جامعة فرحات عباس سطيف

UNIVERSITE FERHAT ABBES SETIF

كلية علوم الطبيعة والحياة

FACULTE DES SCEINCES DE LA NATURE ET DE LA VIE

قسم الكيمياء الحيوية

Département de Biochimie

Mémoire

Présenté par

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Pour l'obtention du titre de

MAGISTER en BIOCHIMIE

Option : Biochimie et Physiologie Expérimentale

THEME

Polyphenols and antioxidant properties of extracts from *Mentha pulegium* L. and *Matricaria camomilla* L.

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2011/2012

ACKNOWLEDGMENTS

In the first place I would like to express my gratitude to my supervisor Dr.Seddik Khennouf for his supervision, advice, and guidance in this thesis as well as giving me extraordinary experiences through out the work. Above all and the most needed, he provided me unflinching encouragement and support in various ways. His truly scientist intuition has made him as a constant oasis of ideas and passions in science, which exceptionally inspire and enrich my growth as a student, a researcher and a scientist want to be. I am indebted to him more than he knows.

I gratefully thank Pr. BAGHIANI Abdrrahmane and Pr. MERBAH Meriem and Dr. DAHAMNA Saliha for their constructive comments on this thesis. I am thankful that in the midst of all their activity, they accepted to be members of the reading committee.

I gratefully acknowledge Miss Saliha Djidel for her advice, supervision, and crucial contribution, which made her a backbone of this work. Her involvement with her originality has triggered and nourished my intellectual maturity that I will benefit from, for a long time to come. Saliha, I am grateful in every possible way and hope to keep up our collaboration in the future.

It is a pleasure to pay tribute also to Saliha Dahamna for her help to bring the plant.

Many thanks go in particular to Professor Pr. Laouer Hocine for the identification of plant species.

I would also like to thank my family for the support they provided me through my entire life and in particular, I must acknowledge my husband and my brother for their encouragement and for their help with their particular skill in handling precisely delicate assistants.

Many thanks go to all those who contributed for the successful of this thesis.

DEDICATION

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

This thesis is dedicated to my husband, Toufik, and my lovely daughter, Nada. I give my deepest expression of love and appreciation for the encouragement that you gave and the sacrifices you made during this graduate program. Thank you for the support and company during late nights of typing.

To my sisters Myriem, Assia and Soumia. My brothers Mohammed and Abdnour. To all those who know Dalila.

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Chapter 3: Results and discussion

SUMMARY

This study was carried out to determine the antioxidant activity of plant extracts and its polyphenols compounds. On one hand, the methanolic extract (MeE) of *Mentha pulegium L* showed the higher yield (14,4%) of extraction. Whereas the aqueous extract (AqE) of *Matricaria chamomilla L* had the highest yield (18,56%) of extraction. Moreover, the ethyl acetate extract of *Mentha pulegium L* contains high amount of total polyphenols; tannins and flavonoids (191,99µg gallic acid equivalent/g of extract; 265,33µg tannic acid equivalent/g of extract; 110,37µg quercetin equivalent/g of extract; 151,11µg rutin equivalent/g of extract) respectively. That is why this extract possessed high antioxidant activity (IC_{50}=0,017mg/ml) in DPPH assay while the chloroformic extract (ChE) is better in the β carotene/linoleic acid assay with (61,07%). On the other hand, the ChE of *Matricaria chamomilla L* contains the higher value of flavonoids (197,43µg quercetin equivalent/g of extract; 273,03µg rutin equivalent/g of extract); the total polyphenols are most in MeE (299,14µg gallic acid equivalent/g of extract) and for tannin, the ChE showed (245,11µg tannic Acid equivalent/g of extract). An increase value of ethyl acetate extract with IC_{50}= 0,0111mg/ml in DPPH assay while chloroformic extract shows appreciable inhibiton of 37,15% with values in some way similar to the methanolic extract 37,04% in β carotene/linoleic acid assay. The analysis of these extracts by deferent methods showed a relationship between the compounds values and effect. These results provide useful information about the utilization of these plants as natural antioxidants in food and in folk medicine.

Key words: oxidative stress, antioxidant activity, DPPH, β carotene/linoleic acid, total polyphenols, flavonoids, tannins.

ملخص

تمت هذه الدراسة لتحديد النشاطية المضاد للأكسدة لمستخلصات النباتات ومكوناتها لعديد الفينول. تحليل هذه المستخلصات عن طريق عدة إختبارات، أظهرت علاقة بين قيمة المكونات والتأثير. أظهر مستخلص الميثانولي (MeE) لنبتة *Mentha pulegium L* أعلى مردودية بقيمة (14.4%) في الاستخلاص. في حين يملك المستخلص المائي (AqE) لنبتة *Matricaria camomilla L* أكبر قيمة للمردودية (18.56%) في الاستخلاص. علاوة على ذلك، يحتوي مستخلص إثيل الاسيتات (AcE) على أكبر كمية من الفينولات والدباغ والفلافونويدات (191.99 ميكروغ مكافئ حمض الغاليك /غ مستخلص؛ 265.33 ميكروغ مكافئ حمض Tannic /غ مستخلص؛ 110.37 مكروغ مكافئ Quercetin /غ مستخلص و151.11 ميكروغ مكافئ Rutin /غ مستخلص) على الترتيب، لهذا السبب يمتلك هذا المستخلص نشاط مضاد للأكسدة بنسبة (IC_{50} = 0.017 مغ/مل) في اختبار إزاحة جذر DPPH. في حين أن مستخلص الكلوروفورم (ChE) أفضل في اختبار تبييض البيتاكاروتين بـ (61.07 %). من جهة أخرى، مستخلص ChE لنبتة *Matricaria camomilla L* يحتوي على أكبر قدر من الفلافونويدات (197.43 ميكروغ مكافئ Quercetin /غ مستخلص و273,03 ميكروغ مكافئ Rutin /غ مستخلص)، الفينولات معظمها في مستخلص MeE (299.14 ميكروغ مكافئ حمض الغاليك /غ مستخلص) أما بنسبة للدباغ، فهوChE (245.11 ميكروغ مكافئ حمض Tannic /غ مستخلص). هناك ارتفاع في نسبة مستخلص إثيل الأسيتات بـ IC_{50} = 0.0111 مغ/مل في اختبار إزاحة جذر DPPH في حين أظهر مستخلص الكلوروفورم تثبيط قدره 37.15% مماثل مع قيمة مستخلص الميثانوليك 37.04% في اختبار تبييض البيتاكاروتين. هذه النتائج توفر معلومات قيمة عن استعمال هذه النباتات كمضادات طبيعية للأكسدة في الغذاء والطب الشعبي.

الكلمات المفاتيح: الإجهاد التأكسدي، مضادات الأكسدة، DPPH، تبييض البيتاكاروتين، الفينولات، الفلافونويدات، الدباغ.

RESUME

Cette étude a été réalisée pour déterminer l'activité antioxydante des extraits des plantes et leurs composés phénoliques. L'analyse de ces extraits par différent tests a révélé une relation entre les valeurs des composées et l'effet. D'une part, l'extrait méthanolique (EBr) de la plante *Mentha pulegium L* est le plus élevés de rendement (14,4%) dans l'extraction. Tandis que l'extrait aqueux (EAq) de *Matricaria chamomilla L* a le rendement le plus élevé (18,56%). Par ailleurs, l'extrait d'acétate d'éthyle a haute teneur en polyphénoles totaux, tanins, et des flavonoïdes (191,99 microg equivalent d'acide gallique/g d'extrait ; 265, 33 microg equivalent d'acide tannique/g d'extrait ; 110,37 microg equivalent quercetine/g d'extrait ; 151,11 microg equivalent rutine/ g d'extrait) respectivement. C'est pour cela cet extrait a possèdé une meilleure activité antioxydante (IC_{50}=0017 mg/ ml) dans le test de DPPH alors que l'extrait chloroformique (ECh) révéle une milleure activité dans le test de β carotène/ l'acide linoléique (61,07%). D'autre part, l'ECh de *Matricaria chamomilla L* a possèdé la valeur la plus élevée en flavonoïdes (197,43 microg equivalent quercetine /g d'extrait ; 273,03 microg equivalent rutine / g d'extrait), les polyphénoles sont les plus dans EBr (299,14 microg/ g d'extrait) et pour le tanin, ECh a montré (245,11 microg equivalent d'acide tannique/ g d'extrait). Une augmentation de la valeur d'extrait d'acétate d'éthyle avec 0,0111 mg/ml dans le test DPPH alors que l'extraire chloroformique a montré une inhibiton appréciable de 37,15% avec des valeurs d'une certaine manière similaire à l'extrait méthanolique 37,04% dans les résultats de test β-carotène/ acide linoléique. Ça fournit des informations utiles sur l'utilisation de ces deux plantes comme antioxydants naturaux dans les aliments et la phytothérapie.

Mots clés: stress oxydatif, l'activité antioxydante, DPPH, β-carotène/ acide linoléique, polyphénoles totaux, flavonoïdes, tanins.

ABREVIATIONS

AcE: Ethyl acetate extract.

$AlCl_3$: Aluminium trichloride.

AqE: Aqueous extract.

BHT: Butylated hydroxyToluene.

ChE: Chloroformic extract.

DPPH: 2, 2-diphenyl-1-picryl-hydrazyl.

FAPy: Formamidopyrimidine.

FAPyG: Formamidopyrimidine derivative of guanine.

H_2O_2: Hydrogen peroxide.

HO_2 ·: Perhydroxyl radical.

HOCl: Hypochlorous acid.

HxE: Hexan extract.

I%: Inhibition percentage.

IC_{50}: The concentration of the substrate which causes the loss of 50% of the activity of the DPPH.

MeE: Methanolic extract.

MeOH: Methanol.

MPO: myeloperoxidase.

NO: The Nitric oxide.

$O_2^{\cdot -}$: Superoxide anion radical.

ROS: Reactive oxygen species.

SOD: Superoxide dismutase.

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INTRODUCTION

INTRODUCTION

Reactive oxygen species (ROS) and their likely involvement in some human physiopathologies have attracted growing interest from the health sector over the last few decades. Recently, the interest increased in occurring naturally antioxidant which can be used to protect the human beings against the damage from the oxidative stress (Scalbert *et al*., 2005). Many plants contain natural antioxidants which act as metabolic response to the endogenous production of free radicals and other oxidizing species (Grassmann *et al*., 2002). These antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids, alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids. For example, the aromatic herbs and spices were used for a long time in the Mediterranean kitchen, not only to improve or modify the taste of food, but also to avoid its deterioration (Proenca *et al*., 2003).

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental techniques to identify natural antioxidants from plants. Several authors have reviewed the beneficial uses of these plant species (Speroni and Scartezzini, 2000; Matkowski., 2008).

