** *Chenopodium murale* Taxonomic Hierarchy.

| **Kingdom** | **Plantae** |
|---|---|
| **Subkingdom** | *Viridiplantae* |
| **Infrakingdom** | *streptophyta* |
| **Superdivision** | *embryophyta* |
| **Division** | *tracheophyta* |
| **Subdivision** | *Spermatophytina* |
| **Class** | *Magnoliopsida* |
| **Superorder** | *Caryophyllanae* |
| **Order** | *Caryophyllales* |
| **Family** | *Amaranthaceae* |
| **Genus** | *Chenopodium* |
| **Species** | *Chenopodium murale* |
| **Arabic name** | **الحريقة الملساء** |

### IV.2.2. Traditional uses

Densities of 248 plants/m² of *C. murale* and *C. album* reduced wheat yields by 16% in Pakistan. *C. murale* is sometimes used to feed cattle in India but can have high nitrate levels and imparts an off-flavor to milk when consumed as young, succulent plants. Interestingly, *C. murale* extracts were the most effective of 29 species tested in inhibiting tobacco and cucumber mosaic viruses. The plant has been used as a leafy vegetable in India and parts of Africa. While rich in minerals, the buildup of high oxalate levels as the plant develops limits its usefulness (Holm 1997).

### IV.2.3. Ethnopharmacology

The importance of *Chenopodium* species was due to their wide variety of medicinal properties. A wide range of medicine application of plants belonging to this genus has been reported (Kokanova-Nedialkova et al. 2009). More specifically, Ibrahim et al. (2007) have reported some important

biological activities of *C. murale.* Anti-inflamatory and analgesic assays revealed an indeniable efficiency about these experiments. The richness of this plant in phenolic compounds is the main cause for these properties. The mechanism of action of flavonoids against inflammation involves the inhibition of cyclooxygenase enzymes, and therefore, prostaglandin synthesis (Neal 1997), thus preventing the generation of free radicals, which cause tissue damage during inflammation (Williamson et al. 1996). Additionaly, the use of the *C. murale* extracts exhibited these medicinal attributes without causing any adverse ulcerogenic effect, which is the case for most of the non-steroidal anti-inflammatory drugs that cause gastrointestinal erosions, pre-ulcerous changes, and bleeding (Neal 1997; Ibrahim et al. 2007). *C. murale* was also reported for its anthelmintic and laxative properties (Ibrahim et al. 2007). As previously stated, these properties are due to the diversity of compounds present in this plant's extracts, such as phenols, sterols, terpens and alkaloids (Dembitsky et al. 2008; Bashir et al. 2003; El-Sayed et al. 1999).

## IV.3. *Ziziphus Lotus*

### IV.3.1. Description

*Zizyphus Lotus* (*Z. Lotus*), also known as jujube, belongs to the angiosperm *Rhamnaceae* family. This family includes about 135–170 species of *Zizyphus* (Maraghni et al. 2010). As a tropical and subtropical plant, *Z. Lotus* grows generally in arid and semiarid countries and is widely distributed in China, Iran, Africa, South Korea, and Europe (Cyprus, Spain, Greece, and Sicily) (Richardson et al. 2004; Gorai et al. 2010; Adeli and Samavati 2015). In Africa, *Z. Lotus* is widely distributed in the Mediterranean region (Algeria, Morocco, Tunisia, and Libya) (Pottier-Alapetite 1979). It is found mainly in the Mediterranean bioclimatic semi-arid and arid zones. It contributes to the landscape formation. It is usually bushy because of the sandy winds often occurring in these regions and the cattle grazing (goats, sheep sans Arabian camels). Frutescent plant from 1.3 to 2.2 m high. Very branched. The twigs are curved downwards, flexuous, greyish white with spines in straight or curved pairs. The leaves are small, alternate, obtuse, crenate, three-veined, glabrous, weakly rigid, 7-9 mm wide and 9-13 mm long, with short petiole. The flowers are solitary or grouped with a single short pedicel, with a funnel-shaped calyx, a pentamer, with a small corolla with 5 petals. 5 epipetal stamens. 2 short styles. Fruits: spherical drupes whose small, round, bilocular bone nuclei are covered with a half-fleshy pulp, very quickly dry.

### IV.3.2. Traditional uses

This plant is employed in nutrition, health, and cosmetics in several forms, for example, honey, tea, jam, juice, oil, loaf, and cake. In addition, in traditional medicine, both in North Africa and Middle East, several parts of *Z. lotus* are given as antiurinary troubles agents, antidiabetes, skin infections, antifever, antidiarrhea, insomnia agents, sedative, bronchitis, and hypoglycemic activities (Adzu et al. 2003; Lahlou et al. 2002; Le Floc'h 1983; Anand et al. 1989). On the other hand, this plant offers a delicious read fruit (jujube) that is consumed fresh, dried, and processed as food by local populations in substantial amounts (Elaloui et al. 2014). Its fruits are used as edible food and traditional Chinese medicine. The fruits are claimed to purify the blood and aid digestion. Among the bioactive constituents, polysaccharides may play an important role. It was reported that polysaccharides from the fruits had anti-complementary activities (Adeli and Samavati 2015). The seeds are edible and frequent eating of kernels, said to increase flesh and strength and cause a sedative effect, is recommended in the case of insomnia. The fruits are claimed to purify the blood and aid digestion. The roots are used in fever and to cure wounds and ulcers. The bark is used as a remedy in diarrhoea (Chopra 1956). A number of cyclopeptide alkaloids have previously been reported from *Z. jujuba* (Tschesche and Kaußmann 1975).

**Tab 4.** *Ziziphus lotus* Taxonomic Hierarchy.

| **Kingdom** | **Plantae** |
|---|---|
| **Subkingdom** | *Viridiplantae* |
| **Infrakingdom** | *streptophyta* |
| **Superdivision** | *embryophyta* |
| **Division** | *tracheophyta* |
| **Subdivision** | *Spermatophytina* |
| **Class** | *Magnoliopsida* |
| **Superorder** | *Rosanae* |
| **Order** | *Rosales* |
| **Family** | *Rhamnaceae* |

| | |
|---|---|
| **Genus** | *Ziziphus Mill - jujube* |
| **Species** | *Ziziphus lotus* |
| **Arabic name** | السدر |

### IV.3.3. Ethnopharmacology

In recent years, several scientific reports have been carried out about the presence of many biologically active molecules from *Z. lotus*, which may have high potential benefit in human nutrition, health, and disease (Chouaibi et al. 2012; Renault et al. 1997). In herbal medicine, the properties of bioactive compounds from plants depend on the part of the plant concerned (root, leaf stalk, pulp, or fruit) and the type of extract used. *Z. lotus* is known for its high content in polyphenols exhibiting antioxidant and antimicrobial, immunomodulatory properties (Ghazghazi et al. 2014; Abdoul-Azize and Souleymane 2016). Importantly, others biologically active molecules, particularly cyclopeptide alkaloids, termed lotusines (Ghedira et al. 1995; Le Crouéour et al. 2002), dammarane saponins (Renault et al. 1997) , and various flavonoids (Borgi et al. 2008) have been isolated from this shrub, along with polyunsaturated fatty acids (oleic acid and linoleic acid), high carbohydrate, and fbers which are abundant in seed extracts and endowed with antiulcerogenic and antioxidants effects (Chouaibi et al. 2012; Abdeddaim et al. 2014).

## IV.4. *Mentha rotundifolia*

### IV.4.1. Description

*Mentha suaveolens*, apple mint, pineapple mint, woolly mint or round-leafed mint (synonyms *M. rotundifolia, Mentha macrostachya, Mentha insularis*) (Austin 2006) is a member of the mint genus *Mentha* that ranges through southern and western Europe and the western Mediterranean region. It is a herbaceous, upright perennial plant that is most commonly grown as a culinary herb or for ground cover. Apple mint is native to southern and western Europe and is naturalised in central and northern parts of Europe. It is found in damp and wet locations. Pineapple mint (*Mentha suaveolens* 'Variegata') is a cultivar of apple mint that has leaves which are banded with white. A hybrid derived from it is grapefruit mint (*Mentha suaveolens x piperata*). Apple mint is called

hierbabuena in Spain and most South American countries, literally meaning "good herb". Apple mint has been used for medicinal purposes for thousands of years in many parts of the world, including Africa, Europe, Asia, and the Americas. Apple mint typically grows to a height of from 40 to 100 centimetres tall and spreads by stolons to form clonal colonies. The foliage is light green, with the opposite, wrinkled, sessile leaves being oblong to nearly ovate, 3 to 5 cm (1.2 to 2.0 in) long and 2 to 4 cm (0.8 to 1.6 in) broad. They are somewhat hairy on top and downy underneath with serrated edges. The flowers develop in terminal spikes 4 to 9 cm (1.6 to 3.5 in) long and consisting of a number of whorls of white or pinkish flowers. Apple mint flowers in mid to late summer. The plant is aromatic with a fruity, minty flavour. The spiciform inflorescences are terminal. The whitish dew flowers are densely arranged in tapered ears, 2 to 5 cm long (Božović et al. 2015). The green calyx (2 mm) is bell-shaped, bordered by 5 triangular teeth. The corolla (2.5 mm) is white, pink, glabrous, with 4 subtle lobes, and the upper emarginate. The ovary glabrous, superior, is formed of 2 boxes each containing 2 ovules. The four stamens are exserted (Arvy and Gallouin 2003). Flowering takes place from July to September. It is a honey plant. The fruits are achenes in 4 smooth parts on the surface and ovoids.

### IV.4.2. Traditional uses

An attractive herb, apple mint is often used as an ornamental plant. It is hardy and easy to grow, preferring full sun to lightly shady conditions. The leaves of this plant can be used to make apple mint jelly, as well as a flavoring in dishes such as apple mint couscous. It is also often used to make a mint tea, as a garnish, or in salads.

Round-leaved mint is traditionally used in the Mediterranean basin for its tonic, stomachic and antispasmodic effects. Herbalists, even if they know perfectly the various species of mints, treat the properties of "Mint" collectively (Lieutaghi 1966).

**Tab 5.** *Mentha rotundifolia* Taxonomic Hierarchy.

| **Kingdom** | **Plantae** |
|---|---|
| **Subkingdom** | *Viridiplantae* |
| **Infrakingdom** | *streptophyta* |
| **Superdivision** | *embryophyta* |
| **Division** | *tracheophyta* |
| **Subdivision** | *Spermatophytina* |
| **Class** | *Magnoliopsida* |
| **Superorder** | *Asteranae* |
| **Order** | *Lamiales* |
| **Family** | *Lamiacea* |
| **Genus** | *Mentha.* |
| **Species** | *Mentha Rotundifolia* |
| **Arabic name** | **تيمجة** |

### IV.4.3. Ethnopharmacology

The leaf of *Mentha suaveolens* contains secondary metabolites such as flavonoids and terpenoids. The essential oil is extracted from the leaves by hydro distillation. Its chemical composition varies greatly with the climatic, edaphic, cultural conditions and the time of the harvest. Several chemotypes were found (Božović et al. 2015). Some *Mentha suaveolens* have the oxide of piperitone and the oxide of piperidone as main components (Lawrence 2006). Other specimens have high percentages of alcohols such as menthol, or ketones such as pulegone, piperitone and dihydrocarvone (Oumzil et al. 2002).

Three types where distinguished as a whole (Oumzil et al. 2002) :

- Oil rich in pulegone ;
- Oil rich in piperidine oxide ;
- Oil containing similar amounts of piperitenone oxide and piperitone oxide.

The analysis of the essential oil of leaves of *Mentha suaveolens* taken from two localities of the Middle Atlas in Morocco gave for one, a dominance of the oxide of piperitenone (with a rate of 74.69% in Azrou) And for the other a richness of piperitone oxide (81.67%) (Zekri et al. 2013). The subspecies *M. suaveolens* subsp. Timija of Morocco (Kasrati et al. 2013) indicates a dominance of menthone (39.4%), pulegone (34.3%) and isomenthone (7.8%). The variety 'Chocolate' grown in Korea is rich in carvone (37.4%), germacrene D (11.9%) (Park et al. 2011). A study carried out in Egypt on the composition of the essential oil showed substantial variations during the seasons (El-Kashoury et al. 2012). Carpone is the major component in spring (31%) in summer (56%) and fall (35%), while limonene becomes the majority (26%) in winter, followed by carvone (25%).

The antibacterial activity of Moroccan sweet mint rich essential oil was evaluated on 19 bacterial strains and three fungi. Pulgone-rich essential oils strongly inhibit all bacteria and those rich in piperidine oxide are less active (Oumzil et al. 2002). Pulgone is the most active aromatic component but unfortunately it is also a hepatotoxic component (Thomassen et al. 1990). Limonene and carvone have moderate antimicrobial activity compared to pulegone and piperitone oxide. The antioxidant activity of mints is due to their polyphenol content. Measurements are therefore made on methanol extracts. A study of 9 species of mint (Ahmad et al. 2012) Showed that the in vitro capacity to trap free radicals of DPPH• (2,2-diphenyl-1-picrylhydrazyl) was highest for *Mentha suaveolens* (82%) followed by *M. longifolia* (79%). The insecticidal activity of *Mentha suaveolens* was tested against rice weevil (*Sitophilus oryzae*). The essential oil of *Mentha suaveolens* is very toxic but considerable differences in insect mortality following oil fumigation are observed depending on concentrations and duration of exposure (Božović et al. 2015). The toxicity by fumigation is related to the abundance of piperitone oxide of the plant.

## V. Problematic overview

Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially polyphenols, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer. The effective extraction and proper assessment of antioxidants from food and medicinal plants are crucial to explore the potential antioxidant sources and promote the application in functional foods, pharmaceuticals and food additives. The present work focuses on four wild plants that are present and abundant in Morocco. These plants have been chosen for several reasons. These plants are used as food in a lot of regions in Morocco. *Ziziphus lotus* fruit for instance, is a snack that is sold and consumed largely all over Morocco. In all cases, these plants have been reknown for their ability to cure digestive system related issues. Three of these plants namely, *Urtica dioica*, *Chenopodium murale* and *Mentha rutondifolia* are small green plants that needs little to no maintenance and can be grown in most regions. *Ziziphus lotus* fruit however is different from the other plants, the aim was to asses the differences in terms of mechanism of action in the assays performed. This could be an addition to the study of the three other plants, in order to better understand the influence of the type of the vegetal material used. Several methods will be used to asses the properties of the extracts of these plants. Quantification techniques such as total phenolic content, total flavonoid content and tannins content are an important primary indicator of the extracts composition. Along with other techniques such as DPPH and ABTS, it will give us a better understanding of the antioxidants work mechanism exhibited by the studied plants. Corrosion inhibition assays were also performed. Althought their main purpose is to study materials surface protection under corrosive conditions, it can also be used as an indicator of the type of compounds present in the inhibitor used. Corrosion inhibition have been linked to secondary metabolites, when vegetal extracts are used as an inhibitor. High accuracy mass spectrometry is a useful tool for chemical identification because it provides accurate mass and structural information especially high-resolution mass spectrometric techniques. It is the perfect tool to complete the previously cited tests. The readings from the results obtained from such methods, can lead to a better understanding of the pharmacological properties of these plants.

# Chapter II

# Materials and methods

# I. Targeted plants

**Urtica dioica**

Kingdom: *Plantae*
Order: *Rosales*
Family: *Urticaceae*
Genus: *Urtica*
Species: *Urtica dioica L.*
Binomial name: *Urtica dioica L.*

**Mentha rotundifolia**

Kingdom: *Plantae*
Order: Lamiales
Family: Lamiaceae
Genus: Mentha
Species: M. suaveolens
Binomial name: Mentha suaveolens

**Chenopodium mural**

Kingdom: *Plantae*
Order: *Caryophyllales*
Family: *Amaranthaceae*
Genus: Chenopodiastrum
Species: *Chenopodium mural L.*
Binomial name: *Chenopodiastrum murale*

**Ziziphus lotus**

Kingdom: *Plantae*
Order: *Rosales*
Family: *Rhamnaceae*
Genus: Ziziphus
Species: *Z. lotus*
Binomial name: *Ziziphus lotus L.*

**Fig 32.** Targeted plants.

## II. Sampling

The studied plants were harvested from their natural wild habitat. The location of harvesting was choosed based on the frequency of usage. For instance, Zaouate cheickh in one of the best-known regions in Morocco for the *Ziziphus lotus* fruit. In khemisset, people use *Mentha rutondifolia* for bread and pies recipes. For *Urtica dioica* and *Chenopodium murale*, they have similar growing conditions, and they are widespread all over the bouskoura forest. The sampling conditions of the studied plants are summurized in Tab 6.

**Tab 6.** Sampling information of the studied plants.

| Scientific name | Date of cultivation | Location | Organs |
|---|---|---|---|
| *Urtica dioica* | Mai 2015 | Bouskoura forest | - Whole plant<br>- Leafs<br>- Barks<br>- Seeds |
| *Chenopodium murale.* | Mai 2015 | Bouskoura forest | - Whole plant<br>- Leafs<br>- Barks<br>- Seeds |
| *Mentha rutondifolia* | August 2015 | Khemisset | - Whole plant<br>- Leafs<br>- Barks<br>- Seeds |
| *Ziziphus lotus* | August 2014 | Zaouate cheikh | - Fruit |

Immediately after harvest, the plants are washed and shade dried to remove all cell debris and organisms potentially responsible for deterioration of their quality. Plant material was air-dried at room temperature. For *Urtica dioica, Chenopodium murale* and *Mentha rutondifolia*, barks, leaves and seeds were selected and blended and powdered afterwards, the whole was also blended for further use. For *Ziziphus lotus*, the whole fruit was used.

## III. Extraction methods

### III.1. Solvent based extracts preparation

The method of extraction of the biomolecules used in the laboratory is sonication, due to its simplicity of execution, saving of time, ambient temperature, in addition to low solvent usage and high yield of extracts (Palma and Barroso 2002; Biesaga 2011). Polyphenols, a class of rather water-soluble molecules, are predominantly extracted by solvents of medium to high polarity. Thus, the solvents, which have been retained for our study, are methanol and ethanol that have an average polarity, and water whose polarity is the highest. Ethanol was used in mixture with water, ethanol/water (80/20, V/V). The choice of these solvents is justified by the several studies reporting great yields of polyphenols using them (Qasim et al. 2016; Do et al. 2014). The extracts were then concentrated using rotary vacuum evaporator (Heidolph G 1, Germany) at 60-75°C under vacuum and dried residues were stored in Eppendorf tubes at -4°C for further studies.


**Fig 33.** Image representation of the experimental setup used for ultrasonic assisted extraction.

### III.2. Oil extraction

A Soxhlet extractor (or Soxhlet apparatus) is a piece of glassware used in analytical chemistry and organic chemistry that allows continuous solvent extraction of a vegetal material in a solid powder state. This device bears the name of its inventor: Franz von Soxhlet.

For oil recovering, in the case of *Ziziphus Lotus*, which contains vegetale oil, hexane is the solvent of choice. Due to its non polar characteristic hexane is able to extract oils, which are also non-polar, easily. And with its low boiling point, the recovery of the oil is rather easy. It has been established by previous studies that it grants the highest yield of recovered oil.

The extraction was carried out for 6 hours with a temperature of 40°C. The extract was then concentrated using rotary vacuum evaporator (Heidolph G 1, Germany) at 40°C under vacuum to recover the oil, that was stored in glass test tubes at -4°C for further studies.

**Fig 34.** Image representation of the experimental setup used for soxhlet extraction.

## IV. Determination of total phenolic content

The determination of total phenols is based on the oxidation of phenolic compounds and the development of a coloration. The most used method for this purpose is that of Folin-Ciocalteu, a spectrophotometric method based on the reduction of a mixture of phosphotungstic and phosphomolybdic acids (Folin-Ciocalteu reagent) in a mixture of blue oxides of tungsten and molybdenum. The disadvantage of the Folin-Ciocalteu method is that it gives overestimated levels due to interference with other compounds such as vitamin C and sugars, possibly present, which also reduces the Folin reagent (Pérez-Jiménez et al. 2010; Ignat et al. 2011). For this reason, Li et

al. (2006) and Georgé et al. (2005) proposed purification of the crude extract prior to determination of total phenol content to remove organic acids and sugars.

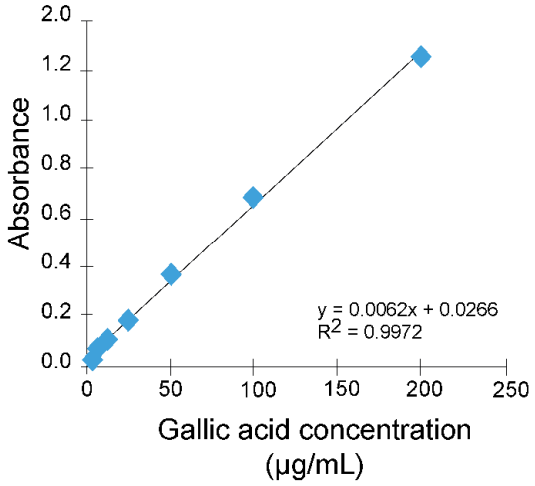



**Fig 35.** Gallic acid standard curve.

The total phenolic contents assay was performed according to the Folin-Ciocalteu method as described by Lister and Wilson (2001). In short, 0.5 mL of the sample solution was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted with distilled water to a ratio of 1:10, then 4 mL of $Na_2CO_3$ (7.5 %, w/v) was added. The obtained solution was then incubated in a water bath at 45°C for 30 minutes. The absorbance was then measured at 765nm using a UV-Vis spectrophotometer against a blank sample. Under the same conditions as above, the standard curve of gallic acid was obtained, using a range of concentrations (0-300 mg/L). The total phenolic content was measured as Gallic acid equivalent (mg GAE/g extract).

## V. Determination of flavonoids content

Several methods are available to measure the content of total flavonoids: ***(i)*** a colorimetric method based on the complexation of phenolic compounds with Al(III), ***(ii)*** the Prussian blue method (spectrophotometric method similar to the Folin method, sometimes used for quantification of tannins) (Pérez-Jiménez et al. 2010). The total flavonoids assay was performed using a colorimetric method (Marinova et al. 2005). Approximately, 1 mL of dissolved sample was placed in a 10 ml volumetric flask. Distilled water was added to a 5 mL volume; 0.3mL of $NaNO_2$ was then added. 0.3 mL of $AlCl_3$ was added after 5 minutes and the obtained solution was allowed to

stand for about 6 minutes. Afterwards, 2 mL of 1 M NaOH was added and the total volume was increased to 10 mL using distilled water. The solution was well homogenised and allowed to stand for 30 minutes. The absorbance was recorded against a blank at 510 nm. The rutin equivalent from the calibration curve of Quercitin standard solutions was used to determine the flavonoid content, and it was then expressed as mg quecetin equivalent (QE)/g extract.

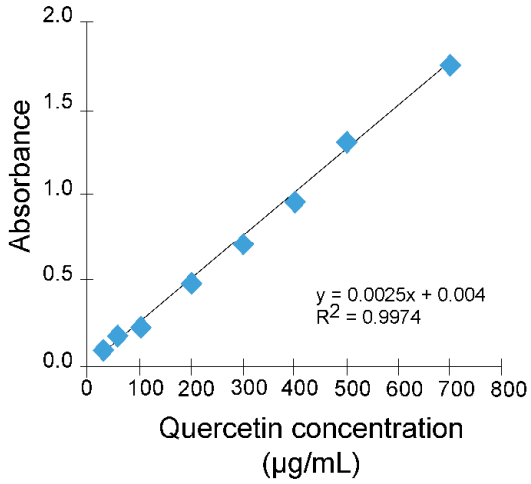



**Fig 36.** Quercetin standard curve.

## VI. Determination of proanthocyanidins content

Condensed tannins or proanthocyanidins content was performed as described by Sun et al. (1998). 500µL of the extract solution was mixed with 3mL of 4% vanillin–methanol solution, 1.5mL hydrochloric acid was then added. The mixture was left to stand for 15 min. The absorbance was measured at 500 nm, while the final result was expressed as mg catechin equivalent (CE)/g extract.

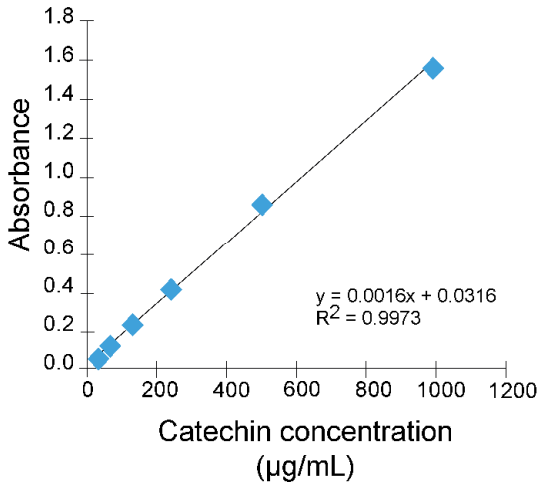



**Fig 37.** Catechin standard curve.

## VII. Antioxidant Activity (AA)

Many methods of measuring the antioxidant power have been developed in recent years. We distinguish the measurements made on living matrices (in vivo measurement) and on food matrices (in vitro). During in vitro measurements, the antioxidant activity is evaluated by measuring the oxidation inhibition of a specific substrate. The substrate is first oxidized under standard conditions, and then the extent of oxidation at an end point is measured. Many methods have been developed combining different substrates, initiations and endpoints.

Nature of the substrate: They are often of a lipid nature: methyl-linoleate (Heinonen et al. 1998), lipoproteins (Davalos et al. 2003) or carotenoids (Tsao et al. 2005).

Starting point: The oxidation of the substrate can be initiated by increasing the temperature (accelerated aging of the lipid compounds), catalyzed by metal ions or linked to the generation of free radicals.

End of measurement point: It is possible to measure the inhibition by an antioxidant compound at the end of the oxidation reaction or during the reaction, to measure the delay in oxidation in the presence of antioxidants.

The antioxidant capacity can also be evaluated as the potential to trap free radicals by directly measuring the inhibition of the radical when adding the antioxidant compound. These tests use

2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable colored radical, or 2,2'-azino-bis- (3-ethylbenzthialozine-6-sulfonic acid) (ABTS), whose radical colored can be generated by enzymatic reaction (Rice-Evans et al. 1996).The discoloration of the radical after addition of the antioxidant is then measured.

### VII.1. DPPH Free radical scavenging activity

DPPH$^{\bullet}$ (2,2-diphenyl-1- picrylhydrazyl) is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Thus, DPPH• does not dimerize, as happens with most free radicals. The delocalisation on the DPPH• molecule determines the occurence of a purple colour, with an absorbtion band with a maximum around 520nm. When DPPH• reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the purple colour. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. The free radical scavenging activity of the stinging nettle extracts was measured by 1.1-diphenyl-2-picryl-hydrazil (DPPH) (Huang et al. 2011). A 0.2 mM solution of DPPH in methanol was prepared, 0.5 mL of this solution was then added to 2.5 mL of plant extract and was left to stand at room conditions for about 30 minutes, and the absorbance was read at 517 nm against blank samples. High free radical scavenging activity is equivalent to low absorbances of the reaction mixture. Eq (1) was used to calculate the radical-scavenging activity (RSA) as a percentage of DPPH discoloration:

$$\%\mathrm{RSA} = \frac{A_D - A_E}{A_D} \times 100 \qquad \text{Eq (1)}$$

Where $A_D$ is the absorbance of the DPPH blank sample, and $A_E$ is the absorbance of the test solution. $A_E$ was calculated as the difference between the absorbance of the test solution and the absorbance of its blank.

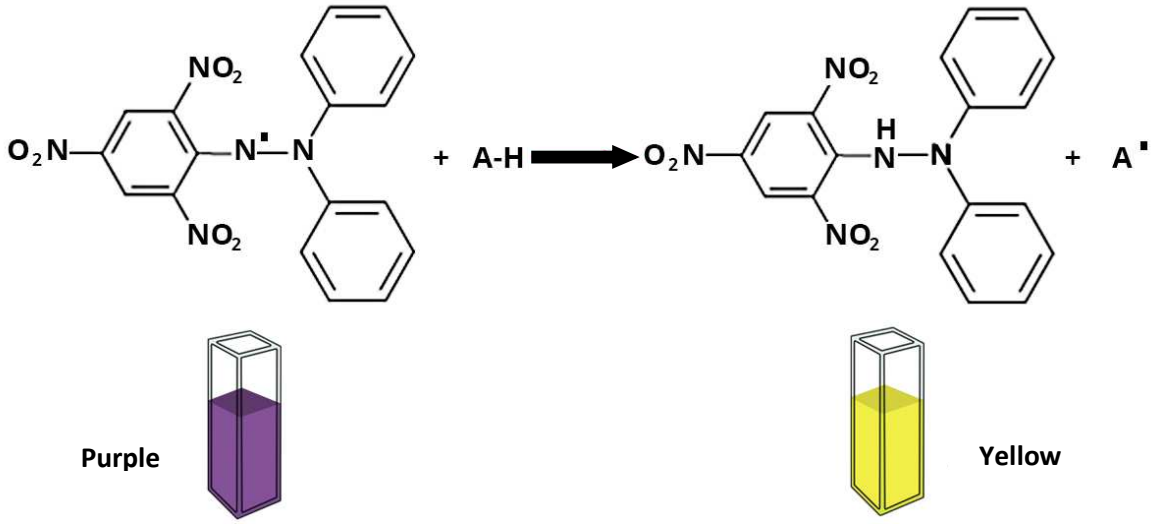



**Fig 38.** Illustration of the DPPH assay work mechanism.

### VII.2. ABTS radical scavenging assay

The ABTS cation radical ($ABTS^{\bullet+}$) which absorbs at 743 nm (giving a bluish-green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). The ABTS free radical decolorization assay was performed according to the method developed by Roberta Re et al (1999). The working solution was prepared by mixing two stocks solutions of 2 mM ABTS solution and 70 mM potassium persulphate solution with equal volumes, the obtained solution was allowed to react for 24 h at room temperature in the dark. Using methanol, the solution was diluted to obtain the absorbance of 0.7 units at 734 nm. Later, 2 mL of the resulting solution was allowed to react with 200µL of the plant extract with different concentrations and the reaction mixture was vortexed and the absorbance was measured at 734 nm with a one minute interval. The free radical scavenging capability was calculated using the Eq (2) and expressed as the percentage of inhibition rate of free radial scavenging compared with the blank.

$$\text{ABTS radical scavenging activity (\%)} = (1 - \text{Abs sample} - \text{Abs blank}) \times 100 \qquad \text{Eq(2)}$$

Where Abs blank is the absorbance of the blank sample, and Abs sample is the absorbance of test sample.

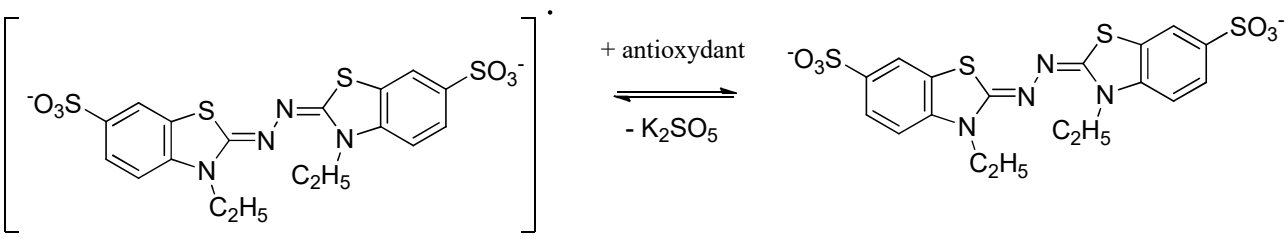



**Fig 39.** Illustration of the ABTS assay reaction.

## VIII. Antibacterial activity

Extracts were tested against the clinically pathogenic gram (-) bacteria, *Escherichia coli*, *Agrobacterium sp.*, *Rhizobium sp.* and gram (+) bacteria, *Bacillus pumilus* and *bacillus subtilis*. Antibacterial activity was determined using agar well diffusion assay according to NCCLS (Cockerill et al. 2012). Petri plates containing 20 ml broth agar medium were seeded with bacterial strains for 24 h. Wells (6 mm diameter) were cut into the agar and 10 µl of extracts were tested in a concentration of 100 mg/mL. The plates were then incubated at 28°C for 24 hours. The inoculum size was adjusted to deliver a final inoculum ~107 colony-forming units (CFU/mL). The antibacterial activity was assayed by measuring the diameter of the inhibition area formed around the well. Minimum inhibitory concentration (MIC) was determined by the micro-dilution method using serially diluted extracts (2-fold) according to NCCLS (Cockerill et al. 2012). Bacterial inoculum were adjusted to contain approximately 107 CFU/mL.

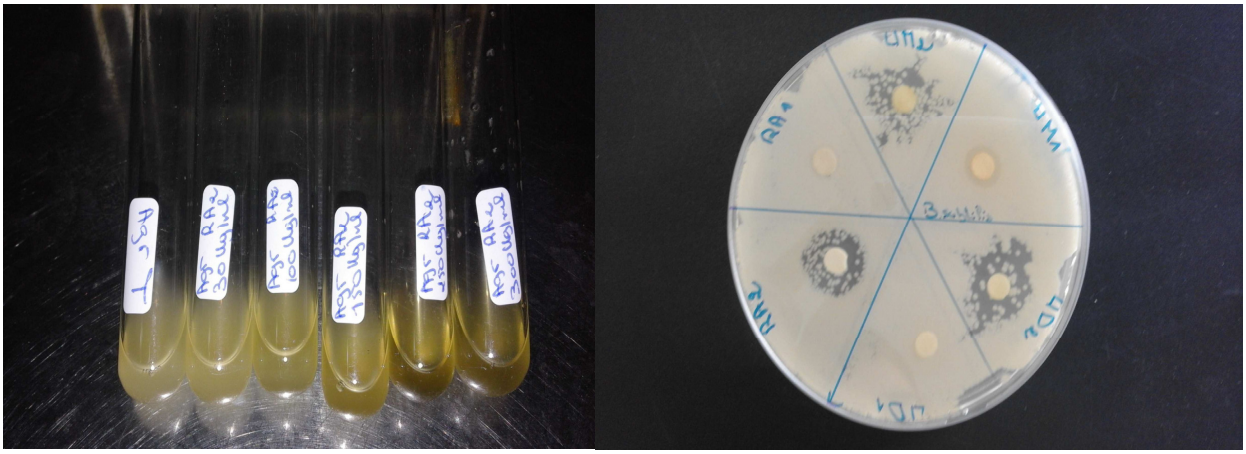


**Fig 40.** Left: Observation of bacterial growth for the MIC assay, Right: Observation of bacterial growth inhibition for the disc difussion method.

## IX. Gas-Chromatography for fatty acids composition

Fatty acids were turned into methyl esters beforehand, by homoginasing a mixture of 0.3 mL of 2N methanolic potassium hydroxide, 60 mg oil and 3 mL of hexane for 25 min. Varian CP-3800 gas chromatograph equipped with an FID detector, was used to identify the FAMEs in the sample. a CP- Wax 52CB column (30 m×0.25 mm) was used. 1mL was the volume injected using a split injector. The rate of the carrier gas, which is Helium, is 1 mL/min. 170 °C was the first column temperature, which was gradually augmented by 4°C/min until reaching the final temperature of 230°C. 230°C was set as the initial injector, and final detector temperature. Relative percentage was given as a result to each fatty acid presents in the sample.

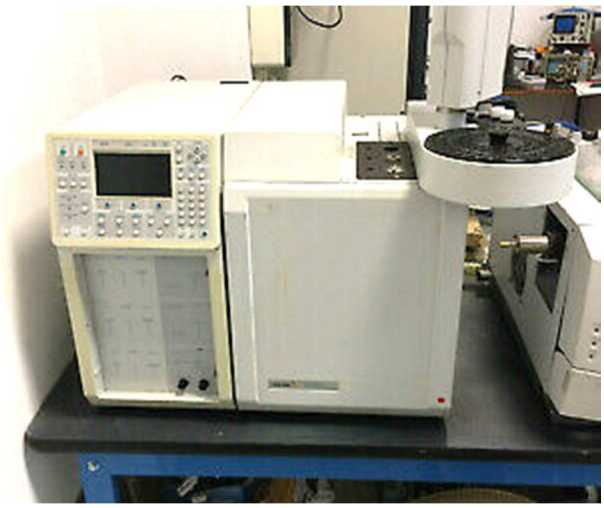


**Fig 41.** Varian CP-3800 gas chromatograph with an FID detector.

## X. Corrosion inhibition assays

### X.1. Material and study medium

Ferrous metals are widely used materials in the industry; their applications range from buildings to food cans, electronic components or the hulls of some boats. Cutting tools and commonly manufactured parts are mostly made of ferrous metals.These materials are subjected to many aggressive external stresses making themvulnerable to corrosion (temperature heating, hydrodynamics, etc.). The material tested in this study is C38 (AFNOR XC38). The composition of C38 steel is given in the Tab 7 below:

**Tab 7.** Chemical composition of C38 carbon steel (wt.%).

| C | Si | Mn | S | Cr | Ti | Ni | Co | Cu |
|---|---|---|---|---|---|---|---|---|
| **0.380** | 0.230 | 0.680 | 0.016 | 0.077 | 0.011 | 0.059 | 0.009 | 0.160 |

The contents of elements Mn, Si, S, Cu, Cr, Ti, Ni, Co and O (a few thousandths of a %) are relatively low. The low proportion of these elements makes it possible to be based on the Iron-Carbon diagram (Fig 42); Borders will be very little displaced by their presence. However, despite their low levels, the "residual" elements considerably affect the mechanical behavior of the steel. An observation, after metallographic attack of the surface of the steel, made it possible to check its composition. In particular, the different constituent phases could be highlighted. The attack is carried out by dipping for a few seconds the steel, previously polished, in a solution of "nital" (mixture of nitric acid and alcohol in a ratio of 3/100). A scanning electron microscope teveals the two phases present. As shown in Fig 42, the ferrite or α phase appears much clearer than the perlite, consisting of ferrite and cementite ($\alpha + Fe_3C$).

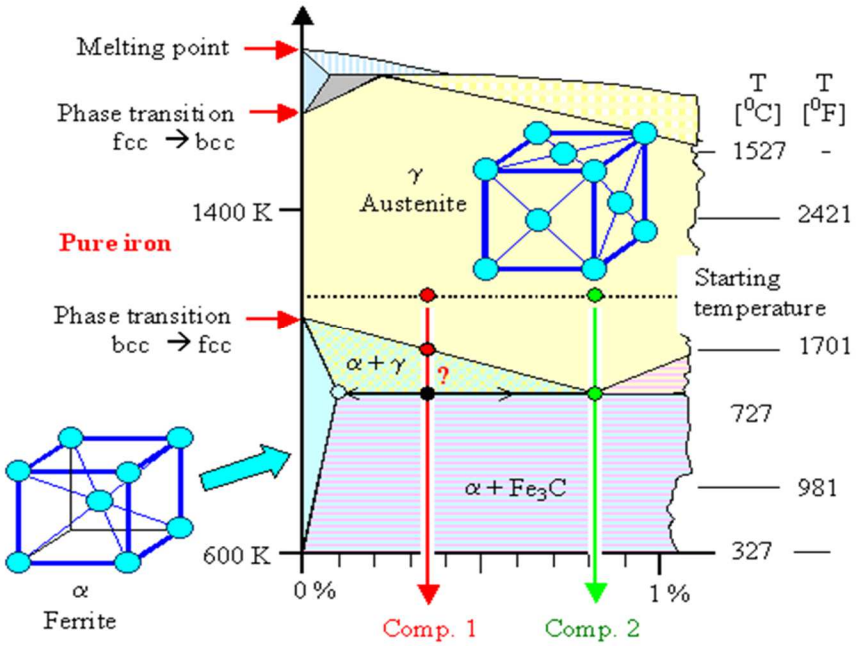



**Fig 42.** Iron-carbon equilibrium phase diagram.

The corrosive solutions that were used are:

- A solution of phosphoric acid $H_3PO_4$ 5.5M, obtained by dilution, with distilled water, of the 85% concentrated commercial acid. This is the typical concentration of phosphoric acid used in the manufacturing industry.
- A solution of hydrocloric acid HCl 1M, obtained by dilution, with distilled water, of the 37% concentrated commercial acid.

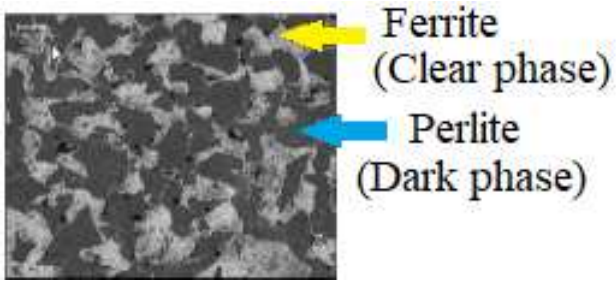



**Fig 43.** Image of the surface of a C38 steel taken by scanning electron microscopy, after nital attack.

## X.2. Immersion test: Gasometric measurements

The gasometric technique is considered as a fast and reliable way to evaluate the protective effect of an inhibitor against corrosion in acid medium. Gasometric methods were conducted at 20°C as described in literature (Oguzie et al. 2006; Umoren et al. 2006) .The ease and efficiency of the gasometric technique, as well as its ability to monitor in situ, any disturbance by an inhibitor of gas evolution in metal / solution systems have been established (Solmaz 2010).
In the case of corrosion of steel in acidic medium, the measurement of the evolution of hydrogen makes it possible to quantify the damage, by using the following relation:

$$V_{corr} = \frac{\Delta V_{H2} \times M}{V_{mol} \times \rho \times S \times \Delta t} \qquad \text{Eq(3)}$$

With $V_{corr}$: corrosion rate (cm / year)

$\frac{\Delta V_{H2}}{\Delta t}$ : Volume of hydrogen released per unit of time (L/an)

$V_{mol}$ : molar volume (24,055 l·mol-1 à 20 °C under an atmosphere)

M : molar mass of steel (g/mol)

$\rho$ : Density of the metal (g/cm$^3$)

S : surface of the sample in contact with the solution (cm$^2$)

Note: The equation above is valid when the cation involved is divalent. In the case where the cation $M^{n+}$ is not divalent, the balance of the charges between the anodic and cathodic reactions must be taken into account.