In this work, we studied the antioxidant and free radicals scavenging effects of plant extracts from *Mentha pulegium* L. and *Matricaria chamomilla* L., which are largely used in the Algerian folk medicine such as encouraging menstruation, in the treatement of painful related to convulsions like the diarrhoeas. The contents of total polyphenols, flavonoids and tannins in these extracts were also determined**.**

Chapter 1: REVIEW OF THE LITERATURE

I. Oxidative stress:

I.1. Oxidative stress:

During the production of reactive species of oxygen (ROS) in the human beings, by endogenous or external sources, for example tobacco smoke, certain pollutants, organic solvents or pesticides, involves an oxydative stress (Gulcin *et al*., 2003).

I.1.1. Definition of stress:

An imbalance between free radical generation and sequestration leads to oxidative stress. ROS generation through normal cellular metabolism and by exogenous stimulus is a constant problems for example which associated with many multifactorial diseases, especially cancers (Kawanishi *et al*., 2002), cardiovascular diseases (Sachidanandame *et al*., 2005) and inflammatory disorders (Bodamyali *et al*., 2000) for which cells have developed multiple defense mechanisms to survive (Ha *et al*.,1998 ; Halliwell,1999).

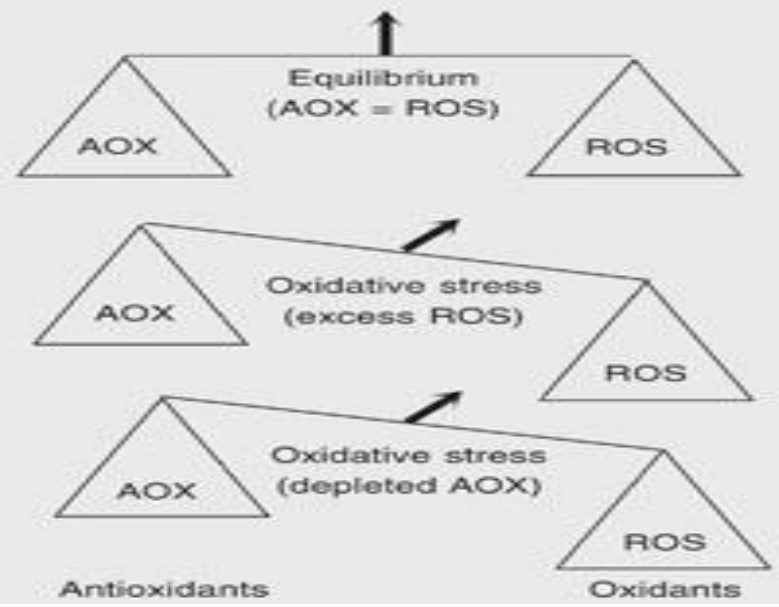


Fig 1. The balance between the oxidant and antioxidant systems (Scandalios, 2005).

I.2. Reactive oxygen species:

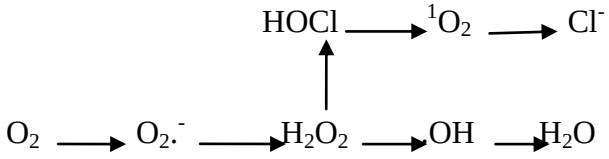
I.2.1. Definition:

A free radical is any species capable of independent existence containing one or more unpaired electrons (Halliwell and Gutteridge, 1984). The unpaired electron alters the chemical reactivity of the molecule/atom, making it more reactive than the corresponding non-radical form. The oxygen free radicals include superoxide anion radical ($O_{2}^{\cdot -}$), singlet oxygen ($^1O_2$), hydroxyl radical (·OH), the nitric oxide (NO), and perhydroxyl radical (HO2 ·) are termed collectively the 'reactive oxygen species' (ROS). The usual route of O_2 metabolism is through its complete reduction to H_2O by accepting four electrons. However, with a single electron reduction several free radicals and hydrogen peroxide (H_2O_2) are formed (Grisham, 1992; Nappi and Vass, 1998).

I.3. Source of free radicals:

In vivo, ROS are generated by oxidant enzymes, phagocytic cells, ionizing radiation, etc. Superoxide anion is believed to be the first radical formed, mainly by the electron transport chain when O_2 picks up a single electron. Radicals such as ·OH, HO2· and H_2O_2 are formed from $O_2^{\cdot -}$ (Grisham, 1992 ; Nappi and Vass, 1998). $O_2^{\cdot -}$ undergoes a dismutation reaction catalysed by the enzyme superoxide dismutase (SOD) to form H_2O_2, which by itself is not reactive enough to cause damage to macromolecules. It is, however, a very important oxidant since it can cross biological membranes and form the highly reactive ·OH by interaction with transition metal ions such as Fe^{2+}or Cu^{+}. H_2O_2 is reduced by three general mechanisms. First, it is a substrate for two enzymes, catalase and glutathione peroxidase, that catalyse its conversion to H_2O and O_2 (Maddipati and Marnett, 1987), a detoxification mechanism. Secondly, H_2O_2 is converted by myeloperoxidase (MPO) in neutrophils to hypochlorous acid (HOCl), a strong oxidant that acts as a bactericidal agent in phagocytic cells. Reaction of

HOCl with H_2O_2 yields $^1O_2$. Thirdly, H_2O_2 is converted in a spontaneous reaction catalysed by transition metal ions to the highly reactive ·OH.



Among the ROS, ·OH is the most potent damaging radical which can react with all biological macromolecules (lipids, proteins, nucleic acids and carbohydrates). It is extremely reactive and can lead to formation of DNA-protein cross-links, single- and double-strand breaks, base damage, lipid peroxidation and protein fragmentation (Lloyd *et al.*, 1997; Stohs and Bagchi, 1995). It may also be generated by ionizing radiation (Ward, 1987):

$H_2O \longrightarrow H_2O\cdot + e^-$

$H_2O + H_2O\cdot \longrightarrow H_3O^+ + \cdot OH$

The cellular generation of ·OH may occur in two steps (Mates *et al.*, 2000):

(i) Reduction of H_2O_2 by the Fenton reaction:

$Fe^{2+} + H_2O_2 \longrightarrow \cdot OH + OH^- + {}^-Fe^{3+}$

$Cu^+ + H_2O_2 \longrightarrow Cu^{2+} + \cdot OH + OH^-$

(ii) Interaction of $O_2^{\cdot -}$ with H_2O_2 by the Haber–Weiss reaction:

$O2^{\cdot -} + H_2O_2 \longrightarrow O_2 + H_2O + \cdot OH$

I.4. The effect of free radicals:

I.4.1. Oxidative damage to lipids:

Among the more susceptible targets of ·OH are polyunsaturated fatty acids. Abstraction of a hydrogen atom from a molecule of polyunsaturated fatty acid initiates the process of lipid peroxidation (Arouma, 1993). The peroxidation reactions differ among these fatty acids depending on the number and position of the double bonds on the acyl chain and the reader is referred to Frankel (1985). A hydrogen atom is abstracted from a second molecule, leading to a new free radical. Aldehydes of lipid peroxidation can react with sulphydryl (cysteine) or basic amino acids (histidine, lysine) affecting their biological characteristics (Arouma, 1993). The peroxidation of lipids involves three distinct steps: initiation, propagation and termination (Bradley and Minn, 1992).

I.4.2. Oxidative damage to proteins:

Oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis. The amino acids in a peptide differ in their susceptibility to attack, and the various forms of activated oxygen differ in their potential reactivity. Primary, secondary, and tertiary protein structures alter the relative susceptibility of certain amino acids. In spite of this complexity, generalisations can be made. Sulphur containing amino acids, and thiol groups specifically, are very susceptible sites. Activated oxygen can abstract an H atom from cysteine residues to form a thiyl radical that will cross-link to a second thiyl radical to form disulphide bridges. Alternatively, oxygen can add to a methionine residue to form methionine sulphoxide derivatives. Reduction of both of these may be accomplished in microbial systems by thioredoxin and thioredoxin reductase (Farr and Kogama, 1991).

Other forms of free radical attack on proteins are not reversible. For example, the oxidation of iron-sulphur centres by superoxide destroys enzymatic function (Gardner and Fridovich, 1991). Many amino acids undergo specific irreversible modifications when a protein is oxidised. For example, tryptophan is readily cross-linked to form bityrosine products (Davies, 1987). Histidine, lysine, proline, arginine, and serine form carbonyl groups on oxidation (Stadtman, 1986). The oxidative degradation of protein is enhanced in the presence of metal cofactors that are capable of redox cycling, such as Fe. In these cases, the metal binds to a divalent cation binding site on the protein. The metal then reacts with hydrogen peroxide in a Fenton reaction to form a hydroxyl radical that rapidly oxidises an amino acid residue at or near the cation binding site of the protein (Stadtman, 1986).

I.4.3. Oxidative damage to DNA:

Similarly, modification of individual nucleotide bases, single strand breaks and cross-linking are the typical effects of ROS on nucleic acids (Arouma, 1993). The damage to DNA by ·OH includes single-strand breaks, base modifications and conformational changes. Nitrogenous bases react preferentially with ·OH rather than sugar moiety by 4–6-fold. Thymine and guanine are most susceptible to modifications followed by cytosine and adenine. Thymine glycol is the major oxidation product, its presence in urine serves as an indicator of endogenous DNA damage. Cytosine glycols are also formed which can undergo deamination to form uracil derivatives that base pair preferentially with adenine, instead of guanine. Reduction of guanine leads to ring opening forming formamidopyrimidine (FAPy) derivative of guanine (FAPyG). Oxidation leads to the formation of 8-oxo-deoxyguanine (8-oxodG), a major product. Its measurement in urine is used as a biomarker of endogenous oxidative DNA damage (Linn, 1998).

I.4.4.Oxidative damage to inflammatory system:

ROS are generated by mitochondria through the electron transport chain as toxic by products of oxidative phosphorylation (Melov *et al.,* 1998). In addition, free radical production and disturbances in redox status can modulate the expression of a variety of immune and inflammatory molecules (Sundaresan *et al.,*1995 ; Kaouass *et al.,*1997 ; Kagaya *et al.,*1992) leading to inflammatory processes, exacerbating inflammation and affecting tissue damage (Tsai *et al.,*1998). It has been suggested that abnormal immunity is related to oxidative imbalance (Chen *et al.,* 1997; Galan *et al.,* 1997) and antioxidant functions are linked to anti-inflammatory and/or immunosuppressive properties (DeWaart *et al.,* 1997; Chen *et al.,* 1998 ; Shankar and Prasad, 1998). Neutrophils, which constitute about 60% of the circulating leucocytes and are the most abundant cellular components of the immune system, produce ROS resulting in oxidative damage and inflammation. The phagocytosis of bacteria, secretion of proteolytic enzymes and immunomodulatory agents are accompanied by 'respiratory burst', involving a sudden increase in oxidative metabolism that results in the production of ROS (PithonCuri *et al.,* 1998).

I.5. Anti-oxidant defense system:

Under stress, our bodies produce more reactive oxygen species (ROS) e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants, especially superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase and thioredoxin systems, which are recognized as being highly efficient in ROS detoxification. The main non-enzymatic antioxidants present in the human organism are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathion, carotenoids, and flavonoids, bilirubin, estrogenic sex hormones, uric acid, coenzyme Q, melanin, melatonin, and lipoic acid. This imbalance leads to cell damage (Aruoma, 1998; Lefer and Granger, 2000; Smith *et al*., 2000; Bhatia *et al*., 2003; Peuchant *et al*., 2004).

I.6. Polyphenolic compounds:

Many medicinal plants contain various bioactive compounds, such as polyphenolic compounds, which are secondary metabolites. From a chemical point of view, polyphenols can react with one-electron oxidants, which prevents free radical formation in biological systems (Huang *et al*., 1992). This class includes phenolic acids, flavonoids and tannins (Bruneton, 1993).

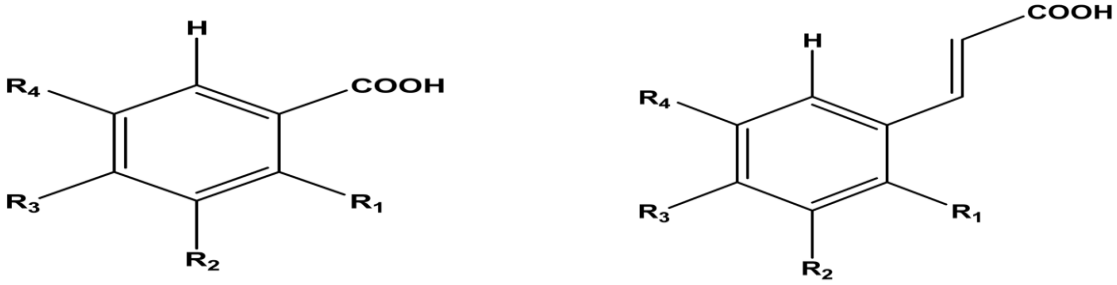
I.6.1. Phenolic acids:

I.6.1.1. Definition:

Phenolic acids are aromatic secondary plant metabolites widely distributed throughout the plant kingdom (Hemmann, 1989). The term "phenolic acids", in general, designates phenols that possess one carboxylic acid functionality. However, when talking about plant metabolites, it refers to a distinct group of organic acids (Shahidi and Wanasundara, 1992; Robbins, 2003).

I.6.1.2. Classification :

These naturally occurring phenolic acids contain two distinctive carbon frameworks: the hydroxycinnamic and hydroxybenzoic structures (Figure a1). Although the basic skeleton remains the same, the numbers and positions of the hydroxyl groups on the aromatic ring make the difference and establish the variety (Table 1). Caffeic, p-coumaric, vanillic, ferulic, and protocatechuic are acids present in nearly all plants (Shahidi and Wanasundara, 1992 ; Robbins, 2003). Other acids are found in selected natural sources (e. g., gentisic, syringic). Pre-eminent amongst cinnamic acids, as far as natural occurrence is concerned, is chlorogenic acid (5-O-caffeoylquinic acid) which is caffeic acid esterified with quinic acid (Harborne, 1986).



Hydroxybenzoic Acids Hydroxycinnamic Acids

Fig a1. The structure of phenolic acids(Harborne, 1986).

Table1. Structures of the prominent naturally phenolic acids (Harborne, 1986).

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Name	**R1**	**R2**	**R3**	**R4**
Bensoic acid	H	H	H	H
p-Hydroxybenzoic acid	H	H	OH	H
Vanillic acid	H	OCH3	OH	H
Gallic acid	H	OH	OH	OH
Protocatechuic acid	H	OH	OH	H
Syringic acid	H	OCH3	OH	OCH3
Gentisic acid	OH	H	H	OH
Veratric acid	H	OCH3	OCH3	H
Salicylic acid	OH	H	H	H

Name	R1	R2	R3	R4
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
Ferulic acid	H	OCH3	OH	H
Sinapic acid	H	OCH3	OH	OCH3
Caffeic acid	H	OH	OH	H

I.6.2. Flavonoids:

I.6.2.1. Definition:

Flavonoids are planar molecules ubiquitous in plants, formed from the aromatic amino acids phenylalanine, tyrosine, and malonate (Harborne, 1986). Flavonoids as flower pigments consist of two aromatic rings (A and B) and a heterocycle (C) with oxygen. Based on the configuration and state of oxidation of the central C3 unit in the molecule, flavonoids are divided into eight groups. The first to suggest this flavonoid structure was Robinson (1936). This hypothesis was further confirmed by the formation and biosynthesis of quercetin in tartary buckwheat (Underhill *et al.,* 1957*;* Watkin *et al.,* 1960).

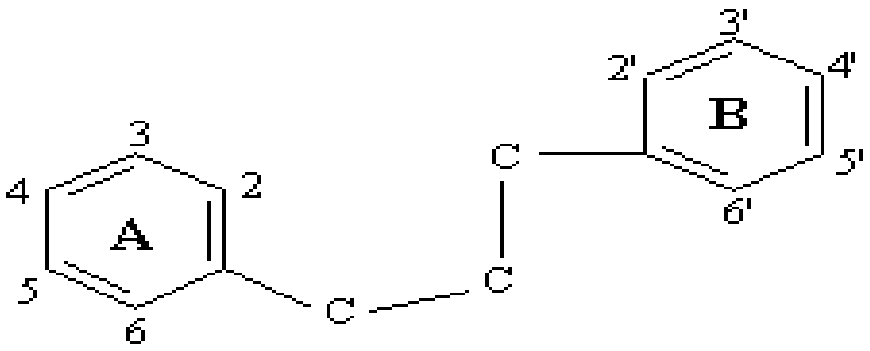


Fig a2. Basic skeleton stucture of flavonoids (Robinson, 1936).

Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid aglycone consists of a benzene ring (A) condensed with a sixmembered ring (C), which in the 2-position carries a phenyl ring (B) as a substituent. The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position) (Harsteen, 1983). Flavonoids are often hydroxylated in position 3, 5, 7, 2', 3', 4', 5'. Methylethers and acetylesters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucor-hamnose, galactose or arabinose (Middleton, 1984)

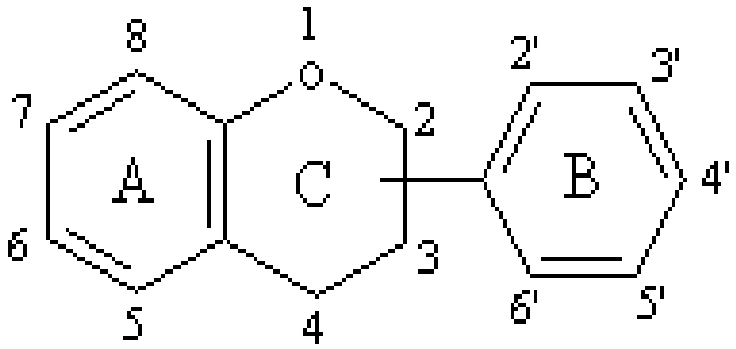


Fig a3. Chemical structure of flavonoids (Hasteen, 1983).

I.6.2.2. Classification:

Over 5000 naturally occurring flavonoids have been characterized from various plants. They have been classified according to their chemical structure (Ververidis *et al*., 2007). According to the oxidation condition of the pyran ring placed at the center of flavonoids, flavonoids can be further subdivided into five major subclass as follows: flavonols, flavanols, flavones, isoflavones, anthocyanidins (Moon *et al*., 2006).

1. Flavones:

Flavone belongs to the flavonoids, which are found from various plant sources and composed of C6-C3-C9 skeleton (Moon *et al*., 2006).

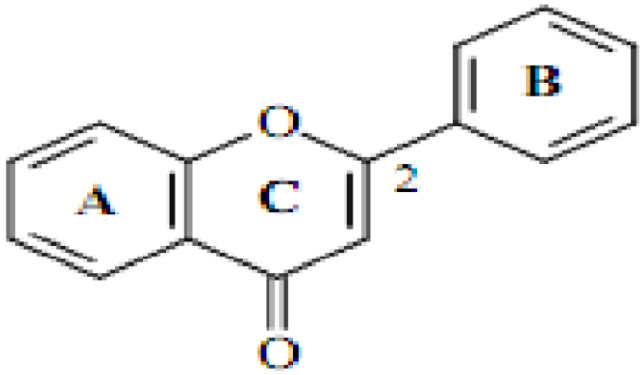


Fig a4. Structure of flavone (Dewick, 1994).

2. Flavonol or or 3-hydroxyflavone : **Flavan-3-ols (also known as flavanols), Flavan-4-ols, Flavan-3,4-diols and Proanthocyanidins:**

Flavonols differ from flavonones by hydroxyl group the 3-position and a C**2**-C**3** double bonds (Harsteen, 1983). Flavan-*3-ols* use the 2-phenyl-3,4-dihydro-2*H*-chromen-*3-ol* skeleton. While Proanthocyanidins are dimers, trimers, oligomers, or polymers of the flavanols.

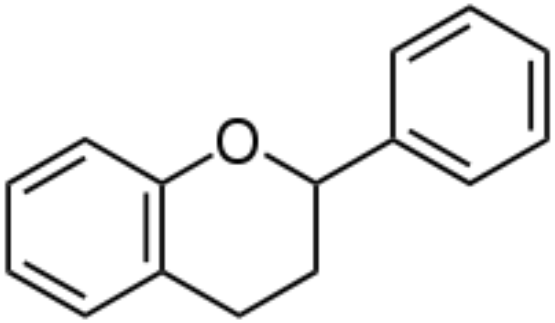
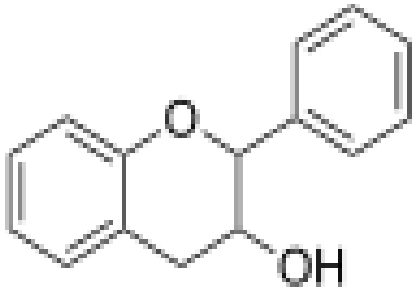
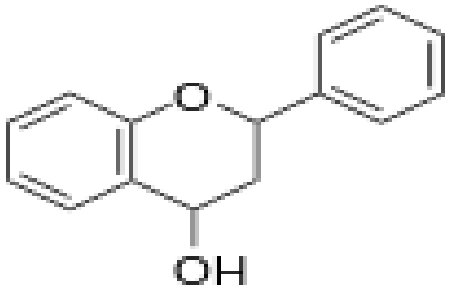


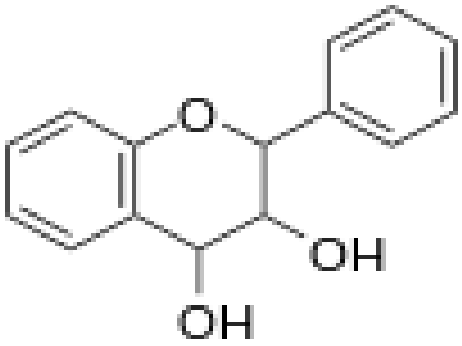
Fig a5. Flavan structure (Ververidis *et al*., 2007)



Flavan-3-ol



Flavan-4-ol



Flavan-3,4-diol

Fig a6. Derivatives of flavan (Ververidis *et al*., 2007).