## X.3. Electrochemical techniques for the corrosion evaluation

### X.3.1. Electrochemical appartus

The study of electrochemical processes requires an apparatus capable of controlling and measuring the potentials and / or the electric currents on an electrochemical interface. An electrochemical cell

with three electrodes is used: working electrode, reference electrode and counter electrode (or auxiliary electrode).

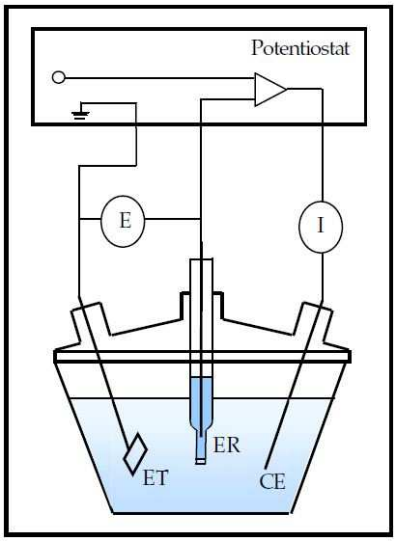



**Fig 44.** Graphical representation of the 3 electrodes setup: ET: Work electrode; ER: Reference electrode and CE: Counter electrode.

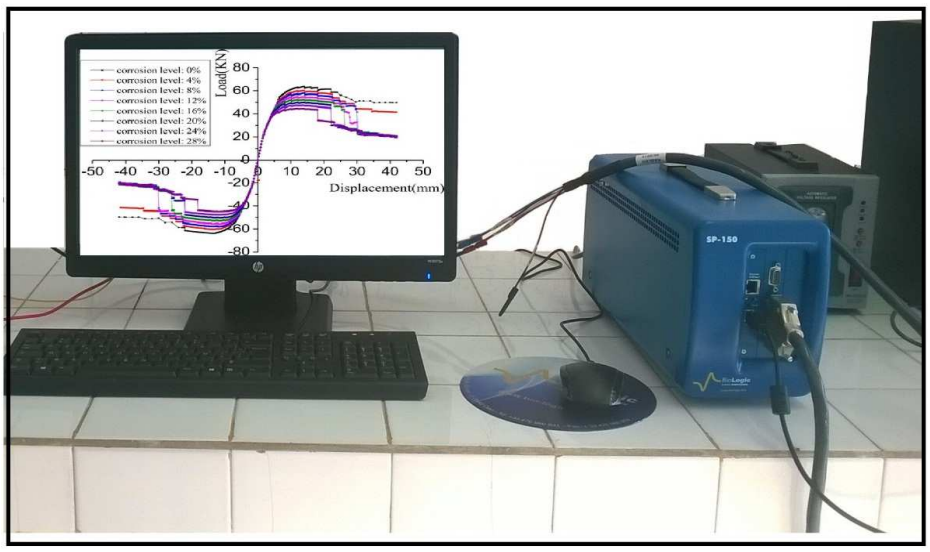



**Fig 45.** The used experimental setup.

- A platinum electrode as an auxiliary electrode;
- The reference electrode used is a saturated calomel electrode (ECS) radiometer analytical, schematized by the electrochemical sequence Hg / $Hg_2Cl_2$ / KClsat. This has a potential of + 0.241 V with respect to the standard hydrogen electrode.
- C38 steel as working electrode. The latter is in the form of a disc is disposed facing the auxiliary electrode.

The electrochemical measurements were carried out using a Biologic instrument of type SP 150, controlled by EC-Lab software V11.01. Before recording the cathodic polarization curves. The potential applied to the sample continuously varies to positive potentials with a scanning speed of 50 mV/mn. We chose a relatively low scanning speed in order to be in quasi-stationary mode. The polarization data was analyzed using the EC-Lab V11.01 software and the Tafel extrapolation method was used to obtain corrosion current density values. From the polarization curves obtained, the inhibition efficiency was evaluated from the measured $I_{corr}$ values using the Eq (4):

$$\%IE = \left(\frac{I^{o}_{corr} - I^{i}_{corr}}{I^{o}_{corr}}\right) \times 100 \qquad \text{Eq(4)}$$

Where $I^{\circ}_{corr}$ and $I^{inh}_{corr}$ are uninhibited and inhibited corrosion current densities, respectively.

Electrochemical impedance spectroscopy (EIS) measurements were performed after 1 hour of open circuit immersion (OCP) immersion in an aerated solution. The sinusoidal wave voltage is 10 mV peak-to-peak at frequencies between 100 kHz and 10 mHz. The linearity of the system was verified by varying the amplitude of the AC signal applied to the sample. Impedance curves were adjusted by EC-Lab software V11.01. Each experiment was performed three times to verify reproducibility. The inhibition efficiency of the inhibitor can be calculated from the charge transfer resistance values using the Eq (5):

$$\%IE = \left(1 - \frac{R^{\circ}_{ct}}{R_{ct}}\right) \times 100 \qquad \text{Eq(5)}$$

Where $R^{\circ}_{ct}$ and $R_{ct}$ are the charge transfer resistance in the absence and presence of different concentrations of inhibitor, respectively.

### X.3.2. Stationary method: Plotting of polarization curves

A metal immersed in any electrolytic medium tends to dissolve and electrically charge with the creation of a double electrochemical layer comparable to an electrical capacitor. After a time long enough for a stationary regime to be established, the metal electrode takes a potential, called the corrosion potential (Ecorr), with respect to the solution. This potential can not be known in absolute value. It is marked with respect to a reference electrode. If, using an external generator and a counter-electrode, a current is passed through the metal electrode, its stationary state is modified, and its surface takes a new potential value. The intensity-potential curves E = f (I) or I = f (E) thus obtained constitute the polarization curves. The device used for the drawing of these curves consists of a conventional three-electrode circuit consisting of a potentiostat, a generator programming the evolution of the potential as a function of time, and a recorder.

The plot of the polarization curves provides information on the kinetics of the slowest step in the overall corrosion process, which consists of different elementary reactions (charge transfer, material transport, species adsorption on the electrode, etc.). Since the speed of the overall reaction is determined by that of the slowest step, the plot of the polarization curves can be used to measure the rate of corrosion. Three types of polarization curves are observed depending on the kinetics of the reaction:

**a) Activation kinetics or charge transfer:**

This first case is verified when the reaction occurring at the Electrode | Electrolyte interface does not cause a significant change in the concentration of the electroactive species in the electrolyte. The agitation of the medium then has no influence on the kinetics. In this case, the relation between the measured transfer current and the electrode overvoltage η (η = E - Ecorr), is given by the simplified formula of the Butler-Volmer fundamental equation:

$$\mathrm{i} = i_a + i_c = i_{corr}\left(\exp\left(\frac{2.303}{b_a}\eta\right) - \exp\left(\frac{2.303}{b_c}\eta\right)\right) \qquad \text{Eq(6)}$$

where ia and ic are the densities of the anodic and cathodic partial currents, icorr the corrosion current, ba and bc the Tafel slopes of the anodic and cathodic reactions in logi = f (E) representation; with:

$$b_a = \frac{2.303 \times RT}{\alpha nF} \text{ et } b_c = \frac{2.303 \times RT}{(1-\alpha)nF} \qquad \text{Eq(7)}$$

Where F is the Faraday constant, R is the universal constant of perfect gases, T is the absolute temperature, n is the number of electrons transferred, and α is the charge transfer coefficient.

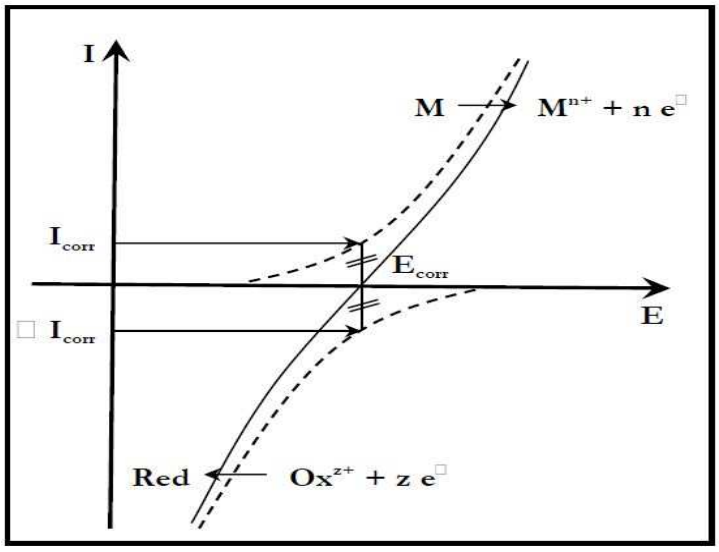



**Fig 46.** Current-potential curve relative to metal M in a solution containing. Red / $Ox^{z+}$ (Control by a process of transfer of charges); dashed lines: partial polarization curves anodic and cathodic.

In the case of strong anodic (ηa) or cathodic (ηc) overvoltages (> 100 mV), one or another of the anode and cathode currents of the Butler-Volmer equation becomes negligible. In this case, after simplification of the Butler-volmer formula, a linear equation between the electrode overvoltage and the logarithm of the measured current density, known as Tafel's law, is obtained:

$$\log i = a + \frac{\eta_i}{b_i} \qquad \text{Eq(8)}$$

(Anodic branches (i=a) and Cathodic branches (i=c))

Where “a” is a constant. The extrapolation, at the corrosion potential, of the cathodic or anodic Tafel line (or both) provides the corrosion current.

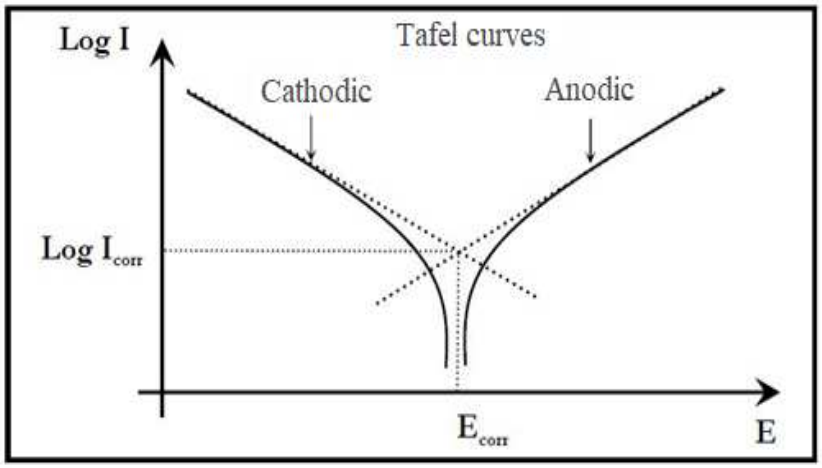



**Fig 47.** Polarization curves in semi-logarithmic and straight lines of Tafel of an equilibrium redox system in the absence of a limitation by a transport material.

**b) Diffusion kinetics or transport material (concentration polarization)**

It occurs when the diffusion rate of a molecule or ion towards the electrode limits the rate of reaction at the electrode. This phenomenon is encountered in particular in a ventilated environment where the consumption of oxygen at the metal / electrolyte interface is not completely compensated by the flow of dissolved oxygen from the core of the solution. The reaction is then limited by the transport of material. In this case, the polarization curves show a diffusion plateau which corresponds to a limit current ($I_L$). The corrosion rate is then equal to the density of the diffusion limit current; it is affected by agitation of the solution or rotation of the electrode.

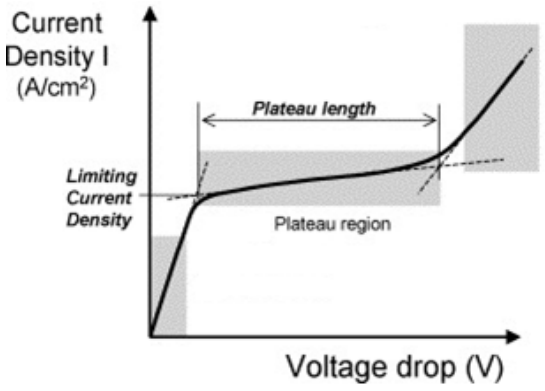



**Fig 48.** Schematic drawing of a typical voltage current curve indicating the diffusion phenomenon characterized by a plateau with a limited current.

### c) Mixed kinetics

Thanks to a correction of the diffusion using the formula:

$$\frac{1}{I} = \frac{1}{I'} + \frac{1}{I_L} \qquad \text{Eq(9)}$$

where I is the measured current, corresponding to the mixed diffusion activation process, I' the corrected current of the diffusion and $I_L$ the limiting diffusion current, one obtains a Tafel-type linear equation and Icorr is obtained by extrapolation to the corrosion potential, as in the case of a pure activation kinetics. The plot of the polarization curves makes it possible to confirm the indications given by the evolution of the corrosion potential and to specify them by distinguishing the influence of the inhibitor on each of the elementary reactions, anodic and cathodic, at the electrode.

This method makes it possible to estimate the corrosion rates fairly quickly and its implementation is relatively easy in the context of a laboratory (by the use of a potentiostat). It must be remembered, however, that:

- The adsorption conditions of the inhibitor at the surface can be modified by an increasing polarization of the electrode: the recovery rate can vary with the applied potential, the inhibitor can be desorbed at a certain potential, etc. The interpretation of the curve I = f (E) must therefore be made taking into account these possibilities;

- The corrosion current measured in the presence of the inhibitor is related to the geometric surface of the sample and does not necessarily give the true dissolution current density of the metal, particularly if the adsorption of the inhibitor leads to localization of the corrosion process (recovery rate θ).
- Its principle is essentially based on the assumption that the anodic and cathodic reactions occupy the entire surface and take into account the mixed potential and not the potential for thermodynamic equilibrium.

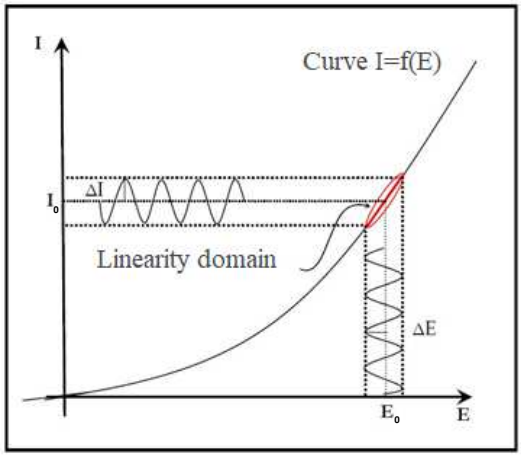



**Fig 49.** Principle of the linearization of a nonlinear electrochemical system subjected to a sinusoidal disturbance around an operating point.

### X.3.3. Transient Methods: Electrochemical Impedance Spectroscopy

This method consists in measuring the response of the electrode to a low amplitude sinusoidal modulation of the potential ΔE (t) as a function of the frequency f. Indeed, electrochemical systems are generally non-linear and non-stationary systems. Their periodic dynamic study can however be carried out around an operating point ($E_0$, $I_0$), which is assumed to be quasi-stationary, using signals of low amplitude for which the behavior of the system can be linearized and during a determined period during which there is no observable evolution of the system. Under these conditions, the behavior of the system can be likened to that of a Linear Time Invariant System (SLIT) (Diard et al. 1996; Gabrielli 2002).

The SLIT is experimentally characterizable by the graph H (ω) of its transfer function H (p) (p being the Laplace operator) which is independent of the shape and amplitude of the excitation

signal used for its tracing. The behavior of a nonlinear electrochemical system is characterized by the set of graphs H (ω) of its transfer function measured along its stationary current-voltage curve. The transfer function is the electrode impedance Z (ω) when the system is current controlled and the electrode admittance Y (ω) = 1 / Z (ω) when it is voltage controlled.
The electrochemical system can indeed be considered as a "black box" which reacts by emitting a signal y (t) when it is subjected to a disturbance x (t).The two signals are then connected by a transfer function H (ω) such that:

$$Y(\omega) = H(\omega)\, X(\omega) \qquad \text{Eq(10)}$$

X (ω) and Y (ω) being respectively the Fourier transforms of x (t) and y (t).

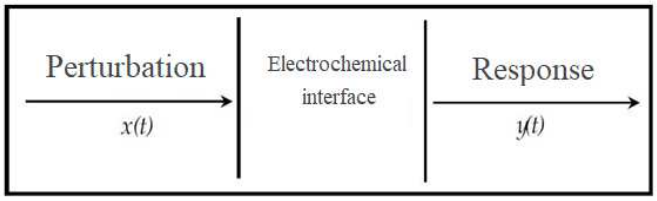



**Fig 50.** Schematic of a transfer function.

Conventionally, the perturbation applied to the DC component of the voltage is sinusoidal. It is provided by a programmable frequency generator. The total expression of the voltage is given by:

$$E = E_0 + \Delta E \sin(\omega t);\ \omega \text{ being the pulsation } (\omega = 2\pi f) \qquad \text{Eq(11)}$$

The sinusoidal current response obtained after a relaxation time is then superimposed on the DC bias current $I_0$ which defines the steady state studied, with a phase shift φ between ΔE and ΔI. Its expression is of the following form:

$$I = I_0 + \Delta I \sin(\omega t + \varphi) \qquad \text{Eq(12)}$$

For each excitation frequency, the impedance Z(ω) around the operating point ($I_0$, $E_0$) is then defined as the ratio between the perturbation signal and the associated response:

$$Z(\omega) = \frac{\Delta E(\omega)}{\Delta I(\omega)} = \frac{|\Delta E| e^{(j\omega t)}}{|\Delta I| e^{(j(\omega t - \varphi))}} = |Z| e^{j\varphi} \qquad \text{Eq(13)}$$

The impedance Z(ω) is a complex number characterized by its Z module and its phase φ. It can be written in the form:

$$Z(\omega) = Z_{Re}(\omega) + jZ_{Im}(\omega) \; avec \; j = \sqrt{-1} \qquad \text{Eq(14)}$$

$$|Z| = \sqrt{(Z_{Re}^2 + Z_{Im}^2)} \qquad \text{Eq(15)}$$

The frequency analysis of the electrochemical impedance will make it possible to differentiate the various elementary phenomena as a function of their characteristic frequency (or time constant). Each disturbed process returns to the steady state with its own response time. The partial reactions occurring at the electrode / electrolyte interface can therefore be differentiated from each other: Fast electrochemical phenomena (charge transfer) are solicited in the high frequency domain, whereas slow phenomena (matter transport: diffusion adsorption) occur at low frequencies.

Several types of diagrams are used to represent the electrochemical impedance Z(ω). The Nyquist diagram is obtained by plotting in the complex plane the impedance graduated in pulsation ω or in frequency. Electrochemists, unlike electricians, carry the opposite of the imaginary part of the impedance $-Z_{Im}(\omega)$ as a function of its real part $Z_{Re}(\omega)$. The Bode diagrams (module and phase) are obtained by drawing respectively logZ(ω) and the phase as a function of log(ω) or log(f). These two modes of representation of the impedance give visualizations of the different results but remain complementary. The presentation of Bode will be preferred when information observed at high frequency is masked by the Nyquist representation. It makes it possible to better visualize the inflection points of the impedance module, the phase variations as well as the different time constants of the phenomena involved. Conversely, the identification of certain characteristic phenomena taking place at the same time. working electrode interface | Electrolyte will be facilitated by the Nyquist representation which allows to determine parameters such as the resistance of the electrolyte (Re), the charge transfer resistance (Rt) and the double layer capacitance ($C_{dl}$).

The impedance of any electrochemical system can be assimilated by analogy to an electrical impedance. It is therefore possible to associate with this system an equivalent electrical circuit or EEC, where each physico-chemical phenomenon taking place at the metal | electrolyte interface can be modeled by an electrical component.

It should be noted, that the interpretation of experimental impedance diagrams via EEC must respect two essential conditions:

- All elements of the circuit must have a precise physical meaning, associated with the physical properties of the system;
- The spectrum simulated from the EEC should be as faithful as possible to the experimental spectrum and the error should not be systematic as a function of frequency.

In dynamic mode, a simple electrolyte electrode interface behaves like a capacitor called an interfacial double layer capacitor and denoted $C_{dl}$; this double layer being described by three principal models: Helmotz, Gouy-Chapman and Stern (Gabrielli 2002; Landolt 1993).The dynamic behavior of a redox reaction in the absence of a concentration gradient of the electroactive species is therefore analogous to that of the electric circuit of Fig 51, called the generalized Randles circuit.

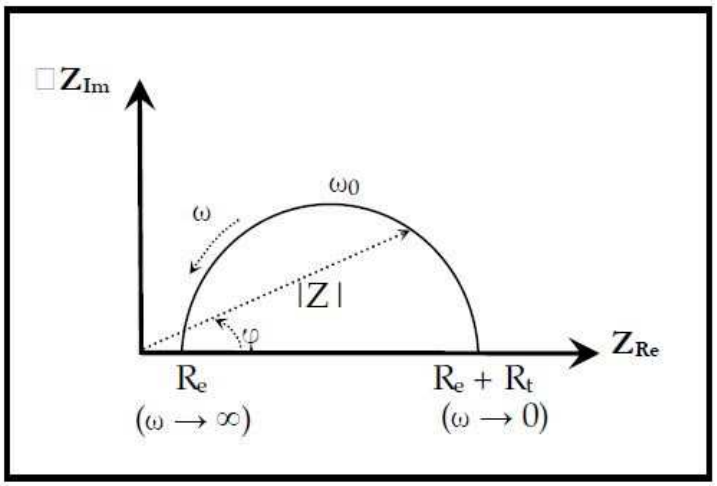



**Fig 51.** Nyquist graph of a parallel RC circuit (reaction with charge transfer without diffusion). The arrow indicates the direction of the increasing frequencies.

This circuit also includes the charge transfer resistance involved in the dissolution (Rt), as well as the resistance of the electrolyte (Re) which represents the ohmic drop in the electrolyte between

the working and reference electrodes when a current passes. Note that $C_{dl}$ and Rt are introduced in parallel to account for the fact that the total current crossing the interface is the sum of the distinct contributions of the faradic process (faradic current which corresponds to the transfer of electrons through the interface and which is due to electrochemical reactions) and the double-layer charge (capacitive current which is due to the change in the distribution of electrical charges at the interface; this current exists even in the absence of an electrochemical reaction at the interface). The Nyquist graph corresponding to this Randles circuit is represented in Fig 52.

For the values $\omega = 0$ and $\omega = \infty$, the imaginary part of the total impedance vanishes, which makes it possible to determine the values of Rt and Re. The value of the double layer capacitance is obtained by the equation:

$$C_{dl} = \frac{1}{\omega_0 R_t} \qquad \text{Eq(16)}$$

With $\omega_0 = 2\pi f_0$; $f_0$ being the frequency for which -Zim reaches a maximum on the Nyquist diagram. This cutoff frequency makes it possible to define the different time constants of the circuit.

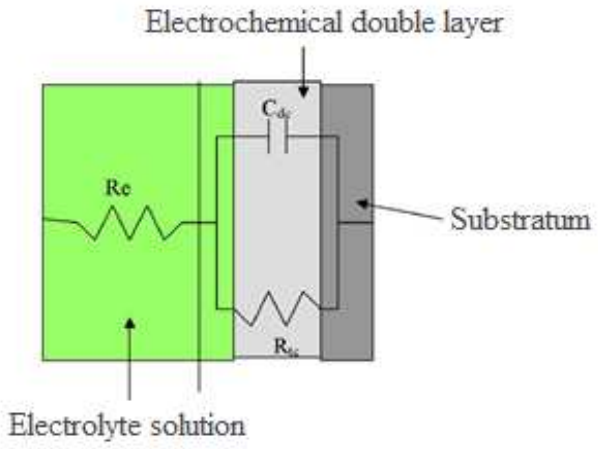



**Fig 52.** Randles circuit of a simple electrochemical interface.

## XI. Plants extracts identification

Ultrahigh-resolution mass spectra were acquired on a Bruker solariX Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) (Bruker Daltonics GmbH, Bremen, Germany) equipped with a 12 Tesla superconducting magnet and an Apollo II ESI source (Bruker Daltonics GmbH, Bremen, Germany) operated in the negative ionization mode. Negative ionization has already been proven to be the preferred ionization mode in fingerprinting plant extracts by FT-ICR-MS. Samples were introduced into the electrospray source at a flow rate of 120 µL $h^{-1}$. The instrument was externally calibrated based on arginine clusters and an internal calibration was performed for each mass spectrum using appropriate reference list, yielding mass accuracies lower than 0.1 ppm in routine day-to-day measurements. Spectra were acquired with a time domain of 4 MW over a mass range from m/z 100 to 1000 and two hundred scans were accumulated for each sample. For molecular formulas, FT-ICR mass spectra were exported to peak lists. From those lists, possible elemental formulas were calculated for each peak in batch mode by a home-made software. The generated formulas were validated by setting sensible chemical constraints (O/C ratio $\leq$ 1, H/C ratio $\leq$ 2n + 2, element counts: C $\leq$ 80, H $\leq$ 80, O $\leq$ 30) in conjunction with an automated theoretical isotope pattern comparison. For sample classification, Hierarchical Clustering Analysis-unsupervised method (HCA) was performed using Hierarchical Clustering Explorer 3.5 software (Maryland, USA) on the normalized data. Pearson metric and average linkages were chosen to measure distance. This method allows samples to be grouped into homogeneous and distinct clusters, without imposing preliminary hypotheses on the data.

# CHAPTER III

# Results and discussion

# Part I

# Antioxidant and antibacterial based bioactivities of plant extracts

# Review paper I

# A bio-phytochemical review on *Urtica dioica*


## Summary

In this review, a focus was dedicated to *Urtica dioica.* (stinging nettle), a wild-growing, annual and perennial plant species from the *Urticaceae* family. *Urtica dioica* is a plant found worldwide, and is the most known of the *Urtica* genus. The interest of this plant comes from the many uses that it accumulated during its history, for skin and nutritional disorders. Recent studies have brought to light the facts justifying these uses, such as the antihyperglycemic, antifungal and anti-inflamatory poperties that it holds. Cardiovascular, immunity and organs protection are the major issues that this plant has an effect on. A large pool of secondary metabolites have been identified from this plant and the major families of compounds are phenolics, flavonoids, anthocyanins, fatty acids and volatile compounds. Hence, the well-known flavonoids such as soquercetin, kaempferol, isoharmnetin and rutin are found in its extracts. Some anthocyanins and volatile compounds identified in *Urtica dioica* are chlorogenic acid, caffeoylmalic acid, p-coumaric acid, peonidin-3-O-glucoside, carvone, naphtalene, anethole and carvacrol. The pharmacological activities reported in this review confirm the therapeutic value of *Urtica Dioica.* Throughout several studies performed on it, *Urtica dioica* is considered as an important source of phytochemicals, especially with a large variety of compounds that are for the most known for being active and for their pharmacological properties.

# A bio-phytochemical review on *Urtica dioica* (under review)


## Abstract

Plants have been used as medication for centuries; and they are still used for their several health benefits. One such plant is *Urtica dioica* from the *Urticacea* family, commonly known as stinging nettle, grows and can be found all over the world. Different studies in various countries showed its health benefits (i.e., China, Morocco, Ireland, etc.). It has a long history as a nutrition source and as medicine. Many bioactive compounds are found in *Urtica dioica*, mainly, phenolics, flavonoids, anthocyanins amongst others. In traditional medicine, it is mostly used for its antioxidant, wound disinfection, galactogogue, antibacterial and antifungal properties. Therefore, we aimed in this review to shed light on the available studies related to the pharmacological properties of *Urtica dioica.*


### 1. Introduction

Generally, different groups of secondary metabolites are found in plants and the most important ones are flavonoids and phenolic acids (Kim et al. 2003). These compounds have antioxidant abilities and can scavenge free radicals, which grants them anti-aging and anticancer properties. It has been reported that flavonoids can help reduce lipid and glucose levels in blood for humans. The pharmaceutical industry relies often on plant bioactive compounds structures to discover immunity enhancing drugs for humans (Atoui et al. 2005).

*Urtica dioica* (stinging nettle) is a wild-growing, annual and perennial plant species from the *Urticaceae* family. Native to Europe, Asia, northern Africa, and North America, *Urtica dioica* is the best-known member of the nettle genus *Urtica* (Bisht et al. 2012). Its extracts were used to treat several common diseases and disorders such as rheumatism, eczema, arthritis, gout, and anaemia for over thousands years (Mills and Bone 2013; Chrubasik et al. 1997). Different types of compounds have been described in several studies from *Urtica dioica*, including phenolic acids, flavonoids, fatty acids and phenolics (Farag et al. 2013; Orčić et al. 2014). Its use in traditional medicine was approved by its efficiency regarding antihyperglycemic activity and noticeable

cytotoxicity (Johnson et al. 2013; Bnouham et al. 2003). On the other hand, its alcoholic extracts have shown antifungal and anti-inflammatory activity (Johnson et al. 2013; Hadizadeh et al. 2009).

In addition to its application in medicine, leafs stinging nettle has been used since ancient times in our food lifestyle. The use of nettle as a nutritional source has been reporter in Canada by Thompson Indians and also in the United kingdom (Vickery 1993; Beith 1995; Richard Mabey 1996; Turner et al. 1990). Grieve (1992) reported the use of nettle juice in cheese making. Likewise, in Cronwall, UK, nettle is used as vegetarian rennet and its leaves as a wrapper for cheese production (Randall 2002). It is also mentioned that it is used as a galactagogue for breast-feeding humans to increase milk supply (Mills 1988). Another use of nettle is the production of green dye. During the second world war period, the British government requested a huge scale collection of these plants for the production of green camouflage dye (Vickery 1993). The aim of this review is mainly to highlight the important medicinal propertiesof *Urtica dioica* plant.

## 2. Medicinal / Therapeutic uses of Urtica

### 2.1. Local application of nettles

The use of nettle leafs sting is a way to treat joint pain (The urtication effect). Several reports have published this mode of use in sevreal countries (i.e., Australia (Czarnetzki et al. 1990), Germany (Weiss 1991), India (Shah and Joshi 1971), North America (Duke 1997), South America (Brisley 1994), Italy (Cappelletti et al. 1982), and the United Kingdom (Thomson 1976; Mills 1991; Bradley 1992; Randall 1994; Richard Mabey 1996; Randall et al. 1999; Randall et al. 2000)). Thus, different methods have been mentioned, for instance beating the painful areas with a quantity of nettles (Pahlow 1992; Vickery 1995), cutaneous application of the leaves (Schauenberg and Paris 1977; Mills 1991; Lust 1983; Stary 1991; Bradley 1992; Duke 1997; Randall et al. 1999; Randall et al. 2000; Randall 2001), the use of nettle juice (Cyran 1981), and boiled leaves (Lal and Yadav 1983). Another application of this type of plants is the use of its stewed liquor as an antiseptic wash for infected wounds (Schauenberg and Paris 1977), burns, cuts, and eczema (Wren 1988). American Native Indians traditionally used nettle extracts for dental pain (Duke 1997). Furthermore, one of the most interesting uses of nettles is as an ingredient to the the world famed kneipp hair remedy (Lust 1983).

### 2.2. Nettle taken as an internal medication

The use of nettle as internal medication is mostly stricted to leaves ingested as teas, capsules or decoction. Lust (1983) reported the use of nettle tea as a cure to breast, lung, stomach and urinary system problems. Considering the fact that this tea is recommended by herbalists for diabetic problems, contradictive studies have shown that it both increased (Oliver-Bever and Zahland 1979) and decreased sugar levels in blood (Wren 1988; Newall et al. 1996), and therefore cannot be recommended (Bisset 1994). The nettle tea is used in Germany as a mild diuretic for hypertension problems (Tyler 1994). Accordingly, Zhang (1994) listed *Urtica doica* as one of the most used nettles for medicinal purposes in China. In rural areas in Europe, boiled leaves are still consumed for their high nutritional value, since it is rich in minerals and vitamins (Castleman 1991; Vickery 1995; Duke 1997). Its high levels of vitamin C and iron supports its use traditionally as a cure for anaemia and scurvy (Stary 1991; Patten 1993; Sapronova 1989).

## 3. Phytochemical compositions

### 3.1. Phenolic compounds

Phenolic compounds contains a wide range of chemicals, and is one of the greatest classes of secondary metabolites. With a variety of structures, these compounds are responsible for organoleptic characteristics of plants and plant derived products. A study conducted by Otles and Yalcin (2012) was focused on phenolic identification using samples from different locations, specifically from the black Sea, Marmara, and the mediterranean region. In this investigation, Otles and Yalcin (2012) have been able to uncover the presence of multiple compounds in different parts of the plant. Roots contained myricetin, rutin, ellagic acid, ferulic and naringin. Myricetin, isorhamnetin, ferulic, naringin, syringic, quercetin and kaempferol were found in stalk extracts. And finally, leaves extracts exhibited the presence of myricetin, quercetin, rutin, ellagic, with also caffeic, chlorogenic and fumaric acids (Otles and Yalcin 2012). In addition, Pinelli et al. (2008) reported that leaves content of this plant contain a large quantity of caffeic acids derivatives, such as chlorogenic and 2-O-caffeoylmalic acid. However, the results showed that depending on the origin of the sample, the composition may vary, where some compounds could be missing or barely present depending on the origin of the nettle sample (Otles and Yalcin 2012). Several studies conducted on *Urtica dioica* have revealed the presence of various phenolic compounds. A study conducted by Orčić et al. (2014), on this plant from Serbia, uncovered the presence of important

quantities of phenolics. Findings in this study suggest that the major compounds present in the 80% methanol extracts are 5-O-caffeoylquinic acid with 3.6% of extract by weight, quercetin 3-O-rhamnosylglucoside (rutin) and quercetine-3-O-glucoside (isoquercitrin). It was also reported that these compounds were mostly concentrated in the inflorescence extracts (Orčić et al. 2014).

Accordingly, minor compounds were also detected in the form of chlorogenic acid, quinic acid and caffeic acid. However, they did not exceed 0.19% of the extract by weight. The phenolics composition in root's extracts differed from the aboveground ones. In this case, the most abundant compounds were secoisolariciresinol, p-coumaric acid, quinic acid and scopoletin. The abundance of phenolics in this part of the plant was significantly lower and did not exceed 0.086% of extract by weight (Orčić et al. 2014). In addition, Grevsen et al. (2008) showed the presence of several phenolic acids and derivatives like 3-O- and 5-O-caffeoylquinic acids, 5-O-feruloylquinic acid and 2-O-caffeoylmalic acid.

### 3.2. Flavonoids

Flavonoids are widely distributed in plants, fulfilling many functions (Tinea et al. 2017). Stalk extracts of *Urtica dioica* showed the presence of flavonoids in the form of glycosides (i.e., quercetin 3-O-glucoside, 3-O-rutinosides of quercetin, kaempferol, and isorhamnetin with Rutin) being the most abundant flavonol detected (Pinelli et al. 2008). Accordingly, other type of flavonoids (i.e., glucoside, diglucoside and rutinoside of quercetin, rutinosides of kaempferol and isorhamnetin) are also reported by Orčić et al. (2014).

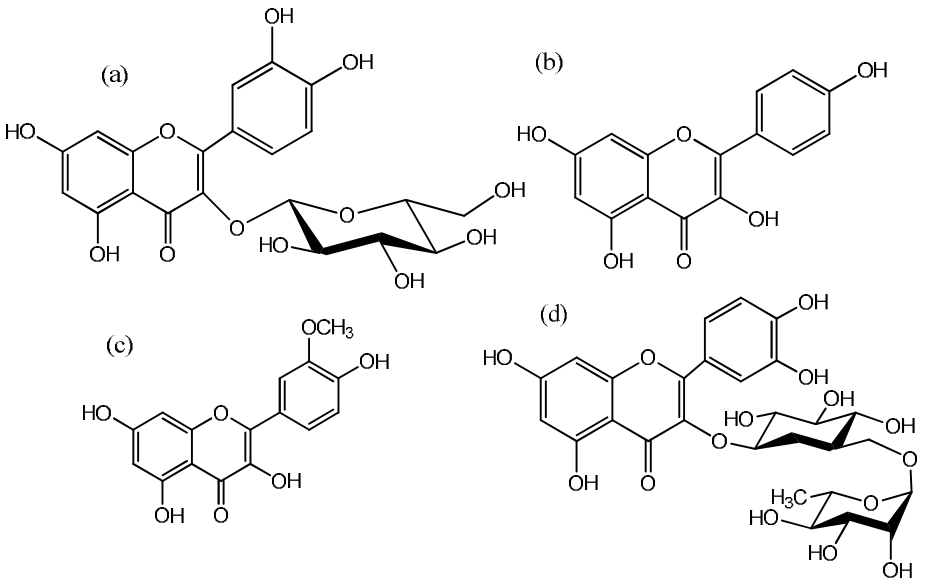



**Fig 53.** Some identified flavonoids in *Urtica dioica* (a): soquercetin, (b): Kaempferol, (c): Isoharmnetin, (d): Rutin.

### 3.3. Anthocyanins

Anthocyanins are important pigments of the vascular plants; they are soluble in water, which makes them interesting for use as natural aqueous colorants (Alexandra Pazmio-Durán et al. 2001). These compounds are responsible of many colors in flowers and plants fruits. Due to their antioxidant activity, they are studied for their neuronal and cardiovascular problems prevention, among other health related issues such as cancer and diabetes (Konczak and Zhang 2004). A spectrophotometric method for anthocyanin glycocides analysis was conducted on *Urtica dioica* from Western Georgia, where Kavtaradze and Alaniya (2003) have successfully detected pelargonidin glycosydes. In addition, Pinelli et al. (2008) reported that anthocyanin compounds are abundant in the aerial parts of nettle. Anthocyanin glycosides compounds were only found in stalk extracts, represented by the presence of chlorogenic acid and another caffeoylquinic acid, 2-O-caffeoyl-malic acid, p-coumaric acid, and a caffeic acid derivative (Pinelli et al. 2008). These anthocyanin glycosides contained peonidin 3-O-rutinoside, peonidin p-coumaroylglucoside, and rosinidin 3-O-rutinoside (Pinelli et al. 2008). Rosinidin 3-O-rutinoside was reported for the first time, as it is rare to find rosinidin glycosides in plants (Iwashina 2000).

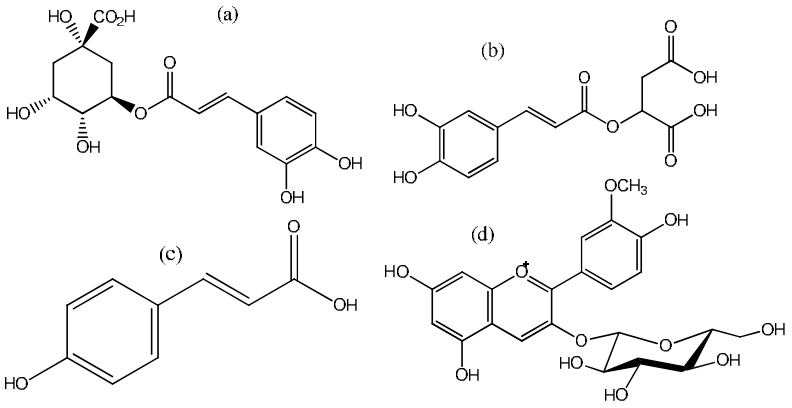



**Fig 54.** Identified anthocyanins in *Urtica dioica* (a): Chlorogenic Acid, (b): Caffeoylmalic Acid, (c): p-Coumaric Acid, (d): Peonidin-3-O-glucoside.

### 3.4. Fatty acids

Fatty acids are an important sources of fuel because, when metabolized, they yield large quantities of ATP. It has been stablished that several fatty acids have important antibacterial and antifungal properties (Russell 1991; Gleńsk and Włodarczyk 2017). Scientific evidence shows that stinging nettle incorporate a fairly large amount of fatty acids in its leaves (Dar et al. 2013). The presence of 24.86% fatty acid esters, in the form of hexyl octyl ester, butyl tetradecyl ester, and 1,2 benzenedicarboxylic acid was also demonstrated (Dar et al. 2013). Another study revealed the presence of other types of fatty acids such as palmitic, linolenic, nonadecanoic acids and their isomers, along with benzofurandicarboxylic acid (Lapinskaya and Kopyt'ko 2009).

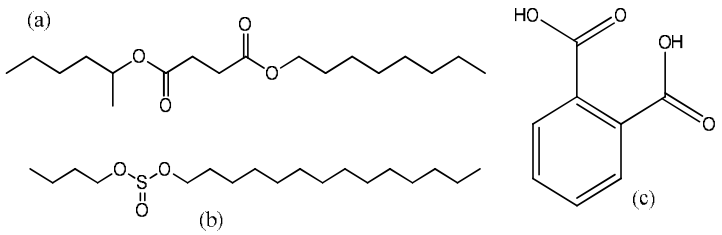



**Fig 55.** Major Fatty Acids found in *Urtica dioica* (a): Succinic Acid, 2-hexyl oxtyl ester, (b): Butyl tetradecyl ester, (c): Phtalic Acid.