3. Flavanonol or 3-Hydroxyflavanone or 2,3-dihydroflavonol:

Among the dietary flavonoids, high levels of flavanols are found in numerous common food stuffs such as grapes, red wine, apples, both green and black teas, and cocoa and cocoa-containing products (Gu *et al*., 2004). They are particularly abundant in cocoa where the number and arrangement of flavanols is distinct, containing both the simple monomeric flavanols (primarily (–)-epicatechin and to a much lesser extent, (+)-catechin) as well as the structurally related dimeric and oligomeric flavanols known as procyanidins (Lazarus *et al*., 1999).

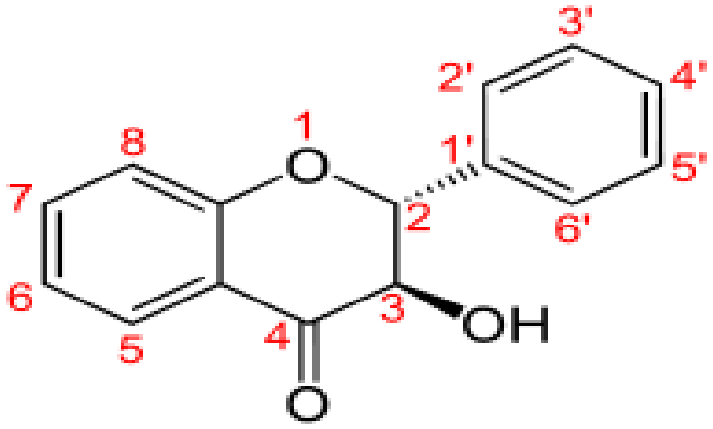


Fig a7. Structure of flavanonol (Ververidis *et al*., 2007).

4. Isoflavones:

Isoflavonoids are a subclass of flavonoids, a large group of diphenolic plant compounds with a phenylchroman skeleton of 15 carbons. The three rings in isoflavonoids (and flavonoids) are labeled A, B, and C, and the numbering starts from oxygen of the heterocyclic ring C. Isoflavonoids differ from flavonoids by position of ring B, which is at C2 in flavonoids and at C3 in isoflavonoids. To aid systematic classification, isoflavonoids have been further divided, according to the oxidation level of the 3phenylchroman skeleton (ring C), into subgroups of isoflavones, isoflavanones, isoflavans, and amethyldeoxybenzoins. Isoflavones constitute the

largest group of natural isoflavonoids, with some 360 known aglycones reported by the year 1994 (Dewick, 1994).

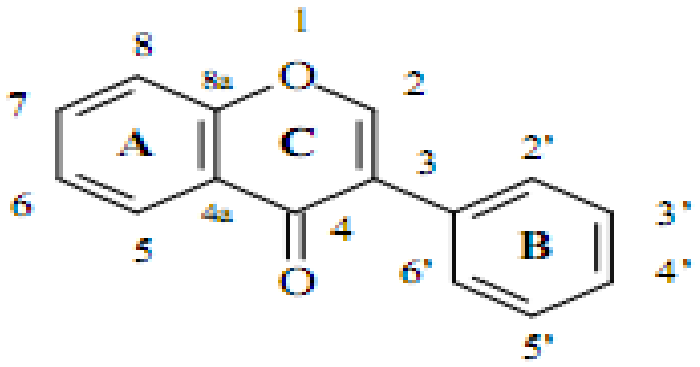


Fig a8. Basic structures of isoflavonoids (Dewick, 1994).

5. Anthocyanidins:

Anthocyanidins are the aglycones of anthocyanins. Anthocyanidins use the flavylium (2-phenylchromenylium) ion skeleton. The most important flavonoid classes with regard to flower colour are anthocyanins, flavonols and flavones, and, in addition, the chalcones and aurones which are biosynthetically closely related to the flavonoids (Forkmann, 1991).

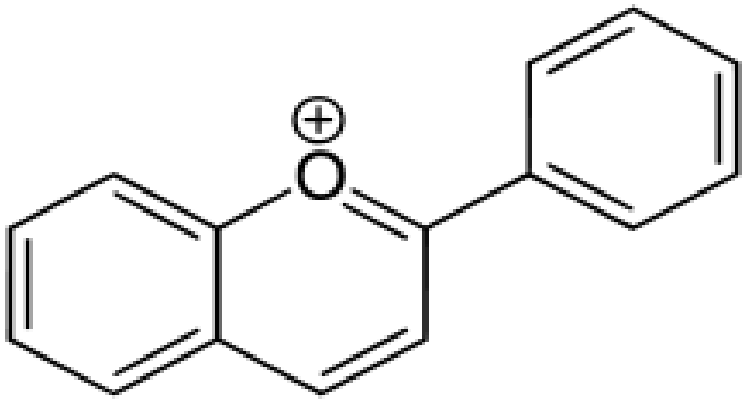


Fig a9. Flavylium skeleton of anthocyanidins (Ververidis *et al*., 2007).

There is other class of flavonoids :

6. Chalcones and Aurones :

Nowadays they are regarded as a biochemically-related but separate group because their chemical structure cannot be derived from the typical flavan structure. Hence, ring numbering in chalcones and flavonoids is divergent and position 3 of chalcones corresponds to position 3' of flavonoids and aurones (Bohm, 1994).

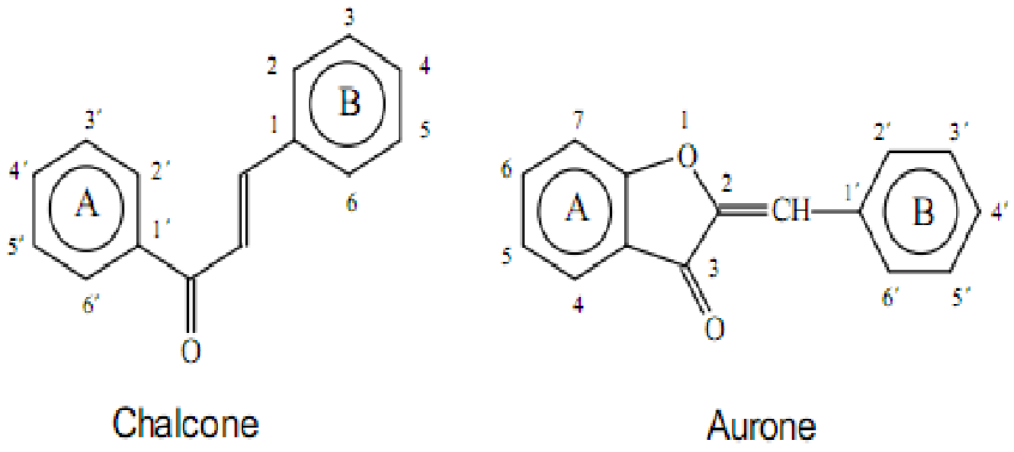


Fig a10. The basic structures of chalcone, aurone (Bohm, 1994).

7. Flavanone:

Six-member ring condensed with the benzene ring is either a α-pyrone (flavonols and flavonones) or its dihydroderivative (flavanols and flavanones) (Harsteen, 1983).

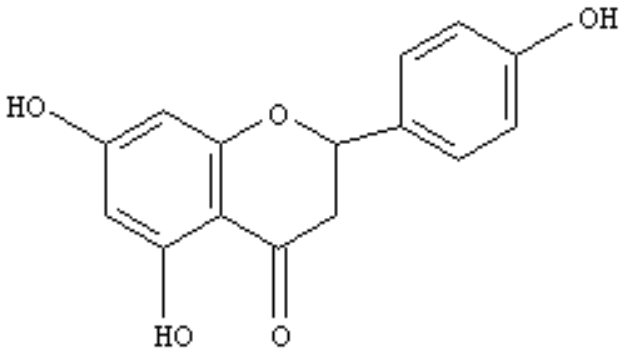


Fig a11. Structure of flavanone (Harsteen, 1983).

I.6.3. Tannins:

I.6.3.1. Definition :

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances.

I.6.3.2. Classification:

1. Hydrolysable tannins:

These are based on esters of phenol carboxylic acids (gallic acid) with a central carbohydrate core for example :

- gallotannins (gallic acid, quinic acid, tannic acid)
- ellagitannins (ellagic acid, castalagin, vescalagin, etc.)
- hydrolysable tannin oligomers (agrimoniin, rugosin D)
- caffeic acid derivatives (chlorogenic acid, caffeetannin, dicaffeoylquinic acid, rosmarinic acid).

2. Condensed tannins:

Structurally related to flavonoids, these tannins are distributed widely in nature and constitute a heterogeneous group. The Cl l5 skeleton of the flavonoids is made up of two distinct units, A ring (consisting of a CEl unit) and B ring (made up of CEl-CS unit). Condensed tannins are chemically oligomers of hydroxyflavan-3-o1 (catechin, epicatechin) and polyhydroxyflavan-3,4-diols (leucoanthocyanidin) or oligomers of a combination of those two compounds. The basic flavonoid structure in condensed tannins is flavan (Santappa *et al.,* 1982*)* ; for example :

- flavan-3-ol (catechin, epicatechin)
- flavan-3,4-diol (leucoanthocyanidin)

3. Complex tannins:

The complex tannins are a series of compounds and found to occur widely in plants containing both hydrolysable and non-hydrolysable or condensed tannins. Complex tannins are shown to contain a hydrolysable tannin moiety in their molecules connected through a carbon-carbon linkage to flavan-3-ol (flavano-ellagitannin), procyanidin (procyanidino-ellagitannin) and flavonoid glucoside (flavono-ellagitannin) moieties ; for example : (stenophyllanin A, acutissimin B, mongolicain A, stenophynin A, etc.)

II. Herbal therapy:

Phytomedicine, also called herbal therapy is an important branch of complementary and alternative medicine and is in fact a traditional therapeutic system which takes advantage of herbal plants to prevent and cure maladies and improve general health (Givens *et al*., 2006). Plants are important source of active natural products which differ widely in terms of structure and biological properties. Many herbs have been used for a long time for claimed health benefits. They are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. However, some can cause health problems, some are not effective and some may interact with other drugs you are taking.

II.1. *Mentha pulegium* L.:

II.1.1. Monograph of *Mentha pulegium* L.:

Scientific name: *Mentha pulegium* L. (*Lamiaceae*)

French vernacular names: Pennyroyal, Pouliot.

Vernacular name: Feliou, Afilgou, Felgou, Moursal, Tamarsa.

Fig 2. **Photograph of *Mentha pulegium* L. (www.google.com).**

II.1.2. Systematic position:

Reign:	***Plantae***
Family:	***Lamiaceae***
Kind:	***Mentha***
Species:	***Mentha pulegium* L.**

II.1.3. Description:

It is a hardy perennial by its rhizomes, low, from 10 to 55 cm in height, frequent in the wet mediums, which expire a strong aromatic odor. The stems divided into leaf, quadrangular, spread out or lying very easily emit adventitious roots with the lower face of the nodes. The floriferous stems are more or less raiser. The sheets, opposite, small, oval almost whole (slightly notched) and are provided with a short petiole. The flowers, which appear the summer, from July at the end of September, are pink lilac, sometimes white, and are grouped

with the armpit of the sheets in clusters (false verticils) spread out along the stem. The fruits are akenes.