### 3.5. Volatil compounds

GC-MS analysis of essential oil extracted from aerial parts of nettle from Turkey revealed the presence of 43 compounds accounting for 95.8% of the essential oil. In this study, major compounds representing 72.2% of the oil were found to be carvacrol (38.2%), carvone (9.0%), naphthalene (8.9%), (E)-anethol (4.7%), hexahydrofarnesyl acetone (3.0%), (E)-geranyl acetone (2.9%), (E)-b-ionone (2.8%) and phytol (2.7%) (Gül et al. 2012).

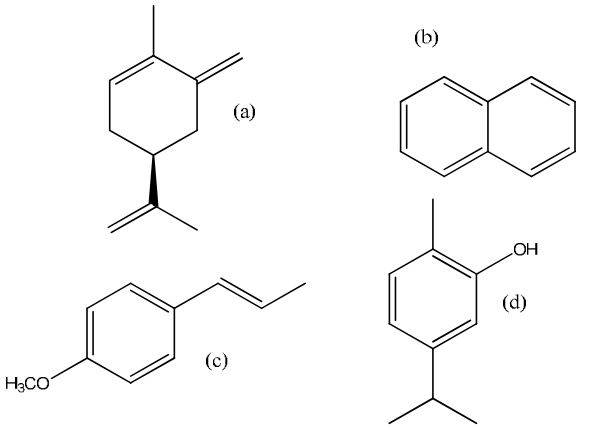



**Fig 56.** Major volatil compounds found in *Urtica dioica* (a): Carvone, (b): Naphtalene, (c): Anethole, (d): Carvacrol.

## 4. Biological Activities

### 4.1. Immunity and immune system

A study on the immunodulatory activity of *Urtica dioica* aerial parts exctracts has shown that neutrophils were stimulated by the flavonoids. Quercetin-3-O-rutinoside was confirmed as a major chemoattractant. Quercetin-3-O-rutinoside, keampherol-3-O-rutinoside and isorhamnetin-3-O-rutinoside were tested with the NBT reduction test and were shown to increase the intracellular killing activity of neutrophils with no statistically significant differences. Along with the flavonoid fraction, these compounds showed significant immuno-stimulating effect on neutrophils. Rutin was determined to be the major chemoattractant and increased significantly the intracellular killing activity of the neutrophils (Akbay et al. 2003). The three compounds (i.e., Quercetin-3-O-rutinoside, keampherol-3-O-rutinoside and isorhamnetin-3-O-rutinoside) were reported to indicate

significant immune-stimulatory activities on neutrophils, they could possibly be useful for treating patients suffering from neutrophil function deficiency to chronic granulomatous disease (Akbay et al. 2003).

The Stinging nettle was also evaluated for its antioxidant properties. Total antioxidant activity of water nettle extract was determined by the thiocyanate method. The water extracts of nettle exhibited effective antioxidant activity. Similar behavior was reported for reducing power of these extracts. Both the reducing power and total antioxidant activity increased concentration dependently. All of the concentrations showed higher activities than the control in a statistically significant manner (Gülçin and Küfrevioğlu 2004). A significant decrease in the concentration of DPPH radical due to the scavenging ability of the water extracts has been reported (Gülçin and Küfrevioğlu 2004). The water extract of nettle antioxidant compound interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine (Gülçin and Küfrevioğlu 2004).

Nettle has been used for inflammatory diseases such as allergies for decades, and is documented, but the role of nettle in the pro-inflammatory pathways that characterize hay fevers has been obscure (Roschek et al. 2009). The plant demonstrated a positive anti-inflammatory activity, where accessions with high phenolic content showed more effective effect (Farag et al. 2013).

Several studies showed that the nettle extract has H1-receptor antagonism and negative agonism activity, mechanisms of action similar to traditional OTC anti- histamines. Since the nettle extract is rich in bioactives that are effective H1-receptor antagonists, it will prevent histamine from binding to the receptor, preseventing the responses to histamine triggered allergic responses (Roschek et al. 2009). Along with the Histamine receptor inhibition assays, the mast cell tryptase inhibition assay was conducted, where the nettle extract effectively inhibits mast cell tryptase activity with an $IC_{50}$ of 172 µg/mL (Roschek et al. 2009). It was also shown that bioactive compounds in the nettle extract such as 4-shogaol, deoxyharringtonine and carnosol are effective inhibitors of COX-1 and COX-2, and possess theoretical $IC_{50}$ values between 40 and 90 µM (Roschek et al. 2009). In another hand, the water extracts were also tested for antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Citrobacter koseri, Enterobacter aerogenes, Staphylococcus aureus, Streptococcus pneumonia, Micrococcus luteus, Staphylococcus epidermidis,* and *Candida albicans*. Of these species, *Staphylococcus aureus* is

one of the most common bacteria causing food poisoning. The extracts exhibited antimicrobial activity against all tested microorganisms (Gülçin and Küfrevioğlu 2004). These aqueous extract have also shown antiviral activity when tested in vitro on feline immunodeficiency viruses, indicated a good inhibition on the development of syncytia with low doses (0.5–1µg/ml) and increased when higher concentration were used. However, at a certain point it starting showing cytotoxic effects with great concentrations (Uncini et al. 2005).

### 4.2. Cardiovascular system

Cardiac and vascular effects of nettle's water extract were investigated on the isolated rat heart and aorta. The results have shown a dose-dependent bradycardia of nettle's water extract without intrinsic modification of contractile capacities. Also, the water extract showed that its effect is independent of the cholinergic pathway, since it persisted in presence of atropine (a muscarinic receptor antagonist) (Legssyer and Ziyyat 2002). The vascular effect of the water extract of nettle was also investigated, on rat isolated aorta. This essay showed that the water extract provoked a dose dependent increase in the basal contraction without relaxing N-Adr- or KCl-precontracted aorta, meaning that the extract has a vasoconstricting and not a vasorelaxing action (Legssyer and Ziyyat 2002).

It was also reported that aqueous extract of *Urtica dioica* had an immediate dose dependent decrease of the arterial pressure. Althought a vasorelaxing effect and a negative inotropic action were observed (Testai et al. 2002). In a different study, the hypotensive effect of nettle was reported, where there has been a correlation between the continuous intravenous perfusion of aqueous extract of *Urtica dioica* and the reduction of the arterial blood pressure suggesting that the *Urtica dioica* treatement had a positive impact on cardiovascular activity. It was also reported that this extract has toxic effect at the higher dose (Tahri et al. 2000). In alloxan induced diabetic rats, the oral administration of the aqueous extract of *Urtica dioica* did not modify the blood glucose level. However, strong antihyperglycemic effect of *Urtica dioica* has been reported for rats under oral glucose tolerance test, a fall of approximately 33% which lasted for 120 min (Bnouham et al. 2003). In the intestinal glucose absorption test, nettle water extract significantly reduced the absorption of glucose without any signs of intestinal irritation (Bnouham et al. 2003).

It has been also reported that the active components in the *Urtica dioica* extract responsible for glucose uptake are a series of cyclic peptides, having a specific repeating NSX motif (Domola et al. 2010). In a different study, *Urtica dioica* was used to treat diabetic mice, and was compared to streptozotocin (a naturally occurring chemical that is particularly toxic to the insulin producing beta cells of the pancreas in mammals). Treatment with *Urtica dioica* significantly reversed the streptozotocin induced alteration in bodyweight, water intake, blood glucose and insulin level (Patel and Udayabanu 2013 and 2014). The *Urtica dioica* treatment also showed significant improvement in spatial and associative memory dysfunction, and also reduced hypoalgesia. These findings suggest that nettle has a great effect in reversing the long standing diabetes induced complications such as central, as well as peripheral neuronal dysfunction (Patel and Udayabanu 2013). It was also reported that *Urtica dioica* extract reversed significantly the corticosteroid mediated depressive and diabetic like state (Patel and Udayabanu 2014).

### 4.3. Organs protection

*Urtica dioica* extracts were investigated for their antiulcer properties. Pretreatment with aqueous nettle extracts were found to inhibit the ethanol induced gastric mucosal Preventive effects in a dose dependent manner (Gülçin and Küfrevioğlu 2004). On the other hand, the petroleum ether extracts of *Urtica dioica* have shown positive results in presenting prostatic hyperplasia along with the ethanoic extract on rats, where a 50 mg/kg dose showed the best activity for the petroleum ether extracts (Nahata and Dixit 2012). *Urtica dioica* treatement showed positive impact for prostatic hyperplasia on humans. On a double blind test, patients had a substantial and lasting favorable effect with the *Urtica dioica* treatment (Safarinejad 2005). It has been proven that *Urtica dioica* extracts have a 5α-reductase inhibitory activity. It was also noticed that the extracts have a positive effect on hypertrophy of the prostate, since it has been noticed that there was a considerable decrease in urinary obstruction (Nahata and Dixit 2012).

The administration of *Urtica dioica* extract to rats have shown that it improves the hepatic damage, decreased a-SMA, cytokeratin- positive ductular proliferation and the activity of TUNEL in the BDL rats, which suggests that nettle extracts may be used as a remedy to cholestatic liver injuries (Sayhan et al. 2012). It was also demonstrated that treatment with *Urtica dioica* prevented ischemia/reperfusion injury in the rat kidney, and showed normal glomeruli and slight mononuclear cell infiltration. Treatment with *Urtica dioica* markedly increased the reactivity of

cell proliferation in the renal cortical tissues. The obtained evidence suggests that *Urtica dioica* extract has a protective effect against proximal tubule damage after ischemia/reperfusion injury in the rat kidney (Sayhan et al. 2012). In other studies, *Urtica dioica* aqeous extracts had diuretic and natriuretic effects on rats, suggesting a direct and efficient action on the renal function (Tahri et al. 2000).

## 5. Conclusion

*Urtica dioica* is a widely traditionally used and potent medicinal plant that is world widely distributed and known for its applications in traditional medicine. The pharmacological activities reported in the present review confirm the therapeutic value of *Urtica Dioica.* Throughout several studies performed by researchers on it. It is an important source of compounds, especially with a large variety of metabolites that are for the most known for being active and for their pharmacological properties. The presence of phytochemical constituents and pharmacological activities proved that the plant has a leading capacity for the development of drugs.

# Paper II

# Phenolic compounds quantification, antioxidant and antibacterial activities of different parts of *Urtica dioica* and *Chenopodium murale*


## Summary

The present paper is an assembly of multiple comparative assays that were performed on *Urtica dioica* and *Chenopodium murale.* Assays performed include DPPH and ABTS antioxidant assays, as well as total phenolics, flavonoids and condensed tannins quantification. Besides those tests, antibacterial assays were done using disc diffusion and micro dilution methods. TPC, TFC and CTC assays have shown that *Urtica dioica* contains higher levels of phenolics than *Chenopodium murale*. These results were in line with the antioxidant assays. UD extracts were more potent with the strongest response in leaves extracts. Nevertheless, CM seeds were the most efficient organ of the plant. The antibacterial ability exhibited by these plants was assessed for gram positive and negative strains, which further confirms their response to bacteria's growth. The most potent inhibitions were observed for *Urtica dioica* extract against "*Bacillus subtilis*" and "*Rhizobium sp.*" with a CMI value of 3 mg/mL. For CM, which is once again less effective than UD extract, the lowest CMI value was of 11 mg/mL against "*Bacillus subtilis*". Still, neither of the extracts used was efficient against the *Agrobacterium sp.* strain.

# Phenolic compounds quantification, antioxidant and antibacterial activities of different parts of *Urtica dioica* and *Chenopodium murale*

Walid Belmaghraoui, Amina Manni, Mourad Harir, Abdelkarim Filali-Maltouf, Souad El Hajjaji
(Research Journal of Pharmacy and Technology, 2019, 12:1)

**Abstract**

In this study, antioxidant and antibacterial activities of *Urtica dioica* (UD) and *Chenopodium murale* (CM) extracts as well as the levels of phenolic compounds (TPC), flavonoids (TFC) and condensed tannins (CTC) were investigated for all parts of these plants. The antioxidant ability was measured in an in-vitro mechanism using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and radical scavenging capacity, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonique) (ABTS) methods. The ability to scavenge radical was done by discoloration assay. Antibacterial tests were done using disc diffusion and micro dilution methods. The results reported here, demonstrate that seeds and leaves extracts of UD has potent antioxidant response. Phenolic compounds level were abundant in leaves extract of UD and seeds extract of CM. While TFC levels were abundant in both plants, CTC values were higher in UD seeds extract and CM leaves extract. In addition, UD extract with the highest content of TPC and TFC exhibited a stronger antibacterial activity than CM, with a minimal concentration of inhibition as little as 3 mg/mL against *Rhizobium sp*. and *Bacillus subtilis* strains, while it was 12 mg/mL and 11 mg/mL for CM for the same strains, respectively. The obtained results for the antibacterial and antioxidant activities were in line with the levels of phenolic compounds, flavonoids as well as condensed tannins in the extracts.

## 1. Introduction

Plants present a large chemical pool of natural antioxidants that can serve for the development of drugs (Sunkar et al. 2018). Antioxidants with a radical-scavenging capacity counteract free radical formation such as reactive oxygen species ROS (i.e., $O_2$, $O^{2-}$, $H_2O_2$, and $^{\bullet}OH$) involved in several health disorders (Premkuma 2018). ROS are mainly created through aerobic metabolism and

present as side products of metabolic pathways. However, higher doses of ROS become harmful for live organisms (Shah et al. 2001; Sharma and Dubey 2005; Mittler 2002; Maheshwari and Dubey 2009; Mishra et al. 2011; Srivastava and Dubey 2011; Verma and Dubey 2003; Meriga et al. 2004). It has been reported that degenerative or pathological diseases (i.e., aging) (Burns et al. 2001) , alzheimer's and cancer diseases (Smith et al. 1996; Diaz et al. 1997) , neurodegenerative disorders, atherosclerosis, cataracts, and inflammation (Aruoma 1998) were observed to be directly linked to ROS formation. Another interesting effect that plants might contribute to, is the antibacterial ability. Plants extracts were studied numerous times and proved to be an important source of antibacterial compounds. The effectiveness duration of any antibiotic is limited, and the need for new bacteria resistant metabolites is of great interest, since bacteria have the genetic capacity to transmit and develop protection from drugs (Cohen 1992).

*Urtica dioica* is a wild, yearly and perennial plant species from the *Urticaceae* family. Besides its use for many decades in foods, UD extracts were used to treat several common diseases and health issues such as rheumatism, eczema, arthritis, gout, and anemia for over a thousand years (Chrubasik et al. 1997; Bone and Mill 1999). Its aqueous extracts have shown an efficiency regarding antihyperglycemic activity and noticeable cytotoxicity (Bnouham et al. 2003; Johnson et al. 2013), while its alcoholic extracts have shown antifungal and anti-inflammatory ability (Hadizadeh et al. 2009; Johnson et al. 2013). On the other hand, *Chenopodium murale* is a widespread weed from the *Amaranthaceae* family. CM leaves are mainly used for their food benefit (De Simone et al. 1990). *Chenopodium* plants are well known to have anthelmintic properties (Vasishta 1989; Lozoya and Lozoya 1982). It has been reported that *Chenopodium* species contain a rich variety of flavonoids (Crawford and Mabry 1978; Aritomi and Kawasaki 1984; De Simone et al. 1990; Jain et al. 1990) and even Gohara and Elmazar (1997) isolated specific flavonoids with hypotensive activity from the aerial parts of CM.

The two plants previously cited have been used for decades as alternative medicine to cure several types of health issues. Some issues linked to oxidative stress and bacterial infections. Interestingly, these two plants have similar appearance for a non trained eye and are mistaken for each other by users. Therefore, it was of interest to extend the available data by assessing the antioxidant and antibacterial activity as well as phenolic compounds (TPC), flavonoids (TFC) and condensed tannins (CTC) content of UD and CM methanol extracts for the different organs of these plants.

The comparison of organs extracts will allow us to locate the most abundant parts for antioxidants extraction. In addition, antibacterial assays were performed on the whole plants extracts in order to determine which plant is the most effective against the bacterial strains used. Gram-positive and gram-negative type bacteria were chosen, for their structural and resistance difference. The evaluation extended purpose was to expand the usage of these plants as a potential source of antioxidant compounds and to define which part of the plant has the most significant antioxidant potential, and which plant is the best antibacterial agent against the studied strains.

## 2. Materials and methods

### 2.1. Plant material

(See chapter II. Materials and methods. II. Sampling).

### 2.2. Methanolic extract

(See chapter II. Materials and methods. III.1.Solvent based extracts preparation).

### 2.3. Determination of total phenolic content

(See chapter II. Materials and methods. IV. Determination of phenolic content).

### 2.4. Determination of flavonoids content

(See chapter II. Materials and methods. V. Determination of flavonoids content).

### 2.5. Determination of proanthocyanidins content

(See chapter II. Materials and methods. VI. Determination of proanthocyanidins content).

### 2.6. Antioxidant Activity (AA)

#### 2.6.1. Free radical scavenging activity

(See chapter II. Materials and methods. VII.1. DPPH Free radical scavenging activity).

#### 2.6.2. ABTS radical scavenging assay

(See chapter II. Materials and methods. VII.2. ABTS radical scavenging assay).

### 2.7. Antibacterial activity

(See chapter II. Materials and methods. VIII. Antibacterial activity).

## 3. Results and discussion

### 3.1. Total phenolics, total flavonoids and condensed tannins content

Phenolic compounds are essential secondary metabolites in plants and are of great interest because of their biological activity and enzyme inhibition capacity (Okudu et al., 1994; Tepe et al., 2006). Tab 8 shows total phenolics, flavonoids and condensed tannins content of UD and CM extracts. These contents were investigated for methanolic extracts including leaves, seeds, barks and the whole part of these plants. The obtained results showed significant level of phenolic compounds in UD leaves and CM seeds with values of 88.25 and 46.88 (mg GAE /g Extract), respectively. The level of flavonoids in leaves was higher in both plants with values of 55.2 (mg QE /g Extract) for UD and 26.8 (mg QE /g Extract) for CM. Unlike to phenolic compounds and flavonoids, the highest content of condensed tannins was found in UD seeds and CM leaves with values of 32.15 and 21.53 (mg CE /g Extract), respectively. These results are in line to those of Otles and Yalcin (2012), where the occurrence of phenolic compounds was confirmed using chromatographic approach for different parts of UD. However, flavonoids were the most abundant class of compounds in UD. In agreement with Komes et al. (2011), where significant yield of flavonoids were found than any other classes of compounds. Even though CM shows a lower content of phenolic compounds than UD, previous studies showed that it hosts also an important amount of phenolic compounds, namely flavonoids (Nowak et al. 2016). Indeed, CM contains also significant level of flavonol glycosides (Jeganathan et al. 2016; El-Sayed et al. 1999). Furthermore, phenolic compounds present either in UD and CM are mainly liable for their health related effects. For instance, the hypotensive effect of CM is due to the quercetin phytochemical, a flavonoid existing in most fruits and vegetables (Perez-Vizcaino et al. 2009). Antioxidant activity, anti-inflammatory activity, and modulation of signaling pathways are among many mechanisms that are health related, and are provided by flavonols that are abundant in these plants (Lago et al. 2014).

**Tab 8.** Total phenolic content (TPC), flavonoid content (TFC) and condensed tannins content (CTC) of *Urtica dioica* and *Chenopodium murale* methanolic extracts.

| | TPC (mg GAE /g Extract) | | TFC (mg QE /g Extract) | | CTC (mg CE /g Extract) | |
|---|---|---|---|---|---|---|
| | *Urtica dioica* | *Chenopodium murale* | *Urtica dioica* | *Chenopodium murale* | *Urtica dioica* | *Chenopodium murale* |
| Seeds | 83.94 | 46.88 | 46.20 | 23.50 | 32.15 | 19.81 |
| Leafs | 88.25 | 39.62 | 55.20 | 26.80 | 18.87 | 21.53 |
| Barks | 28.21 | 24.95 | 17.80 | 14.80 | 11.06 | 5.28 |
| Whole plant | 56.20 | 32.69 | 35.20 | 19.26 | 11.53 | 12.31 |

**Tab 9.** DPPH $IC_{50}$ and ABTS $IC_{50}$ values of the different parts of *Urtica dioica* and *Chenopodium murale* methanolic extracts.

| | DPPH [$IC_{50}$ (µg/mL)] | | ABTS [$IC_{50}$ (µg/mL)] | |
|---|---|---|---|---|
| | *Urtica dioica* | *Chenopodium Murale* | *Urtica dioica* | *Chenopodium Murale* |
| Seeds | 90.10 | 345.34 | 552.36 | 1175.63 |
| Leafs | 337.60 | 1707.12 | 432.60 | 1650.39 |
| Barks | 877.42 | >2000 | >2000 | >2000 |
| Whole plant | 483.98 | 882.60 | 1064.30 | 1835.70 |

## 3.2. Antioxidant activities

### 3.2.1. DPPH scavenging activity

The DPPH assay is a simple and fast method frequently used to evaluate the ability of antioxidants to scavenge free radicals. It gives solid data of the antioxidant capacity of the assessed extracts, to play the role of free radical scavengers or hydrogen donors (Huang et al. 2005). As mentioned earlier, phenolic compounds and their free radical scavenging ability were liable to decreased risk of oxidative stress diseases such as cancer and cardiovascular disease using antioxidants rich diets (Hatamnia et al. 2014; Li et al. 2006; Govindarajan et al. 2007). In this study, radical scavenging activity was studied for all parts of UD and CM plants. The free radical scavenging activity determined by DPPH was expressed using $IC_{50}$ values (the concentration of extract required to inhibit 50% of the initial DPPH free radical) (Tab 9). The results showed that DPPH values increase with increasing concentrations of plants extracts. Seed extracts exhibited the most potent DPPH scavenging activity while bark extracts showed the lowest activity for both plants. The values were comparable for all parts of the plant extracts. UD seed extract was the most efficient in DPPH assay, followed by leaves extract. Moreover, the extract of the whole plant of UD was more efficient than the barks extract. These results indicate that the level of phenolic compounds in leaves, and especially seeds, are significantly more important than those of barks and the whole plant. CM extracts fall behind when it comes to antioxidant activity. However, within this plant, seeds extract shows the highest antioxidant property, indicating that the phytochemicals present in this part of the plant is rich in antioxidants.

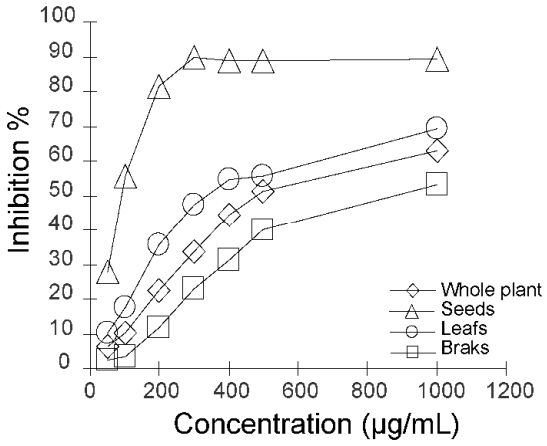



**Fig 57.** DPPH Free radical scavenging activity of *Urtica dioica* extracts.

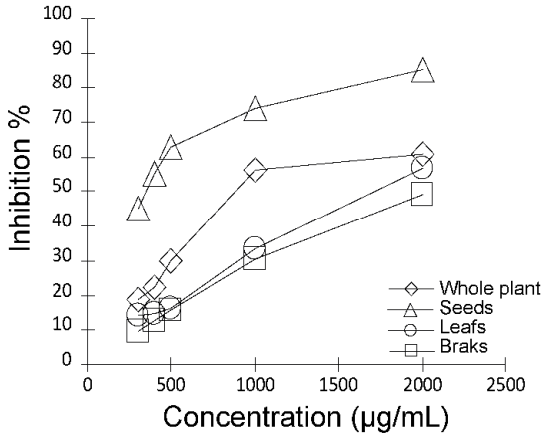



**Fig 58.** DPPH Free radical scavenging activity of *Chenopodium murale* extracts.

### 3.2.2. Radical cation ABTS+ scavenging activity

ABTS assay is an excellent approach for investigating the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants. The extract effectively scavenged ABTS radicals resulting from the reaction between ABTS and ammonium persulfate (Fig 60, 61). It is obvious that ABTS values increase with increasing plant concentrations. Seeds extract demonstrated the most potent ABTS radical scavenging activity for CM, while leaves extracts had the highest efficiency. For both plants, the bark extracts showed the lowest response. Likewise, to DPPH assay, UD extracts were more potent with the strongest response in leaves extracts. Nevertheless, CM seeds were the most efficient part of the plant. These results are in line with those obtained for the phenolic compounds, flavonoids and condensed tannins contents, in which seeds and leaves had the higher doses of these compounds, and explicate the concentration related efficiency observed in the antioxidant experiments. However, UD leaves had the highest values of phenolics content and unlike, seeds extracts showed the highest antioxidant potential in the DPPH assay. These indicates that phenolic compounds in seeds extracts are more efficient. The values obtained for UD are in line to those previously reported, where $IC_{50}$ numbers were ranging from 1060 µg/mL to 530 µg/mL, depending on the extraction method used (Sidaoui et al. 2015).

Previous studies conducted on the UD antioxidant power showed that it has an effective oxidative stress inhibitor (Kaledaite et al. 2011; Gülçin and Küfrevioğlu 2004; Komes et al. 2011; Kukrić et al. 2012). Similarly, for *Chenopodium* species, the extracts have been also studied for their antioxidant potential. The results obtained were hopeful toward the use of this plant as a source of natural antioxidants and to be further studied for identification of potent phytochemicals that can further enrich the pharmaceutical compounds database (Nowak et al. 2016; Lone et al. 2017).

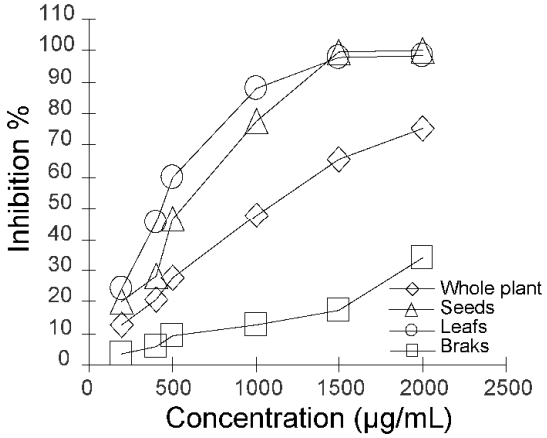



**Fig 59.** ABTS radical scavenging activity of *Urtica dioica* extracts at different concentrations.

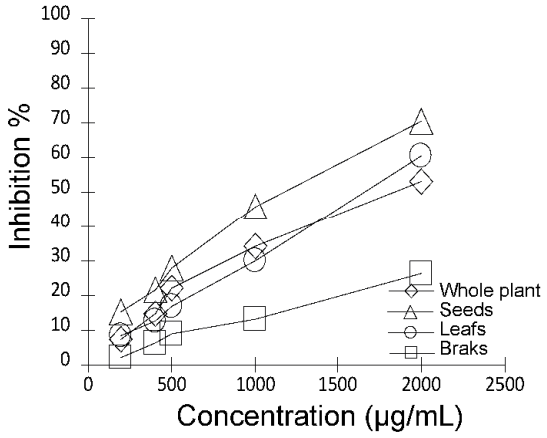



**Fig 60.** ABTS radical scavenging activity of *Chenopodium murale* extracts at different concentrations.

### 3.3. Antibacterial activity

The antibacterial activity was studied using two different methods, i.e., disc diffusion and micro dilution methods. Tab 10 shows the results for the disc diffusion method. As shown in Tab 10, the tested extracts were not effective against *Agrobacterium sp.* Strains. However, the UD extract had a stronger antibacterial effect than CM extract. Indeed, the largest inhibition circle was observed for UD against *Bacillus pumilus* strain. Additionally, the micro-dilution method was performed to confirm the disc diffusion one and the results are in Tab 11. The most potent inhibitions were observed for *Urtica dioica* extract against "*Bacillus subtilis*" and "*Rhizobium sp.*" with a CMI value of 3 mg/mL. For CM, which is once again less effective than UD extract, the lowest CMI value was of 11 mg/mL against *"Bacillus subtilis"*. In line with Kukrić et al. (2012), where UD leaves extract exhibited low efficiency inhibition of *E. Coli* strains. Gram-negative strains showed more resistance to the used extracts, namely *E. Coli* and *Agrobacterium*, due to their permeability limitation provided by their cell wall. This structural advantage makes the gram-negative class more resistant than gram-positive bacteria (Abu-Shanab et al. 2004; Adwan and Abu-Hasan 1998). In general, many types of phytochemicals were found to be liable to the antibacterial activity in plants, mainly organic acids and phenolic compounds attached to carbohydrates (Manach et al. 2004) . The occurance of these moieties with phenolic compounds able their interaction with proteins, macromolecules like cellulose and pectin (McLeod 1974) and also some multiple physiological properties such as antimicrobial effect (Abah and Abah 2010; Balasundram et al. 2006; Funatogawa et al. 2004; Karou et al. 2005; Tavassoli and Djomeh 2011). The efficiency antibacterial of UD extract compared to CM is a proof that it's rich in bacterial growth inhibiting compounds as well as other type of related metabolites.

**Tab 10.** Antibacterial activity (inhibition zone diameter in mm) of plant extracts on different bacteria using disc diffusion method.

| Test agents | Bacterial species | | | | |
|---|---|---|---|---|---|
| | *E.Coli* | *Agrobacterium sp* | *Rhizobium sp* | *Bacillus pumilus* | *Bacillus subtilis* |
| *Urtica dioica* (30 mg/mL) | 16 | - | 12 | 19 | 7 |

| | | | | | |
|---|---|---|---|---|---|
| *Chenopodium murale* (30 mg/mL) | 6 | - | 5 | 5 | 4 |

**Tab 11.** Minimal inhibitory concentration using micro dilution method (MIC in mg/mL) of the methanol extracts.

| Test agents | Bacterial species | | | | |
|---|---|---|---|---|---|
| | *E.Coli* | *Agrobacterium sp* | *Rhizobium sp* | *Bacillus pumilus* | *Bacillus subtilis* |
| *Urtica dioica* MIC (mg/mL) | 3.65 | - | 3.00 | 3.20 | 3.00 |
| *Chenopodium murale* MIC (mg/mL) | 12.00 | - | 12.00 | 11.00 | 12.00 |

## 4. Conclusion

Phenolics, flavonoids and condensed tannins contents, along with the antioxidant activity of different part of *Urtica dioica* and *Chenopodium murale*, as well as antibacterial ability of the whole plants were investigated. TPC, TFC and CTC assays have shown that *Urtica dioica* contains higher levels of phenolics than *Chenopodium murale*. These results were in line with the antioxidant assays. Indeed the extracts where phenolic content was highest, demonstrated the most potent antioxidant power. Even, most parts of these plants have shown a good antioxidant property. Additionally, *Urtica dioica* seeds and leaves extract have shown the strongest response. As for the antibacterial activity, *Urtica dioica* had the upper hand over *Chenopodium murale*. The antibacterial ability exhibited by these plants was assessed for gram positive and negative strains, which further confirms their response to bacteria's growth. Still, neither of the extracts used was efficient against the *Agrobacterium sp.* strain. The data obtained demonstrate that these plants have potential uses that are still to be revealed and studied in depth (ongoing investigation).

# Paper III

# Total phenolic and flavonoid content, antioxidant and antibacterial activity of *Ziziphus lotus* from Morocco


## Summary

The present paper brought an insight into the antioxidant and antibacterial potential of *Ziziphus lotus* (*Rhamnaceae*), a widely spread fruit in the Mediterranean zone. The intrest to this plant was due to the vertues reported for its use. In traditional medicine, it is reportedly used to treat sore throats, alleviate stress and helps common colds. *Ziziphus lotus* is also claimed to purify blood, help digestion and diet. Total phenolic and flavonoid content as well as in vitro antioxidant and antimicrobial activity of the methanolic extract of *Ziziphus lotus* were evaluated using different methods. A total phenolic content value of 143.12 mg/g GAE was recorded for the methanolic extract of *Ziziphus lotus.* Furthermore, *Ziziphus lotus* showed higher antioxidant potency. DPPH $IC_{50}$ values of 131.01 µg/mL compared to 50.67 µg/mL for BHT, and ABTS $IC_{50}$ values of 52.42 µg/mL compared to 63.44 µg/mL for Trolox. Antibacterial tests showed that the methanolic extract exhibited a powerful antibacterial effect against the bacterial strains with MIC values of 400 and 320 µg/ml against *E.coli* and *Bacillus pumilus*, respectively. The above cited results indicate that *Ziziphus lotus* fruit has an important nutritional value as a source of phenolic compounds well-known for their impact in huma.

# Total phenolic and flavonoid content, antioxidant and antibacterial activity of *Ziziphus lotus* from Morocco

Walid Belmaghraoui, Nadia El Madani, Amina Manni, Mourad Harir, Abdelkarim Filali-Maltouf, Souad El Hajjaji (Pharmacologyonline, 2018, 3: 176-183)

**Abstract**

In this study, total phenolic and flavonoid content as well as in vitro antioxidant and antimicrobial activity of the methanolic extract of *Ziziphus lotus* were evaluated using different methods. The total phenol and flavonoid content in the extract were determined by Folin Ciocalteu and $AlCl_3$ assays, while antioxidant and antibacterial activities were studied using DPPH free radical-scavenging, ABTS, disc diffusion and micro-dilution methods. The results showed that the phenols amount (143.12 mg/g) in the methanolic extract of *Ziziphus lotus* were thirty-five fold higher than the flavonoids (4.28 mg/g). Furthermore, *Ziziphus lotus* showed higher antioxidant potency as determined by DPPH and ABTS methods, (DPPH $IC_{50}$ values of 131.01 µg/mL compared to 50.67 µg/mL for BHT, and ABTS $IC_{50}$ values of 52.42 µg/mL compared to 63.44 µg/mL for Trolox). Accordingly, antibacterial tests showed that the methanolic extract exhibited a powerful antibacterial effect against the bacterial strains with MIC values of 400 and 320 µg/ml against *E.coli* and *Bacillus pumilus*, respectively. These results indicated that *Ziziphus lotus* fruit could be taken as a potential source of phenolic compounds well-known for their impact in human health as well as nutrition.

## 1. Introduction

An antioxidant is considered to be any chemical substance that prevents the oxidation of a substrate in presence of an oxidisable compound (Halliwell et al. 1995). Polyphenols, carotenoid and traditional antioxidant vitamins such as vitamin C and E, are considered among the major

phytochemicals liable to the antioxidant activity in plant materials. Phenolic substances have been studied rigorously for their human health benefits, and have been considered the most bioactive phytochemicals for this purpose (Cao et al. 1996). Their antioxidant power is mainly due to their redox properties of the phenolic hydroxyl groups and the chemical structure (Bors and Saran 1987; Visioli et al. 1998; Ramiréz-Aristizabal et al. 2016). It has been proven that antioxidant substances are associated with many biological benefits, such as the maintenance of the immune functions, the diminution of lipid peroxidation and DNA damages (Gropper et al. 2007). Because of their ability to scavenge free radicals, antioxidant substances have attracted great interest (Saeed et al. 2012). Many disorders in human organism (e.g., neurodegeneration, alzheimer disease, cancer and inflammation) may result from increased concentrations of these free radicals in organisms (Ferguson 2010; Halliwell 2006; Halliwell 2007). Furthermore, the antioxidant substances probably prevent human organism against several diseases, for instance, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gülçin 2012). Accordingly, natural antioxidants present in plants have no side effects, whereas chemically synthetized antioxidants were found to have multiple genotoxic effects (Chen et al. 1992; Kahl and Kappus 1993). It is the main reason behind the consistent and great effort that the researchers put into finding new natural antioxidants and thus, safe, effective and cheap alternatives to the synthetic products (Mundhe et al. 2011).

*Ziziphus lotus (Rhamnaceae)* is a widely spread fruit in the Mediterranean zone. It is used in traditional medicine to treat sore throats, alleviate stress and helps common colds (Harisson A.P. and Bartels E.M. 2006). *Ziziphus lotus* is also claimed to purify blood, help digestion and diet (Tripathi et al. 2001). The most present metabolites in the *Ziziphus* genus are the phenolic substances. Several metabolites, namely flavonoids, tannins and alkaloids showed great pharmacological properties (Naili et al. 2010). These metabolites are widespread in plants where they act as antioxidants and free radical scavenger (Park et al. 2004; Vaya et al. 2003; Yokozawa et al. 1998). Previous studies showed strong relationship between total phenolic content and antioxidant activity in different seeds, fruits and greens (Yang et al. 2009). The main aim of this study was to determine the total phenolic (TPC) and flavonoid (TFC) content, the antibacterial and antioxidant activity (AA) of the *Ziziphus lotus* fruit.

## 2. Methods

### 2.1.Collection and preparation of extracts

(See chapter II. Materials and methods. II. Sampling).

(See chapter II. Materials and methods. III.1.Solvent based extracts preparation).

### 2.2.Antibacterial activity

(See chapter II. Materials and methods. VIII. Antibacterial activity).

### 2.3.Determination of total phenolic content

(See chapter II. Materials and methods. IV. Determination of phenolic content).

### 2.4.Determination of flavonoids content

(See chapter II. Materials and methods. V. Determination of flavonoids content).

### 2.5.1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay

(See chapter II. Materials and methods. VII.1. DPPH Free radical scavenging activity).

### 2.6.ABTS radical scavenging assay

(See chapter II. Materials and methods. VII.2. ABTS radical scavenging assay).

## 3. Results and discussion

### 3.1.Antibacterial activity

The antibacterial proprieties of *Ziziphus lotus* extracts in aqueous and organic solutions were evaluated using disc diffusion method as well as micro-dilution method against different microorganisms (*E.Coli, Agrobecterium sp, Rhizobium sp, Bacillus pumilus* and *Bacillus subtilis*) and the results are shown in Tab 12. These methods were used to test the susceptibility of bacteria to the *Ziziphus lotus* extracts. It seems that the microorganisms tested in this study were not as sensitive to aqueous solution as compared to organic solution. In line with previous study where no inhibitory effect was observed in aqueous extracts (Cowan 1999; Abu-Shanab et al. 2004).

Furthermore, the results showed that the *Ziziphus lotus* extracts were more active towards the gram-positive bacteria. This was not surprising since gram-negative bacteria are more resistant to plant extracts as compared to gram-positive bacteria (Abu-Shanab et al. 2004). Such behavior could be explained by the permeability barrier provided by the cell wall or to the membrane accumulation mechanism that is more effective in gram negative bacteria due to the presence of the outer membrane in their structure in contrast to gram positive bacteria (Adwan and Abu-Hasan 1998).

**Tab 12.** Antibacterial properties by disc difusion method (inhibition zone diameter in mm) of *Ziziphus lotus* extracts as well as its methanol extract antibacterial activity by micro dilution method on different bacteria.

| Test agents | Bacterial species | | | | |
|---|---|---|---|---|---|
| | *E.Coli* | *Agrobacterium sp* | *Rhizobium sp* | *Bacillus pumilus* | *Bacillus subtilis* |
| A (94 mg/mL) | - | - | - | - | - |
| B (3 mg/mL) | 11 | 11 | 16 | 12 | 7 |
| MIC (µg/ml) in B | 400 | 400 | 3.2 | 320 | 340 |

A: water extract; B: methanol extract

Accordingly, the largest zone of inhibition was recorded for the methanolic extract against *Rhizobium sp.* with a diameter of 16 mm, followed by *Bacillus pumilus, Agrobacterium sp., E.coli* and *Bacillus subtillis* with diameters of 12, 11, 11 and 7 mm respectively. The most potent inhibition with the micro-dilution method was observed in case of *Rhizobium sp* with a MIC=3.2 µg/mL. It has been reported that antimicrobial activities of *Rhamnacea* species are mainly attributed to its most active ingredients (i.e., polyphenols and alkaloids) (Harisson A.P. and Bartels

E.M. 2006; Tripathi and Tripathi 2014; Han et al. 1990; Devi et al. 1987). In this study, the water extracts were discarded in the micro-dilution method since they haven't shown any inhibitory effect in the disc diffusion method.

### 3.2. Total phenolic and total flavonoid content

The total phenolic content of the extracts were determined using Folin-Ciocalteu (FC) colorimetric method. This method allows the estimation of all flavonoids, anthocyanins and other phenolics compounds (non-flavonoids) present in the extracts. The total flavonoid content was assessed by precipitating the crude extract with aluminum chloride ($AlCl_3$), allowing $Al^{3+}$ to bind with the ketone and hydroxyl group of the flavonoids through electron transfer reaction. A yellow colour is then observed under UV spectrophotometer at the maximum absorbance of 510 nm (Abdullah et al. 2012). Fig 62 shows the TPC and TFC results of the methanolic extract of *Ziziphus lotus* and the calculated values of TPC and TFC were 143.12 mg/g GAE and from 4.281 mg/g QE, respectively. It has been reported that hydroxyls included in flavonoids has direct relationship with the radical scavenging effect, while phenols inhibits action of reactive oxygen species in the plants (Vaya et al. 2003; Younes and Siegers 1981; N.P. and T.A. 1990; Roya and Fatemeh 2013). In addition, flavonoids are shared constituents of phenolics mainly synthetized by the phenylpropanoid metabolic pathway (Ververidis et al. 2007). Accordingly, each of phenolics and flavonoids contribute widely to human health by their antioxidant and anticancer properties (Ghasemzadeh and Ghasemzadeh 2011).

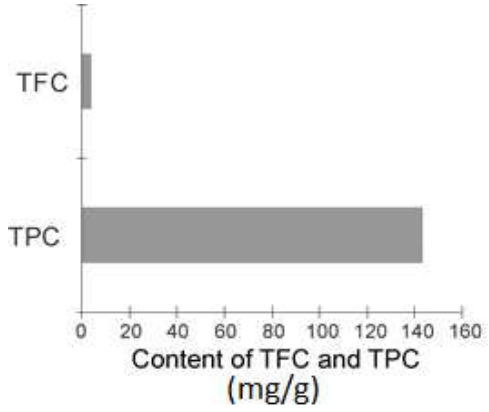



**Fig 61.** Phenolic and flavonoid content of *Ziziphus lotus* in methanolic extract.

### 3.3.Antioxidant activities

Phytochemical diversity of secondary metabolites including alkaloids, terpenoids, phenols and flavonoids present antioxidant activity because of their red/ox properties as well as structural diversity. In this study, the antioxidant activity of *Ziziphus lotus* was assessed using DPPH assay (Fig 63). Due to ease of reaction, the DPPH radical method is extensively used to evaluate free radical scavenging activity and the concentration of a species to scavenge 50% DPPH radical ($IC_{50}$) was mainly determined to get better estimation on sample antioxidant efficiency (Tab 13). In DPPH assay, the $IC_{50}$ value was found to be 131.01 µg /mL in methanolic extract. Furthermore, the BHT values are higher than those of the methanolic extract. Nevertheless, with greater concentrations, the free radical scavenging activity of *Ziziphus lotus* fruit is closely similar to BHT, as the inhibition percentage of the extract is near the values of BHT.

**Tab 13.** DPPH's and ABTS's $IC_{50}$ of the *Ziziphus lotus* extract.

| | $IC_{50}$ (µg/ml) |
|---|---|
| *DPPH* | |
| Methanolic extract | 131.01 |
| BHT | 50.67 |
| *ABTS* | |
| Methanolic extract | 52.42 |
| Trolox | 63.44 |

In addition, radical inhibition activity of *Ziziphus lotus* extract was determined using ABTS radical decolorization assay (Fig 64). Here, the percentage of the ABTS radical inhibition activity reached a peak of 95.85% at a concentration of 200 µg/mL. We found that the ABTS values of the methanolic extract are higher than those of Trolox leading to the conclusion that *Ziziphus lotus* fruit has good antioxidant properties (Fig 64). It has been reported that *Ziziphus jujube* had good antioxidant activity against DPPH as well as a straight correlation with phenols content (Li et al.

2005). Furthermore, the potential antioxidant of *Ziziphus lotus* could be also related with the content and type of metabolites present in its extract (Cheng et al. 2000; Pawlowska et al. 2009). Alkaloids are amongst the well-known metabolites for their antioxidant activity; they are spread in the entire parts of the plant. For instance, betulinic acids, which are natural pentacyclic triterpenoids extensively disseminated in all parts of the plant, showed high antioxidant activity (Adesanwo et al. 2013). However, further phytochemical study focusing on the isolation and characterization of the constituents in *Ziziphus lotus* extract liable to its bioactivity is still required.

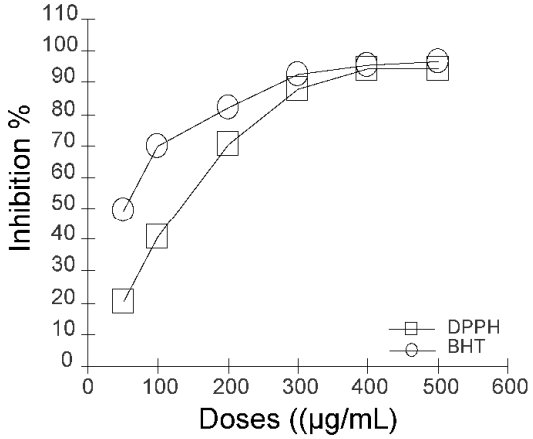



**Fig 62.** DPPH free radical scavenging activity of *Ziziphus lotus* in methanolic extract.

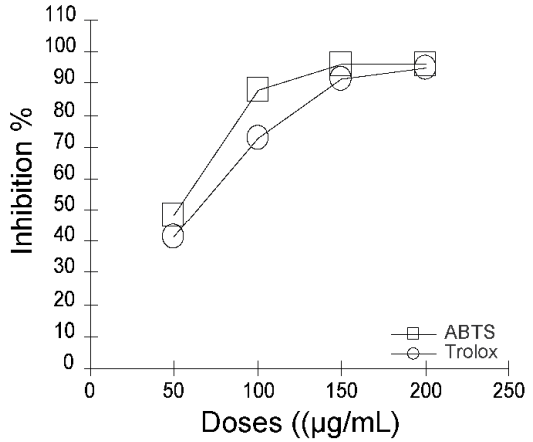



**Fig 63.** ABTS radical scavenging activity of *Ziziphus lotus* in methanolic extract with different concentrations.

## 4. Conclusion

Our study showed that *Ziziphus lotus* fruit can be a source of plant antioxidants with the possibility of usefulness in foodomics, cosmetics and pharmaceutical fields. The phenolic compounds might be the major active substances responsible for the highest antioxidant activity. Furthermore, the richness of *Ziziphus lotus* fruit and its potent antioxidant effect may explain its efficiency against bacteria. This could be also explained by the usefulness of this plant in treating several infections and health issues which is interesting for designing an antibacterial agent from vegetable source.

# Paper IV

# Antioxidant activity and phenolic contents of *Mentha rotundifolia* organs extracts from Morocco


## Summary

*Mentha rotundifolia* is a native aromatic species belonging to the *Mentha* genus of *Lamiaceae* family. This study was dedicated to his plant for the many traditional uses in our food habits. In this study, the contents of phenolic compounds (TPC), flavonoids (TFC) and condensed tannins (CTC) were investigated for all parts of *Mentha rotundifolia* (MR), as well as their antioxidant activity using DPPH and ABTS assays. These assays were performed on extracts using two solvents, methanol and 80% ethanol. Seeds and leaves extracts of MR are the most potent in antioxidant assays. However, seeds were the most interesting part in MR, due to its high content of phenolic compounds (398.4 mg GAE /g Extract). The antioxidant efficiency for *Mentha rotundifolia* extracts reached up to 97% of inhibition at a concentration of 40 µg/mL for all parts. Furthermore, seeds extracts showed the lowest $IC_{50}$ values in both solvents and assays, with values as low as 5.98 µg/mL with DPPH. Seeds extracts exhibited the strongest in-vitro antioxidant response with methanolic extracts compared to ethanolic (80%) extracts. The previously reported results and observations are an important testimony for the beneficial health related power, the seeds of the *Mentha rotundifolia* species holds.

# Antioxidant activity and phenolic contents of *Mentha rotundifolia* organs extracts from Morocco

(To be submitted)

**Abstract**

In this study, the contents of phenolic compounds (TPC), flavonoids (TFC) and condensed tannins (CTC) were investigated for all parts of *Mentha rotundifolia* (MR), as well as their antioxidant activity using DPPH and ABTS assays. The antioxidant activity was measured using a discoloration in-vitro test with 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate (ABTS). The results showed that seeds and leaves extracts of MR has potent antioxidant response. However, seeds were the most interesting part in MR, due to its high content of phenolic compounds (398.4 mg GAE /g Extract), followed by the whole plant, leafs and barks respectively (336, 336 and 196.305 mg GAE /g Extract). Similar behavior for flavonoids with a highest value for seeds (265.6 mg QE /g Extract), followed by the whole plant, leafs and barks respectively (224, 189.6 and 130.87 mg QE /g Extract). The antioxidant efficiency for *Mentha rotundifolia* extracts reached up to 97% of inhibition at a concentration of 40 µg/mL for all parts. Furthermore, seeds extracts showed the lowest $IC_{50}$ values in both solvents and assays, with values as low as 5.98 µg/mL with DPPH, while whole plant, leafs and barks fell behind with values of 6.93, 13.2, 14.48 µg/mL, respectively . The obtained antioxidant activities results were in line with the content of phenolic and flavonoid compounds.

## 1. Introduction

*Mentha rotundifolia* is a native aromatic species belonging to the *Mentha* genus of *Lamiaceae* family (Riahi et al. 2013; Ladjel et al. 2011). It grows in wet areas near low watercourses and middle mountain, and includes various chemotypes (Lorenzo et al. 2002). *Mentha rotundifolia* is a hybrid plant between *M. longofolia* and *M. suaveolens* (Lorenzo et al. 2002). This species is present in Morocco as a domestic and wild plant. Its leaf have a distinctive odor to it, and has many traditional uses in our food habits. The *Lamiaceae* family possess great pharmacological and commercial significance. The *Lamiaceae* species gained popularity throughout centuries over the world and in Morocco, due to its many traditional uses in traditional medicine, food, cosmetic and

the world of spices. In Morocco, *Mentha rotundifolia* is used in traditional medicine for its digestive, antiseptic, expectorant, antispasmodic and tonic properties in winter (Lieutaghi 1966). *Mentha rotundifolia* is also known for its diuretic, carminative, antiflatulent, expectorant, antitussive and antioxidant properties (Oumzil et al. 2002; Ahmad et al. 2012; Thomassen et al. 1990).

Medical Pharmacists has highlighted the use of medicinal plants by the extraction of active ingredients for pharmaceutical industry. In this process, the industry finds an innovation base for the development of new products for medical, veterinary and cosmetic practice. It has been shown that a variety of medical herbs have been investigated for their ability to produce a large varieties of phytochemicals with antifungal and microbicide activity (Leal et al. 2013). Medical herbs are rich in tannins, alkaloids, flavonoids and phenolic compounds that are useful in treating severe degenerative disorders (Gul et al. 2015). Actually, most of natural commercialized compounds are of plant origins. However, there is still a significant gap for unknown biologically active compounds. Our concern in this work was to investigate the extraction efficiency of two different solvents (i.e., methanol and ethanol 80%) for *Mentha rutondifolia* plant (including leafs, seeds and barks) and to evaluate their potential pharmacologic uses trough quantification of phenolic, flavonoid and condensed tannin compounds as well as the antioxidant activity.

## 2. Material and methods

### 2.1. Plant material

(See chapter II. Materials and methods. II. Sampling).

### 2.2. Preparation of extracts

(See chapter II. Materials and methods. III.1.Solvent based extracts preparation).

### 2.3. Determination of total phenolic content

(See chapter II. Materials and methods. IV. Determination of phenolic content).

### 2.4. Determination of flavonoids content

(See chapter II. Materials and methods. V. Determination of flavonoids content).

### 2.5. Determination of proanthocyanidins content

(See chapter II. Materials and methods. VI. Determination of proanthocyanidins content).

### 2.6. Antioxidant Activity (AA)

#### 2.6.1. Free radical scavenging activity

(See chapter II. Materials and methods. VII.1. DPPH Free radical scavenging activity).

#### 2.6.2. ABTS radical scavenging assay

(See chapter II. Materials and methods. VII.2. ABTS radical scavenging assay).

## 3. Results and discussion

### 3.1. Total phenolics, total flavonoids and condensed tannins content

Phenolic compounds are important secondary metabolites in medicinal plants and are of great interest because of their biological activity and enzyme inhibition capacity (Ho et al. 1994; Tepe et al. 2006). Phenolic composition is highly complex in term of structural variety from rather than simple structures. Some families have a widespread diet while others are limited or particularly abundant in specific foods (Cheynier 2005). Tab 14 shows the total phenolics, flavonoids and condensed tannins content in MR ethanolic (80%) and methanolic extracts for leafs, barks and seeds organs as well as their mixture. Thus, the highest content of phenolic compounds was found in seeds MR methanolic extract with a value of 398.4 (mg GAE /g Extract). A value of 256.8 (mg GAE /g Extract) in 80% ethanolic seed extract. In addition, the content of flavonoid compounds in seeds was higher in both extracts. However, methanolic extract was as two fold higher than ethanolic (80%) extract with values corresponding to 265.6 and 171.2 (mg QE /g Extract), respectively. In general, the content phenolic and flavonoid compounds was abundant in methanol rather than in ethanol (80%). Unlike, the content of tannin compounds, it was eighteen fold higher in leafs 80% ethanolic as compared to methanolic extract with values as 91 and 5 (mg CE /g Extract), respectively. Herein ethanolic (80%) and methanolic extracts were compared to evaluate their ability to extract specific secondary metabolites (i.e., phenolic, flavonoid and condensed

tannin compounds). We found that ethanol (80%) was able to extract more condensed tannins than methanol solvent. A previous study on the other hand, where three medicinal plants i.e., (*Ribes rubrum var. Rondom*), black currant (*Ribes nigrum var. Rosenthal Falch*) and grape (*Vitis vinifera var. Pinot Noire*) were used, reported that an extract of methanol (70%) was able to get more anthocyanins than ethanol (70%) (Lapornik et al. 2005).

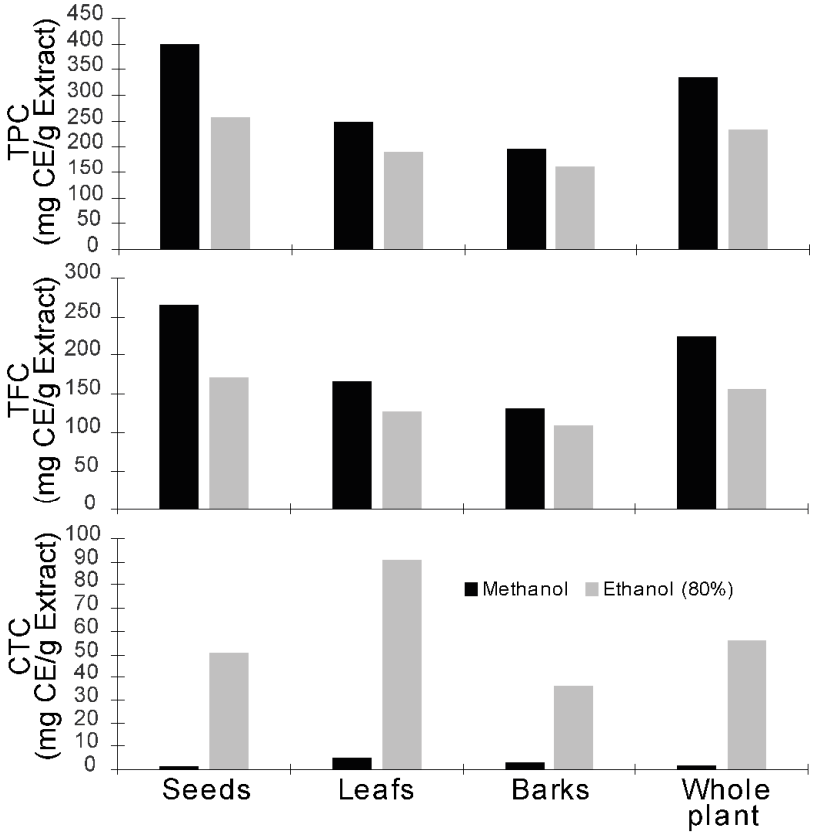



**Fig 64.** TPC, TFC and TTC values of *Mentha rutondifolia* methanol and ethanol (80%) extracts for the different parts of the plant.

**Tab 14.** Total phenolic content (TPC), flavonoid content (TFC) and condensed tannins content (CTC) of *Mentha rutondifolia*.

| | TPC (mg GAE /g Extract) | | TFC (mg QE /g Extract) | | CTC (mg CE /g Extract) | |
|---|---|---|---|---|---|---|
| | Methanol | Ethanol (80%) | Methanol | Ethanol (80%) | Methanol | Ethanol (80%) |
| Seeds | 398.4 | 256.8 | 265.6 | 171.2 | 1.36 | 51 |
| Leafs | 249.6 | 189.6 | 166.4 | 126.4 | 5 | 91 |
| Barks | 196.305 | 162.99 | 130.87 | 108.66 | 3 | 36 |
| Whole plant | 336 | 232.8 | 224 | 155.2 | 1.46 | 56 |

## 3.2. Antioxidant activities

### 3.2.1. DPPH scavenging activity

In this study, radical scavenging activity was studied for all organs of *Mentha rutondifolia*. As shown in Tab 15, the free radical scavenging activity determined by DPPH was expressed using $IC_{50}$ values (the concentration of extract required to inhibit 50% of the initial DPPH free radical). We found that DPPH values increase with increasing concentrations of plants extracts. Seed extracts exhibited the most potent DPPH scavenging activity while bark extracts showed the lowest activity in both solvents. Second to the seeds in efficiency, the whole plant extract is the most efficient, followed by leafs and barks, respectively. The lowest obtained $IC_{50}$ value was that of the methanolic seeds extract with a value of 5.98 (µg/mL). The results obtained here are in line with the ones obtained for the TPC and TFC assays, in which the content of phenolic compounds were abundant in seeds extract.

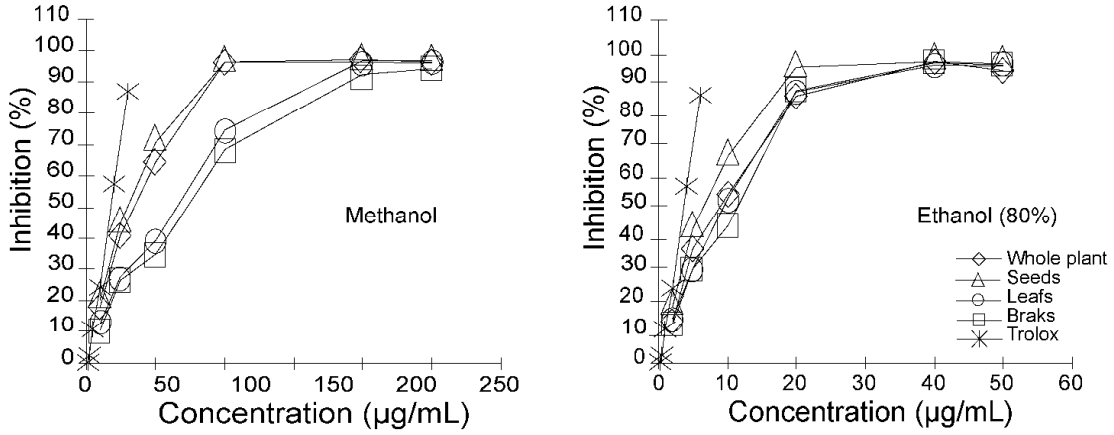



**Fig 65.** DPPH radical scavenging activity of *Mentha rutondifolia* methanol and ethanol (80%) extracts at different concentrations.

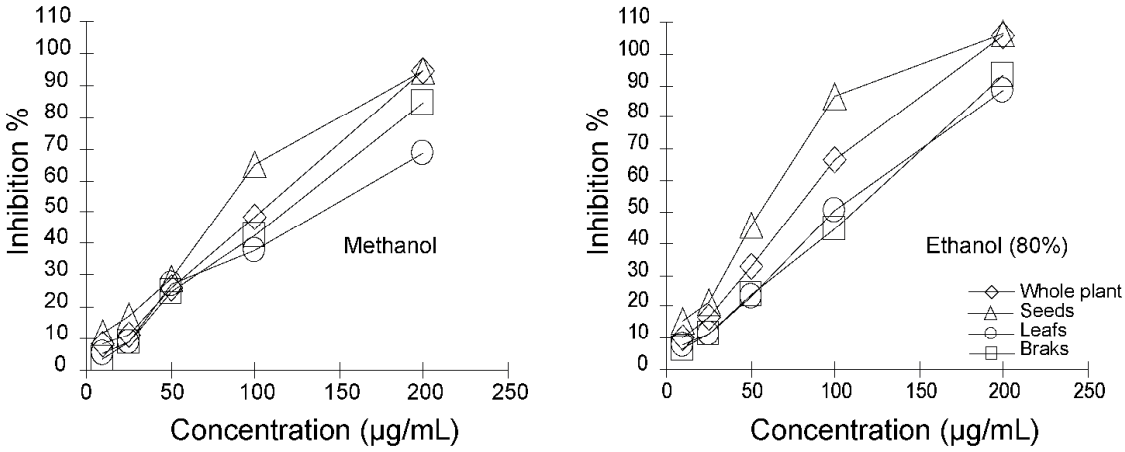



**Fig 66.** ABTS radical scavenging activity of *Mentha rutondifolia* methanol and ethanol (80%) extracts at different concentrations.

### 3.2.2. Radical cation $ABTS^{+}$ scavenging activity

The results of ABTS assays were in line with those obtained for DPPH scavenging activity. Here, seeds showed the most potent ABTS radical scavenging activity in both solvents, followed by leafs methanolic extract and the whole plant ethanolic (80%) extract. These results are also in line with those obtained for the phenolics, flavonoids and condensed tannins contents, in which seeds and the whole plant extracts showed the highest content of phenolic compounds as well as the highest antioxidant efficiency. We found that seeds extract is extremely potent against free radicals. This

finding suggests that this plant organs is a large pool of secondary metabolites with extraordinary antioxidant potential that can be used as pharmaceuticals.

**Tab 15.** DPPH $IC_{50}$ and ABTS $IC_{50}$ values of the different parts of *Mentha rutondifolia* methanol and ethanol (80%) extracts.

| | DPPH [$IC_{50}$ (µg/mL)] | | ABTS [$IC_{50}$ (µg/mL)] | |
|---|---|---|---|---|
| | Methanol | Ethanol (80%) | Methanol | Ethanol (80%) |
| Seeds | 5.98 | 6.33 | 79.17 | 55.23 |
| Leafs | 13.02 | 9.16 | 139.93 | 99.51 |
| Barks | 14.48 | 11.16 | 118.17 | 111.38 |
| Whole plant | 6.93 | 8.57 | 103.33 | 75.51 |

The previous results obtained suggest that the seeds extract of this plant is similar in antioxidant power to that of Trolox. For instance, the DPPH $IC_{50}$ value for the seed methanolic extract is 5.98 µg/mL, and 3.54 µg/mL for Trolox; while the ABTS $IC_{50}$ value for the seeds ethanolic (80%) extract is 55.23 µg/mL and 78.49 µg/mL for trolox. In fact, the seeds are so potent in the antioxidant tests, that the second most potent extract is that of the whole plant. Making it clear that seeds content in phenolic compounds, brings up the efficiency of the whole plant extract, to exceed that of leafs and barks. The specificity of this plant is mainly due to its composition. It has been reported that *Mentha rutondifolia* contains a vast variety of compounds such as phenolic acids ( Caffeic, p-hydroxybenzoic, ferulic and p-coumaric acid) and flavonoids ( Thymonin, ladanein, apigenin, esculetin, etc.) (Hachimi et al. 2015; Zaidi et al. 1998). Furthermore, its essential oil hold great antimicrobial and antioxidant properties (Riahi et al. 2013; Ladjel et al. 2011; El Arch et al. 2003). *Mentha rutondifolia* oil was also reported for its antibacterial power against gram + and gram – bacteria. The minimal inhibitory concentration against *E. coli* was that of 0.025 (% v/v). This oil was also reported to have, in the DPPH assay, an $IC_{50}$ value of 26.11 ± 1.04 µg/ml (Riahi et al. 2013).

**4. Conclusion**

We reported in this study, the contents of polyphenolic, flavonoid and condensed tannin compounds, in parallel with two antioxidant assays for *Mentha rutondifolia* organs extracts obtained using methanol and ethanol (80%). The results showed that seeds contain high amount of phenolic compounds, and the strongest in-vitro antioxidant response in methanolic extracts versus ethanolic (80%) extracts. On the other side, Leafs showed the highest amount of condensed tannins in ethanol (80%) extract. In line with Trolox results, phenolic compounds in seeds extracts showed the strongest oxidation inhibition power either in DPPH or ABTS assays. The previously reported results and observations, are an important testimony for the beneficial health related power, the seeds of the *Mentha rotundifolia* species holds within it. That is why it must be considered for further experiments. Specifically, isolation and identification of new secondary metabolites, in order to enrich the antioxidant compounds database and help to synthetize new drugs with medicinal properties.

# Part II

# Corrosion inhibitory assays using various plant extracts

# Paper V

# Investigation of corrosion inhibition of C38 steel in 5.5 M $H_3PO_4$ solution using *Ziziphus Lotus* Oil Extract: An application model


## Summary

*Ziziphus lotus* (ZL) fruit, is the only plant from the studied group that contains vegetal oil, which can be extracted using hexane. In a previous study, dried materiel of *Ziziphus lotus* fruit was used as green inhibitor of copper corrosion in natural sea water and showed ~93% of inhibition efficiency at 5 g/L extract concentration. This is a testimony for its corrosion inhibition ability, which draws a futher intrest in studying this ability. The hexane extract was used in the present study to asses its ability to protect C38 steel in phosphoric acid medium with a concentration of 5.5M by impedance and potentiodynamic polarization techniques. An identification of the fatty acids present in the extracted oil has been also performed to shed some light on the components that can intervene in this study. The oil was found to be rich in oleic acid, linoleic acid, palmitic acid, stearic acid, linolenic acid and arachidic acid with percentages of 57.2%, 14.98%, 11.21%, 4.92%, 4.45 and 1.96, respectively. Accordingly, the oil extract was found to act as a cathodic type inhibitor. Furthermore, inhibition efficacity of *Ziziphus lotus* oil extract increase with oil concentrations and achieve ~70.5% at 3 g/L solution of *Ziziphus lotus* oil. This green inhibitor has a great potential, taking into consideration that the study was conducted, on a highly concentrated medium. The applications of such inhibitor, original from a largely present wild plant, are tremendous especially in industrial areas and related domain.

# Investigation of corrosion inhibition of C38 steel in 5.5 M $H_3PO_4$ solution using *Ziziphus Lotus* Oil Extract: An application model

Walid Belmaghraoui, Aimad Mazkour, Hicham Harhar, Mourad Harir, Souad El Hajjaji (Anti-Corrosion Methods and Materials, 2018, 66:1).

**Abstract**

This study aims to investigate the corrosion inhibition effect of extracted oil from *Ziziphus lotus* fruit on corrosion of C38 carbon steel in 5.5 M $H_3PO_4$ solution using potentiodynamic polarization and impedance techniques. Oil composition was determined using gas chromatography, and the results showed that oleic and palmitic acids present approximately 84.0% of its total chemical content. Electrochemical impedance spectroscopy (EIS) data were analyzed by adapting it to a well-developed electric circuit model. The inhibition efficiency of *Ziziphus lotus* oil was calculated and compared using Tafel polarization and EIS. Accordingly, the oil extract was found to act as a cathodic type inhibitor. Furthermore, inhibition efficiency of *Ziziphus lotus* oil extract increase with oil concentrations and achieve approximately 70.5% at 3 g/L solution of *Ziziphus lotus* oil. The results obtained from different tested methods were in line, and the oil was able to reduce significantly the kinetics of the corrosion process of C38 carbon steel.

## 1. Introduction

Metals and alloys are used in different areas and environments. The reaction of these metals and alloys with their usage surroundings can result sometimes in corrosion. The phenomenon of corrosion consists of a return of the metal to its stable oxide state by reacting with the surrounding environment (Finšgar and Jackson 2014). In addition to our daily encounters with this form of degradation, corrosion is wasting precious resources, inducing product loss or contamination, yield reduction, high maintenance costs, and expensive over-sizing. It can also compromise safety and inhibit technological progress. Moreover, structures such as storage tanks, pipelines, ships, wagons, tank trucks and nuclear waste processing facilities that store and/or transport potentially

corrosion. The weakening of the structural assembly of these installations by corrosion phenomenon can threaten in certain cases public safety and the environment.

For the above cited facts, the mastery of metal condition and its deterioration is significant (Kesavan et al. 2012). The aggressive nature of the corrosion phenomenon requires the search for effective protection methods capable of stopping or slowing the rate of degradation of the metals. The use of inhibitors is an original method that provides protection by acting on the aggressive environment (Raja et al. 2016). However, synthetic chemicals used for this purpose can solve a problem while posing an environmental one, due to their heavy metals and toxic compounds content (Rani and Basu 2012; Saufi et al. 2015; Aziate et al. 2015; Stephen and Adebayo 2018). Therefore, seeking green alternative inhibitors is a potential cure. Behind its health issue, oil rich fatty acids was found to have a great anti-corrosion property (Chebli et al. 2017).

*Ziziphus Lotus (Rhamnaceae)* is a widely spread fruit in the Mediterranean zone. It is used as a medicine to treat sore throats, alleviate stress and helps common colds (Harisson A.P. and Bartels E.M. 2006). *Ziziphus Lotus* is claimed to purify blood, help digestion and diet (Tripathi et al. 2001). *Zizyphus lotus* is also utilized for its antidiabetic, sedative and hypoglycemic properties. Each segment of the plant hold different medicinal properties, for instance the fruit is utilized for its emollient, while the leaves are suitable for health issues in the boils. Furthermore, the barks are most known for the antidiabetic properties (Anand et al. 1989; Glombitza et al. 1994). In addition, the variations in biological properties of *Ziziphus lotus* are mainly attributed to its diverse active compounds such as flavonoids, saponins, and alkaloids. It has been recorded that *Ziziphus lotus* alkaloids exhibited significant antifungal and antibacterial properties (Renault et al. 1997; Le Crouéour et al. 2002).

In a previous study, dried materiel of *Ziziphus lotus* fruit was used as green inhibitor of copper corrosion in natural sea water and showed ~93% of inhibition efficiency at 5 g/L extract concentration (Oukhrib et al. 2017). However, gape on the constituents that provide inhibitive action needs still attention. Our aim here, is to assess *Ziziphus Lotus* oil extract corrosion inhibition properties for C38 steel in 5.5 M $H_3PO_4$ solution by potentiodynamic polarization and impedance techniques.

## 2. Experimental

### 2.1. Plant material and extraction protocol

(See chapter II. Materials and methods. II. Sampling).

(See chapter II. Materials and methods. III.2.Oil extraction).

### 2.2. Gas-Chromatography for fatty acids composition

(See chapter II. Materials and methods. IX. Gas-Chromatography for fatty acids composition).

### 2.3. Electrochemical experiment

(See chapter II. Materials and methods. X.3. Electrochemical techniques for the corrosion evaluation).

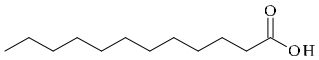
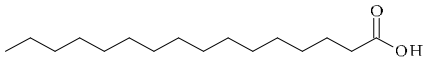
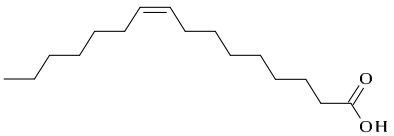
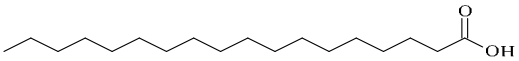
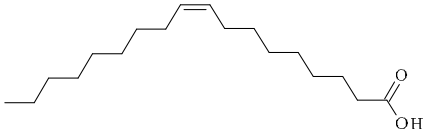
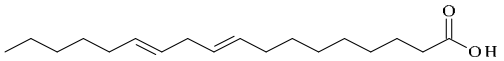
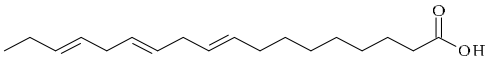
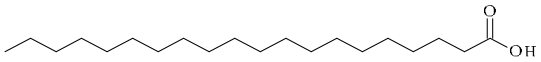
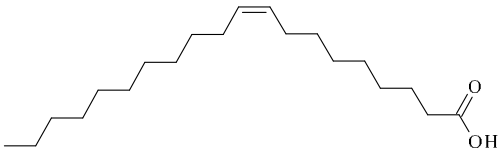
## 3. Results and discussion

### 3.1. Fatty acids composition in *Ziziphus Lotus* oil extract

The experimental result concerning fatty acids composition of *Ziziphus lotus* oil is summarized in Tab 16. The major fatty acids identified were oleic acid, linoleic acid, palmitic acid, stearic acid, linolenic acid and arachidic acid with the percentages i.e., 57.2%, 14.98%, 11.21%, 4.92%, 4.45 and 1.96, respectively. These results are in accordance with previously reported *Ziziphus Lotus* seed oil fatty acid composition where percentages were of, oleic acid (61.93%), linoleic acid (18.31%) and Palmitic acid (9.14%) (Chouaibi et al. 2012). Amongst the compounds identified, palmitic acid occurred as well as a mixture of fatty acids with 18 carbons mainly oleic acid, but also linoleic acid, stearic acid and linoleic acid with decreased percentages. Thus, the total unsaturated fatty acids (e.g., palmitoleic, oleic, linoleic, linolenic and strearic acids) content was up to 79%. In addition, the content of fatty acids with more than 20 carbon atoms in their chemical skeleton was negligible and the most prevalent unsaturated fatty acid was oleic acid. The total mono unsaturated fatty acid of *Ziziphus Lotus* oil extract is 63.27% which makes it a strong resistant to oxidative rancidity. These results are in line with the chemical compounds reported previously (Chouaibi et al. 2012). However, the percentage of each compound is different which might be due to the geographical site of the plant (Chouaibi et al. 2012). In general, vegetal oil can be obtained from other parts of the plant than its fruit and their chemical composition is somewhat

similar and doesn't exhibit major changes in content (Abdoul-Azize and Souleymane 2016). Numerous investigations demonstrated that all parts of *Ziziphus lotus* and particularly, seeds, pulp, fruits, leaves, almond, root, and stem, contained a great amount of palmitic, stearic, linoleic, and oleic acids (Chouaibi et al. 2012; Ghazghazi et al. 2014; Abdeddaim et al. 2014; Benammar et al. 2010).

**Tab 16.** Acids compositions in *Ziziphus Lotus* oil extract as followed by gas chromatography analysis.

| Compounds name | Chemical Structures of Acids | C:D | Percentage |
|---|---|---|---|
| Lauric acid |  | C12 :1 | 0.57±0.06 |
| Palmitic Acid |  | C16 :0 | 11.2±0.57 |
| Palmitoleic Acid |  | C16 :1 | 1.05±0.05 |
| Stearic Acid |  | C18 :0 | 4.92±0.06 |
| Oleic Acid |  | C18 :1 | 57.2±0.98 |
| Linoleic Acid |  | C18 :2 | 14.9±0.55 |
| Linolenic Acid |  | C18 :3 | 1.05±0.02 |
| Arachidic Acid |  | C20 :0 | 1.96±0.01 |
| Gadoleic Acid |  | C20 :1 | 4.45±0.01 |
| SFA | | 18.08 | |
| UFA | | 79.22 | |

## 3.2. Electrochemical experiment

### 3.2.1. Potentiodynamic Polarization Study

The current-potential relationships (cathodic and anodic) for C38 carbon steel in concentrated phosphoric acid with different concentrations of *Ziziphus Lotus* oil are shown in Fig 68.

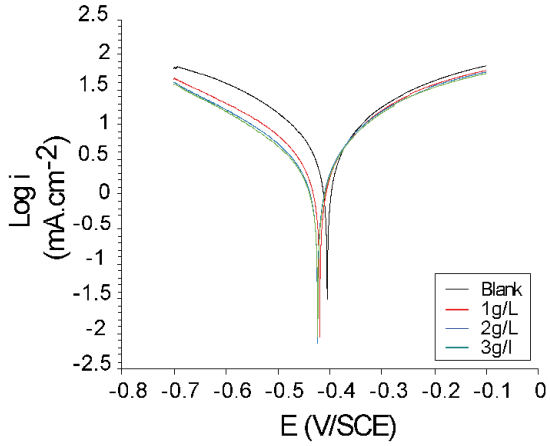



**Fig 67.** Potentiodynamic polarization curves of C38 carbon steel in 5.5 M $H_3PO_4$ in the presence of different concentrations of *Ziziphus lotus* at room temperature.

The following electrochemical indices: mixed potential ($E_{corr}$), corrosion current density ($I_{corr}$) and inhibition efficency (%) are extracted from EI curves and gathered in Tab 17.

**Tab 17.** Electrochemical parameters of C38 carbon steel in 5.5 M $H_3PO_4$ solution without and with concentrations of *Ziziphus lotus* oil extract.

| Concentration (g/L) | $E_{corr}$ (V/SCE) | $I_{corr}$ (mA/cm$^2$) | Efficiency (%) |
|---|---|---|---|
| Blank | -405.5 | 5.46 | - |
| 1 | -420.5 | 2.45 | 55.13 |
| 2 | -423.5 | 2.19 | 59.9 |
| 3 | -424.5 | 1.61 | 70.5 |

The potentiodynamic polarization curves, with and without addition of *Ziziphus Lotus* oil, exhibit the same general shape, indicating that the mechanism of anodic and cathodic reactions is

unaffected by the addition of the inhibitor and that the surface undergoes a non-reactive blockade by the inhibitor. The cathodic curves rise to Tafel lines indicating that the reduction reaction of hydrogen at C38 carbon steel surface is done by a pure activation mechanism.

The potentiodynamic polarization curves in Fig 68 suggest that the presence of *Ziziphus Lotus* oil clearly reduces the cathodic hydrogen evolution reaction of C38 carbon steel in concentrated phosphoric acid by being adsorbed and blocking active sites on its surface (Ferreira et al. 2004), whereas the anodic dissolution of C38 carbon steel remains intact. This result implies that *Ziziphus Lotus* oil is a cathodic type inhibitor for C38 carbon steel in 5.5M phosphoric acid. It is apparent from the gathered results that inhibition efficiency is greater with higher concentrations of *Ziziphus Lotus* oil. The corrosion current density diminishes with higher concentrations of *Ziziphus Lotus* oil. The inhibition efficiency reached a value of 55.13% for just 1g/L solution of oil extract.

### 3.2.2. Impedance

The EIS tests were performed to examine the different corrosion processes that takes place on C38 carbon steel and 5.5M phosphoric acid interface with different concentrations of *Ziziphus lotus* oil (0, 1, 2 et 3g/L). Fig 69 shows Nyquist plots for C38 carbon steel electrode in 5.5M phosphoric acid solution with different concentrations of *Ziziphus lotus* oil extract, at OCP potential. As shown in Fig 69, the diameter of the half circle augments with higher concentrations of the extract in 5.5M phosphoric acid, exhibiting a higher resistance of C38 carbon steel to corrosion process. In line with previous study (Ghareba and Omanovic 2010).

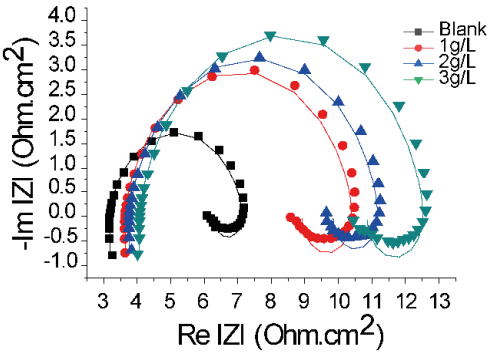



**Fig 68.** Nyquist plots of experimental values for C38 carbon steel in 5.5M phosphoric acid solution in presence of various concentrations of *Ziziphus lotus* oil at room temperature (Theoretical values in solid lines and experimental values in symbols).

To better exploit impedance analysis, an adjustment procedure was developed using an electrical circuit model. Therefore, the corresponding electrical circuit appearing in Fig 70 exhibits a favourable fitting of EIS data in all cases. The results of the adjustment parameters namely : The electrolyte resistance, the faradaic reaction resistance and C38/ 5.5M $H_3PO_4$ interfacial double layer capacitance values are presented in Tab 18 and fitting curves are presented as solid lines in the Nyquist diagrams of Fig 69.

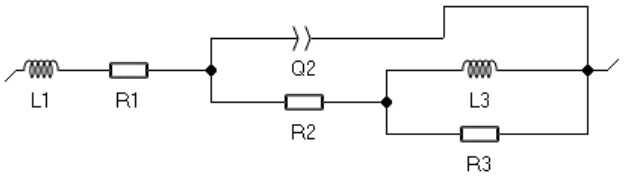



**Fig 69.** Equivalent electrical circuit tested for the modeling data of the EIS experiments.

From $f_{max}$ value which corresponds to the maximum frequency of the highest value in the imaginary part and the $R_{ct}$, the calculation of the double layer capacitance value at the interface C38/ 5.5M $H_3PO_4$ will be possible by employing the following relation (Jeyaprabha et al. 2005).

$$C_{dl} = \frac{1}{2\pi f_m R_t} \qquad \text{Eq(17)}$$

Where $C_{dl}$ Double layer capacitance (μF.cm$^{-2}$); $f_m$: maximum Frequency (Hz) and $R_t$: Charge transfer resistance (Ω.cm$^2$).

**Tab 18.** Impedance data of C38/ 5.5M $H_3PO_4$ interface with and without addition of different concentrations of *Ziziphus lotus* at room temperature.

| concentration | $L_1$ (μH) | $R_1$ (Ohm) | $Q_2$ x10$^{-3}$ (Ω$^{-1}$.cm$^{-2}$.s$^n$) | $a_2$ | $R_2$ (Ohm) | $L_3$ (H) | $R_3$ (Ohm) | Efficiency % |
|---|---|---|---|---|---|---|---|---|
| Blank | 1.236 | 3.179 | 0.369 | 0.91 | 3.102 | 0.354 | 0.862 | - |
| 1 | 1.21 | 3.63 | 0.284 | 0.91 | 5.791 | 0.647 | 1.523 | 46.43 |
| 2 | 1.13 | 3.763 | 0.282 | 0.91 | 6.582 | 0.679 | 1.37 | 52.9 |
| 3 | 1.22 | 4.06 | 0.273 | 0.91 | 7.953 | 1.11 | 1.71 | 61 |

It is noticed that adding more *Ziziphus lotus* oil concentration helps increasing the corrosion resistance and leads to a reduction in $C_{dl}$ value. The diminishing in $C_{dl}$ values occurs because of the gain in thickness of the interfacial double layer, which is in accordance with previous study (Harmaoui et al. 2015). The rise in corrosion resistance is mainly because of the appearance of a protective film on the C38/5.5M $H_3PO_4$ interface (Muralidharan et al. 1995; Bentiss et al. 2000). This observation suggests that the inhibitor molecules interact by adsorption on the C38 carbon steel surface and thereby causing decreases in $C_{dl}$ values and increases in Rct values. The high frequency inductive behavior may be due to the mutual inductance of the wires connecting potentiostat to the cell. The AC current that goes through the counter electrode and working electrode leads to creating a magnetic field around the wires which is important if the electrode impedance is low (Rodgers and Eggers 1993).