II.1.4. Botanical description:

The leaves of Pennyroyal are generally small, ovate, slightly serrate, slightly hairy, and opposite. For the record, the leaf of the non-creeping pennyroyal can be up to 3cm or 1.5 in long and may be entire rather than slightly toothed. The color depends on the variety and whether wild or cultivar. The small flowers are produced in distinctive, dense whorls (similar to corn or fieldmint and gingermint in bloom). The tight, axillary clusters appear in July-August with colors ranging from reddish -purple to lilac. There are few flowering stems on the prostate form; they lie on top of what appears to be "a dense green turf". Seed is light brown, very small and oval.

II.1.5. Origin and distribution:

Pennyroyal (*Mentha pulegium*) is an aromatic Perennial and is common wild or garden plant. It is a spontaneous species in the whole of Europe, found in wet grounds around the Med and the west of Asia (from Chypre to Turkmenistan) and the north of Africa (from Morocco to Egypt). In France, this plant is very common up to 1800 m of altitude.

Mentha pulegium L among the vegetables recommended in capitulary De Villis with the Middle Ages.

II.1.6. Traditional medicinal uses:

Iranian peaple usually uses the plant *Mentha pulegium* L. against the infectious diseases and finds to be effective against these problems without any scientific base to explain this action. The increase in resistance to antibiotics of the pathogenic agents associated the infectious diseases as well as undesirable side effects of antibiotics suggested the use of oil of *Mentha*

pulegium L. like antibiotic or of replacement. An Additional uses of this plant is well regarded as an insect repellent, for both humans and pets. However, additional research is necessary to evaluate the practical values of therapeutic application (www.google.com).

II.1.7. Medicinal uses:

A good digestive tonic, it stimulates digestive juices, relieves flatulence and colic; a good remedy for headaches and for minor respiratory infections helping to keep fever and congestion in check; a powerful stimulant to the uterine muscle encouraging menstruation; externally it can be used to relieve chine and rheumatic conditions including gout (www.google.com).

II.1.8. Chemical constituents:

Ingredients of *Mentha pulegium* L. were subjected to a certain number of studies which showed a difference of its commissar according to the area of culture and it have some variations in the components of various countries. El-Ghorab (2006) was noted that the oil of *Mentha pulegium L*. coming from Egypt contains pulegone (43,5%), piperitone (12,2%); from Tunisia (Mkaddem *et al,* 2007), pulegone(8%), isomenthone (11,3%). These studies showed three chimiotypes of *Mentha pulegium L* with the following major components oils (1) pulegone, (2) piperitenone and/or piperitone and (3) isomenthone/neoisomenthol (Topalov and Dimitrov, 1969; Cook *et* a*l,* 2007). Although the air part of flowering of this plant is usually used because of its disinfectants and pharmaceutical properties.

II.2. Camomille romaine:

II.2.1. Monograph of Matricaria chamomilla L.:

Scientific name: Chamaemelum nobile L.

French vernacular names: Camomile roman, Camomile noble, Anthemis noble, Anthemis odorous, Camomile of Anjou.

Algerian name vernacular: Habak, Babounj.

II.2.2. Systematic position:

Reign:	***Plantae***
Family:	***Asteraceae***
Kind:	***Chamaemelum***
Species:	**Matricaria chamomilla L.**

Fig 3. Photograph of *Matricaria chamomilla* L. (Nemtanu *et al*., 2008).

II.2.3. Description:

According to Julve 1998, Roman camomile (*Chamaemelum nobile* L.) is a long-lived herbaceous plant of the family of Asteracees. One finds it in the dry and sandy grounds rich in silica until 1.000m of altitude. It is long-lived herbaceous plant of 10 with 30cm top. Its hairy stems are initially lying to be rectified thereafter. They end in flowerheads floral odorous, solitary. Of green color whitish, its sheets are finely divided into short and narrow lobes. The fruits are yellowish, small and corded akenes (Julve, 1998).

II.2.4. Botanical description:

The camomile has a soft, grassy aroma and slightly fruit-loft. Its flowers resemble the daisies, with yellow centers (roughly 1-1.5 cm diameter) formed of tubulous flowers, and petals white (between 12 and 20 in a number). It is among the plants whose liquid infusions, extracts and essential oils are made of the heads of the fresh or dried flowers. Two species of the camomile are generally used in traditional herb trade, *Matricaria chamomilla* (*Chamomilla recutita;* German camomile) and *Chamaemelum nobile* (Roman camomile). The two species belong to the family of Asteracees/Composed, and are similar in physical appearance, the chemical properties as well as the general applications. However, the German camomile (*Matricaria chamomilla*) most familiar and is most commonly used.

II.2.5. Origin and distribution:

The current origin of *Matricaria camomilla* L. is in the meadows of the East and the South of Europe, in the West of Siberia, Med Asia, the mountains of the Caucasus, in Iran, Afghanistan and India. After its introduction, it became common in North America, South America, New Zealand and Australia (Schultze-Motel, 1986).

II.2.6. The traditional use of *Matricaria camomilla* L.:

The camomile is known during centuries and is well established in therapy. In traditional popular medicine, it is used in the form of camomile tea, is drunk internally in the event of gastric and intestinal diseases painful related to convulsions like the diarrhoeas and the distensions, as it is used for the inflammatory,gastric and intestinal diseases (Franke and Schilcher, 2005). In external use, the camomile is applied in the form of compress heats against the badly healed wounds, like a bath for the abscesses, the female furoncles, hemorrhoids and genital diseases, as rinsing of the mouth reached of ignitions of the oral cavity and the pharynx, like vapor inhaled for the treatment of the acne, the nasal flow and bronchitides and like baths of babies in order to soften the skin. In the countries of Rome, the use of the camomile tea was spread out at the restaurants and in the bars, and finally, it is used even in the form of concentrated coffee or espresso. This last use constitutes a good way of fight against the upheavals of the stomach following a sumptuous meal, full of alcohol or with nicotine (Franke and Schilcher, 2005). The camomile is an annual plant largely recognized in the culture of the West. Its medical use shows again antiquity or notable Hippocrates, Galen, and Asclepius referred of it. It is most commonly used in the form of herb tea due to its calming, carminative and spasmolytic properties, as it is used for health topics and in the beauty products due for its lenitive purposes and anti-inflammatory drugs on the skin.

II.2.7. Medicinal properties:

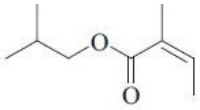
At the Average Age, one also knew his properties analgesics, tonics and stimulative, febrifuge, and stomatic. Current natural medicine also uses it as anti-inflammatory drug, disinfectants, and like emmenagogue (Bardin, 2004). Its flower is used out of herb tea, only or in mixture, and the herbalists allot to him with moderate amount an effectiveness against insomnia.

Currently, the camomile is used in a general way to treat all the disorders where the spasm occupies a significant place. In particular, in the case of functional digestive disorders: difficult digestions (painful digestive spasms) or of dysmenorrhoea. According to Julve (1998) one prepares compress and drops with 20 gr. of flowers for one liter of ebullient water, to look after the conjunctivites and the ignitions of the eyelids. Furoncles, whitlow and the suppuration of the wounds and to relieve certain aches (Bardin, 2004). In beauty care it is always present in lotions, creams, shampoos (Julve, 1998).

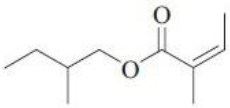
II.2.8. Chemical constituents:

More than 120 components were identified in the flowers of the camomile (Pino, 2002). The flowers of the German camomile contain 0,24 to 2% of volatile oil of blue color.The majority of the secondary components of *Mr. chamo-milla* belong to three various chemical classes: sesquiterpenes, coumarins and flavonoides (McKay *et al*., 2006). The two major components of oil essence are sesquiterpenes (-)-αbisabolole andα-farnesene of which the percentage is 0.4%. The polyphenoles constitute also most of this plant, represented by coumarins and the flavonoides. Coumarins: herniarine, umbelliferone, and the esculetine cover 0,1% of the total components. The major flavonoides are the apigenine, the luteoline and quercetin accounting for 16.8, 1.9 and 9.9% respectively of total flavonoides (Kato *et al*., 2008). These coumarins and flavonoides are hot water soluble, and their quantities obtained by frequent herb tea consumption are not negligible (Kato *et al*., 2008). Other components of the oil of the camomile include: (-)-alpha-bisabolole oxide A and B, (-)-alpha-bisabolone oxide A, the spiroetheres (cis- and trans in-yn-dicycloethere), cadinene, furfurale, spathulenole, and proazulene (matricarine and matricine). The chamazulene is also one of the major components of the plant and is formed of the matricine during the distillation of oil. Thus, the output depends on the origin and the age of the flowers. The camomile contains also more than 8% of flavone glycosides (apigénine 7-glycoside and its 6' acetyl derivative) and of

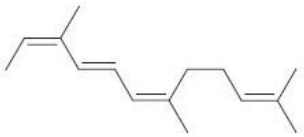
favonoles (luteoline glucosides, quercetin glycosides, and isohame-tine); more than 10% mucilage of polysaccharides and more than 0.3% of choline. Finally, tannins constitute only half of 1% of the components of the camomile. The structures of the most significant components of *Mr. chamomilla* are represented in the following figure.



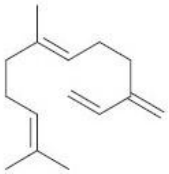
1. Isobutyl angelate



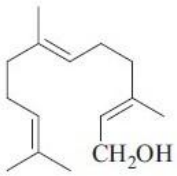
2. 2-Methylbutyl angelate



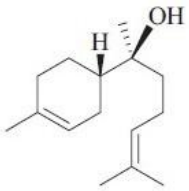
3. Farnesene



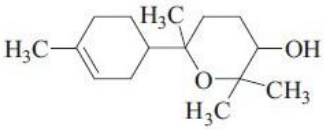
4. β-Farnesene



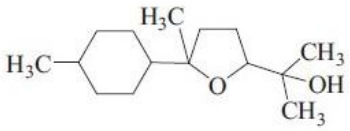
5. Farnesol



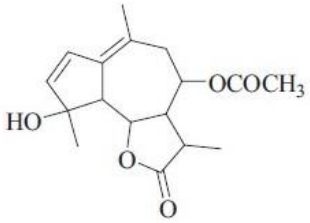
6. (-)-α-bisabolol



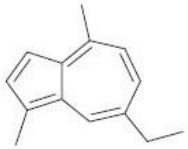
7. Bisabolol oxide A



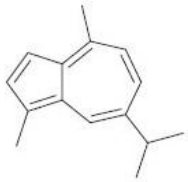
8. Bisabolol oxide B



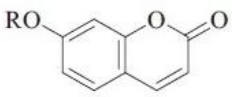
9. Matricin



10. Chamazulene

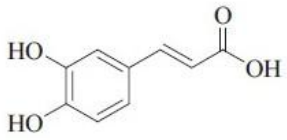


11. Guaiazulene

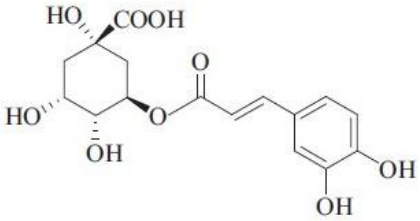


12. Umbelliferone (R=H)

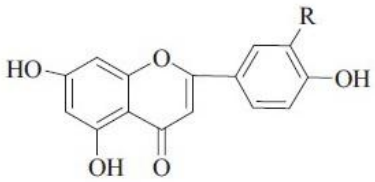
13. Herniarin (R=CH_3)



14. Caffeic acid

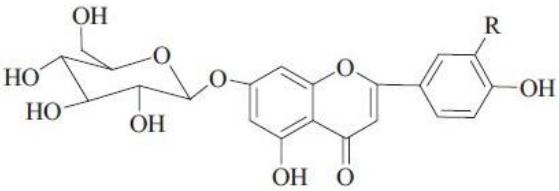


15. Chlorogenic acid



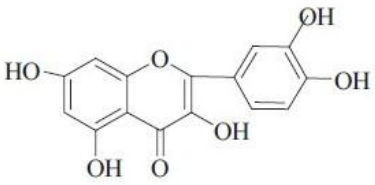
16. Apigenin (R=H)

17. Luteolin (R=OH)

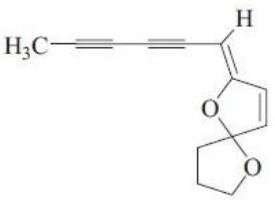


18. Apigenin-7-O-glucoside (R=H)

19. Luteolin-7-O-glucoside (R=OH)



20. Quercetin



21. Z-Enyne dicycloether

Fig 4. The structures of the major components of *Matricaria chamomilla* L. (Gupta *et al*., 2010).