In fact, even a loop of wire, or the electrode, has some self inductance. This inductance can be clearly seen if we are dealing with low impedances (<1 ohm) at high frequencies (> 10 kHz). It is also possible to estimate the inductance of a simple wire or rod by the following relation:

$$L = 2I\left[2.303log\left(\frac{4I}{d}\right) - 1 + \frac{\mu}{4} + \frac{d}{2I}\right] \qquad \text{Eq(18)}$$

Where L is the inductance (nH), l and d are the length and the diameter of the wire respectively (cm). μ is the material permeability.

From the material used in our case (copper wire with L = 0.8 m, d = 0.8 mm and μ = 1.0), the estimated inductance value is about 1.2 μH. It is similar to the results found by EIS, taking into account that the displacement of the wires can significantly change the observed inductance (Hodgman 1963; Grover 2004).

At low frequencies, the manifestation of an inductive loop is due to the relaxation of the adsorbed species at the surface of the electrode (Corrosion product, $H^+$, etc.) (Amin et al. 2009).

Corrosion of metals and alloys in aggressive medium have been the interest of several studies seeking new routes to prevent this phenomenon. The use of inhibitors to decrease rate of corrosion is a commonly used practice and organic compounds are sought for their corrosion inhibition capabilities. In general, compounds bearing N, S and O atoms are most efficient against corrosion.

However, most of them are expensive and toxic (Raja and Sethuraman 2008). For this reason, protection of environment and the best cheap alternatives to common industrial inhibitors are always suggested. Plant extracts have become important because of their availability as well as renewability and related phytochemicals have a tendency to be effective against corrosion. It has been reported that vegetal oils demonstrated great potential against corrosion (Aziate et al. 2015; El-lateef et al. 2012; Komatsu et al. 2010) and acidic solutions are widely used in many industrial processes mainly fertilizers production (Migahed et al. 2003; Gunasekaran and Chauhan 2004; Noor 2005). However, only few studies are conducted on naturally occurring substances in $H_3PO_4$ medium as corrosion inhibitors (Zarrouk et al. 2013). The present study leads to summarize that oil extract from *Ziziphus lotus* fruit have an anticorrosion ability, which may be used as an alternative corrosion preventive method for metals, using other or similar plant extracts. Furthermore, this application allows us to add a new application as a green corrosion inhibitor besides its known medicinal use.

## 4. Conclusion

We have investigated in this work the effect of *Ziziphus lotus* oil extract on the corrosion of C38 carbon steel in a 5.5 M phosphoric acid medium at various concentrations with a couple of techniques which are, potentiodynamic polarization and electrochemical impedance spectroscopy (EIS). The *Ziziphus lotus* oil extract demonstrated a good potential as an inhibitor for corrosion of C38 carbon steel. The inhibition proficiency rose by augmenting oil extract concentrations, and reached an efficiency of ~70.5% for the polarization study and a ~61% efficiency for EIS measurements at 3g/L oil concentration. Furthermore, this oil extract acts as an cathodic type inhibitor which is explained by the adsorption of the inhibitor on the carbon steel surface. This green inhibitor has a great potential, taking into consideration that the study was conducted on a highly concentrated medium. The applications of such inhibitor, original from a largely present wild plant, are tremendous especially in industrial areas and related domain.

# Paper VI

# The corrosion inhibitory effect of *Ziziphus lotus* oil extract on C38 carbon steel in 1M HCl medium


## Summary

The present study is a follow up to the previous work, where *Ziziphus lotus* oil extract was used for C38 protection in a 1M HCl medium. Taking into consideration that in a more corrosive medium (5.5M $H_3PO_4$) the extract was able to reach an efficiency of ~70.5% for the polarization study and ~61% efficiency for EIS measurements at 3g/L oil concentration, it was interesting to observe the behavior of such extract in a less corrosive medium. 1M HCl was chosen for this study. The investigation was conducted in relation to the concentration of the inhibitor, by hydrogen gas evolution, polarization curves and electrochemical impedance spectroscopy (EIS). Potentiodynamic polarization curves indicate that *Ziziphus lotus* oil extract behaves as a mixed type inhibitor by inhibiting both the dissolution of the anodic metal and the evolutionary reaction of the cathodic hydrogen. The use of this oil extract revealed that it has a good mixed type inhibition with a maximum efficiency of 98.28% for a concentration of 4g/L. The temperature's effect on the corrosion behavior was also evaluated with the addition of the optimal concentration of *Ziziphus lotus* in the range from 293 to 323 K. The inhibition efficiency increases slightly with the increase of temperature.

# The corrosion inhibitory effect of *Ziziphus lotus* oil extract on C38 carbon steel in 1M HCl medium (To be submitted)

**Abstract**

*Ziziphus lotus* is a widely spread wild plant that has many traditional uses. In the present study, the effect of *Ziziphus lotus* oil extract on the corrosion of C38 carbon steel in 1 M HCl has been investigated in relation to the concentration of the inhibitor, by hydrogen gas evolution, polarization curves and electrochemical impedance spectroscopy (EIS). The use of this oil extract revealed that it has a good mixed type inhibition with a maximum efficiency of 98.28% for a concentration of 4g/L. The temperature's effect on the corrosion behavior was also evaluated with the addition of the optimal concentration of Ziziphus lotus in the 293 to 323 K range. The inhibition efficiency increases slightly with the increase of temperature. Changes in impedance parameters (charge transfer resistance, $R$ct, and double layer capacitance, $C_{dl}$) were indicative of an adsorption of *Ziziphus lotus* oil extract on the metal surface, leading to the formation of a protective layer. The commonly used adsorption isotherms have not shown satisfactory results for modeling the adsorption of *Ziziphus lotus* oil extract on C38 carbon steel. However, some thermodynamic functions of dissolution and adsorption processes have shown that the organic content of this oil adsorb to the metal surface with strong bonds (chemisorption).

## 1. Introduction

Corrosion is a major issue related to steel made structures, vehicles and products that influences their durability. The corrosion of iron, or rust, can cause structural damage and lead to changes in the mechanical and chemical properties of plants, vessels, pipes, and other processing equipment. Such problems could pose a tremendous amount of materials losses and costs, thus study and research of this phenomenon is mandatory. Various industries, such as chemical and petrochemical processing plants, that use steel in acidic environments could benefit from the corrosion preventive methods.

The use of inhibitors to avoid degradation of materials is widely applied and it has become a necessity. In fact, organic inhibitors were applied extensively to protect metals from corrosion in many aggressive acidic medium (e.g. in the acid pickling and cleaning processes of metals) (Badr 2009; Stanly and Parameswaran 2010). Organic compounds bearing N, S and O atoms were found to be good corrosion inhibitors of metals particularly for active metals like Fe, Zn, and Mg (Larif et al. 2013). Furthermore, molecular structure, size, mass, heteroatoms present and adsorptive tendencies are in part responsible for the efficiency of organic inhibitors. Under specific conditions, the electronic structure of the organic inhibitors has a key influence on the corrosion inhibition efficiency to the metal. The inhibitors influence the kinetics of the electrochemical reactions, which constitute the corrosion process and thereby modify the metal dissolution in acids. Synthetic compounds have demonstrated a good anticorrosion potential. However, the efficiency of these compounds comes with a high harmfulness for the environment. That is why the use of ecofriendly and natural substances are considered as green corrosion inhibitors.

Oils and plant extracts have become an excellent source of ecological inhibitors that ensure high efficiency at a lower price. The extracts obtained from natural sources consist of a large amount of various chemical constituents containing aromatics, carbonyl (-C=O), carboxylic (-COOH), hydroxyl (-OH) and amine (-NH, $-NH_2$) groups. As a result, these compounds can be potential inhibitors for retarding the corrosion process of mild steel in acidic medium. The aim of the present study is to assess the feasibility of using the naturally occurring, cheap and environmentally safe oil extract of *Ziziphus lotus* for the corrosion inhibition of C38 carbon steel in acidic medium. For this purpose, potentiodynamic polarization curves, electrochemical impedance spectroscopy (EIS) and measurements of the hydrogen evolution (gasometry) were used. The use of this extract is expected to achieve simultaneously, the economic and environmental goals.

## 2. Materials ans methods

### 2.1 Materials

(See chapter II. Materials and methods. X.1. Material and study medium).

### 2.2 Oil extraction

(See chapter II. Materials and methods. III.2.Oil extraction).

### 2.3 Gasometric measurements

(See chapter II. Materials and methods. X.2. Immersion test: Gasometric measurements).

### 3.4 Electrochemical tests

(See chapter II. Materials and methods. X.3. Electrochemical techniques for the corrosion evaluation).

## 3. Results and discussion

### 3.1. Gasometry

By observing the direct relationship between the volume of hydrogen evolved and the rate of corrosion, the measurement of the hydrogen gas that emerges at the cathodic sites, as a function of time, can give valuable information on the process of the corrosion. The evolution of the hydrogen gas evolved during the corrosion reaction of C38 steel in the 1M HCl medium without and with the addition of different concentrations of *Ziziphus lotus* oil extract was monitored for 48 hours (Fig 71). The results reveals that hydrogen gas evolved on C38 steel surface decreases significantly with a greater inhibitor concentration, indicating that the protection ability of *Ziziphus lotus* oil extract was depending on the concentrations. Moreover, a linear corrolation is observed over time for the hydrogen gas, which is reduced in presence of the inhibitor. Furthermore, Fig 72 shows the results of the calculation of the loss in thickness and inhibitor efficiency of C38 steel in 1M HCl, considering that the reaction modeling the general process of the corrosion in these conditions R8:

$$Fe + 2H^+ \rightarrow Fe^{2+} + H_2 \qquad R8$$

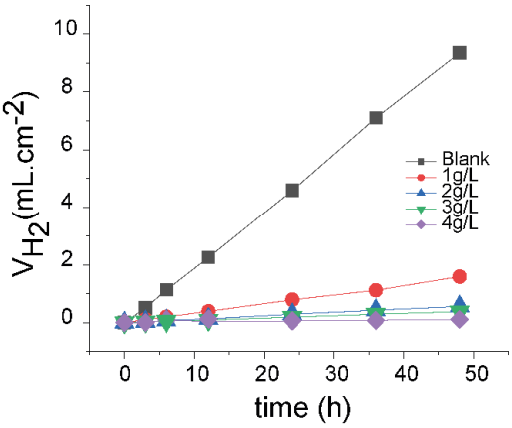



**Fig 70.** Evolution of the volume of hydrogen gas evolved on C38 steel as a function of time in a 1M HCl medium with and without different concentrations of *Ziziphus lotus* oil extract.

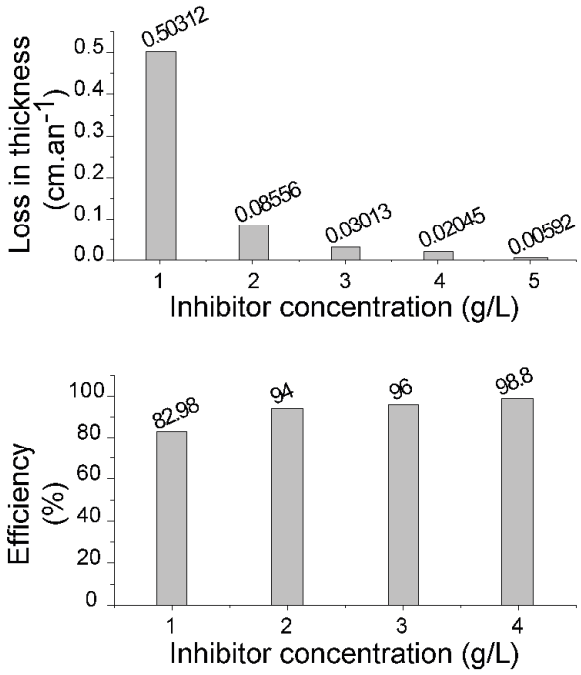



**Fig 71.** The evolution of the thickness loss of C38 steel and efficiency of *Ziziphus lotus* oil extract as a function of its concentration.

The high value (0.5 cm/year) of the corrosion rate of C38 steel in HCL 1M medium shows that this metal is not resistant to the degradation in contact with this aggressive medium. The presence of the inhibitor implies a progressive decrease in the rate of corrosion with the increase of its

concentration. The addition of the inhibitor allowed reducing this rate by a factor of 84, for a concentration of 4 g/L (0.0059 cm/year), which corresponds to an efficiency of 98.8%. As a result, the rate of corrosion becomes comparable to that of high alloy steels. This method has the advantage of being a simple implementation, not requiring an important apparatus, but does not allow the approach of the mechanisms involved during corrosion.

### 3.2.Tafel polarization

The current potential correlation of C38 steel in 1M HCl solution in the absence and presence of various concentrations of *Ziziphus lotus* oil extract at 20°C are shown in Fig 73. The extrapolation of tafel straight line allows the calculation of the corrosion current density ($I_{corr}$). The values of $I_{corr}$, the corrosion potential ($E_{corr}$), cathodic tafel slopes (βc) and the percentage of inhibition efficiency (IE %) are given in the Tab 19.

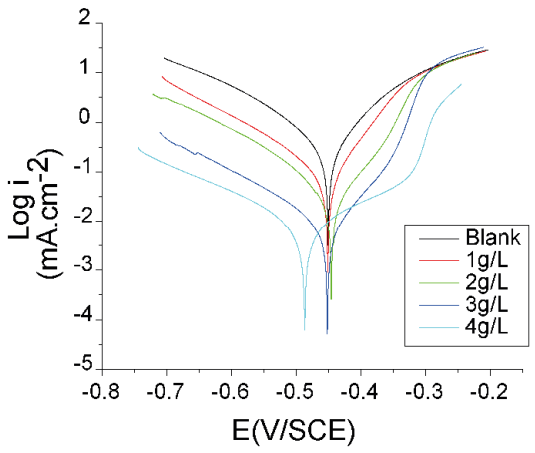



**Fig 72.** Polarization Curves of C38 Steel in 1M HCl Solution containing different concentrations of *Ziziphus lotus* oil extract.

It is evident from Fig 73 that cathodic Tafel slopes (βc) remain almost unchanged with increasing inhibitor concentration. This indicates that hydrogen evolution is activation controlled and the addition of the inhibitor did not change the mechanism of the cathodic hydrogen evolution reaction. On the other hand, the anodic branch shape significantly changed. This means that the inhibitor molecules adsorption resulted in the significant change of the iron dissolution mechanism (W. H. Li et al. 2008; Soltani et al. 2012). Both anodic and cathodic branches shifted to lower

current densities, reflecting the mixed inhibition effects of *Ziziphus lotus* oil extract on mild steel in 1M HCl. In fact, if the displacement in E(i) is > 85 mV with respect to E, the inhibitor can be seen as a cathodic or anodic type, (ii) if the displacement in E is < 85, the inhibitor can be seen as mixed type. In our study, the maximum displacement is less than 85, which confirms that *Ziziphus lotus* oil extract is a mixed-type inhibitor.

**Tab 19.** Electrochemical parameters of C38 Steel in 1M HCl solution without and with addition of *Ziziphus lotus* oil extract at different concentrations.

| Concentration g/L | E (mV) | βa (mV) | - βc (mV) | Icorr ($\mu A.cm^{-2}$) | IE % |
|---|---|---|---|---|---|
| Blank | -0.452 | 109.1 | 135.1 | 368 | - |
| 1 | -0.454 | 146.5 | 138.6 | 58.3 | 84.15 |
| 2 | -0.445 | 179.1 | 141.7 | 21.7 | 94.1 |
| 3 | -0.451 | 198 | 137.1 | 10.4 | 97.17 |
| 4 | -0.486 | 116.4 | 129.5 | 6.7 | 98.17 |

As shown in Tab 19, the $i_{corr}$ remarkably decreased with the increase in *Ziziphus lotus* oil extract concentration. However, in the presence of *Ziziphus lotus* oil extract the $E_{corr}$ slightly shifted to cathodic branch direction. Therefore, it can be said that the dominant effect of the inhibitor is on the cathodic hydrogen evolution reaction rate. Inhibition efficiency (IE %) increases with increase in concentration of the inhibitor and reaches a maximum of 98.17 % for a concentration of 4g/L.

### 3.3. Electrochemical impedance spectroscopy

The electrochemical impedance spectroscopy was used to better understand the interface C38 steel and 1M HCl solution characteristics. The EIS measurements were recorded in potentiostatic conditions after 30 minutes of immersion in absence and presence of *Ziziphus lotus* oil extract. The results are shown in Fig 74.

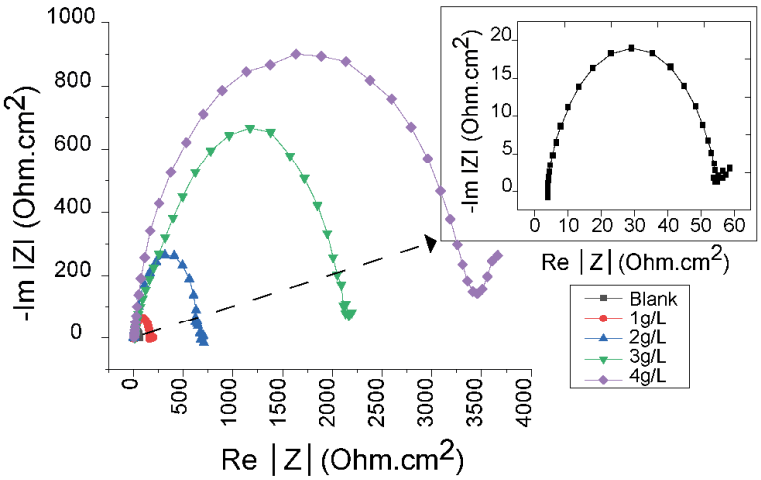



**Fig 73.** The Nyquist plots for C38 steel in 1M HCl with and without different concentrations of *Ziziphus lotus* oil extract at 20°C.

The Nyquist diagrams show single depressed semicircles over the frequency range studied, indicating that the dissolution process is controlled by a charge-transfer reaction. This behavior can be also interpreted as an ideal capacitor deviation behavior. Therefore, a constant phase element (CPE) was used to simulate the non-ideal behavior of the capacitive elements. This behavior was attributed to several factors (i.e., surface inhomogeneity and roughness, adsorption of compounds, dislocations, grain boundaries, the formation of porous layers and the presence of impurities). The impedance of this element is defined as described by Eq (19) (BenSalah et al. 2014; Escrivà-Cerdán et al. 2013):

$$Z_{cpe} = A^{-1}(i\omega)^{-n} \qquad \text{Eq (19)}$$

Where A is the CPE constant, $\boldsymbol{\omega}$ is the angular frequency (rad.s$^{-1}$), i$^2$ = -1 is the imaginary number and n is the CPE exponent (BenSalah et al. 2014). For the value of n = 1, 0, -1 the CPE is reduced respectively to a plane capacitor (C), resistance (R) and an inductor (L). When n = 0.5, it is the Warburg impedance (W).

To account for the corrosion behavior of C38 steel in 1M HCl with the presence of *Ziziphus lotus* oil extract and to simulate the metal/acidic solution interface, a simple Randles circuit presented in Fig 75 consisting of solution resistance (Rs), a constant phase element (CPE) in parallel to a charge transfer resistance Rt gives a good fitting of EIS data in all cases.

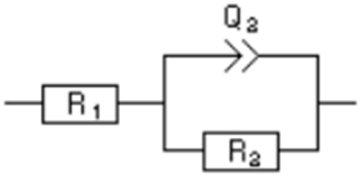



**Fig 74.** Equivalent circuit mode.

**Tab 20.** Impedance data of C38 steel in 1M HCl with and without addition of *Ziziphus lotus* oil extract at various concentrations.

| Concentration g/L | R1 ($\Omega.cm^2$) | R2 ($\Omega.cm^2$) | Q2 10-3 ($\Omega^{-1}.cm^{-2}.sn$) | $a^2$ | IE % |
|---|---|---|---|---|---|
| Blank | 3.57 | 51.73 | 0.29 | 0.83 | - |
| 1 | 4.042 | 165 | 0.175 | 0.84 | 68.64 |
| 2 | 4.004 | 668.8 | 0.08 | 0.84 | 92.2 |
| 3 | 10.76 | 2139 | 0.072 | 0.7 | 97.58 |
| 4 | 6.108 | 3015 | 0.011 | 0.78 | 98.28 |

The theoretical parameters for the simulated impedance were calculated by the EC-Lab software, and the results are summarized in Tab 20. The decrease of double layer capacitance may be due to the decrease of the local dielectric constant or the increase of the thickness of the electrical double layer, indicating that *Ziziphus lotus* oil extract adsorbed on the C38 steel surface. The increase of charge transfer resistance is resulting from the formation of protective film on the C38 steel/solution interface. The inhibition efficiencies recorded by EIS is 98.28% for C38 steel in 1M HCl with 4 g/L of *Ziziphus lotus* oil extract. This result also is in good agreement with the results obtained from gasometric technique and tafel measurement.

### 3.4. Adsorption isotherm and temperature effect

Adsorption isotherms are very important in understanding the mechanism of inhibition of corrosion reaction of metals and alloys. The most frequently used adsorption isotherms are Frumkin, Temkin, Freundlich, Flory Huggins, Bockris-Swinkel, El-Awardy and Langmuir isotherms. All these isotherms can be represented as shown in Eq (20):

$$f(\theta, x)\exp(-2a\theta) = kC \qquad Eq\ (20)$$

Where $f$(θ,x) is the configuration factor which depends upon the physical model and the assumptions underlying the derivation of the isotherm. θ is the degree of surface coverage, C is the inhibitor concentration in the electrolyte, X is the size ratio, a is the molecular interaction parameter and k is the equilibrium constant of the adsorption process. Attempts to adjust the data obtained from the previous results in different adsorption isotherms reveal that the data do not correspond to any of these adsorption isotherms. The use of adsorption isotherms implies that there is a non-reactive blockage of the surface of the steel. In this case, the inhibitor intervenes only by subtracting a portion of the surface from one of the elementary reactions, anodic or cathodic, without modifying the activation energy of these reactions. This amounts to decreasing the corresponding reaction surface. Therefore, the recovery rate will be directly proportional to the inhibitory efficacy. Not all these considerations are longer applied in our case. The polarization curves clearly reveal the change in the reaction mechanism of the iron oxidation reaction after the addition of extracts. When there is not simply blocking of the preferred sites of dissolution of the metal by adsorption of the inhibitor, one must imagine an intervention of the inhibitor at the level of the reaction intermediates accompanying the various stages of the dissolution of a metal.

Temperature is one of the most important factors to be considered because of its complex effect on the metal/inhibitor interaction (Obot and Obi-Egbedi 2010). Changes that may occur as a result of an increase in temperature (Yüce and Kardaş 2012). For this purpose, we made polarization experiments with temperatures ranging from 20 to 50°C (Fig 76), in the absence and presence of 4g/L of *Ziziphus lotus* oil extract after 30 minutes of immersion. The corresponding data are shown in Tab 21.

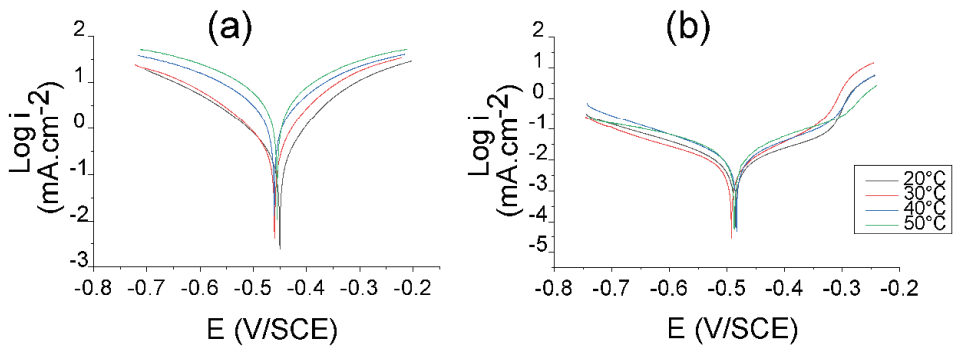



**Fig 75.** Effect of temperature on polarization curves of C38 corrosion rate in free (a) and inhibited (b) acid solution.

**Tab 21.** Various corrosion parameters for C38 steel in 1M HCl in absence and presence of optimum concentration of *Ziziphus lotus* oil extract at different temperatures.

| T (°C) | Blank | | With 4 g/L | | IE% |
|---|---|---|---|---|---|
| | E (mV/SCE) | i (µA.cm$^{-2}$) | E (mV/SCE) | i (µA.cm$^{-2}$) | |
| 20 | -0.452 | 368 | -0.486 | 6.7 | 98.17 |
| 30 | -0.459 | 489 | -0.493 | 8.8 | 98.20 |
| 40 | -0.462 | 1879 | -0.484 | 14.5 | 99.22 |
| 50 | -0.454 | 2119 | -0.487 | 17.8 | 99.15 |

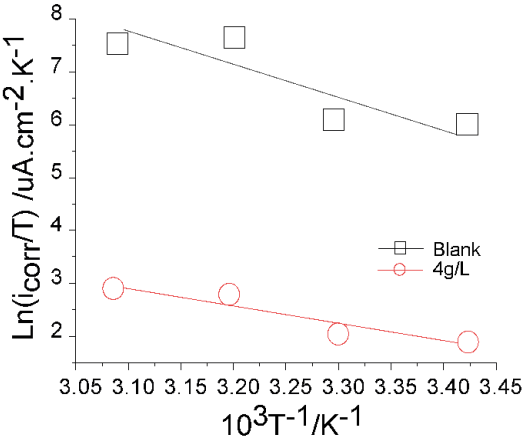



**Fig 76.** Arrhenius plots of C38 steel in HCl 1M with and without 4g/L of *Ziziphus lotus* oil extract.

The inhibition efficiencies were found to increase with increasing temperature from 20-50°C. Adsorption of inhibitor is aided by increasing temperature. This proves that the inhibition occurs through the adsorption of the inhibitor on the metal surface. Enhancement in inhibition competence with growing temperature is ascribed to a change in the nature of adsorption, wherein the inhibitor is materially adsorbed at inferior temperature while chemisorption is favoured at higher temperature. The activation parameters for the corrosion process were calculated from the Arrhenius type plot according to the Eq (21):

$$\mathrm{I_{corr}} = \mathrm{A}\exp\left(-\frac{\mathrm{E_a}}{\mathrm{RT}}\right) \qquad Eq(21)$$

Where $E_a$ is the activation energy of the corrosion process, is the Arrhenius pre-exponential factor, $T$ is the absolute temperature, and $R$ is the universal gas constant. The values of $E_a$ for C38 steel in 1M HCl without and with *Ziziphus lotus* oil extract are obtained from the slope of the plot of log $I_{corr}$ versus 1/$T$ (Fig 77) and are shown in Tab 22.

$E_a$ value for inhibited system is lower than this for the uninhibited system suggest that this type of inhibitor shows an increase of the protective character with temperature. The organic molecules of the inhibitor adsorb to the metal surface by strong bonds (chemisorption) (Radovico 1990).

Alternative Arrhenius plots of log $I_{corr}/T$ versus 1/$T$ (Fig 78) for C38 steel dissolution in HCl medium in the absence and presence of 4g/L of *Ziziphus lotus* oil extract was used to calculate the values of activation thermodynamic parameters such as enthalpy of activation ($\Delta H°_a$) and entropy of activation ($\Delta S°_a$) using the Eq (22):

$$I_{corr} = \frac{RT}{Nh}\exp\left(\frac{\Delta S_a^\circ}{R}\right)\exp\left(-\frac{\Delta \mathrm{H}_a^\circ}{RT}\right) \qquad Eq(22)$$

The values of $E_a$, $\Delta H_a^\circ$ and $\Delta S_a^\circ$ were estimated from the slopes of the straight lines and given in Tab 22. Where $E_a$ is the activation energy of the corrosion process, A is the pre-exponential factor, R the general gas constant, h is the plank's constant, N is Avogadro's number, $\Delta S_a^\circ$ is the apparent

entropy of activation and $\Delta H_a^\circ$ is the apparent enthalpy of activation. Fig 78 showed a plot of Ln ($I_{corr}$/T) versus 1/T. The straight lines are obtained with a slope $\left(-\frac{\Delta H_a^\circ}{RT}\right)$ and an intercept of $\left(Ln\frac{RT}{Nh}+\frac{\Delta S_a^\circ}{R}\right)$ from which the values of $\Delta H_a^\circ$ and $\Delta S_a^\circ$ are calculated and are given in Tab 22.

The positive values of enthalpies reflect the endothermic nature of steel dissolution process. It is well noticed that the values of $E_a$ are larger than the analogous values of $\Delta H_{ads}$ indicating that the corrosion process might have involved a gaseous reaction, like the hydrogen evolution reaction, associated with a decrease in the total reaction volume (Andreani et al. 2016). The negative values of entropies showed that the activated complex in the rate determining step represents an association rather than a dissociation step. It is clear that $\Delta S_{ads}$ decreased in value in the presence of *Ziziphus lotus* oil extract compared to uninhibited acid. In uninhibited solution the transition state of the rate determining recombination step represents a more orderly arrangement relative to the initial state, so a high value for the entropy of activation is obtained. In the presence of *Ziziphus lotus o*il extract, however, the rate-determining step is the discharge of hydrogen ions to form adsorbed hydrogen atoms. Since the surface is covered with extract molecules, this will retard the discharge of hydrogen ions at the metal surface causing the system to pass from a random arrangement, and hence entropy of activation is decreased (Dahmani et al. 2010).

**Tab 22.** Activation parameters for C38 steel dissolution in 1M HCl in the absence and the presence of *Ziziphus lotus* oil extract at optimum concentration.

| Inhibitor | Linear regression coefficient ($R^2$) | Ea (kJ/mol) | $\Delta H_a^\circ$ (kJ/mol) | $\Delta S_a^\circ$ ($J/mol^{-1}$) |
|---|---|---|---|---|
| HCl 1M | 0.89 | 51.95 | 49.4 | -27.60 |
| HCl 1M + 4g/L of inhibitor | 0.98 | 27 | 24.45 | -145.41 |

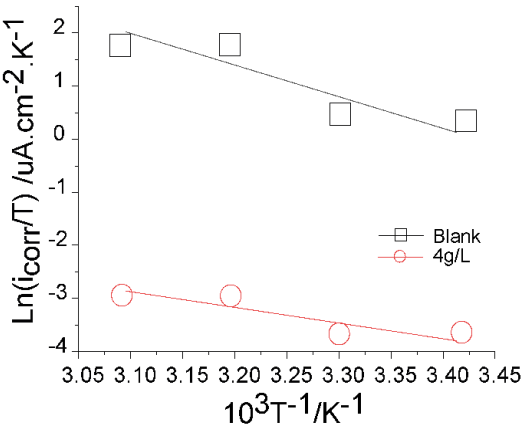



**Fig 77.** Arrhenius plots of Ln Icorr/T vs. 1/T for C38 steel in 1M HCl in the absence and the presence of *Ziziphus lotus* oil extract at optimum concentration.

## 4. Conclusion

In this work, we studied the inhibitory effect of *Ziziphus lotus* oil extract on the corrosion of C38 steel in 1M HCl medium, using chemical and electrochemical techniques. The results obtained showed that the extract has excellent effect of inhibiting the corrosion of C38 steel in 1M HCl medium. Its inhibition efficiency depends on both concentration and temperature. The high inhibition efficiency of the extract was attributed to the adherent adsorption of the inhibitory molecules on the metal surface. Potentiodynamic polarization curves indicate that Ziziphus lotus oil extract behaves as a mixed type inhibitor by inhibiting both the dissolution of the anodic metal and the evolutionary reaction of the cathodic hydrogen. According to the values obtained from the kinetic and thermodynamic parameters, the adsorption of the extract is carried out according to a chemisorption mechanism and obeys none of the adsorption isotherms usually used.

# Paper VII

# The corrosion inhibitory effect of extracts from *Urtica dioica*, *Chenopodium murale* and *Mentha rutondifolia* on C38 carbon steel in 5.5 M $H_3PO_4$ medium


## Summary

*Urtica dioica, Chenopodium murale* and *Mentha rutondifolia* are three wild abundant plants from Morocco. The plant materiel was extracted using two different solvents (i.e., Methanol and 80% Ethanol). Plants extracts were used in this study to asses their ability to protect C38 steel in phosphoric acid medium with a concentration of 5.5 M by impedance and potentiodynamic polarization techniques. The use of the chosen solvents enables to gather mainly polyphenols in these plants, which are known by their high content in polyphenols. These extracts were found to act for the most as mixed type inhibitor. Thus, the inhibition efficiency of these extracts increase with the concentrations and achieve ~91 % at 3 g/L of *Chenopodium murale*, 80% ethanol extract. The highly concentrated medium makes the results obtained even more significant, proving that these green inhibitors have great potential. The applications of such inhibitor are tremendous especially in industrial areas and related domain.

# The corrosion inhibitory effect of extracts from *Urtica dioica*, *Chenopodium murale* and *Mentha rutondifolia* on C38 carbon steel in 5.5 M $H_3PO_4$ medium (To be submitted)

**Abstract**

In the current study, corrosion inhibition effect of three wild plants extracts from Morocco was tested on corrosion of C38 steel in phosphoric acid medium with a concentration of 5.5 M by impedance and potentiodynamic polarization techniques. These extracts were prepared using two different solvents, methanol and 80% ethanol. Electrochemical impedance spectroscopy (EIS) data was analyzed by adapting it to a well developed electric circuit model. The inhibition proficiences of these extracts were assessed and compared using tafel polarization and EIS, respectively. Accordingly, the extracts were found to act as a mixed type inhibitor. Furthermore, inhibition efficacity of these extracts increases with the concentrations to achieve as high as ~ 91% at 3 g/L solution of *Chenopodium murale*, 80% ethanol extract. The results gathered from different tested methods were in line, and the extracts were able to reduce significantly the kinetic of the corrosion phenomena of C38 carbon steel.

## 1. Introduction

Metals and alloys are used in different areas and environments. The reaction of these metals and alloys with their usage surroundings can result sometimes in corrosion. The phenomenon of corrosion consists of a return of the metal to its stable oxide state by reacting with the surrounding environment (Finšgar and Jackson 2014).This issue that is present in multiple industries, such as phosphoric acid production, cannot be avoided for most cases (Finšgar and Jackson 2014). A more practical approach would be to apply a product that would slow this phenomenon. The occurrence of this phenomenon can, over time, alter the properties of the metal surface and its surrounding environment. Alterations such as pH changes, oxides appearance and electrochemical potential variations (Rani and Basu 2012).

Synthetic compounds have demonstrated a good anticorrosion potential. However, the efficiency of these compounds comes with a high toxicity for the environment. That is why the use of eco-friendly and natural substances such as vegetal oils or plant extracts are considered as green corrosion inhibitors. Oils and plant extracts have become an excellent source of ecological inhibitors that ensure high efficiency at a lower price. The extracts obtained from natural sources consist of a large amount of various chemical constituents containing aromatic rings, carbonyl (-C=O), carboxylic (-COOH), hydroxyl (-OH) and amine (-NH, $-NH_2$) groups. As a result, these compounds can be potential inhibitors for retarding the corrosion process of mild steel in acidic medium. The aim of the present work is to assess the feasibility of using the naturally occurring, cheap and environmentally safe extracts from three different plants, namely *Urtica dioica, Chenopodium murale and Mentha rutondifolia*, prepared by two different solvents, methanol and 80% ethanol, for the corrosion inhibition of C38 carbon steel in acidic medium. For this purpose, open circuit potential, potentiodynamic polarization curves and electrochemical impedance spectroscopy (EIS) were used. The use of this extracts is expected to achieve simultaneously, the economic and environmental goals.

## 2. Material and methods

### 2.1. Plant material and extraction protocol

(See chapter II. Materials and methods. II. Sampling).

(See chapter II. Materials and methods. III.1.Solvent based extracts preparation).

Abbreviations were attribuated to each extract. The abbreaviations “UDEA”, “CMEA” and “MREA” were given to 80% Ethanol extracts of *Urtica dioica, Chenopodium murale* and *Mentha rutondifolia*, respectively. The abbreaviations “UDMA”, “CMMA” and “MRMA” were given to methanol extracts of *Urtica dioica, Chenopodium murale* and *Mentha rutondifolia*, respectively.

### 2.2. Electrochemical experiment

(See chapter II. Materials and methods. X.3. Electrochemical techniques for the corrosion evaluation).

## 3. Results and discussion

### 3.1. Open circuit potential

The evolution of the OCP potential of C38 acid in 5.5M $H_3PO_4$ medium in the presence of 3 wild plants extracts by two different solvents was studied. The results are shown in Fig 79. Almost the same tendency is observed for all plants extracts. There is an increase of the potential of C38 in 5.5M $H_3PO_4$ as a function of the time which can be linked to the formation of a layer of iron oxide, which persists on the surface of the metal and contributes to the ennobling of steel. For all extracts; whatever the solvent, there is a gradual decrease in the steady state OCP potential with the addition of different concentrations of the inhibitor; which suggests the presence of a direct link between potential and the quantity of the used inhibitor.

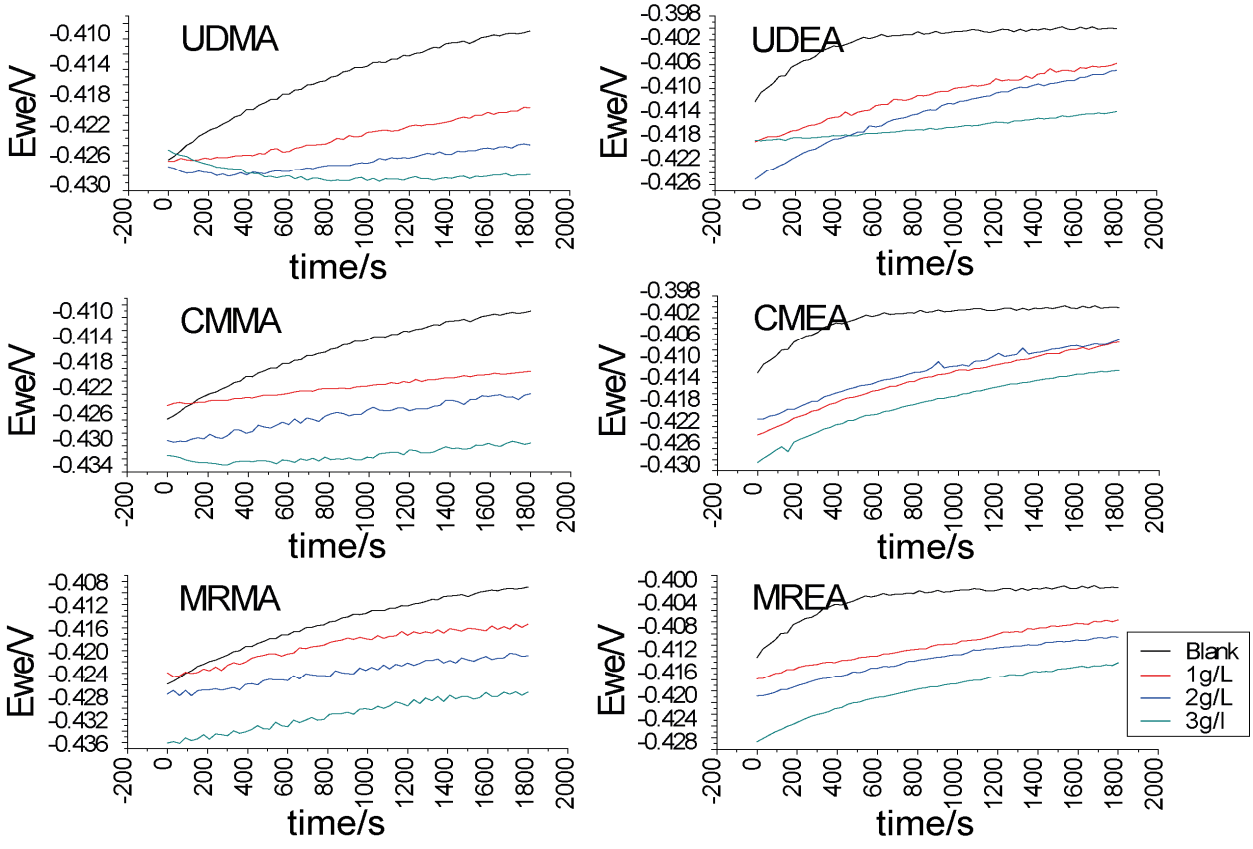



**Fig 78.** Potential time curve for different concentrations of *Urtica dioica, Chenopodium murale* and *Mentha rutondifolia*, 80% ethanol and methanol extracts.

In relation with previous studies, the difference between the interfacial potential of the inhibited system compared to the free system may give an indication of the type of the inhibitor. If the difference is greater than 85mV, the inhibitor can be treated as a cathodic or anodic type. In the opposite case (<85 mV), it is considered as a mixed type inhibitor (Jeroundi et al. 2016; O. L. Riggs Jr. 1973; Arukalam et al. 2014; Verma et al. 2017). In our study, the maximum displacement is lower than 85 mV, which indicates that these extracts act as a mixed type inhibitor.

### 3.2. Potentiodynamic Polarization Study

The current-potential relationships (cathodic and anodic) for C38 carbon steel in concentrated phosphoric acid with different concentrations of the different extracts are shown in Fig 80.

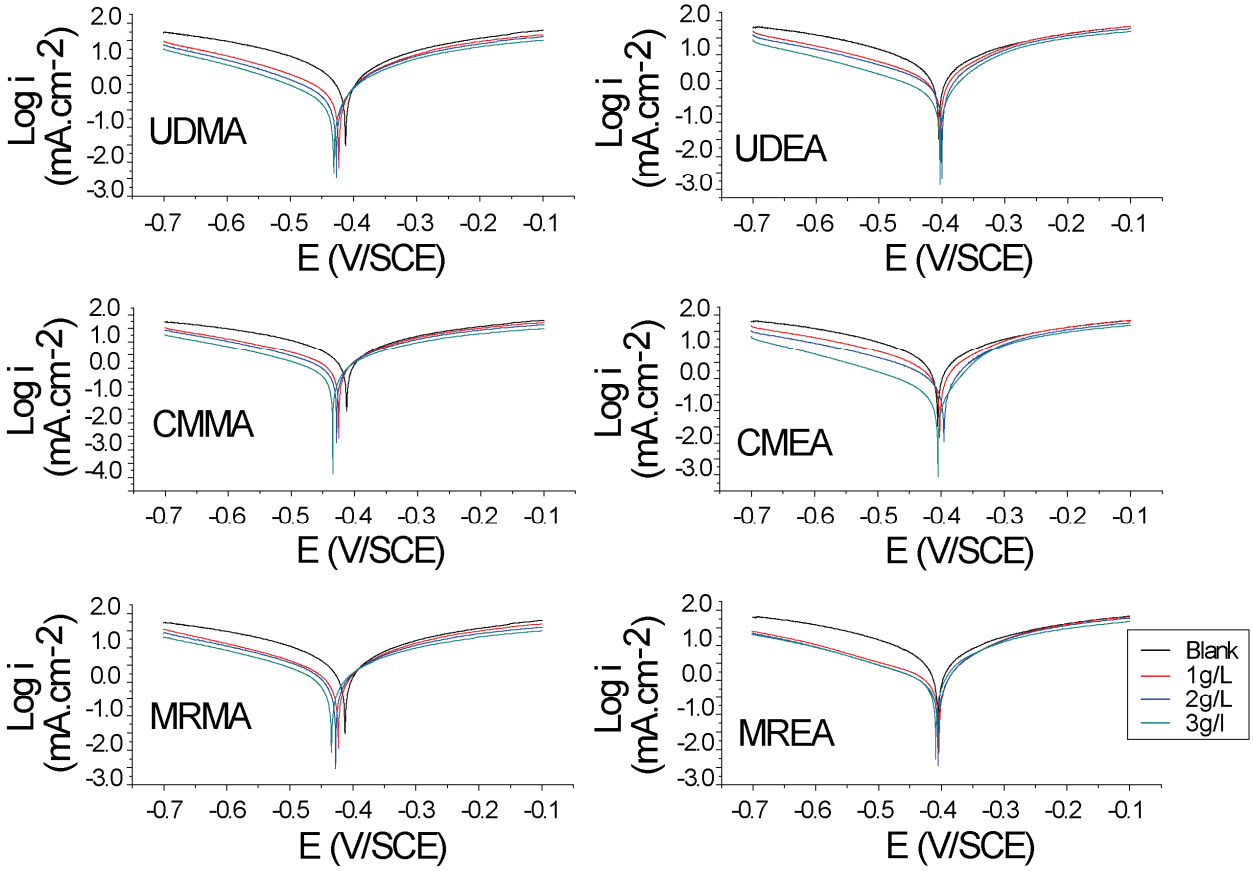



**Fig 79.** Tafel curve for different concentrations of *Urtica dioica, Chenopodium murale* and *Mentha rutondifolia*, 80% ethanol and methanol extracts.

The following electrochemical indices: mixed potential (Ecorr), corrosion current density (Icorr) and inhibition Efficency (%) are extracted from EI curves and gathered in Tab 23.

**Tab 23.** Electrochemical parameters of C38 carbon steel in 5.5 M $H_3PO_4$ solution with and whitout inhibitor.

| Sample | Concentration ( g/L) | Ecorr (mV/SCE) | Icorr (mA/cm$^2$) | Efficiency (%) | Sample | Concentration ( g/L) | Ecorr (mV/SCE) | Icorr (mA/cm$^2$) | Efficiency (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Blank | -412.66 | 5.20 | - | | Blank | -405.37 | 5.72 | - |
| UDMA | 1 | -423.11 | 2.25 | 56.73 | UDEA | 1 | -403.34 | 2.85 | 50.17 |
| | 2 | -427.15 | 1.79 | 65.58 | | 2 | -399.90 | 2.12 | 62.94 |
| | 3 | -431.60 | 1.35 | 74.04 | | 3 | -403.00 | 1.33 | 76.75 |
| CMMA | 1 | -424.56 | 2.62 | 49.62 | CMEA | 1 | -402.06 | 2.82 | 50.69 |
| | 2 | -428.53 | 2.38 | 54.23 | | 2 | -395.24 | 1.93 | 66.26 |
| | 3 | -434.43 | 1.63 | 68.65 | | 3 | -404.96 | 1.59 | 72.20 |
| MRMA | 1 | -423.65 | 3.05 | 41.35 | MREA | 1 | -403.95 | 1.76 | 69.23 |
| | 2 | -427.21 | 2.52 | 51.54 | | 2 | -405.50 | 1.89 | 66.96 |
| | 3 | -433.80 | 2.36 | 54.62 | | 3 | -445.89 | 1.95 | 65.91 |

It can be observed from the potential current curves that the cathodic portion reflecting the reduction of the hydrogen ions evolves exponentially, which indicates that the hydrogen reduction at the cathode sites is done with an activation mechanism. This observation is valid for all the extracts of our chosen plants regardless of the solvent. A decrease in cathodic current density is observed with increasing inhibitor concentration. For the “MREA” case, above a concentration of 1g/L, adding more inhibitor does not have a significatif effect on the curve shape. The anodic current represents the metal oxidation, and diminishes with increasing inhibitor’s concentration. It can be observed from the curves that the current diminution in the anodic sites are less than those

of the cathodic sites. From these observations, it can be concluded that all the used extracts have a mixed type tendencies, with a predominance for the cathodic process.

It should be mentioned that the Tafel slope (anodic and cathodic) exhibit a noticeable change with the addition of the extracts in all cases. This suggests that a change in the mechanism of the oxidation reaction of metal and hydrogen reduction occurs. The following reactions R9, R10 and R11, are used to explain the mechanism of protons reduction.

$$Fe + H^+ \leftrightarrow (FeH^+)_{ads} \quad (R9)$$

$$(FeH^+)_{ads} + e^- \leftrightarrow (FeH)_{ads} \quad (R10)$$

$$(FeH)_{ads} + H^+ + e^- \leftrightarrow Fe + H_2 \quad (R11)$$

The effect of addition of the extracts used in this study can be explained by a potential substitution of the hydrogen ions by the inhibitor molecules which can form a barrier that stops the access of the hydrogen ions to the metal surface. For methanolic extracts, the corrosion potential decreases with the increase of inhibitor concentration, which is in agreement with the results of the OCP curves; which is not the case for ethanolic extracts. For ethanolic extracts an increase in corrosion potential with an increase in inhibitor concentration. The inhibition efficiency reached values of 74.07%, 68.65%, 54.62%, 76.75%, 72.20% and 65.91% for 3g/L concentration of UDMA, CMMA, MRMA, UDEA, CMEA and MREA, respectively.

### 3.3. Impedance

The EIS tests were performed to examine the different corrosion processes that takes place on C38 carbon steel/5.5M phosphoric acid interface with different concentrations of the green inhibitors used (0, 1, 2 et 3g/L). Fig 82 shows Nyquist plots for C38 carbon steel electrode in 5.5M phosphoric acid solution with different concentrations of extracts, at OCP potential. As shown in Fig 82, the diameter of the half circle augments with higher concentrations of the extract in 5.5M phosphoric acid, exhibiting a higher resistance of C38 carbon steel to corrosion process.

To better exploit impedance analysis, an adjustment procedure was developed using an electrical circuit model. Therefore, the corresponding electrical circuit appearing in Fig 82 exhibits a favorable fitting of EIS data in all cases. The results of the adjustment parameters namely: The

electrolyte resistance (R1), the faradic reaction resistance (R2) and C38/ 5.5M $H_3PO_4$ interfacial double layer capacitance (Q2) and resistance (R3), the inductance (L3) and resistance (R3) of the layer composed by different adsorbed elements (from th solution or the metal itself) on the metal surface are presented in Tab 24 and fitting curves are presented as solid lines in the Nyquist diagrams of Fig 82.

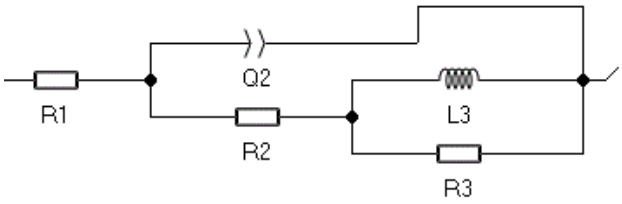



**Fig 80.** Equivalent electrical circuit tested for the modeling data of the EIS experiments.

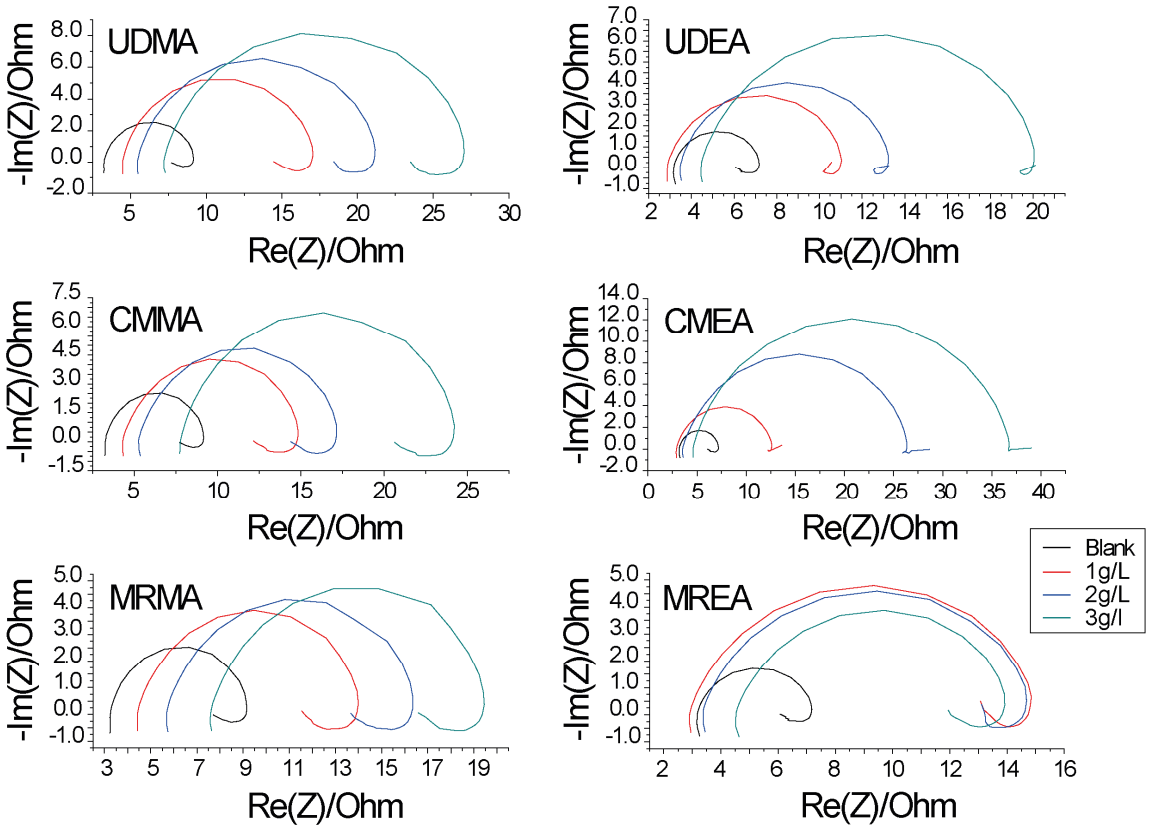



**Fig 81.** Impedance curve for different concentrations of *Urtica dioica*, *Chenopodium murale* and *Mentha rutondifolia* whole plant 80% ethanol and methanol extracts.

frequency inductive behavior may be due to the mutual inductance of the wires connecting

potentiostat to the cell. The AC current that goes through the counter electrode and working electrode leads to creating a magnetic field around the wires which is important if the electrode impedance is low (Rodgers and Eggers 1993). From $f_{max}$ value which corresponds to the maximum frequency of the highest value in the imaginary part and the $R_{ct}$, the calculation of the double layer capacitance value at the interface C38/ 5.5M $H_3PO_4$ will be possible.

**Tab 24.** Impedance data of C38/5.5 $H_3PO_4$ interface with and without inhibitors.

| | | C (g/L) | R1 (Ohm.cm$^2$) | Q2 x 10-3 ($\Omega^{-1}$.cm$^{-2}$.s$^n$) | a1 | R2 (Ohm.cm$^2$) | L3 (H) | R3 (Ohm.cm$^2$) | Efficiency (%) |
|---|---|---|---|---|---|---|---|---|---|
| Methanol | Blank | | 3.26 | 0,298 10-3 | 0.91 | 4.8 | 0.57 | 1.05 | - |
| | *Urtica dioica* | 1 | 4.47 | 0.238 | 0.89 | 10.63 | 1.1 | 1.92 | 54.84 |
| | | 2 | 5.46 | 0.237 | 0.88 | 13.46 | 1.4 | 2.27 | 64.34 |
| | | 3 | 7.15 | 0.237 | 0.87 | 16.98 | 1.8 | 2.81 | 71.73 |
| | *Chenopodium murale* | 1 | 4.32 | 0.292 | 0.86 | 8.44 | 0.99 | 1.87 | 43.13 |
| | | 2 | 5.28 | 0.285 | 0.87 | 9.78 | 1.04 | 2.1 | 50.92 |
| | | 3 | 7.76 | 0.26 | 0.86 | 13.75 | 1.31 | 2.29 | 65.09 |
| | *Mentha rutondifolia* | 1 | 3.26 | 0,298 | 0.91 | 4.8 | 0.57 | 1.05 | 38.85 |
| | | 2 | 4.44 | 0.294 | 0.87 | 7.85 | 0.85 | 1.95 | 43.66 |
| | | 3 | 5.62 | 0.274 | 0.86 | 8.52 | 1.31 | 2.09 | 50.05 |
| 80% Ethanol | Blank | | 3.17 | 0,369 10 | 0.9 | 3.1 | 0.3539 | 0.8621 | - |
| | *Urtica dioica* | 1 | 2.88 | 0.259 | 0.89 | 7.42 | 0.21 | 0.8 | 58.22 |
| | | 2 | 3.47 | 0.27 | 0.88 | 9.17 | 0.18 | 0.7 | 66.19 |
| | | 3 | 4.44 | 0.259 | 0.86 | 15.15 | 0.19 | 0.77 | 79.54 |
| | *Ch en* | 1 | 2.899 | 0.293 8 | 0.868 5 | 9.316 | 0.175 5 | 0.474 8 | 66.66 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 0.474 8 | 0.366 9 | 0.819 6 | 22.45 | 0.320 9 | 0.901 9 | 86.19 |
| | | 3 | 4.457 | 0.345 | 0.787 6 | 33.58 | 0.45 | 1.88 | 90.76 |
| | *Mentha rutondifolia* | 1 | 2.91 | 0.262 | 0.85 | 10.57 | 0.52 | 1.47 | 70.67 |
| | | 2 | 3.38 | 0.239 | 0.86 | 9.91 | 0.39 | 1.5 | 68.72 |
| | | 3 | 4.55 | 0.24 | 0.87 | 7.81 | 0.6 | 1.57 | 60.31 |

It is noticed that adding more extract concentration helps increasing the corrosion resistance and leads to a reduction in $C_{dl}$ value. The diminishing in $C_{dl}$ values occurs because of the gain in thickness of the interfacial double layer, which is in accordance with previous study (Harmaoui et al. 2015). The rise in corrosion resistance is mainly because of the appearance of a protective film on the C38/5.5M $H_3PO_4$ interface (Muralidharan et al. 1995; Bentiss et al. 2000). This observation suggests that the inhibitor molecules interact by adsorption on the C38 carbon steel surface and thereby causing decreases in $C_{dl}$ values and increases in Rct values. The decrease of capacities with increase of concentration of inhibitors, can result in a decrease of dielectric constant and / or an increase in thickness of a double layer, according to model of Helmholtz Eq (23):

$$C_{dl} = \frac{\varepsilon\varepsilon_0 A}{\delta} \qquad \text{Eq (23)}$$

Where ε is the dielectric constant, $\varepsilon_0$is the permittivity of the medium, A is the surface of the electrode and δ is the thickness of the protective layer.

At low frequencies, the manifestation of an inductive loop is due to the relaxation of the adsorbed species at the surface of the electrode (Corrosion product, $H^+$, etc.) (Amin et al. 2009). The present study leads to summarize that extract from *Urtica dioica*, *Chenopodium murle* and *Mentha rotondifolia* plant have an anticorrosion ability which may be used as an alternative corrosion preventive method for metals, using other or similar plant extracts. Furthermore, this application allows us to add a new application as a green corrosion inhibitor besides their known medicinal use.

## 4. Conclusion

We have investigated in this study the effect of methanol and 80% ethanol extracts from three different wild, medicinal plants on the corrosion of C38 carbon steel in a 5.5 M phosphoric acid medium at various concentrations with different techniques (i.e., open circuit potential, potentiodynamic polarization and electrochemical impedance spectroscopy (EIS)). The used extracts showed a good potential as an inhibitor for corrosion of C38 carbon steel. The inhibition proficiency rose by augmenting extracts concentrations, and reached efficiency ~72 % for the polarization study and ~91% efficiency for EIS measurements at 3g/L extract concentration of 80% ethanol extract of *Chenopodium murale*. Furthermore, extracts act as mixed type inhibitors. These green inhibitors have great potential, taking into consideration that the study was conducted on a highly concentrated medium. The applications of such inhibitors, original from largely present wild plants, are tremendous especially in industrial areas and related domain.

# Part III

# Compounds screening and identification using high accuracy mass spectrometry (FT-ICR MS) of *Urtica dioica*, *Chenopodium murale* and *Mentha rotundifolia* plants


## Summary

For decades, plants extracts played an important role in human health care and their use in the pharmaceutical trade become actually of great interest. Therefore, the identification and characterization of their chemical constituent namely secondary metabolites is crucial in the current drug development as well as human life style. In general, secondary metabolites are directly liable to the plant survive in the environment but they are not participating in its growth and progress. Thus, we highlighted in this part a metabolomics approach for profiling and analyzing secondary metabolites (also known as phytochemicals) in different plants extracts (i.e., *Urtica dioica, Chenopodium mural* and *Mentha rutondifolia*) including the whole plants extracts as well as their organs extracts using high-resolution Fourier transform ioncyclotron resonance mass spectometry FT-ICR MS. In parallel, Hierarchical cluter anylsis (HCA) is also used to construct a hierarchy of clusters related to the chemical similarities and dissimilarities of the chosen plants extracts. The correlation between FT-ICR MS and HCA assisted us to classify and annotate compounds corresponding to the assigned m/z ions signals. We found that the classification of the plants extracts was depending on the type of the plants and the annotated compounds showed that each plant has its specific chemical fingerprint.

# Compounds screening and identification using high accuracy mass spectrometry (FT-ICR MS) of *Urtica dioica*, *Chenopodium murale* and *Mentha rotundifolia* plants (To be submitted)

**Abstract**

In this part, we further investigated the chemical diversity of *Urtica dioica* (UD), *Chenopodium murale* (CM) and *Mentha rotundifolia* (MR) plants including their leafs, seeds, stems as well as the whole plants organs using Fourier transform ioncyclon resonance mass spectrometry (FT-ICR MS) approach. The chemical composition identified suggests that each plant extract has its uniqueness. MR extract showed the highest richness in its compounds constituents as compared with UD and CM extracts. While CM extracts showed the lowest features abundance, UD extracts were in the middle. Hierarchical cluster analysis (HCA) base on the profile search selected m/z ions showed that only five features were abundant in CM extracts, while 286 and 54 features were abundant in MR and UD extracts, respectively. The comparison of these abundant chemical m/z ions revealed specific signatures of each plant extract that highlight the importance of this analysis approach. In addition, this study not only tackle the chemical specificity of each plant extracts but give also a fast inside into chemical complexity of these plants which needs several steeps and time consuming in order to resolve and identify their chemical constituents.

## 1. Introduction

Herbal medicines have been widely consumed since early times and are used by different inhabitants during human growth. According to World Health Organization, about 80% of residents in Asian and African countries consume herbal medicine as their daily dietary supplements (Aziz et al. 2016; Gu et al. 2013). In general, plants are valuable basis of a wide range of chemical compounds and represent an unlimited source of phytochemicals either primary or secondary metabolites. Herbal extracts as pure compounds or standardized extracts, offer unlimited potentials for the discovery of new drugs because of the unparalleled availability of chemical diversity (Cos et al. 2006; Sasidharan et al. 2011). This why understanding the chemical

compositions of plants provides a basis for the challenges of their protection and uses. To our knowledge, most of the investigations in phytochemistry were performed using nuclear magnetic resonance (NMR) spectroscopy. However, gas and liquid chromatography has proved also to be a competent tool for the analysis of phytochemicals in plant extracts and has contributed to a better knowledge of their rules in natural processes (Seeka et al. 2016; Marshall et al. 2018). The identification and characterization of secondary metabolites have shown great potential for exploring the chemical complexicity and benefit of medicinal plants in different fields.

Plant secondary metabolites are important in pharmaceutical industry, foodomics and several industrial materials (Zhaoa et al. 2004). They are considered as useful compounds originated from plants secondary metabolism and are mainly classified based on their chemical structure, elemental compositions, solubility in various solvents as well as their biosynthetic pathways (Harborne n.d.; Tiwari and Beriha 2015). Only little is known about the chemical signatures in medicinal plants. Studies using Ultrahigh-resolution mass spectrometry FT-ICR mass spectrometry analysis provides an exceptional picture for their chemical diversity and complexity on a molecular sense. Furthermore, FT-ICR MS has recently emerged as a highly powerful tool for molecular characterisation of organic compounds from different origins (Marshall et al. 2018). Several exceptional FT-ICR MS-based metabolomics profiling approaches have been reported (Takahashi et al. 2008). Because of its high resolution ($m/\Delta m50\ \% \geq 400{,}000$, where $\Delta m50\ \%$ is the mass spectral peak full width at half-maximum peak height) and mass accuracy ($\leq$200 ppb), ultrahigh-resolution FT-ICR MS makes molecular identification plausible, and is suitable for the desired molecular-level characterization (Guigue et al. 2016).

We aimed in this part to explore the potential of ultrahigh resolution mass spectrometry to profile secondary metabolites in three different plant extracts (i.e., *Urtica dioica*, *Chenopodium murale* and *Mentha rotundifolia*) including stems, seeds, leafs as well as their mixture. Here, we introduce the usefulness of electrospray ionization as a soft ionization tool with increased resolution and mass exactness of FT-ICR-MS in detecting several hundred of m/z mass signals in the investigated plant extracts. We were able to make screening and identification of known/unknown compounds feasible, and examples are presented.

## 2. Materials and methods

### 2.1. Methanol extracts preparation

(See chapter II. Materials and methods. III.1.Solvent based extracts preparation).

### 2.2.ESI(-) FT-ICR-MS

(See chapter II. Materials and methods. XI. Plants extracts identification).

### 2.3.Molecular formulas

(See chapter II. Materials and methods. XI. Plants extracts identification).

### 2.4.Samples classification

(See chapter II. Materials and methods. XI. Plants extracts identification).

## 3. Results and discussion

### 3.1.CHO-chemical diversity in plants extracts

A total of three plants (i.e., *Urtica dioica* UD, *Chenopodium murale* CM and *Mentha rotundifolia* MR) and their corresponding organs (i.e., leafs, seeds and stems) were extracted using methanol and were analysed using Fourier transform ioncyclotron resonance mass spectrometry FT-ICR MS. The spectra show the abundance of detected m/z ions signals in a mass range from m/z 100 to 1000 Da with several thousands of non-volatile signals (Fig 83). As shown in Fig 83, each spectrum can be considered as a symbolic pattern of these plants. Full spectra allow a direct visual comparison of the organic chemical content of these plant extracts. The observed differences within the used mass range may originated from the chemical diversity of each plant and organ extract. However, Fig 83 also shows that there is consistency in the chemical diversity mainly depending on the plant type. The corresponding van Krevelen diagram provides a qualitative visual picture of the nature of the non-volatile compounds characteristic of each plant as well as their related organs Fig 84. A significantly great richness of CHO-elemental formulas was observed for MR with an average assigned value of m/z ion ~ 1073 compounds followed by UD with an average

assigned value if m/z ion ~ 632 compounds. Thus, the lowest average assigned values of m/z ions was observed for CM with ~ 270 compounds.

**Tab 25.** Number of m/z ions, assigned formulas and database hits found for mass the spectra of *Chenopodium murale* (CM), *Mentha rotundifolia* (MR) and *Urtica dioica* (UD) extracts.

| Sample type | Signals assigned to elemental formulae containing C,H,O | Database annotations |
|---|---|---|
| CM whole plant | 262 | 97 (37%) |
| CM stems | 225 | 77 (34%) |
| CM seeds | 280 | 101 (36%) |
| CM leafs | 315 | 115 (37%) |
| UD whole plant | 618 | 243 (39%) |
| UD stems | 368 | 139 (38%) |
| UD seeds | 709 | 288 (41%) |
| UD leafs | 836 | 355 (43%) |
| MR whole plant | 1426 | 656 (46%) |
| MR stems | 865 | 380 (44%) |
| MR seeds | 701 | 315 (45%) |
| MR leafs | 1303 | 622 (48%) |

Annotations using LipidMAPS and HMDB databases provided different hits with a percentages relatively greater that 33% for all plants and organs (Tab 25). Commonly saturate compounds, carbohydrates, lignins, anthocyanins, flavonols, ellagitannins and tannins (Fig 84B). More than 50% of the assigned molecular formulas found in the studied extracts were not annotated in these databases signifying that many of these compounds are unknown and need other procedures for their identification. To evaluate better the uniqueness of the m/z ions found in each plant and organ, Venn diagram that uses overlapping rings or other figures to represent the consistent connexion between two or more datasets was used (Fig 84C). As shown in the Venn diagram, we found 231, 224 and 131 of unique m/z ions signals in leafs of UD, MR and CM, respectively. The order of this unique m/z signals is as follow: unique signals (UD) > unique signals (MR) > unique signals (CM). This highlights the molecular diagenesis occurring in leafs organ. Furthermore, the unique m/z ions found in MR seeds extract were the lowest in comparison with UD and CM seeds extracts. While the unique m/z ions of CM-stems and UD-stems were quite similar, the m/z ions in MR-stems were two fold higher (Fig 84C). Accordingly, the unique m/z ions found in the whole plant extract of MR was two-fold higher than of those found in the whole plant extracts of UD and CM.

Generally, these distinctions in the elemental compositions uniquely found either in the whole plant extract and the corresponding organs can be consequences of several unknown processes.

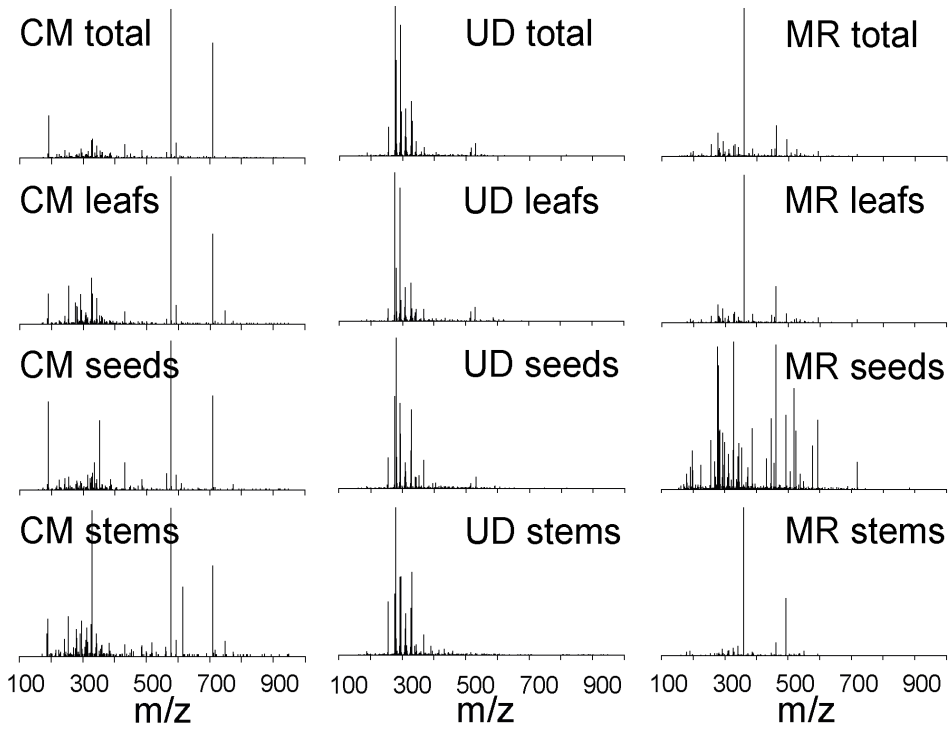



**Fig 82.** Visualization of ESI(-) FT-ICR MS spectra of the extracts of the whole plants and their corresponding organs in the mass range from 100-1000 Da.

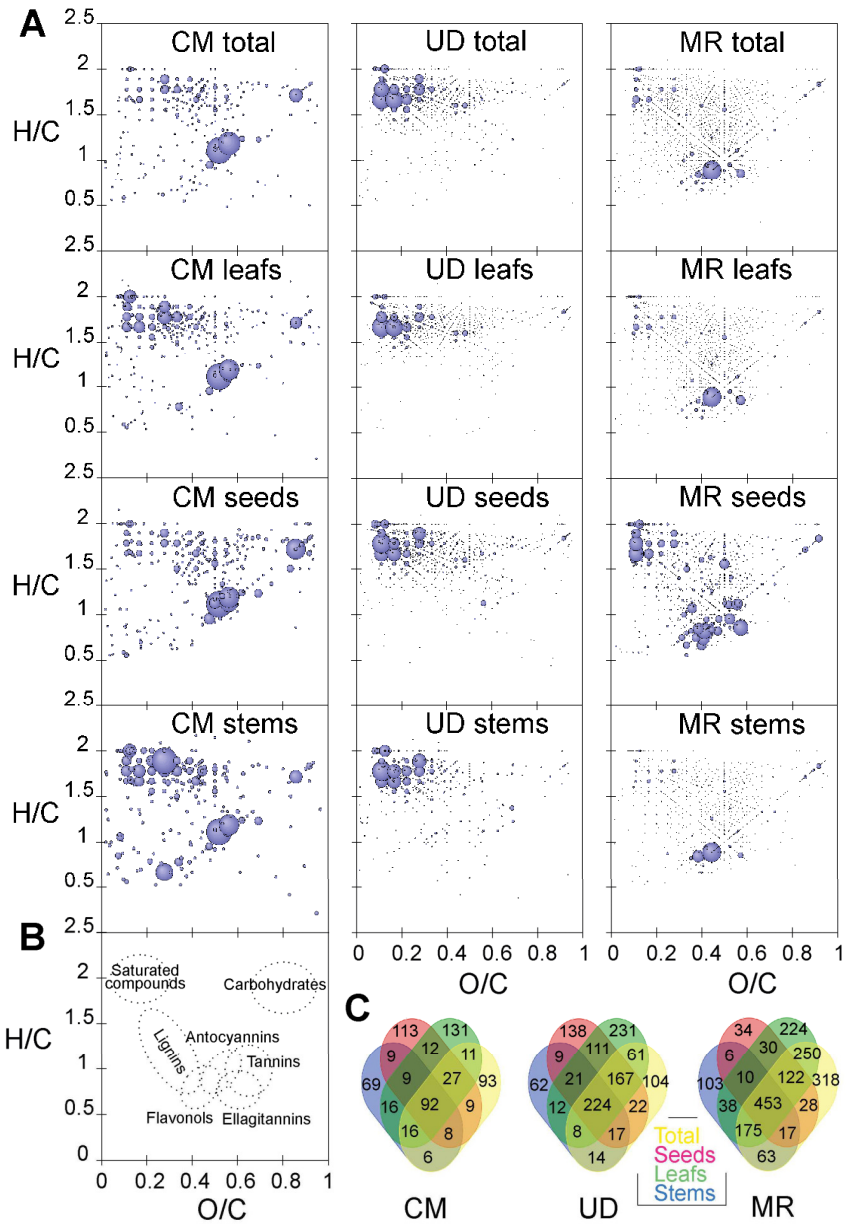



**Fig 83.** (A) van Krevelen diagram (H/C vs O/C atomic ratios). (B) van Krevelen diagram model showing the abundant compound classes of the assigned secondary metabolites (Roullier-Gall, et al. 2018). (C) Venn diagram summarizing count of common and unique secondary metabolites in the whole plants extracts of CM, UD and MR, as well as their corresponding organs extracts (i.e., leafs, stems and seeds).

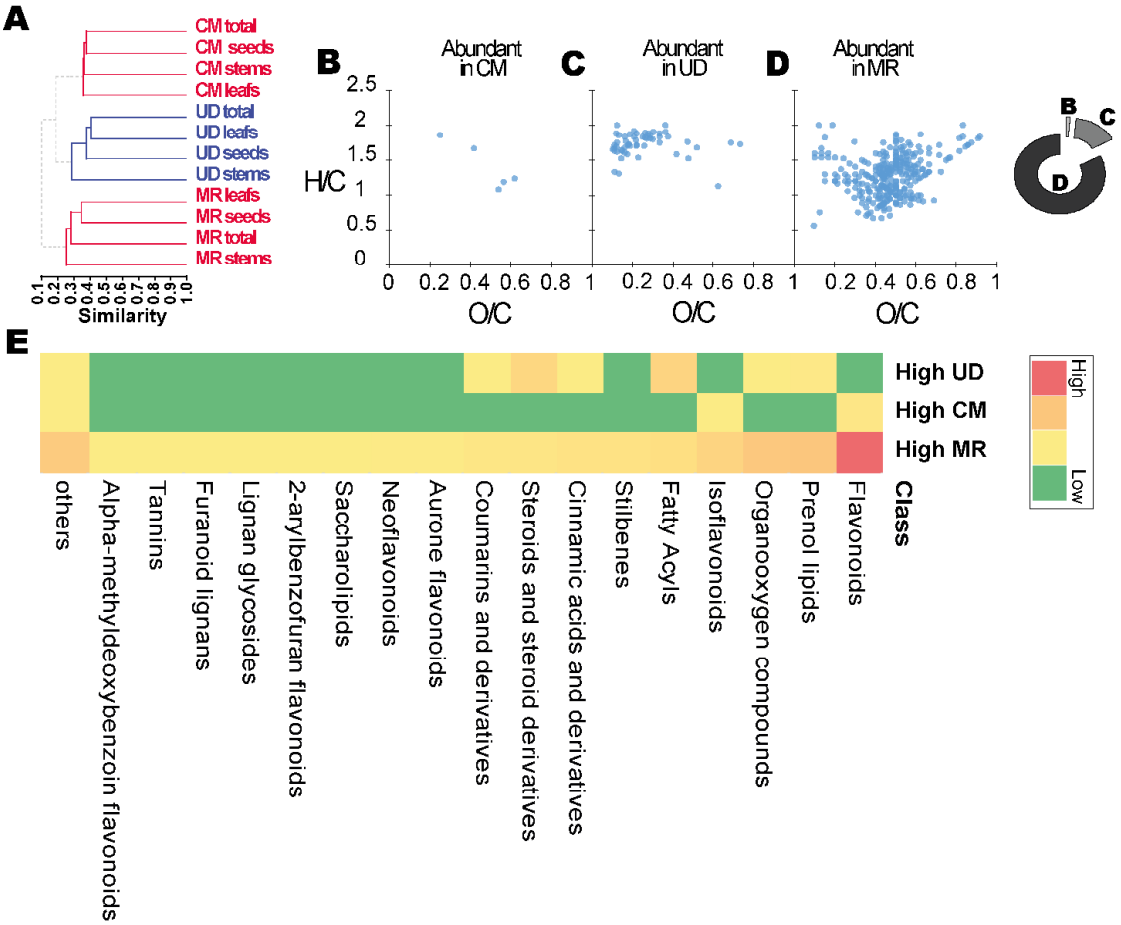



**Fig 84.** (A) Hierarchical cluster analysis of the assigned ESI(-) FT-ICR-MS derived molecular formulas observed in all extracts. (BCD) van Krevelen diagrams depict the most abundant molecular formulas in all plant extracts including only CHO-compounds. (E) Compounds classification of the most abundant annotated m/z ions signals present in CM, UD, MR and their corresponding organs.

### 3.2. Mass spectrometric characterization of the chemical space of UD, CM, MR plants extracts

In general, principal of annotating metabolites mainly includes spectrometric and biological metadata annotation. Actually, its description take in consideration investigational conditions that assist to discover bio-roles of metabolites mainly based on their stability/alterations response to some processes. In this present thesis, profiling and molecular identification using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) of the whole plants extracts of UD, CM and MR as well as the extracts of their organs allows a comprehensive description of their content metabolic. FT-ICR-MS shows the significant and direct insight into molecular compositions and changes in each plant extract. The initial screening using FT-ICR-MS resulted

in a set of 2,603 m/z values (S/N ≥ 4) found across all samples. In this regards, we explored the covariability of each plant extract dataset (i.e., UD, CM and MR) generated from the FT-ICR-MS analysis based on a statistical analysis approach. This approach provides detailed metabolite profiling of these plant extracts and an exact comparison of the metabolites compositions. According to the detected m/z ions signals (highly polar compounds), the comparison of these plants extracts was done using hierarchical cluster analysis (HCA) including leafs, seeds, stems as well as the whole plants organs, according to their likeness in order to find the key changes in their molecular composition (Fig 85). HCA was applied to better visualize the indirect resemblances and variances between the chemical compositions assigned in each plant extract.

Accordingly, HCA analysis showed the presence of three dissimilar subclusters as shown in Fig 85. Cluster formation resembles the widest to the type of plant more than to the plants organs. The wide chemical dissimilarity between the subclusters is visible from the ring chart shown in Fig 85 which representing the distribution of the CHO-elemental compositions, along with the van Krevelen plots of the ESI(-) FT-ICR-MS derived molecular formulas. The van Krevelen plots and ring chart display the most illustrative elemental compositions for each subcluster and show separate chemical space characteristic for each plant type. We found that up to 286 m/z ions revealed an increasing intensity together in MR extracts and occupied essentially the chemical space of saturated compounds, carbohydrates, lignins and polyphenols in the van Krevelen plot (Fig 85). In contrast and as shown in Fig 85, up to 54 m/z ions revealed an increasing abundance together in UD extracts occupying mostly the saturated area in van Krevelen plot. The lowest number of elemental composition was observed in CM extracts.