Chapter 2: MATERIALS AND METHODS

I. MATERIAL:

I.1. Plant material:

Mentha pulegium L. leaves were collected in September,2011 from the capital of Algeria and the flowers of *Matricaria chamomilla* L. were collected in the end of May and the beguining of June from Res-El-Oued. BBA. The two plants were identified by professor Pr. Laouer Hocine from the Faculty of Sciences. Department of Ecology. University Ferhat Abbass, Setif, Algeria. The leaves and the flowers were separated from the other parts and dried at room temperature.

The plant samples were air dried in shadow and finely powdered in a rotating knife grinder. The powder was sieved through a 1mm mesh to remove large fragments. Each plant powder was then used for the extraction procedure.

I.2. Chemicals:

Methanol (MeOH) , Hexan , Ethyl acetate , Chloroform , Tannic acid, aluminium trichloride (AlCl3) , the various polyphenols (Gallic acid, Quercetin and Rutin) ; Folin ; 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Butylated hydroxytoluene (BHT) , Tween 40 , β carotene , Linoleic acid and Carbonate.

The various products used were purchased from Aldrich and Sigma.

II. METHODS:

II.1. Choice of solvent:

The extraction of polyphenols from *Mentha pulegium* L. leaves and *Matricaria chamomilla* L. flowers were performed by methanol-distilled water. The extraction yields was calculated for each sample extracted with solvent system as the percentage of weight of resulting powder to the weight of extracted material. Total polyphenols in all extracts were detemnined.

II.1.1. Polyphenols extraction procedures :

100g of *Matricaria chamomilla* L. flowers or the leaves of *Mentha pulegium* L. are macerated to the 1 liter methanol/ water (85% methanol), the mixture is subjected to an agitation (700 tours/minute) during three days (72h) at ambient temperature. The whole is filtered thereafter on funnel (N03) by the cotton and the filter paper of Wattman, the solvent (methanol) is eliminated from the filtrate by rotary evaporation in Rotavapor (BÜCHI). The extraction is remade for the second time (maceration with 50% methanol, followed by an agitation during 4heurs). The second filtrate is mixed with the first. The extract obtained is of a sunk brown color, it is regarded as being the rough extract of the leaves or the flowers of the two plants. 50 ml of this extract are dried using a drying oven in order to determine their dry matter value as well as the output of extraction. Volume remaining will be split later on.

Fractionation is carried out according to the method of (Gilani *et al,* 2001), after modifications, by using solvents with increasing polarity. The rough extract is initially mixed with the hexan (1 V/V), the mixture is let elutriate, and the higher organic phase is recovered. The extraction is remade several times until the solvent (hexan) becomes transparent. The hexane is evaporated thereafter and the resulting extract is regarded as being the fraction of

hexan. The residual aqueous phase is subjected to another extraction by chloroform, and finally by the ethyl acetate while following the same stages as the first extraction by hexan.

The series of extractions makes it possible to obtain five fractions; the extract crude methanolic (MeE), fraction of the hexan (HxE), fraction of chloroform (ChE), fraction of the ethyl acetate (AcE) and aqueous fraction (AqE) residual. These fractions are subjected to a freeze-drying and are preserved at -20C until use.

Ultraviolet–visible (UV–Vis) spectra and k-maximum values were obtained for *Matricaria chamomilla* L. or *Mentha pulegium* L. extracts, using a Varian Cary 1E UV–Vis spectrophotometer (Varian Australia, Melbourne, Australia). Figure 5 summarizes the stages followed in the fractionation of the rough extract.

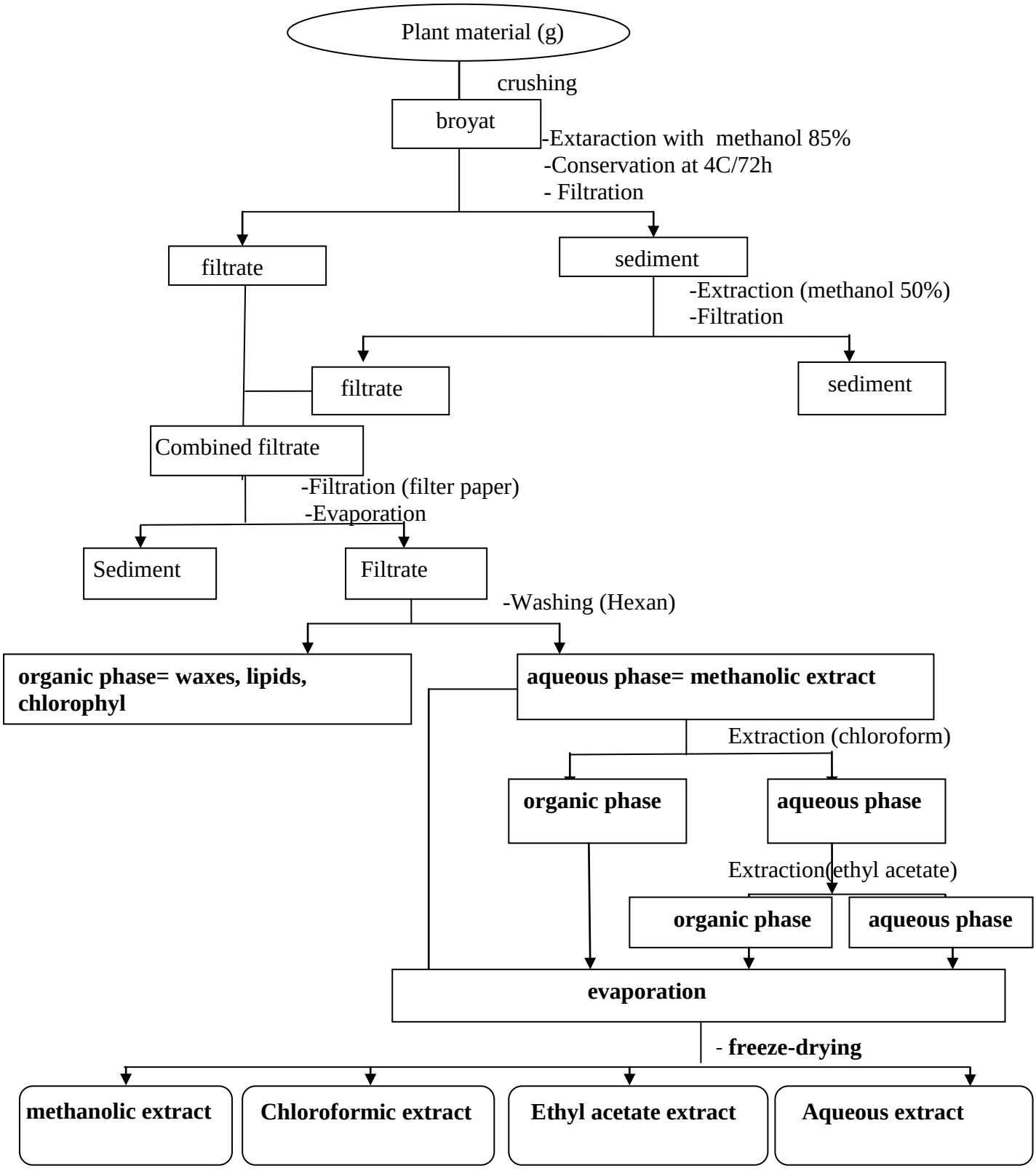


Fig 5. Schematic diagram represents the process of extraction.

II.2.2. Dosage of the metabolites in plants extracts:

II.2.2.1 Determination of total polyphenols:

In order to measure phenolic compounds in plant extracts (the water, chloroform, methanolic and ethyl acetate extracs) at different occasions, we used the Folin–Ciocalteu assay. The reagent of Folin–Ciocalteu consists of a mixture of acid phosphotungstic and phosphomolybdic acid. During oxidation, it is reduced to a mixture of blue oxide. The color produced is proportional to the amount of polyphenols present in the extract analyzed (kassemi, 2006).

According to the method of Miliauskaset *et al* (2004) after slight modifications, 50 µ L of each extract of the plant (MeE, AqE, ChE, AcE) diluted and is mixed with 250 µl folin-ciocalteu (2M) diluted 10 times and 250 µl of sodium carbonate (Na_2CO_3) with concentration of 7.5g/ 100ml. The absorbance is measured at 765 nm, after incubation for 1 hour and 30 min at ambient temperature against a methanol blank. A standard curve of gallic acid was created using an adequately range of gallic acid concentration from 150 to 5 µg/ml. The results were expressed as mg gallic acid equivalent/ gram fresh material. All measurements are repeated 3 times (figure 6).