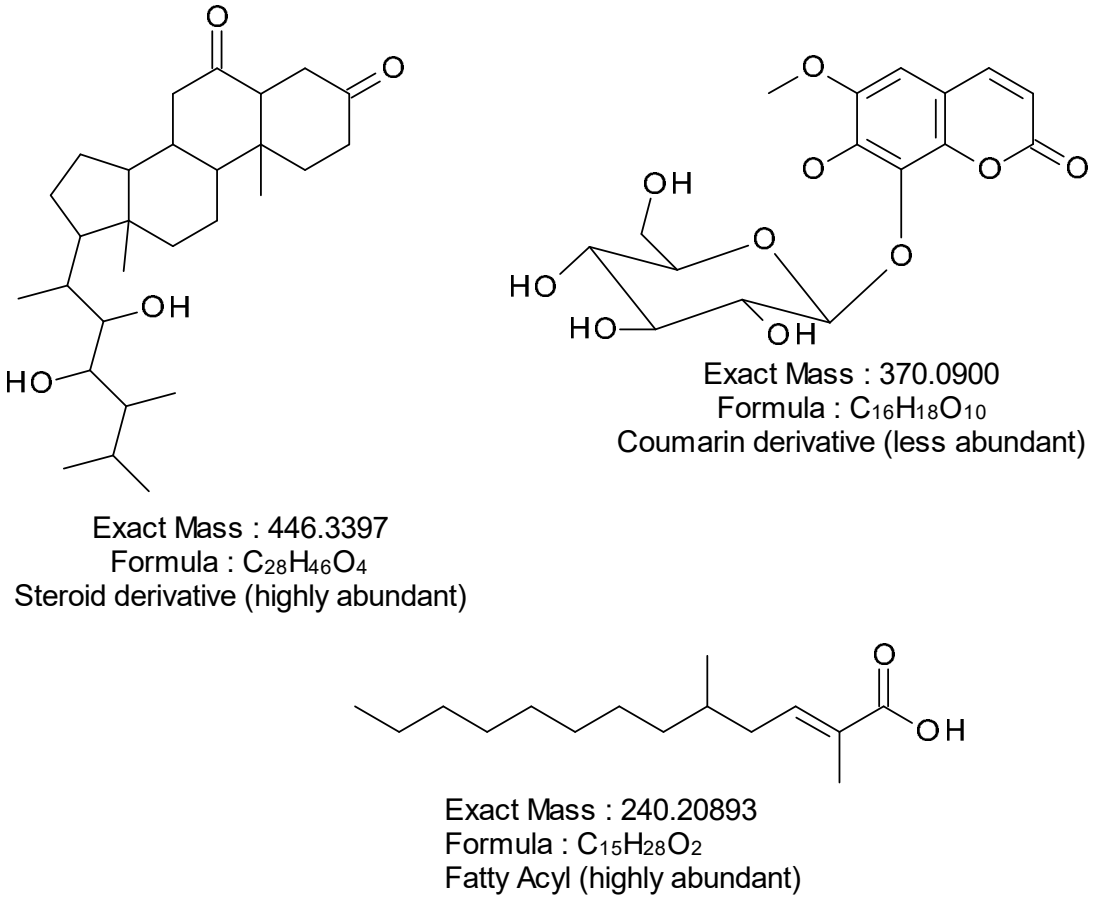



**Fig 85.** Putative structure example of the most and less abundant compounds found in UD extract.

### 3.3. Metabolites classification

In order to assess and explore metabolites classification and annotation, a chemical fingerprint of each plant extracts was statically sorted and evaluated. Thus, the abundant annotated m/z ions computed from ESI(-) FT-ICR mass spectrometry of CM, UD and MR plants extracts were divided into seventeen different classes (Fig 85E). Commonly, the main classes of phytochemicals reported for UD were classified as flavonoids, tannins, volatile compounds, fatty acids, carbohydrates, isolectins, sterols, terpenes, protein, vitamins and minerals (Joshi et al. 2014; Krystofova et al. 2010; WETHERILT 1992; Bombardelli and Morazzoni 1997; Kudritsata et al. 1986; Rafajlovska et al. 2001). In our case, the most abundant classes observed were classified as fatty acyls and steroids -(and steroid derivatives) in UD plant extracts (Fig 85E). However, we

found also that the classes (i.e., prenol lipids, organooxygen compounds, stilbenes, cinnamic acids -(and cinnamic acids derivatives) were abundant in MR plant extract.

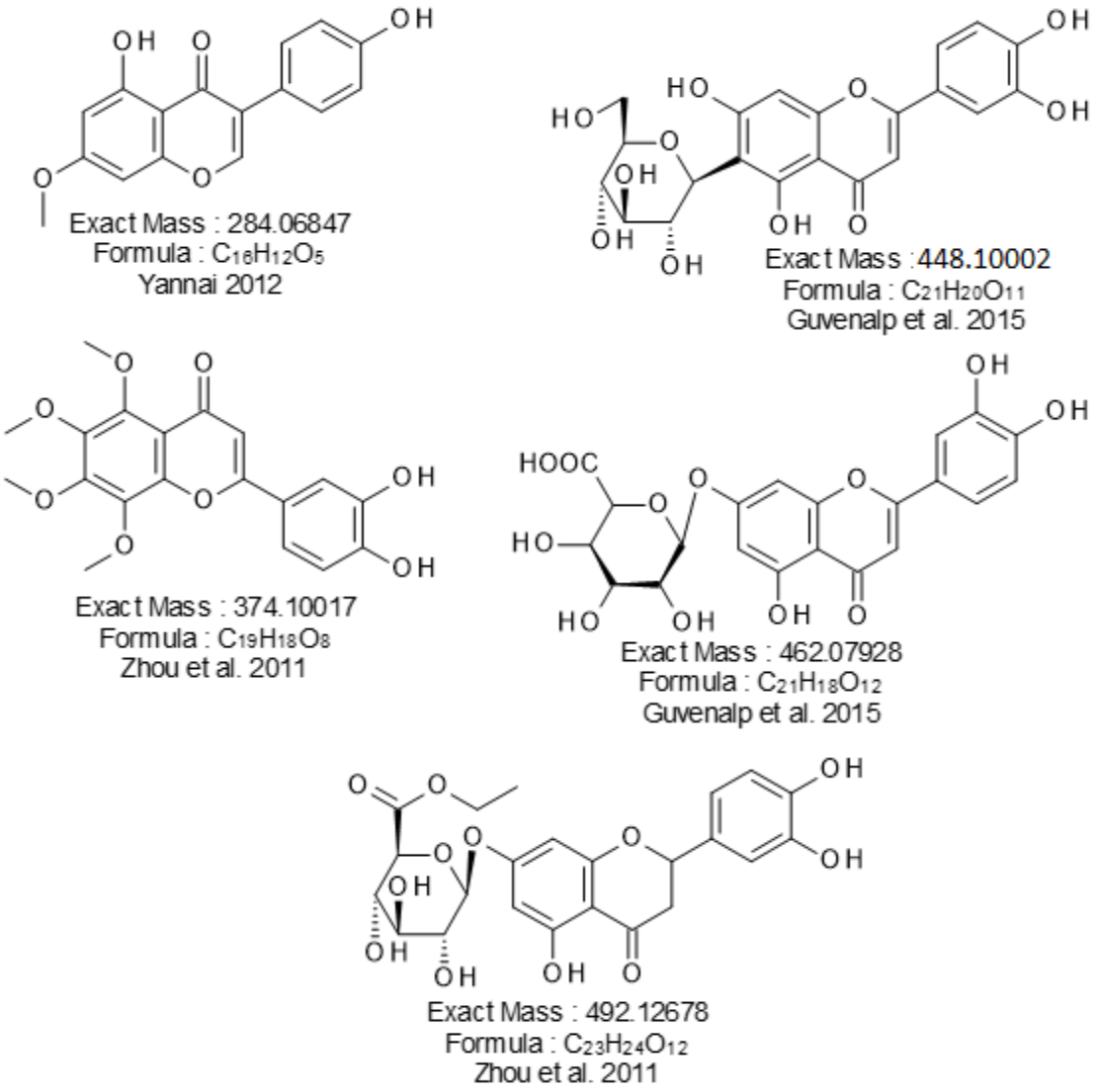



**Fig 86.** Putative structure of the most abundant phytochemicals found in MR extracts as computed from ESI(-) FT-ICR mass spectra for singly charged ions.

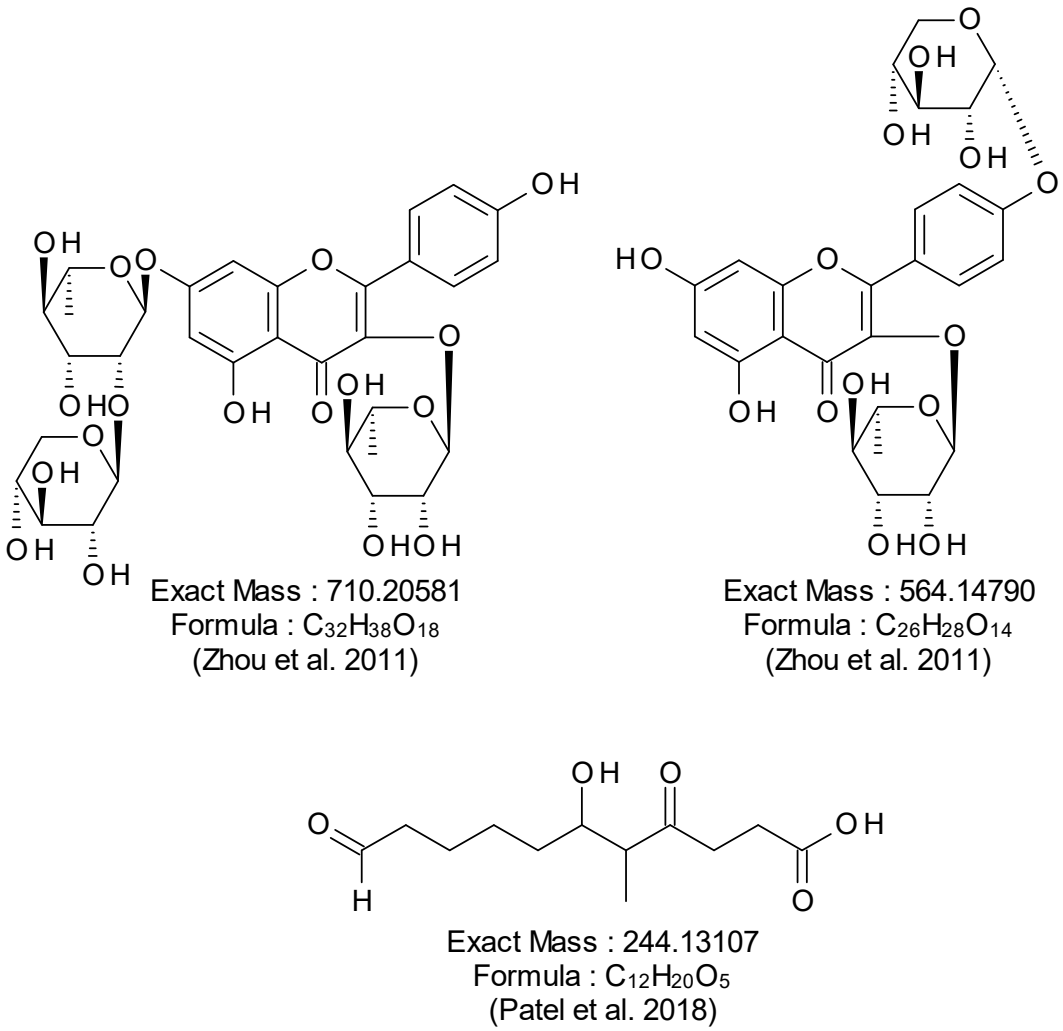



**Fig 87.** Putative structure of the most abundant phytochemicals found in CM extracts as computed from ESI(-) FT-ICR mass spectra for singly charged ions.

In addition, the following classes, which include coumarins -(and coumarins derivatives), aurone flavonoids, neoflavonoids, saccharolipids, 2-arylbenzofuran flavonoids, lignan glycosides, furanoid lignans, tannins and alpha-methyldeoxybenzoin flavonoids, were solely representative in MR extracts. Thus, the annotated m/z ions in UD and MR extracts were ~ 65% and 82%, respectively. However, about 35% and 18% of the assigned molecular formulas were not annotated in UD and MR extracts. The most obvious class of compounds found in UD extracts was classified as fatty acyls and steroids (and steroids derivatives) (Fig 85E). The less abundant was coumarins (and coumarins derivatives), cinnamic acids (and cinnamic acids derivatives), organooxygen

compounds and prenol lipids. Accordingly, Fig 86 shows putative structure example of two annotated m/z ions signals corresponding to the molecular formulas (i.e., $C_{15}H_{28}O_2$ and $C_{16}H_{18}O_{10}$) with high and less abundance, respectively. However, the proposed structure of each class is one example of several possible structures of these m/z ions.

Based on the abundant phytochemicals present in the whole plants extracts and organs extracts, MR extracts showed the highest content of flavonoids as compared to CM and UD extracts. The lowest abundance was detected for UD extracts. Around 1528 and 63 annotations were assigned to the flavonoids in MR and CM extracts, respectively. Structural elucidation examples of the most abundant flavonoids found in MR extracts are shown in Fig 87. It has been reported that luteolin metabolites (corresponding to the formulas $C_{21}H_{20}O_{11}$ and $C_{21}H_{18}O_{12}$ in Fig 87) are likely liable to luteolin bioactivity (Kure et al. 2016).

Accordingly, the number of phytochemical compounds found with high abundance in CM extracts was five metabolites. This number of metabolites was the lowest among the three studied plants. As shown in Fig 88, one compound of these five phytochemicals was belonging to the class of organic compounds known as hydroxy fatty acids with the corresponding chemical formula $C_{12}H_{20}O_5$ (Patel et al. 2018), while another two phytochemical were identified as flavonoids derivatives with the corresponding chemicals formulas $C_{26}H_{27}O_{14}$ and $C_{32}H_{37}O_{18}$ (Zhou et al. 2011). Unlike, compounds with the corresponding molecular formulas $C_{21}H_{25}O_{13}$ and $C_{28}H_{27}O_{14}$ were assigned as unidentified compounds. Even the highest annotation of compounds reached using FT-ICR MS approach; it is still challenging to assess the bioactivity effect of the total plant extract to one or few active compound. Therefore, either high and/or low content of organic compounds should affect strongly the activity of plant extracts, which is the consequence of the interaction of their chemical constitution. In this regards, further investigations talking the isolation and characterization of these metabolites is still of great interest for these plants.

## 4. Conclusion

High-resolution FT-ICR mass spectrometry analysis of the twelve plants extracts including the whole plants extracts and their corresponding organs (i.e., leafs, seeds and stems) allowed a classification into three classes. Thus, FT-ICR MS in combination with a statistical based analysis using hierarchical cluster analyses allowed us to unravel the chemical complexity of the chosen

plants and their corresponding organs regardless of their origins. We were able to profile and identify potential chemical markers consistent in each plant and organ extract. Also, our results further demonstrated that the large number of m/z ions signals present in CM, UD, MR and their corresponding organs (i.e., leafs, seeds and stems) point out the molecular diagenesis occurring in these plants and their comparison revealed specific plant signature.

# Part IV

# Conclusions and remarks

The previously cited studies had an ultimate purpose to highlight the different properties and potential uses of the wild aromatic plants studied using different approaches and methods. In most cases these plants had significant amounts of phytochemically active compounds, namely polyphenols that displayed useful properties. When it comes to antioxidant properties, all plants displayed a significant radical reducing power trough DPPH and ABTS assays. The results were in line and concentration dependent. When compared, UD have shown that it contains higher levels of phenolics than CM. These results were in line with the antioxidant assays. Indeed the extracts where phenolic content was highest, demonstrated the most potent antioxidant power. *Ziziphus lotus* fruit was also proved to have important amounts of antioxidants. However, MR was the strongest of the four. It was recorded that its seeds extract were so efficient, that it was close to that of Trolox, a well known synthetic antioxidant. The methanol extract of this organ had an $IC_{50}$ value as low as 5.98 µg/mL.

UD, CM and ZL were also studied for their antibacterial potential. Their extracts exhibited an growth inhibition for gram positive and gram negative bacterial strains such as E. Coli, Rhizobium sp., Bacillus pumilus and Bacillus subtilis, but were reluctant against Agrobacterium sp.The richness of these extracts in phenolics and their potent antioxidant effect may explain their efficiency against bacteria. This could be also explained by the usefulness of this plant in treating several infections and health issues related to bacteria, reported trough studies and traditional medicine.

ZL was the prefect candidate for corrosion inhibition studies, since it holds within its seeds vegetale oil that is known for its anticorrosion properties on C38 steel. This organic material was test in phosphoric acid and hydrochloric acid mediums. In both cases it demonstrated important inhibition properties. In the phosphoric acid case, the reached efficiency exceeded 50% for a concentration as low as 1 g/L of oil. It might seem like a low efficiency value, but phosphoric acid is a highly corrosive environment. Subsequently, this same material was highly effective in hydrochloric acid medium ~ 98 % efficiency for 3g/L oil concentration. Its inhibition efficiency depends on both concentration and temperature. The high inhibition efficiency of the extract was attributed to the adherent adsorption of the inhibitory molecules on the metal surface.

Additionaly, methanol extracts of UD,CM and MR were subjected to FT-ICR-MS analysis. The interpretation of the results obtained using classification methods, as well as Krevelen diagram,

was useful in order to understand the chemical composition of these extracts. Several types of compounds were identified such as carbohydrates, lignins, anthocyanins, flavonols, ellagitannins and tannins. However, coressreference with metabolites database showed that there are a lot more unknown compounds that need further analysis for their identification. This observation opens up opportunities for valorization and drug discovery from the studied plants.

## References


Alkurd, A., Takruri H.R. and Heba A.S., 2010. Tannin contents of selected plants used in jordan. Jordan Journal of Agricultural Sciences, 4(3): 265-274.

Abah, S.E. and Abah, G., 2010. Antimicrobial and antioxidant potentials of agaricus bisporus. Advances in Biological Research, 4(5): 277–282.

Abdeddaim, M. et al., 2014. Biochemical characterization and nutritional properties of Zizyphus lotus L. fruits in Aures region, northeastern of Algeria. Annals Food Science and Techgnology, 15(1): 75–81.

Abdoul-Azize, S. and Souleymane, 2016. Potential Benefits of Jujube ( Zizyphus Lotus L.) Bioactive Compounds for Nutrition and Health. Journal of Nutrition and Metabolism, 2016 (1): 1–13.

Abdullah, N. et al., 2012. Assessment on the antioxidant and antibacterial activities of selected fruit peels. International Journal of ChemTech Research, 4(4): 1534–1542.

Abu-Shanab, B., Adwan, G. and Abu-Safiya, D., 2004. Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. Turkish Journal of Biology, 28(2004):99–102.

Adeli, M. and Samavati, V., 2015. Studies on the steady shear flow behavior and chemical properties of water-soluble polysaccharide from Ziziphus lotus fruit. International Journal of Biological Macromolecules, 72:580–587.

Adesanwo, J.K., Makinde, O.O. and Obafemi, C.A., 2013. Phytochemical analysis and antioxidant activity of methanol extract and betulinic acid isolated from the roots of Tetracera potatoria. Journal of Pharmacy Research, 6(9): 903–907.

Adwan, K. and Abu-Hasan, N., 1998. Gentamicin resistance in clinical strains of Enterobacteriaceae associated with reduced gentamicin uptake. Folia microbiologica, 43: 438–440.

Adzu, B. et al., 2003. Evaluation of the antidiarrhoeal effects of Zizyphus spina-christi stem bark in rats. Acta tropica, 87(2): 245–50.

Ahamet, S., 2003. Etudes phytochimiques et des activités biologiques de Balanites aegyptica (Balanitaceae). Université de Bamako.

Ahmad, N. et al., 2012. Free radical scavenging (DPPH) potential in nine Mentha species. Toxicology and Industrial Health, 28(1): 83–89.

Akbay, P., Basaran, A.A. and Basaran, N., 2003. In vitro Immunomodulatory Activity of Flavonoid Glycosides from Urtica dioica L., 17(1):34–37.

Alais, C., Linden, G. and Miclo, L., 2003. Biochimie alimentaire, Dunod.

Alexandra Pazmio-Durán, E. et al., 2001. Anthocyanins from Oxalis triangularis as potential food

colorants. Food Chemistry, 75(2): 211–216.

Amin, M.A. et al., 2009. Polyacrylic acid as a corrosion inhibitor for aluminium in weakly alkaline solutions. Part I: Weight loss, polarization, impedance EFM and EDX studies. Corrosion Science, 51(3):658–667.

Anand, K.K. et al., 1989. Effect of Zizyphus sativa leaves on blood glucose levels in normal and alloxan-diabetic rats. Journal of ethnopharmacology, 27(1–2):121–7.

Andreani, S. et al., 2016. Study of Corrosion Inhibition for Mild Steel in Hydrochloric Acid Solution by Limbarda crithmoides ( L .) Essential Oil of Corsica. , 7(1):187–195.

Aritomi, M. and Kawasaki, T., 1984. Three highly oxygenated flavone glucuronides in leaves of Spinacia oleracea. Phytochemistry, 23(9):2043–2047.

Arukalam, I.O. et al., 2014. Acid Corrosion Inhibition and Adsorption Behaviour of Ethyl Hydroxyethyl Cellulose on Mild Steel Corrosion. Journal of Materials, 2014:1–11.

Aruoma, O.I., 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists' Society, 75(2):199–212.

Arvy, M.-P. and Gallouin, F., 2003. Epices, aromates et condiments, Belin.

Atoui, A.K. et al., 2005. Tea and herbal infusions: Their antioxidant activity and phenolic profile. Food Chemistry, 89(1):27–36.

Austin, D.F., 2006. CRC World Dictionary of Grasses. Common Names, Scientific Names, Eponyms, Synonyms, and Etymology,

Aziate, G. et al., 2015. Study of jojoba vegetable oil as inhibitor of carbon steel C38 corrosion in different acidic media. Journal of Materials and Environmental Science, 6(7):1877–1884.

Aziz, Z.A.A. et al., 2016. Application of Herbal extract and its medicinal value. Der Pharmacia Lettre, 8(9):161–167.

Badr, G.E., 2009. The role of some thiosemicarbazide derivatives as corrosion inhibitors for C-steel in acidic media. Corrosion Science, 51(11):2529–2536.

Baiano, A. and Del Nobile, M.A., 2016. Antioxidant Compounds from Vegetable Matrices: Biosynthesis, Occurrence, and Extraction Systems. Critical Reviews in Food Science and Nutrition, 56(12):2053–2068.

Balasundram, N., Sundram, K. and Samman, S., 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry, 99(1):191–203.

Barros, M.P. de et al., 2008. Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. Journal of Ethnopharmacology, 120(3):372–377.

Bashir, A. et al., 2003. Phytochemical Evaluation of Chenopodium murale Linn. Asian Journal of Plant Sciences, 2(15):1072–1078.

Beith, M., 1995. Healing Threads. Traditional Medicines of the Scottish Highlands and Islands. In Polygon Press. Edinburgh:214.

Benammar, C. et al., 2010. Zizyphus lotus L. (Desf.) modulates antioxidant activity and human T-cell proliferation. BMC complementary and alternative medicine, 10:54.

Bendahou, M., Benabdellah, M. and Hammouti, B., 2006. A study of rosemary oil as a green corrosion inhibitor for steel in 2 M H 3PO 4. Pigment and Resin Technology, 35(2):95–100.

BenSalah, M. et al., 2014. Passivity of Sanicro28 (UNS N-08028) stainless steel in polluted phosphoric acid at different temperatures studied by electrochemical impedance spectroscopy and Mott-Schottky analysis. Corrosion Science, 86:61–70.

Bentiss, F., Traisnel, M. and Lagrenee, M., 2000. The substituted 1 , 3 , 4-oxadiazoles : a new class of corrosion inhibitors of mild steel in acidic media. Corrosion Science, 42:127–146.

Bermness, L., 2005. Plantes Aromatiques et Médicinales, Larousse.

Bhar, H. and Balouk, A., 2011. Plantes Aromatique et médicinales du Maroc. L'espace marocain.

Biesaga, M., 2011. Influence of extraction methods on stability of flavonoids. Journal of Chromatography A, 1218(18):2505–2512.

Bisht, S., Bhandari, S. and Bisht, N., 2012. Urtica dioica (L): an undervalued, economically important plant. Agricultural Science Research Journal, 2012; 2 (5): 250–252.

Bisset, N.G., 1994. Herbal Drugs and Phytopharmaceuticals. Translated from Prof Dr Max Wichtl's original German edition., London, UK.: CRC Press.

Bnouham, M. et al., 2003. Antihyperglycemic activity of the aqueous extract of Urtica dioica. Fitoterapia, 74(7–8):677–681.

Bombardelli, E. and Morazzoni, P., 1997. Urtica dioica L. Fitoterapia, 68:387–402.

Bone, K. and Mill, S., 1999. Principles and practices of phytotherapy: Modern herbal medicine, London, Churchill Livingstone.

Borgi, W. et al., 2008. Anti-inflammatory and analgesic activities of flavonoid and saponin fractions from Zizyphus lotus (L.) Lam. South African Journal of Botany, 74(2):320–324.

Bors, W. and Saran, M., 1987. Radical Scavenging by Flavonoid Antioxidants. Free Radical Research Communications, 2(4–6):289–294.

Bothi, R.P. and Sethuraman, M.G., 2008. Inhibitive effect of black pepper extract on the sulphuric acid corrosion of mild steel. Materials Letters, 62(17–18):2977–2979.

Božović, M., Pirolli, A. and Ragno, R., 2015. Mentha suaveolens Ehrh. (Lamiaceae) Essential Oil and Its Main Constituent Piperitenone Oxide: Biological Activities and Chemistry. Molecules, 20(5):8605–8633.

Bradley, P., 1992. British Herbal Compendium (1). In Exeter, England: British Herbal Medicine

Association: 166–7.

Brisley, G., 1994. Ecuador Indians' use of nettle sting for joint pains (letter):289.

Bruneton, J., 1993. Pharmacognosie et phytochimie plantes médicinales. In Paris, France: Lavoisier: 278–279.

Burns, J. et al., 2001. Extraction of Phenolics and Changes in Antioxidant Activity of Red Wines during Vinification. Journal of Agricultural and Food Chemistry, 49(12):5797–5808.

Cai, Y. et al., 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences, 74(17):2157–2184.

Cao, G., Sofic, E. and Prior, R.L., 1996. Antioxidant Capacity of Tea and Common Vegetables. Journal of Agricultural and Food Chemistry, 44(11):3426–3431.

Cappelletti, E.M., Trevisan, R. and Caniato, R., 1982. External antirheumatics from herbs in Northern Italy. Journal of Ethnopharmacology, 6(2):161–90.

Castleman, M., 1991. The Healing Herbs, Melbourne: Schartz Books.

Cavin, A., 1999. Investigation phytochimique de trois plantes indonésiennes aux propriétés antioxydantes et antiradicalaires: Tinospora crispa (Menispermaceae) Merremia emarginata (Convolvulaceae) et Orophea enneandra (Annonanceae). Indonisia university.

Chebli, H. et al., 2017. Corrosion Inhibition of Steel in 1 M Hydrochloric Acid Medium by Thymus leptobotrys Essential Oils. Applied Journal of Environmental Engineering Science, 3(2):131–143.

Chen, C., Pearson, A.M.M. and Gray, J.I.I., 1992. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. Food Chemistry, 43(3):177–183.

Cheng, G. et al., 2000. Flavonoids from Ziziphus jujuba Mill var. spinosa. Tetrahedron, 56(45):8915–8920.

Chevallie, A., 2001. Encyclopedia of Médicinal Plants (2nd Edition), Larousse.

Cheynier, V., 2005. Polyphenols in foods are more complex than often thought. The American Journal of Clinical Nutrition, 81(1):223-229.

Choi, H.J. et al., 2009. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. Antiviral Res, 81(1):77–81.

Chopra, R.N., 1956. Glossary of Indian medicinal plants, New Delhi: Council of Scientific and Industrial Research.

Chouaibi, M. et al., 2012. Nutritional composition of Zizyphus lotus L. seeds. Journal of the Science of Food and Agriculture, 92(6):1171–1177.

Chrubasik, S. et al., 1997. Evidence for antirheumatic effectiveness of Herba Urticae dioicae in acute arthritis: A pilot study. Phytomedicine : international journal of phytotherapy and

phytopharmacology, 4:105–8.

Cockerill, F.R. et al., 2012. Performance standards for antimicrobial disk susceptibility tests ; approved atandard. Clinical and Laboratory Standars Institute - NCCLS, 32(1):76.

Cohen, M.L., 1992. Epidemiology of Drug Resistance: Implications for a Post--Antimicrobial Era. Science, 257(5073):1050–1055.

Conrad, J. et al., 1998. Two triterpene esters from Terminalia macroptera bark. Phytochemistry, 48(4):647-650.

Cos, P. et al., 2006. Anti-infective potential of natural products: How to develop a stronger in vitro "proof-of-concept." Journal of Ethnopharmacology, 106:290–302.

Cowan, M.M., 1999. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews, 12:564–582.

Crawford, D.J. and Mabry, T.J., 1978. Flavonoid chemistry of Chenopodium fremontii. Infraspecific variation and systematic implications at the interspecific level. Biochemical Systematics and Ecology, 6(3):189–192.

Cwalina, B., 2014. Biodeterioration of concrete, brick and other mineral-based building materials. Understanding Biocorrosion:281–312.

Cyran, H.B., 1981. The Australian and New Zealand Book of Herbs., Sydney: Child and Henry Publishing.

Czarnetzki, B.M., Thiele, T. and Rosenbach, T., 1990. Immunoreactive leukotrienes in nettle plants (Urtica urens). International Archives of Allergy and Immunology, 91:43–6.

Dahmani, M., Hammouti, B. and Bouyanzer, A., 2010. Corrosion Inhibition of C38 Steel in 1 M HCl : A Comparative Study of Black Pepper Extract and Its Isolated Piperine, 5:1060–1069.

Dar, S.A. et al., 2013. Pharmacological and toxicological evaluation of Urtica dioica. Pharmaceutical biology, 51(2):170–80.

Dariva, C.G. and Galio, A.F., 2014. Corrosion Inhibitors - Principles Mechanisms and Applications. Developments in Corrosion Protection:365–379.

Davalos, R.V., Rubinsky, B. and Mir, L.M., 2003. Theoretical analysis of the thermal effects during in vivo tissue electroporation. Bioelectrochemistry, 61(1–2).

De Simone, F. et al., 1990. Two flavonol glycosides from Chenopodium quinoa. Phytochemistry, 29(11):3690–3692.

Dembitsky, V., Shkrob, I. and Hanus, L.O., 2008. Ascaridole and related peroxides from the genus Chenopodium. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia, 152(2):209–15.

Deng, G.-F. et al., 2012. Potential of Fruit Wastes as Natural Resources of Bioactive Compounds. International Journal of Molecular Sciences, 13(7):8308–8323.

Devi, S. et al., 1987. Peptide alkaloids from Zizyphus species. Phytochemistry, 26(12):3374–3375.

Dewick, P.M., 2017. Isoflavonoids. In The Flavonoids: Advances in Research since 1986 : 117–238.

Diard, J.P., Le Gorrec, B. and Montella, C., 1996. Cinétique électrochimique, Paris, France: Hermann.

Diaz, M.N. et al., 1997. Antioxidants and Atherosclerotic Heart Disease F. H. Epstein, ed. New England Journal of Medicine, 337(6):408–416.

Do, Q.D. et al., 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3):296–302.

Domola, M.S. et al., 2010. Insulin mimetics in Urtica dioica: structural and computational analyses of Urtica dioica extracts. Phytotherapy research, 24(S2):S175-82.

Duke, J.A., 1997. The Green Pharmacy, Florida: CRC Press.

Ekoumou, C., 2002. Etude phytochimique et pharmacologique de 5 recettes traditionnelles utilisees dans le traitement des infections urinaires et de la cystite. Universite de Bamako.

Elaloui, M. et al., 2014. Chemical compositions of the Tunisian Ziziphus jujuba oil. Emirates Journal of Food and Agriculture, 26(7):602.

El Arch, M. et al., 2003. Composition chimique et activités antimicrobienne et insecticide de l'huile essentielle de Mentha rotundifolia du Maroc. Acta Botanica Gallica, 150(3):267–274.

El-Etre, A.Y., Abdallah, M. and El-Tantawy, Z.E., 2005. Corrosion inhibition of some metals using lawsonia extract. Corrosion Science, 47(2):385–395.

El-Kashoury, E.-S.A. et al., 2012. Chemical composition and biological activities of the essential oil of Mentha suaveolens Ehrh. Zeitschrift fuer Naturforschung, C: Journal of Biosciences, 67(11/12):571–579.

El-lateef, H.M.A. et al., 2012. Development of New Eco-Friendly Corrosion Inhibitors Based on Vegetable Oils for Protection from CO2 Corrosion. Chemistry Journal, 2(2):38–49.

El-Sayed, N.. et al., 1999. A flavonol triglycoside from Chenopodium murale. Phytochemistry, 51(4):591–593.

Escrivà-Cerdán, C. et al., 2013. Effect of temperature on passive film formation of UNS N08031 Cr-Ni alloy in phosphoric acid contaminated with different aggressive anions. Electrochimica Acta, 111:552–561.

Fang, Y.-Z., Yang, S. and Wu, G., 2002. Free radicals, antioxidants, and nutrition. Nutrition, 18(10):872–879.

Farag, M. a. et al., 2013. Phytochemical, phylogenetic, and anti-inflammatory evaluation of 43 Urtica accessions (stinging nettle) based on UPLC-Q-TOF-MS metabolomic profiles.

Phytochemistry, 96:170–183.

Ferguson, L.R., 2010. Chronic inflammation and mutagenesis. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis, 690(1–2):3–11.

Ferreira, E.S. et al., 2004. Evaluation of the inhibitor effect of l-ascorbic acid on the corrosion of mild steel. Materials Chemistry and Physics, 83:129–134.

Finšgar, M. and Jackson, J., 2014. Application of corrosion inhibitors for steels in acidic media for the oil and gas industry: A review. Corrosion Science, 86:17–41.

Franceschini, P., 1994. La peau et son vieillissement., paris: Ed Flammarion Dominos.

Fu, L. et al., 2010. Antioxidant Capacities and Total Phenolic Contents of 56 Wild Fruits from South China. Molecules, 15(12):8602–8617.

Fu, L. et al., 2011. Total Phenolic Contents and Antioxidant Capacities of Herbal and Tea Infusions. International Journal of Molecular Sciences, 12(4):2112–2124.

Fukuyama, Y. et al., 1996. Two New Benzofuran-Type Lignans from the Wood of Viburnum awabuki. Chemical and Pharmaceutical Bulletin, 44(7):1418–1420.

Funatogawa, K. et al., 2004. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against Helicobacter pylori. Microbiology and Immunology, 48(4):251–261.

Gàbor, M. et al., 1988. Plants Flavonoids in biology and Medecine II; Biochemical, Cellular and Medecinal properties, New York.

Gabrielli, C., 2002. Méthodes électrochimiques, mesures d'impédances, Techniques de l'ingénieur 95, traité Analyse et caractérisation, PE2210,

Genovese, S. et al., 2012. Screening for oxyprenylated anthraquinones in Mediterranean Rhamnus species. Biochemical Systematics and Ecology, 43:125–127.

Georgé, S. et al., 2005. Rapid Determination of Polyphenols and Vitamin C in Plant-Derived Products. Journal of Agricultural and Food Chemistry, 53(5):1370–1373.

Ghareba, S. and Omanovic, S., 2010. Interaction of 12-aminododecanoic acid with a carbon steel surface: Towards the development of "green" corrosion inhibitors. Corrosion Science, 52(6):2104–2113.

Ghasemzadeh, A. and Ghasemzadeh, N., 2011. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research, 5(31):6697–6703.

Ghazghazi, H. et al., 2014. Fatty acids composition of Tunisian Ziziphus lotus L. (Desf.) fruits and variation in biological activities between leaf and fruit extracts. Natural Product Research, 28(14):1106–1110.

Ghedira, K. et al., 1995. Four cyclopeptide alkaloids from Zizyphus lotus. Phytochemistry, 38(3):767–772.

Gleńsk, M. and Włodarczyk, M., 2017. Determination of Oleanolic and Ursolic Acids in Sambuci flos Using HPLC with a New Reversed-phase Column Packed with Naphthalene Bounded Silica. Natural Product Communications, 12(12):1839–1841.

Glombitza, K.-W. et al., 1994. Hypoglycemic and Antihyperglycemic Effects of Zizyphus spina-christi in Rats. Planta Medica, 60(3):244–247.

Gohara, A.A. and Elmazar, M.M.A., 1997. Isolation of hypotensive flavonoids fromChenopodium species growing in Egypt. Phytotherapy Research, 11(8):564–567.

Gorai, M., Maraghni, M. and Neffati, M., 2010. Relationship between phenological traits and water potential patterns of the wild jujube Ziziphus lotus (L.) Lam. in southern Tunisia. Plant Ecology and Diversity, 3(3):273–280.

Govindarajan, R., Singh, D.P. and Rawat, K.S., 2007. High-performance liquid chromatographic method for the quantification of phenolics in "Chyavanprash" a potent Ayurvedic drug. Journal of Pharmaceutical and Biomedical Analysis, 43:527–532.

Grevsen, K., Frette, X.C. and Christensen, L.P., 2008. Concentration and composition of flavonol glycosides and phenolic acids in aerial parts of stinging nettle (Urtica dioica L.) are affected by nitrogen fertilization and by harvest time. European Journal of Horticultural Science, 73(1):20–27.

Grieve, M.M., 1992. A Modern Herbal, London: Penguin Books.

Gropper, S.S. et al., 2007. The freshman 15-a closer look. Journal of American college health : J of ACH, 58(3):223–231.

Grover, F.W. (Frederick W., 2004. Inductance calculations : working formulas and tables, Mineola N.Y.: Dover Publications.

Gu, R. et al., 2013. Characterization of AMT-Mediated High-Affinity Ammonium Uptake in Roots of Maize (Zea mays L.). Plant and Cell Physiology, 54(9):1515–1524.

Guignard, J.L., 1996. Abrégé de biochimie végétale Masson., paris.

Guigue, J. et al., 2016. Ultrahigh-resolution FT-ICR mass spectrometry for molecular characterisation of pressurised hot water-extractable organic matter in soils. Biogeochemistry, 128(3):307–326.

Gul, H., Abbas, K. and Qadir, M.I., 2015. Gastro-protective effect of ethanolic extract of mentha longifolia in alcohol- and aspirin-induced gastric ulcer models. Bangladesh Journal of Pharmacology, 10(1):241–245.

Gül, S. et al., 2012. Chemical composition and in vitro cytotoxic, genotoxic effects of essential oil from Urtica dioica L. Bulletin of environmental contamination and toxicology, 88(5):666–71.

Gülçin, I., 2012. Antioxidant activity of food constituents: An overview. Archives of Toxicology, 86(3):345–391.

Gülçin, I. and Küfrevioğlu, Ö., 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle ( Urtica dioica L.). Journal of Ethnopharmacology, 90(2–3):205–215.

Gunasekaran, G. and Chauhan, L.R., 2004. Eco friendly inhibitor for corrosion inhibition of mild steel in phosphoric acid medium. Electrochimica Acta, 49(25):4387–4395.

Gurbuz, I., Yesilada, E. and Ito, S., 2009. An anti-ulcerogenic flavonol diglucoside from Equisetum palustre L. Journal of Ethnopharmacology, 121(3):360–365.

Gutierrez-Orozco, F. and Failla, M.L., 2013. Biological activities and bioavailability of mangosteen xanthones: A critical review of the current evidence. Nutrients, 5(8):3163–3183.

Hachimi, F. El et al., 2015. Comparaison des huiles des graines et de la teneur en acides gras de différentes populations marocaines de jujubier, de grenadier et de figuier de barbarie, 6(5):1488–1502.

Hackerman, N. and Makrides, A.C., 1954. Action of Polar Organic Inhibitors in Acid Dissolution of Metals. Industrial and Engineering Chemistry, 46(3):523–527.

Hadizadeh, I., Peivastegan, B. and Kolahi, M., 2009. Antifungal activity of nettle (Urtica dioica L.), colocynth (Citrullus colocynthis L. Schrad), oleander (Nerium oleander L.) and konar (Ziziphus spina-christi L.) extracts on plants pathogenic fungi. Pakistan Journal of Biological Sciences: PJBS, 12:58–63.

Halliwell, B., 2007. Biochemistry of oxidative stress. Biochemical Society Transactions, 35(Pt 5):1147–1150.

Halliwell, B., 2006. Oxidative stress and neurodegeneration: Where are we now?. Journal of Neurochemistry, 97(6):1634–1658.

Halliwell, B. et al., 1995. The characterization of antioxidants. Food and Chemical Toxicology, 33(7):601–617.

Han, B.H., Park, M.H. and Han, Y.N.A.M., 1990. Cyclic peptide and peptide alkaloids from seeds of Zizyphus vulgaris. Phytochemistry, 29(10):3315–3319.

Harborne, J.B., Plant Secondary Metabolism. In Plant Ecology. Oxford, UK: Blackwell Publishing Ltd.: 132–155.

Harisson A.P. and Bartels E.M., 2006. A modern appraisal of ancient Etruscan Herbal Pratices. American Journal of Pharmacology and Toxicology, 1(2):26–29.

Harmaoui, A. et al., 2015. Study of triazolotiazepinon as a corrosion inhibitor in 1M hydrochloric acid. Journal of Materials and Environmental Science, 6(9):2509–2519.

Hatamnia, A.A., Abbaspour, N. and Darvishzadeh, R., 2014. Antioxidant activity and phenolic profile of different parts of Bene (Pistacia atlantica subsp. kurdica) fruits. Food Chemistry, 145:306–311.

Haudecoeur, R. and Boumendjel, A., 2012. Recent Advances in the Medicinal Chemistry of Aurones. Current Medicinal Chemistry, 19(18):2861–2875.

Heinonen, I.M., Lehtonen, P.J. and Hopia, A.I., 1998. Antioxidant Activity of Berry and Fruit Wines and Liquors. Journal of Agricultural and Food Chemistry, 46(1):25–31.

Heldt, H.-W. et al., 2011. Secondary metabolites fulfill specific ecological functions in plants. Plant Biochemistry:399–408.

Hirata, T. et al., 2009. Identification and physiological evaluation of the components from Citrus fruits as potential drugs for anti-corpulence and anticancer. Bioorganic and Medicinal Chemistry, 17(1):25–28.

Ho, C.-T. et al. eds., 1994. Food Phytochemicals for Cancer Prevention II, Washington, DC: American Chemical Society.

Hodgman, C., 1963. CRC Handbook of chemistry and physics : a ready-reference book of chemical and physical data 44. ed., Cleveland Ohio: The Chemical Rubber Publishing.

Holm, L.G., 1997. World weeds : natural histories and distribution, Wiley.

Huang, B. et al., 2011. Extracts of Halenia elliptica exhibit antioxidant properties in vitro and in vivo. Food and Chemical Toxicology, 49:185–190.