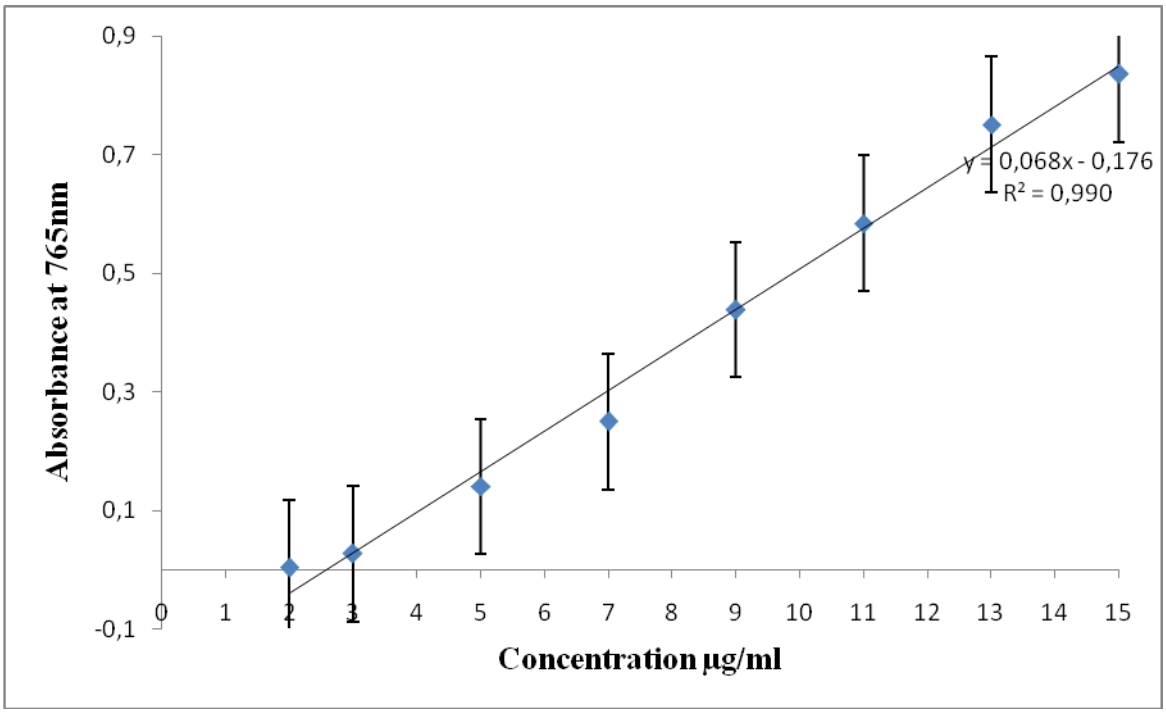


Fig 6. Standard curve of Gallic acid for the determination of total polyphenols. (mean ± SD of three measurements).

II.2.2.2 Determination of flavonoids:

According to the method of aluminium trichloride ($AlCl_3$), the proportioning of the flavonoids is carried out (Bahorum *et al.*, 1996); 1ml of each extracts (to prepare suitable dilutions in methanol or distilled water) by adding 1ml of AlCl3 solution (2% in methanol). After 10 minutes of incubation, the absorbance is measured at 430 nm. Calculates concentration of the flavonoids is established by using the typical standard curve for the reactivity of Quercetin and Rutin (0-40 µg/ml) with AlCl3 solution, which is shown in figure7 and it is expressed as milligram of Quercetin or Rutin equivalent per gram of extract.

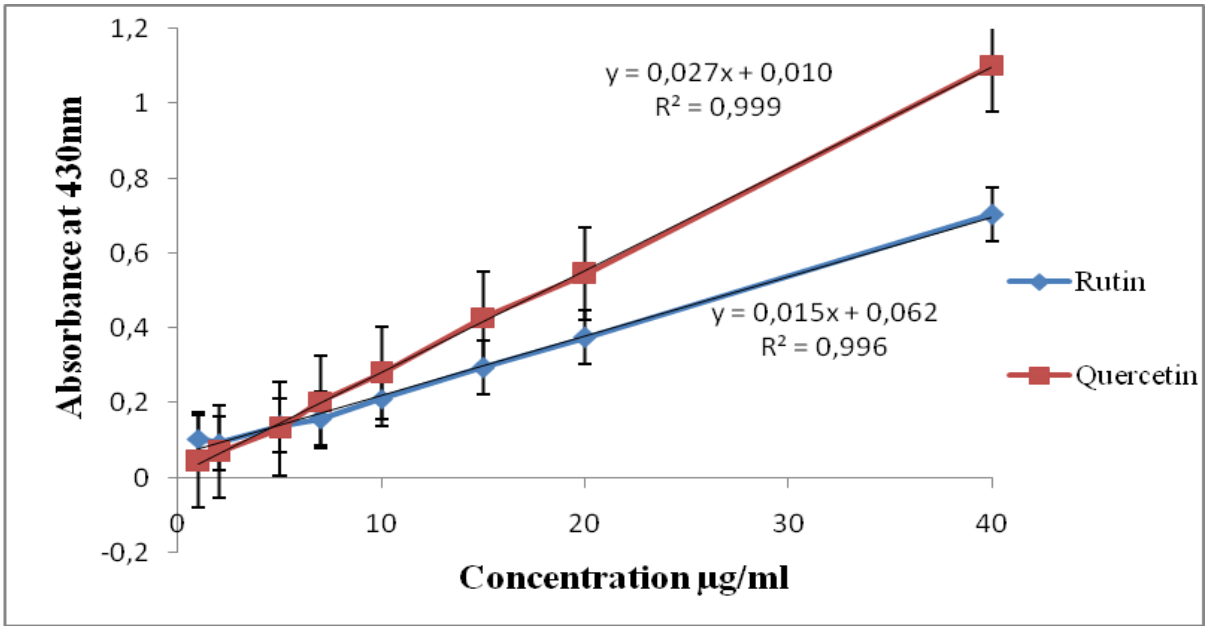


Fig 7. Standard curve of Quercetin and Rutin for the determination of total flavonoids. (mean ± SD of three measurements).

II.2.2.3 Determination of tannins:

Tannins had the capacity to precipitate proteins which binding with phenolic acid, so the test of haemoglobin precipitation was used or the method of Bate (1973). Briefly a volume of each plant extract was diluted to give a concentration of total polyphenols approximately 500 µg/ml and mixed with an equal volume of haemolysed sheep blood (Absorbance equal to 1.6), after 10 minutes this solution was centrifuged for 20 minutes and the absorbance of the supernatant was measured at 576 nm against the white (distilled water DW). Different concentrations of tannic acid were also mixed with an equal volume of haemolysed blood and the absorbance is measured in the same manner. A typical standard curve of precipitation of haemoglobin by tannic acid is shown in figure 8 and the effectiveness of the precipitation of the solutions tested is expressed as µg tannic acid equivalent/ g extract (figure 8).

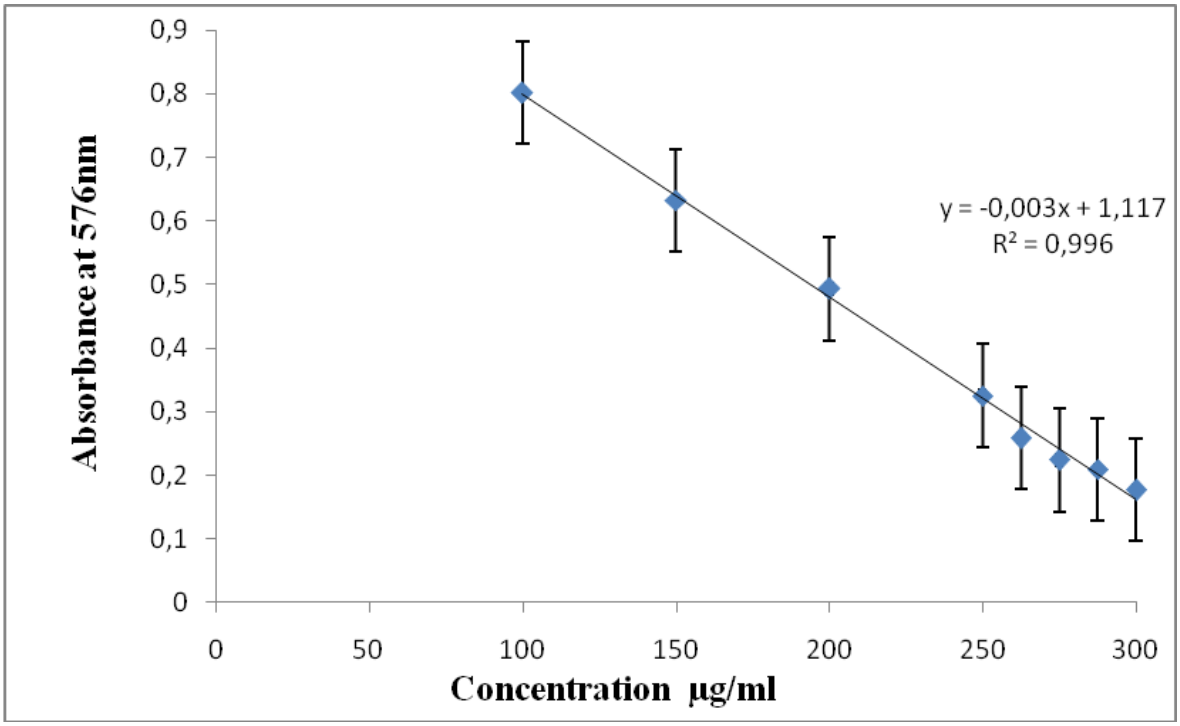


Fig 8. Standard curve of tannic acid for the determination of tannins. (mean ± SD of three measurements).

II.2.3. Determination of the antioxidant activity of plant extracts:

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the distruction of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdalla and Roozen, 1999). There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants (Sa′ nchez-Moreno, 2002; Schwarz *et al.*, 2001).

The antioxydant activity is a complex process which can occur by the means of several mechanisms. Because of its complexity more than one test must be carried out during the evaluation of the antioxydant activity of the pure or extracted compounds (Aruoma, 2003). One such method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH).

II.2.3.1 DPPH radical scavenging activity of plant extract:

The antioxidant capacity of our extracts which is expressed by the donation of an electron or a hydrogen atom to radical free 2,2'-diphenyl-1-picrylhydrazyl (DPPH), as a reagent, was measured by a spectrophotometric (Burits *et al,* 2000).

The experiment was carried out according to the method described by (Güllüce *et al.*, 2003). 50µl of various concentrations of the extracts is added to 5ml solution of the DPPH of concentration 0,004%. After 30 minutes of incubation at ambient temperature and in the darkness, the absorbance is read with a wavelength at 517nm.

Negative control is represented by the methanolic solution of the DPPH and the positive control is represented by the BHT.

The antioxidant activity, which expresses the capacities to trap the free radical one is estimated by the percentage of discolouration of the DPPH in solution in methanol (Inhibition % or I%) according to the formula:

$$\textbf{Inhibition \% = (ABS}_{\textbf{control}} \textbf{ - ABS}_{\textbf{test}}\textbf{) x 100 / ABS}_{\textbf{control.}}$$

Where:

ABS $_{control}$: Absorbance of control at the wavelength 517nm;

ABS $_{test}$: Absorbance of the sample at the wavelength 517nm.

The Value IC_{50} is defined as being the concentration of the substrate which causes the loss of 50% of the activity of the DPPH (color), or, it is the concentration of the sample required to give a reduction of 50% of the absorbance of the solution controls to constitute methanol and DPPH. The values of IC_{50} were calculated by the linear regression where the X-coordinate is represented by the concentration of the compounds tested and ordered by (I %) the percentage of inhibition (Mensor *et al.*, 2001).

The concentrations of the extracts in the reactional medium lie between 0,1-1 mg/ml, 3-12 mg/ml, 3-0,03 mg/ml and 0,1-4 mg/ml for AqE, ChE, AcE, and MeE respectively for *Matricaria chamomilla* L. while *Mentha pulegium* L. were betweenn 10-0,25 mg/ml, 30-0,5 mg/ml, 5-0,025 mg/ml for AqE, ChE and (AcE, MeE) respectively.