Huang, D., Boxin, O.U. and Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53:1841–1856.

Ibrahim, L.F. et al., 2007. A comparative study of the flavonoids and some biological activities of two Chenopodium species. Chemistry of Natural Compounds, 43(1):24–28.

Ignat, I., Volf, I. and Popa, V.I., 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chemistry, 126(4):1821–1835.

Igor, P.L.B., 2003. Etude des activités biologiques de fagara zanthoxyloides Lam (Rutaceae). Université de Bamako.

Ito, C. et al., 2005. Chemical constituents of Murraya siamensis: Three coumarins and their anti-tumor promoting effect. Phytochemistry, 66(5):567–572.

Iwashina, T., 2000. The Structure and Distribution of the Flavonoids in Plants. Journal of Plant Research, 113(3):287–299.

J B Harborne, 1967. Comparative biochemistry of the flavonoids, London: New York, Academic Press.

Jain, N. et al., 1990. Two flavonol glycosides from Chenopodium ambrosioides. Phytochemistry, 29(12):3988–3991.

Jeganathan, B. et al., 2016. Genetic Variation of Flavonols Quercetin, Myricetin, and Kaempferol in the Sri Lankan Tea (Camellia sinensis L.) and Their Health-Promoting Aspects. International journal of food science, 2016.

Jeroundi, D. et al., 2016. Physicochemical study and corrosion inhibition potential of dithiolo[4,5-b][1,4]dithiepine for mild steel in acidic medium. Journal of Materials and Environmental

Science, 7(11):4024–4035.

Jeyaprabha, C. et al., 2005. Influence of poly(aminoquinone) on corrosion inhibition of iron in acid media. Applied Surface Science, 252(4):966–975.

Ji, H.-F., Li, X.-J. and Zhang, H.-Y., 2009. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO reports, 10(3):194–200.

Johnson, T. a et al., 2013. Lipophilic stinging nettle extracts possess potent anti-inflammatory activity, are not cytotoxic and may be superior to traditional tinctures for treating inflammatory disorders. Phytomedicine : international journal of phytotherapy and phytopharmacology, 20(2):143–7.

Joshi, B.C., Mukhija, M. and Kalia, A.N., 2014. Pharmacognostical review of Urtica dioica L. International Journal of Green Pharmacy, 8(4).

Jutiviboonsuk, A. et al., 2005. Bioactive constituents from roots of Bursera tonkinensis. Phytochemistry, 66(23):2745–2751.

Kahl, R. and Kappus, H., 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Zeitschrift fur Lebensmittel-Untersuchung und -Forschung, 196(4):329–338.

Kaledaite, R. et al., 2011. Investigation of antiradical activity of Salvia officinalis L ., Urtica dioica L ., and Thymus vulgaris L . extracts as potential candidates for a complex therapeutic preparation. Journal of Medicinal Plants Research, 5(25):6090–6096.

Kalkhambkar, R.G. et al., 2007. Synthesis of novel triheterocyclic thiazoles as anti-inflammatory and analgesic agents. European Journal of Medicinal Chemistry, 42(10):1272–1276.

Kanter, M. et al., 2003. Effects of Nigella sativa L. and Urtica dioica L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl4-treated rats. Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine, 50(5):264–268.

Karou, D. et al., 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. Journal of Biotechnology, 4(August):823–828.

Kasrati, A. et al., 2013. Essential oil composition and antimicrobial activity of wild and cultivated mint timija (Mentha suaveolens subsp. timija (Briq.) Harley), an endemic and threatened medicinal species in Morocco. Natural Product Research, 27(12):1119–1122.

Kavtaradze, N.S. and Alaniya, M.D., 2003. Anthocyan glucosides from Urtica dioica. Chemistry of Natural Compounds, 39(3):315.

Kesavan, D., Gopiraman, M. and Sulochana, N., 2012. Green Inhibitors for Corrosion of Metals : A Review. Chemical Science Review and Letters, 1(1):1–8.

Khaled, K.F. and Al-Qahtani, M.M., 2009. The inhibitive effect of some tetrazole derivatives towards Al corrosion in acid solution: Chemical, electrochemical and theoretical studies.

Materials Chemistry and Physics, 113(1):150–158.

Kim, D.O., Jeong, S.W. and Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry, 81(3):321–326.

Kim, J.Y. et al., 2009. In vitro anti-inflammatory activity of lignans isolated from Magnolia fargesii. Bioorganic and Medicinal Chemistry Letters, 19(3):937–940.

Kokanova-Nedialkova, Z., Nedialkov, P.T. and Nikolov, S.D., 2009. The Genus Chenopodium : Phytochemistry , Ethnopharmacology and Pharmacology. Pharmacognosy Reviews, 3(6):280–306.

Komatsu, D. et al., 2010. Effect of antioxidants and corrosion inhibitor additives onthe quenching performance of soybean oil. Strojniski Vestnik/Journal of Mechanical Engineering, 56(2):121–130.

Komes, D. et al., 2011. Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis. Phytochemical analysis : PCA, 22(2):172–80.

Konczak, I. and Zhang, W., 2004. Anthocyanins - More than nature's colours. Journal of Biomedicine and Biotechnology, 2004(5):239–240.

Krystofova, O. et al., 2010. Effects of Various Doses of Selenite on Stinging Nettle (Urtica dioica L.). International Journal of Environmental Research and Public Health, 7(10):3804–3815.

Kubata, B.K. et al., 2005. Kola acuminata proanthocyanidins: a class of anti-trypanosomal compounds effective against Trypanosoma brucei. International Journal for Parasitology, 35(1):91–103.

Kudritsata, S.E. et al., 1986. Carotenoids of Urtica dioica. Chemistry of Natural Compounds, 22(5):604–605.

Kukrić, Z.Z. et al., 2012. Characterization of antioxidant and antimicrobial activities of nettle leaves (Urtica dioica L.). Acta Periodica Technologica, 43:257–272.

Kure, A. et al., 2016. Metabolic Fate of Luteolin in Rats: Its Relationship to Anti-inflammatory Effect. Journal of Agricultural and Food Chemistry, 64(21):4246–4254.

Ladjel, S., Gherraf, N. and Hamada, D., 2011. Antimicrobial Effect of Essential Oils From the Algerian Medicinal Plant Mentha Rotundifolia L., Journal of Applied Sciences Research, 7(11):1665-1667.

Lago, J. et al., 2014. Structure-Activity Association of Flavonoids in Lung Diseases. Molecules, 19(3):3570–3595.

Lahlou, M., El Mahi, M. and Hamamouchi, J., 2002. Evaluation of antifungal and mollusuicidial activities of Moroccan Zizyphus lotus (L.) Desf. Annales pharmaceutiques francaises, 60(6):410.

Lal, D.S. and Yadav, B.K., 1983. Folk medicines of Kurukshetra district (Haryana), India.

Economic Botany, 37:299–305.

Landolt, D., 1993. Corrosion et chimie de surfaces des métaux, Lausanne: Presses polytechniques et universitaires romandes.

Lapinskaya, E.S. and Kopyt'ko, Y.F., 2009. Composition of the lipophilic fraction of stinging nettle (Urtica dioica L. and Urtica urens L.) homeopathic matrix tinctures. Pharmaceutical Chemistry Journal, 42(12):699–702.

Lapornik, B., Prošek, M. and Golc Wondra, A., 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering, 71(2):214–222.

Larif, M. et al., 2013. An investigation of carbon steel corrosion inhibition in hydrochloric acid medium by an environmentally friendly green inhibitor. Research on Chemical Intermediates, 39(6):2663–2677.

Lawrence, B.M., 2006. Mint: The genus Mentha. Taylor and Francis Group, Florida, USA, 44:576.

Le Crouéour, G. et al., 2002. Lotusine G: a new cyclopeptide alkaloid from Zizyphus lotus. Fitoterapia, 73(1):63–68.

Leal, F. et al., 2013. Fungicide Activity of Thymus mastichina and Mentha rotundifolia in Plants In Vitro. Journal of Medicinal Food, 16(4):273–273.

Left, D.B. et al., 2013. Review : oils and extracts plants as corrosion inhibitors for different metals and alloys in hydrochloric acid m. , 4(6):855–866.

Le Floc'h, É.A., 1983. Contribution à une étude ethnobotanique de la flore tunisienne, Imprimerie officielle de la République Tunisienne. Tunis.

Le Perchec, P. and Malik, R., 1994. Les molécules de la beauté, de l'hygiène et de la protection : une introduction à la science cosmétologique, Nathan.

Legssyer, A. and Ziyyat, A., 2002. Cardiovascular effects of Urtica dioica L. in isolated rat heart and aorta. Phytotherapy research, 16(6):503–507.

Lemieux G. and Germain D., 2002. Le Bois Rameal Fragmenté: la clé de la fertilité durable du sol. Université Laval.

Li, B.B., Smith, B. and Hossain, M.M., 2006. Extraction of phenolics from citrus peelsI. Solvent extraction method. Separation and Purification Technology, 48:182–188.

Li, F. et al., 2008. Cytotoxic constituents from Brazilian red propolis and their structure-activity relationship. Bioorganic and Medicinal Chemistry, 16(10):5434–5440.

Li, J.W., Ding, S.D. and Ding, X.L., 2005. Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. Process Biochemistry, 40(11):3607–3613.

Li, S. et al., 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. International Journal of Molecular Sciences, 16(11):26087–26124.

Li, W.H. et al., 2008. Some new triazole derivatives as inhibitors for mild steel corrosion in acidic medium. Journal of Applied Electrochemistry, 38(3):289–295.

Lieutaghi, P., 1966. Le livre des Bonnes Herbes R. Morel, ed., Marabout service.

Lister, E. and Wilson, P., 2001. Measurement of Total Phenolics and ABTS Assay for Antioxidant Activity (Personal Communication). In Crop Research Institute Lincoln, New Zealand.

Lone, B.A. et al., 2017. Evaluation of anthelmintic antimicrobial and antioxidant activity of Chenopodium album. Tropical Animal Health and Production, 49(8):1597–1605.

Lorenzo, D. et al., 2002. Essential oils of Mentha pulegium and Mentha rotundifolia from Uruguay. Brazilian Archives of Biology and Technology, 45(4):519–524.

Lozoya, J. and Lozoya, M., 1982. In Flora medicinal de Mexico. Primera parte: plantas in digenas. In Mexico: Instituto Mexicano del Seguro Social:31.

Lust, B., 1983. About Herbs, Medicines from the Meadows, London: Thorsons Pub.

Macheix, J.-J., Fleuriet, A. and Jay-Allemand, C., 2006. Les composés phénoliques des végétaux. Sciences des aliments, 26:189–190.

Maheshwari, R. and Dubey, R.S., 2009. Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings. Plant Growth Regulation, 59(1):37–49.

Manach, C. et al., 2004. Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition, 79(5):727–747.

Mandal, S. et al., 2011. Assessment of the Antioxidant and Reactive Oxygen Species Scavenging Activity of Methanolic Extract of Caesalpinia crista Leaf. Evidence-Based Complementary and Alternative Medicine, 2011:1–11.

Mansfeld, F., 1985. Corrosion Inhibition in Neutral, Aerated Media. Journal of The Electrochemical Society, 132(2):290.

Maraghni, M., Gorai, M. and Neffati, M., 2010. Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of Ziziphus lotus. South African Journal of Botany, 76(3):453–459.

Marinova, D., Ribarova, F. and Atanassova, M., 2005. Total phenolics and total flavonoids in bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy, 40:255–260.

Marshall, J.W. et al., 2018. Monitoring chemical changes during food sterilisation using ultrahigh resolution mass spectrometry. Food Chemistry, 242:316–322.

Martens, S. and Mithöfer, A., 2005. Flavones and flavone synthases. Phytochemistry, 66(20):2399–2407.

Masquelier, J., Dumon, M. and Dumas, J., 1979. Stabilisation des collagènes par des oligomères procyanidoliques. Acta thérapeutique, 1:101–104.

Matsuura, H.N. and Fett-Neto, A.G., 2017. Plant Alkaloids: Main Features, Toxicity, and Mechanisms of Action. In Springer, Dordrecht: 243–261.

Maurya, R. et al., 2009. Constituents of Tinospora sinensis and their antileishmanial activity against Leishmania donovani. Natural Product Research, 23(12):1134–1143.

McLeod, M., 1974. Plant tannins-their role in forage quality. Nutrition Abstracts and Reviews, 44(11):801–814.

Melagraki, G. et al., 2009. Synthesis and evaluation of the antioxidant and anti-inflammatory activity of novel coumarin-3-aminoamides and their alpha-lipoic acid adducts. European Journal of Medicinal Chemistry, 44(7):3020–3026.

Meriga, B. et al., 2004. Aluminium-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (Oryza sativa). Journal of Plant Physiology, 161(1):63–68.

Mieyal, J.J., 1978. Mecanism of enzyme like reactions involving human hemoglobin. In Bioorganic chemistry, vol IV. New York: Academic Press: 315–348.

Migahed, M.A., Mohamed, H.M. and Al-Sabagh, A.M., 2003. Corrosion inhibition of H-11 type carbon steel in 1 M hydrochloric acid solution by N-propyl amino lauryl amide and its ethoxylated derivatives. Materials Chemistry and Physics, 80(1):169–175.

Mills, S. and Bone, K., 2013. Principles and Practice of Phytotherapy - Modern Herbal Medicine (Book Review).

Mills, S.Y., 1988. The Dictionary of Modern Herbalism., Rochester, Vermont: Healing Art Press.

Mills, S.Y., 1991. The Essential Book of Herbal Medicine., London: Penguin.

Mishra, S., Jha, A.B. and Dubey, R.S., 2011. Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. Protoplasma, 248(3):565–577.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science, 7(9):405–410.

Le Moal, M.A. and Truffa-Bachi, P., 1988. Urtica dioica agglutinin, a new mitogen for murine T lymphocytes: Unaltered interleukin-1 production but late interleukin 2-mediated proliferation. Cellular Immunology, 115(1):24–35.

Muanda, F.N., 2010. Identification de polyphenols, évaluation de leur activité antioxydante et étude de leurs propriétés biologiques. Université de lorraine.

Mundhe, K.S. et al., 2011. Evaluation of phenol, flavonoid contents and antioxidant activity of Polyalthia longifolia. Journal of Chemical and Pharmaceutical Research, 3(1):764–769.

Muralidharan, S. et al., 1995. Polyamino-Benzoquinone Polymers: A New Class of Corrosion Inhibitors for Mild Steel. Journal of The Electrochemical Society, 142(5), p.1478.

N.P., D. and T.A., P., 1990. Effects of flavonoids on thermal autooxidation of palm oil: structure–

activity relationship. Journal of the American Oil Chemists' Society, 67:255–258.

Nahata, A. and Dixit, V.K., 2012. Ameliorative effects of stinging nettle (Urtica dioica) on testosterone-induced prostatic hyperplasia in rats. Andrologia, 44 Suppl 1:396–409.

Naili, M.B. et al., 2010. Evaluation of antibacterial and antioxidant activities of Artemisia campestris (Astraceae) and Ziziphus lotus (Rhamnacea). Arabian Journal of Chemistry, 3(2):79–84.

Neal, M.J., 1997. Medical Pharmacology at a Glance. In 3rd:103.

Nebeling, L., 2002. Phytochemicals, the color of a Healthy Diet, Maryland: Health Promotion Research Branch National Cancer Institute.

Newall, C.A., Anderson, L.A. and Phillipson, J.D., 1996. Herbal Medicines: A Guide for Health-care Professionals., London: The Pharmaceutical Press.

Noor, E.A., 2005. The inhibition of mild steel corrosion in phosphoric acid solutions by some N-heterocyclic compounds in the salt form. Corrosion Science, 47(1):33–55.

Nowak, R. et al., 2016. Antioxidative and cytotoxic potential of some Chenopodium L. species growing in Poland. Saudi Journal of Biological Sciences, 23(1):15–23.

Nurmi, T. et al., 2003. Liquid chromatography method for plant and mammalian lignans in human urine. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 798(1):101–10.

Nuutinen, T., 2018. Medicinal properties of terpenes found in Cannabis sativa and Humulus lupulus. European Journal of Medicinal Chemistry, 157:198–228.

O. L. Riggs Jr., 1973. Corrosion Inhibitors 2nd Edition. C. C. Nathan, ed., Houston.

Obot, I.B. and Obi-Egbedi, N.O., 2010. Adsorption properties and inhibition of mild steel corrosion in sulphuric acid solution by ketoconazole: Experimental and theoretical investigation. Corrosion Science, 52(1):198–204.

Oguzie, E.E. et al., 2006. Corrosion inhibition and adsorption behaviour of bismark brown dye on aluminium in sodium hydroxide solution. Materials Letters, 60(28):3376–3378.

Okamura, H. et al., 1993. Antioxidant activity of tannins and flavonoids in Eucalyptus rostrata. Phytochemistry, 33(3):557–561.

Oliver-Bever, B. and Zahland, G.R., 1979. Plants with oral hypoglycaemic activity. Quarterly Journal of Crude Drug Research, 17:139–96.

Orčić, D. et al., 2014. Quantitative determination of plant phenolics in Urtica dioica extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. Food chemistry, 143:48–53.

Otles, S. and Yalcin, B., 2012. Phenolic compounds analysis of root, stalk, and leaves of nettle. The Scientific World Journal, 2012:564367.

Oukhrib, R. et al., 2017. Ziziphus lotus as Green Inhibitor of Copper Corrosion in Natural Sea Water. Portugaliae Electrochimica Acta, 35(4):187–200.

Oumzil, H. et al., 2002. Antibacterial and antifungal activity of essential oils of Mentha suaveolens. Phytotherapy Research, 16(8):727–731.

Ozen, T. and Korkmaz, H., 2003. Modulatory effect of Urtica dioica L. (Urticaceae) leaf extract on biotransformation enzyme systems, antioxidant enzymes, lactate dehydrogenase and lipid peroxidation in mice. Phytomedicine : International Journal of Phytotherapy and Phytopharmacology, 10(5):405–415.

Pahlow, M., 1992. Healing Plants (translation from German). In Hauppauge, New York: Baron's Educational series, p. 72.

Palma, M. and Barroso, C.G., 2002. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. In Analytica Chimica Acta. pp. 119–130.

Park, K.W. et al., 2011. A Multivariate Statistical Approach to Comparison of Essential Oil Composition from Three Mentha Species. , 29(July):382–387.

Park, K.Y. et al., 2004. Antimutagenic activity of flavonoids from the heartwood of Rhus verniciflua. Journal of Ethnopharmacology, 90(1):73–79.

Patel, M., Das, S. and Thakur, J., 2018. GC-MS-Based Analysis of Methanol: Chloroform-extracted Fatty Acids from Plant Tissues. Bio-protocol, 8(18).

Patel, S.S. and Udayabanu, M., 2013. Effect of Urtica dioica on memory dysfunction and hypoalgesia in an experimental model of diabetic neuropathy. Neuroscience Letters, 552:114–9.

Patel, S.S. and Udayabanu, M., 2014. Urtica dioica extract attenuates depressive like behavior and associative memory dysfunction in dexamethasone induced diabetic mice. Metabolic brain Disease, 29(1):121–30.

Patten, G., 1993. Urtica. Australian Journal of Medical Herbalism, 5(1):5–13.

Pawlowska, A.M. et al., 2009. Flavonoids of Zizyphus jujuba L. and Zizyphus spina-christi (L.) Willd (Rhamnaceae) fruits. Food Chemistry, 112(4):858–862.

Peng, C. et al., 2014. Biology of ageing and role of dietary antioxidants. BioMed Research International, 2014, p.831841.

Pérez-Jiménez, J. et al., 2010. Identification of the 100 richest dietary sources of polyphenols: An application of the Phenol-Explorer database. European Journal of Clinical Nutrition, 64:112–120.

Perez-Vizcaino, F. et al., 2009. Antihypertensive effects of the flavonoid quercetin. Pharmacological Reports, 61(1):67–75.

Piironen, V. et al., 2000. Plant sterols: Biosynthesis, biological function and their importance to

human nutrition. Journal of the Science of Food and Agriculture, 80(7):939–966.

Pinelli, P. et al., 2008. Extraction and HPLC analysis of phenolic compounds in leaves, stalks, and textile fibers of Urtica dioica L. Journal of agricultural and food chemistry, 56(19):9127–32.

Pottier-Alapetite, G., 1979. Flora of Tunisia Angiospermes-Dicotyledones, Apetales-Dialypetales. Programme Flore et Végétation Tunisiennes, Tunis: Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture.

Premkuma, B., 2018. Antioxidant Defense and Disease activity in Rheumatoid Arthritis. Research Journal of Pharmacy and Technology, 11(5):1810–1814.

Qasim, M. et al., 2016. Effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes, Pakistan Journal of Botany, 48(2): 621-627.

Radovico, O., 1990. Proc. 7th th European Symposium on Corrosion Inhibitors, Ann. Univ. In Proc. 7th th European Symposium on Corrosion Inhibitors. Ferrara, Italy., p. 330.

Rafajlovska, V., Najdenova, V. and Cvetkov, L., 2001. Influence of some factors at chlorophyll extraction from stinging nettle (Urtica dioica L.). Herba Polonica .

Raimundo, N. and Shadel, G.S., 2009. A "radical" mitochondrial view of autophagy-related pathology. Aging, 1(4):354–356.

Raja, P.B. et al., 2016. Reviews on Corrosion Inhibitors: A Short View. Chemical Engineering Communications, 203(9):1145–1156.

Raja, P.B. and Sethuraman, M.G., 2008. Natural products as corrosion inhibitor for metals in corrosive media - A review. Materials Letters, 62(1):113–116.

Ramiréz-Aristizabal, L.S., Ortiz, A. and Restrepo-Aristizabal, M.F. Salinas-Villada, J.F., 2016. Evaluation of the antioxidant capacity and content of polyphenols obtained from tea (camellia sinensis) of four brands sold in colombia by extraction at room temperature. PharmacologyOnLine, 1:20–30.

Randall, C.F. et al., 1999. Nettle sting of Urtica dioica for joint pain – an exploratory study of this complementary therapy. Complementary Therapies in Medicine, 7:126–31.

Randall, C.F., 2002. Personal Observation by Author, during Visit to Cheese Factory.

Randall, C.F. et al., 2000. Randomized con- trolled trial of nettle sting for treatment of base-of-thumb pain. Journal of the Royal Society of Medicine, 93:305–9.

Randall, C.F., 1994. Stinging nettles for osteoarthritis pain of the hip (letter). British Journal of General Practice, 44:533–4.

Randall, C.F., 2001. Treatment of Musculoskeletal Pain with the Sting of the Stinging Nettle: Urtica dioica. University of Plymouth, Plymouth, UK.

Rani, B.E.A. and Basu, B.B.J., 2012. Green Inhibitors for Corrosion Protection of Metals and Alloys: An Overview. International Journal of Corrosion, 2012:1–15.

Rao, Y.K. et al., 2009. The constituents of Anisomeles indica and their anti-inflammatory activities. Journal of Ethnopharmacology, 121(2):292–296.

Re, R. et al., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26:1231–1237.

Renault, J.H. et al., 1997. Dammarane saponins from Zizyphus lotus. Phytochemistry, 44(7):1321–7.

Riahi, L. et al., 2013. Phytochemistry, antioxidant and antimicrobial activities of the essential oils of Mentha rotundifolia L. in Tunisia. Industrial Crops and Products, 49:883–889.

Ribereau, G.P., 1968. Les composés phénoliques des végétaux, paris: Dunod.

Rice-Evans, C.A., Miller, N.J. and Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7):933–56.

Richard Mabey, 1996. Flora Britannica: The Definitive New Guide to Britain's Wild Flowers, Plants and Trees, Vintage Publishing.

Richardson, J.E. et al., 2004. Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 359(1450).

Richardson, P.M., Harborne, J.B. and Mabry, T.J., 1984. The Flavonoids: Advances in Research. Brittonia, 36(1):85.

de Rijke, E. et al., 2006. Analytical separation and detection methods for flavonoids. Journal of Chromatography A, 1112(1–2):31–63.

Rodgers, R.D. and Eggers, W.J., 1993. Correction for potentiostat response in impedance measurements. In 183rd Meeting of the Electrochemistry Society. Honolulu, HI, USA: 16–21.

Roschek, B.J. et al., 2009. Nettle extract (Urtica dioica) affects key receptors and enzymes associated with allergic rhinitis. Phytotherapy Research, 23(7):920–926.

Roya, K. and Fatemeh, G., 2013. Screening of total phenol and flavonoid content, antioxidant and antibacterial activities of the methanolic extracts of three Silene species from Iran. The International Journal of Agriculture and Crop Sciences, 5(3):305–312.

Rozenfel′d, I.L. (Iosif L.), 1981. Corrosion inhibitors, New York: McGraw-Hill.

Russell, A.D., 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. Journal of Applied Bacteriology, 71(3):191–201.

Saeed, N., Khan, M.R. and Shabbir, M., 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, 12(1), p.221.

Safarinejad, M.M.R., 2005. Urtica dioica for treatment of benign prostatic hyperplasia: a

prospective, randomized, double-blind, placebo-controlled, crossover study. Journal of herbal pharmacotherapy, 5(4).

Sakagami, H. et al., 2005. Molecular requirements of lignin-carbohydrate complexes for expression of unique biological activities. In Phytochemistry:2108–2120.

Sannomiya, M. et al., 2005. Flavonoids and antiulcerogenic activity from Byrsonima crassa leaves extracts. Journal of Ethnopharmacology, 97(1):1–6.

Santhos A. S., Caetano L.C. and Santana A.E.G., 1998. A 12a – hydroxyrotenoid from roots of Boerhaavia coccinea. Phytochemistry, 49(1):255–258.

Sapronova, N.N., 1989. The content of vitamin K and certain microelements in Urtica dioica. Rastitel'nye Resursy, 25:243–7.

Sasidharan, S. et al., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary, and alternative medicines, 8(1):1–10.

Saufi, H. et al., 2014. Evaluation of the hexanoic Anise extract as inhibitor for dental amalgam in synthetic saliva. Journal of Materials and Environmental Science, 5(S1):2129–2132.

Saufi, H. et al., 2015. Evaluation of vegetable oil of nigel as corrosion inhibitor for iron in NaCl 3% medium. Journal of Materials and Environmental Science, 6(7):1845–1849.

Sayhan, M.B. et al., 2012. Protective effect of Urtica dioica L. on renal ischemia/reperfusion injury in rat. Journal of molecular histology, 43(6):691–8.

Schauenberg, P. and Paris, F., 1977. Guide to Medicinal Plants, London: Lutterworth Press.

Schofield, P., Mbugua, D.. and Pell, A.N., 2001. Analysis of condensed tannins: a review. Animal Feed Science and Technology, 91(1–2):21–40.

Seeka, C. et al., 2016. Prenylfuranocoumarin–HMGA–flavonol glucoside conjugates and other constituents of the fruit peels of Citrus hystrix and their anticholinesterase activity. Phytochemistry, 127:38–49.

Shah, K. et al., 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Science, 161(6):1135–1144.

Shah, N.C. and Joshi, M.C., 1971. An ethnobotanical study of the Kumaon region of India. Economic Botany, 25:414–22.

Sharma, P. and Dubey, R.S., 2005. Drought Induces Oxidative Stress and Enhances the Activities of Antioxidant Enzymes in Growing Rice Seedlings. Plant Growth Regulation, 46(3):209–221.

Sidaoui, F. et al., 2015. Study of Tunisian Nettle Leaves (Urtica dioica L.): Mineral Composition and Antioxidant Capacity of their Extracts Obtained by Maceration and Supercritical Fluid Extraction. International Journal of Pharmacognosy and Phytochemical Research, 7(4):707–

713.

Smith, M.A., Richey and Sayre, L., 1996. Test for oxidative damage in Alzheimer's. Nature, 382:120–121.

Smyth, T., Ramachandran, V.N. and Smyth, W.F., 2009. A study of the antimicrobial activity of selected naturally occurring and synthetic coumarins. International Journal of Antimicrobial Agents, 33(5):421–426.

Sohn, H.Y. et al., 2004. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: Morus alba L., Morus mongolica Schneider, Broussnetia papyrifera (L.) Vent, Sophora flavescens Ait and Echinosophora koreensis Nakai. Phytomedicine, 11(7–8):666–672.

Solmaz, R., 2010. Investigation of the inhibition effect of 5-((E)-4-phenylbuta-1,3-dienylideneamino)-1,3,4-thiadiazole-2-thiol Schiff base on mild steel corrosion in hydrochloric acid. Corrosion Science, 52(10):3321–3330.

Soltani, N. et al., 2012. Green approach to corrosion inhibition of 304 stainless steel in hydrochloric acid solution by the extract of Salvia officinalis leaves. Corrosion Science, 62:122–135.

Srivastava, S. and Dubey, R.S., 2011. Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings. Plant Growth Regulation, 64(1):1–16.

Stalikas, C.D., 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of Separation Science, 30(18):3268–3295.

Stanly, K. and Parameswaran, G., 2010. Corrosion inhibition of mild steel in hydrochloric acid solution by Schiff base furoin thiosemicarbazone. Corrosion Science, 52(1):224–228.

Stary, F., 1991. Medicinal Herbs and Plants, London: Treasure Press.

Stephen, J.T. and Adebayo, A., 2018. Inhibition of Corrosion of Mild Steel in Hydrochloric Acid Solution Using Akee Apple Seed Extract. Journal of Failure Analysis and Prevention, 18(2):350–355.

Stöckigt, J. et al., 2002. High-performance liquid chromatographic, capillary electrophoretic and capillary electrophoretic-electrospray ionisation mass spectrometric analysis of selected alkaloid groups. Journal of Chromatography A, 967(1):85–113.

Sun, B., Ricardo-da-Silva, J.M. and Spranger, I., 1998. Critical Factors of Vanillin Assay for Catechins and Proanthocyanidins. Journal of Agricultural and Food Chemistry, 46(10):4267–4274.

Sunkar, S. et al., 2018. Phytochemical analysis and isolation of endophytic bacteria from Bauhinia purpurea. Research Journal of Pharmacy and Technology, 11(5):1867–1876.

Sutradhar, R.K. et al., 2008. Bioactive flavones of Sida cordifolia. Phytochemistry Letters,

1(4):179–182.

Tahri, a et al., 2000. Acute diuretic, natriuretic and hypotensive effects of a continuous perfusion of aqueous extract of Urtica dioica in the rat. Journal of Ethnopharmacology, 73(1–2):95–100.

Takafuta, T. and Fujimura, K., 2017. Steroids. In Autoimmune Thrombocytopenia. pp. 145–151.

Takahashi, H. et al., 2008. Metabolomics approach for determining growth-specific metabolites based on Fourier transform ion cyclotron resonance mass spectrometry. Analytical and Bioanalytical Chemistry, 391(8):2769–2782.

Tavassoli, S. and Djomeh, Z.E., 2011. Total phenols, antioxidant potential and antimicrobial activity of methanol extract of rosemary (Rosmarinus officinalis L.). Global Veterinaria, 7(4):337–341.

Tepe, B. et al., 2006. Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chemistry, 95(2):200–204.

Testai, L. et al., 2002. Cardio vascular effects of Urtica dioica L . ( Urticaceae ) roots extracts : in vitro and in vivo pharmacological studies. Journal of Ethnopharmacology, 81(1):105–109.

Thomassen, D., Slattery, J.T. and Nelson, S.D., 1990. Menthofuran-dependent and independent aspects of pulegone hepatotoxicity: roles of glutathione. Journal of Pharmacology and Experimental Therapeutics, 253(2):567–572.

Thomson, W., 1976. Herbs That Heal., London: Adam and Charles Black.

Tinea, Y. et al., 2017. LC-MS/MS Analysis of Flavonoid Compounds from Zanthoxylum zanthoxyloides Extracts and Their Antioxidant Activities. Natural Product Communications, 12(12):1865–1868.

Tiwari, S. and Beriha, S.S., 2015. Pantoea species causing early onset neonatal sepsis: a case report. Journal of Medical Case Reports, 9(1):188.

Tripathi, M. et al., 2001. Cyclopeptide alkaloids from Zizyphus jujuba. Fitoterapia, 72(5):507–510.

Tripathi, P. and Tripathi, S., 2014. Ziziphus jujuba: A Phytopharmacological Review. International Journal of Research and Development in Pharmacy and Life Sciences, 3(3):959–966.

Tripoli, E. et al., 2007. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chemistry, 104(2):466–479.

Tsao, M.-L., Tian, F. and Schultz, P.G., 2005. Selective Staudinger Modification of Proteins Containing p-Azidophenylalanine. ChemBioChem, 6(12):2147–2149.

Tschesche, R. and Kaußmann, E.U., 1975. Chapter 4 The Cyclopeptide Alkaloids:165–205.

Türkdoğan, M.K. et al., 2003. The role of Urtica dioica and Nigella sativa in the prevention of carbon tetrachloride-induced hepatotoxicity in rats. Phytotherapy Research, 17(8):942–946.

Turner, N. et al., 1990. Thompson Ethnobotany: Knowledge and Usage of Plants by the Thompson Indians of British Columbia. Memoir No. 3, Victoria, Canada: Royal British Columbia Museum.

Tyler, V.E., 1994. Herbs of Choice: The Therapeutic use of Phytomedicinals., New York: Pharmaceutical Products Press.

Umoren, S.A., Ogbobe, O. and Ebenso, E.E., 2006. Synergistic inhibition of aluminium corrosion in acidic medium by gum arabic and halide ions. Transactions of the Society for Advancement of Electrochemical Science and Technology, 41(2):74–81.

Uncini, R.E., Zaccaro, L. and Tomei, P.E., 2005. Antiviral activity in vitro of Urtica dioica L., Parietaria diffusa M. et K. and Sambucus nigra L. Journal of Ethnopharmacology, 98(3):323–7.

Vafeiadou, K. et al., 2009. The citrus flavanone naringenin inhibits inflammatory signalling in glial cells and protects against neuroinflammatory injury. Archives of Biochemistry and Biophysics, 484(1):100–109.

Vasishta, P.C., 1989. In Taxonomy of angiosperms. In Ramchand, p. 648.

Vaya, J. et al., 2003. Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. Phytochemistry, 62(1):89–99.

Verma, C. et al., 2017. Corrosion inhibition of mild steel in 1M HCl by D-glucose derivatives of dihydropyrido [2,3-d:6,5-d'] dipyrimidine-2, 4, 6, 8(1H,3H, 5H,7H)-tetraone. Scientific reports, 7:44432.

Verma, S. and Dubey, R.S., 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science, 164(4):645–655.

Ververidis, F. et al., 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. Biotechnology Journal, 2(10):1214–1234.

Vickery, R., 1995. A Dictionary of Plant-Lore,

Vickery, R., 1993. Nettles; their uses and folklore in the British Isles. Folk Life, 31:88–93.

Visioli, F., Bellomo, G. and Galli, C., 1998. Free radical-scavenging properties of olive oil polyphenols. Biochemical and biophysical research communications, 247(1):60–64.

Wagner, H., Willer, F. and Kreher, B., 1989. Biologically active compounds from the aqueous extract of Urtica dioica. Planta Med, 55:452–454.

Wang, F. et al., 2016. Natural Products for the Prevention and Treatment of Hangover and Alcohol Use Disorder. Molecules, 21(1):64.

Wang, Y.F. et al., 2002. New rotenoids from roots of Mirabilis jalapa. Helvetica Chimica Acta, 85(8):2342–2348.

Wangensteen, H. et al., 2005. Rotenoids and isoflavones from Sarcolobus globosus. Planta Medica, 71(8):754–758.

Weiss, R.F., 1991. Herbal Medicine (English Translation-Meuss AR), Beaconsfield, England.: Beaconsfield Pub. Ltd.

Wetherilt, H., 1992. Evaluation of Urtica Species as Potential Sources of Important Nutrients. Developments in Food Science, 29:15–25.

Williamson, E.M., Okpako, D.T. and Evans, F.J., 1996. Selection, preparation, and pharmacological evaluation of plant material, J. Wiley.

Win, N.N. et al., 2008. Novel anticancer agents, kayeassamins C-I from the flower of Kayea assamica of Myanmar. Bioorganic and Medicinal Chemistry, 16(18):8653–8660.

Wollgast, J. and Anklam, E., 2000. Review on polyphenols in Theobroma cacao: Changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Research International, 33(6):423–447.

Wren, C., 1988. Potters New Cyclopaedia of Botanical Drugs and Preparations., Bradford, England: Health Sciences Press.

Yang, J., Liu, R.H. and Halim, L., 2009. Antioxidant and antiproliferative activities of common edible nut seeds. LWT - Food Science and Technology, 42(1):1–8.

Yerragunta, V. et al., 2013. A review on Chalcones and its importance. PharmaTutor, 1(2):54–59.

Yokozawa, T. et al., 1998. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. Biochemical Pharmacology, 56(2):213–222.

Younes, M. and Siegers, C.P., 1981. Mechanistic aspects of enhanced lipid peroxidation following glutathione depletion in vivo. Chemico-Biological Interactions, 34(3):257–266.

Yüce, A.O. and Kardaş, G., 2012. Adsorption and inhibition effect of 2-thiohydantoin on mild steel corrosion in 0.1M HCl. Corrosion Science, 58(0):86–94.

Zaidi, F. et al., 1998. Free flavonoid aglycones from leaves of mentha pulegium and mentha suaveolens (labiatae). Phytochemistry, 48(6):991–994.

Zarrouk, A. et al., 2013. Evaluation of N-containing organic compound as corrosion inhibitor for carbon steel in phosphoric acid. Journal of Materials and Environmental Science, 4(2):177–192.

Zekri, N. et al., 2013. Phytochemical study and fumigant toxicity of Mentha suaveolens Ehrh essential oil from Morocco against adults of S . oryzae ( L .). Australian Journal of Basic and Applied Sciences, 7(14):599–606.

Zenk, M.H. and Juenger, M., 2007. Evolution and current status of the phytochemistry of nitrogenous compounds. Phytochemistry, 68(22–24):2757–2772.

Zhang, H.K., 1994. Synopsis of Traditional Chinese Medicine Sources Z. H. Yuan and Z. Z. Ying,

eds., Beijing: Science Press.

Zhaoa, J., Nana, P. and Zhong, Y., 2004. Chemical Composition of the Essential Oils of Clausena lansium from Hainan Island, China. Zeitschrift für Naturforschung C, 59(3–4):153–156.

Zhou, C. et al., 2011. Flavonoids, phenolics, and antioxidant capacity in the flower of eriobotrya japonica lindl. International Journal of Molecular Sciences, 12(5):2935–2945.

Zhou, Y. et al., 2016. Alcoholic Beverage Consumption and Chronic Diseases. International Journal of Environmental Research and Public Health, 13(6), p.522.

Zrira, S., 2017. Some Important Aromatic and Medicinal Plants of Morocco. In Springer, Dordrecht: 91–125.

## *Résumé*

La biodiversité du Maroc a récemment été reconnue comme d'intérêt mondial. Cette riche biodiversité fournit des services à l'homme, notamment par le biais des plantes aromatiques et médicinales, utilisées traditionnellement. Les quatres plantes etudier, à savoir, *Urtica dioica, Chenopodium murale, Mentha rutondifolia* et *Ziziphus lotus*, sont bien connue des Marocains et largement consommée pour leurs propriétés aromatiques, médicinales et alimentaires.

Le présent travail a pour objectif d'étudier certains aspects de la biologie et la chimie de ces espèces. Ainsi, les activités biologiques, anti-corrosive et la composition métabolomique ont été étudiés.

Les extraits présentaient dans la plupart des cas de bonnes propriétés antibactériennes et antioxydantes. L'extrait de graines de *Mentha rutondifolia*, par exemple, avait une IC50 de 5,98 µg /mL, comparable aux antioxydants synthétiques. De plus, l'inhibition de la corrosion de ces extraits a atteint une efficacité ~ 72% pour l'étude de polarisation et ~ 91% pour les mesures EIS à 3 g / L. Quelques classes intéressantes de composés ont été identifiées à l'aide de la spectrométrie de masse à résonance cyclotronique ionique (FT-ICR-MS). Les résultats concourent à améliorer la connaissance de ces espèces, et fournissent une base solide pour leur mise en valeur, ainsi que pour l'exploitation pharmacologique appropriées.

*Mots-clés: Inhibiteur de corrosion, Urtica dioica, Chenopodium murale, Mentha rotundigolia, Ziziphus lotus.*

## *Abstract*

Morocco's biodiversity has recently been recognized as of global interest. This rich biodiversity provides services to mankind, especially through the aromatic and medicinal plants traditionally used. The four plants used for this study, namely*, Urtica dioica, Chenopodium murale, Mentha rutondifolia* and *Ziziphus lotus*, are well known to Moroccans and are widely consumed for their aromatic, medicinal and nutritional properties.

An integral part of this project, aims to study some biological and chemical aspects of these species. Thus, biological activities, anti-corrosive properties and metabolomic composition were studied.

The extracts exhibited for most cases good antibacterial and antioxidant properties. *Mentha rutondifolia* seeds extract for instance, had an IC50 value of is 5.98 µg/mL, which is comparable if not better than synthetic antioxidant. Whatsmore, the corrosion inhibition of these extracts reached an efficiency of ~72 % for the polarization study and ~91% efficiency for EIS measurements at 3g/L. Accordingly, interesting classes of compounds were in parallel identified using ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR- MS). The results helped to improve the knowledge of these species, and provided a solid basis for their development, as well as for the appropriate pharmacological exploitation.

*Keywords: Corrosion inhibition, Urtica dioica, Chenopodium murale, Mentha rotundigolia, Ziziphus lotus.*



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