II.2.3.1. β-carotene/ linoleic acid assay:

In this test, the antioxydant capacity of the extracts is given by measuring the inhibition of the decomposition oxydative of β-carotene (discolouration) by the products of oxidation of the linoleic acid according to the method described by (Kartal *et al*, 2007). The emulsion of β-carotene/ linoleic acid is prepared by solubilization of β-carotene 0,5mg in 1ml of chloroform, 25µl of the linoleic acid and 200mg of Tween 40 are added, chloroform is completely evaporated with the rotavapor, thereafter 100ml of distilled water saturated with oxygen are added, the resulting emulsion is agitated vigorously. 350µl of solution of extracts or antioxydants of reference (BHT) solubilized in methanol (2mg/ml) is added with 2,5ml with the preceding emulsion.

The kinetics of discolouration of the emulsion in presence and absence of antioxidant (negative control in which the sample is replaced by 350µl methanol and distilled water) is followed to 490 nm with intervals of regular times during 48 heurs (after : 1heure, 2h, 3h, 4h, 6h, 24h, and 48h) of incubation at ambient temperature and in the darkness.

The percentage of inhibition of the extracts antioxidant is measured as follows:

$$\mathbf{AA\% = ABS_{test} / ABS_{BHT} \times 100}$$

AA%: Percentage of the antioxidant activity;

ABS_{test}: Absorbance in the presence of the extract (test);

ABS_{BHT}: Absorbance in the presence of positive control BHT.

Chapter 3: RESULTS AND DISCUSSION

III.1. Preparation of extracts from plants:

The different extract of *Matricaria chamomilla* L. and *Mentha pulegium* L. were obtained following the extraction method described by (Markham, 1982). This method is based on the degree of solubility of polyphenols in organic solvents. It takes place in four stages:

1)- solubilization of polyphenols in methanol.

2)- defatting of the extract by adding hexan.

3)- the addition of chloroform to obtain the flavonoid aglycons.

4)- the addition of ethyl acetate to obtain the glucoflavonoids.

This method allowed us to obtain five different fractions yield an extract variable to another (tables 1, 2). The yield was calculated on the total weight of the crushed flowers or leaves of *Matricaria chamomilla* L. and *Mentha pulegium* L. respectively**.**

III.1.1. Extracts from *Mentha pulegium* L.:

In the leaves of *Mentha pulegium* L, the ME had the higher yield (14,4%) and for other extracts were lower than these values in this order: AqE (13,872%), AcE (4,324%), ChE (1,012%) and HxE (0,46%) as shown in table 1.

Table 2: Yeild of various extracts of *Mentha pulegium* L.

Extract	**Yield(%)**
Methanolic Extract (ME)	**14,4%**
Chloroformic Extract (ChE)	1,012%
Ethyl acetate Extract (AcE)	4,324%
Aqueous Extract (EAq)	13,872%
Hexane (HxE)	0,46%

In the work of Mata *et al* (2007), ethanol extracts were obtained by extracting the plant material three times at room temperature and removal of the solvent by vacuum distillation at 50 _C. Aqueous extracts were obtained by boiling 5 g of dried plant material, cut into small pieces, in 100 ml of distilled water for 20 min, followed by filtration. For the enzymatic tests, aliquots of 1 ml were used immediately, or frozen and used when necessary. An aliquot of each water extract was evaporated to dryness to obtain the equivalent dry weight (dry wt). So, the yields obtained presented the following increasing order of occurrence: essential oils (0.7) < ethanol extract (8.2) < water extract (49.8). The values obtained for the last extract were above 25%. The essential oils obtained by hydrodistillation presented the lowest yields (<1%) for all plants under study. While the fresh flowering aerial parts of *Mentha pulegium* L. were subjected to hydrodistillation and a yield of 0.27% (v/w) was obtained.

III.1.2. Extracts from *Matricaria chamomilla* L.:

Table 3 gives the content of analyzed components of the investigated flowers of plant studies. As can be seen in Table 3, aqueous extract (AqE) of *Matricaria chamomilla* L. had the highest yield (18,56%) followed by methanolic extract or (ME) (17 ,18%), ethyl acetate extract or (AcE) (2,2%), chloroformic extract or (ChE) (0 ,54%) and Hexan extract or (HxE) (0,22%).

Table 3: Yeild of various extracts of *Matricaria chamomilla* L.

Extract	**Yield(%)**
Methanolic Extract (ME)	17 ,18%
Chloroformic Extract (ChE)	0 ,54%
Ethyl acetate Extract (AcE)	2,2%
Aqueous Extract (AqE)	**18,56%**
Hexan (HxE)	0,22%

According to the method of extraction of *Matricaria chamomilla* L. from Djibouti by Fatouma (2011), followed an extraction protocol similar to that described by Lin (1999). The yields of the essential oil and methanol extract of *M. chamomilla* were respectively 0.25% (w/v) and 2.35% (v/v) in contrast of, in our work, (17,18%). So, Differences in yields of the two extractions could be due to extraction conditions as well as the geographical origin (Algeria, Djibouti) of the plant used and we can find various extracts yields in the same species.

III.2. Determination of total polyphenols, flavonoids and tannins in plants extracts:

III.2.1. Determination of total polyphenols, flavonoids and tannins in *Mentha pulegium* L. extracts:

Based on the absorbance value of the plant extracts solution reacting with Folin-Ciocalteu phenol reagent and compaired with the absorbance values of standard solutions of gallic acid, total phenolics content of the plant extracts was estimated in this order: AcE (191,99±0,016 µg GAE/g of extract)> ME (183,45±0,125 µg GAE/g of extract)> ChE (119,73±0,036 µg GAE/g of extract)> AqE (88,84±0,112 µg GAE/g of extract). This values indicate that each milligram of the plant extracts contains phenolic compounds equivalent to about 191,99; 183,45; 119,73; 88,84 µg of pue gallic acid respectively.

As can be seen in the table 4, AcE had the higher contents of tannins (265,33±0,030 µg TAE/gE) followed by ChE (209±0,017 µg TAE/gE) then ME (149,33±0,0046 µgTAE/gE) and the AqE (137,22±0,029 µg TAE/gE) with lower content.

In the $AlCl_3$ method, the flavonoid results measured either by: the Quercetin equivalent which as the following order: AcE(110,37±0,023 µg QE/gE)> ME (59,87±0,005 µg QE/gE)> ChE (19,50±0,013 µg QE/gE) > AqE (1,19±0,004 µg QE/gE) or the Rutin equivalent which revealed that the extracts had: AcE (151,11±0,023 µg RE/gE), ME (60,92±0,005 µg RE/gE), AqE (1,41±0,004 µg RE/gE) and ChE (0,37±0,013 µg RE/gE).

Table 4: Total polyphenols, flavonoids and tannins in *Mentha pulegium* L. extracts.

Extract	Flavonoid [(a)]		Polyphenol [(b)]	Tannin [(c)]
	Quercetin	Rutin		
Methanolic	59,87±0,005	60,92±0,005	183,45±0,125	149,33±0,0046
Chloroform	19,50±0,013	0,37±0,013	119,73±0,036	209±0,017
Ethyl acetate	**110,37±0,023**	**151,11±0,023**	**191,99±0,016**	**265,33±0,030**
Aqueous	1,19±0,004	1,41±0,004	88,84±0,112	137,22±0,029

[(a)] µg Quercetin or Rutin equivalent per gramme of extract.

(b) µg Gallic acid equivalent per gramme of extrait.

(c) µg Tannic acid equivalent per gramme of extract.

The values present the mean of three measurements ± SD.

The total phenolic compound contents in the ethanol of *Mentha pulegium* L. (71.7 ± 2.1 mg GAE per g of extract) and water extracts (57.9 ± 1.6 mg GAE per g of extract) were determined by colorimetric assays, using the Folin-Ciocalteu reagent (Oktay *et al*., 2003) and pyrogallol as a standard. Pennyroyal (*M. pullegium*) and mint (*M. spicata*) contain flavonoids that may account for the high antioxidant activity observed for the polar extracts of these aromatic herbs (Justesen and Knuthsen, 2001; Zaidi *et al*., 1998).

III.2.2. Determination of total polyphenols, flavonoids and tannins in *Matricaria chamomilla* L extracts:

The total flavonoid contents of different *Matricaria chamomilla* L. fractions were reported as µg QE and µg RE per g of extract. The results show that the fractions have the following order: ChE had the higher value (197,43±0,033 µg QE/gE)> AcE (173,33±0,007 µg QE/gE)> ME (35,16±0,028 µg QE/gE)> AqE (27,65±0,007 µg QE/gE). While the Rutin equivalent represent that ChE (273,03±0,033 µg RE/gE), AcE (202,22±0,007 µg RE/gE), ME (29,62±0,028 µg RE/gE) and AqE (18,37±0,007 µg RE/gE).

Unlike the flavonoid, our results represent that the polyphenols in extracts are ME (299,14±0,102 µg GAE/g of extract), AcE (2079,65±0,048 µg GAE/g of extract), AqE (146,97±0,046 µg GAE/g of extract) and ChE (104,53±0,033 µg GAE/g of extract).

Table 5 shows the relative contents of tannins in our extracts which are: ChE (245,11±0,039 µg TAE/gE), AcE (201,66±0,165 µg TAE/gE), ME (145,55±0,067 µg TAE/gE) and AqE (132,22±0,023 µg TAE/gE).

Table 5: Total polyphenols, flavonoids and tannins in *Matricaria chamomilla* L. extracts.

Extract	**Flavonoid [(a)]**		**Polyphenol [(b)]**	**Tannin [(c)]**
	Quercetin	**Rutin**		
Methanolic	35,16±0,028	29,62±0,028	299,14±0,102	145,55±0,067
Chloroform	**197,43±0,033**	**273,03±0,033**	104,53±0,033	**245,11±0,039**
Ethyl acetate	173,33±0,007	202,22±0,007	**2079,65±0,048**	201,66±0,165
Aqueous	27,65±0,007	18 ,37±0,007	146,97±0,046	132,22±0,023

[(a)] µg Quercetin or Rutin equivalent per gramme of extract.

(b) µg Gallic acid equivalent per gramme of extrait.

(c) µg Tannic acid equivalent per gramme of extract.

The values present the mean of three measurements ± SD.

Flavonoid glycosides represent the major fraction of water-soluble components in chamomile. Apart from the glycosides, flavonoid aglyca were found in great variety among the lipophilic constituents. Chamomile flavonoids were recognized to be spasmolytic and antiphlogistic and are therefore of great interest. Apigenin was the first flavone to be isolated from chamomile (Franke and Schilcher, 2005).

Substances that interfere with the analysis of flavonoids (e.g., carotinoids) are usually removed by extraction. It has to be taken into account that the majority of apolar flavonoids will also be removed in this step, e.g., by the extraction with carbon tetrachloride (Franke and Schilcher, 2005). Moreover, extraction by hot water results in 30–45% lower values for

flavonoids compared to methanol extraction. It is therefore not possible to avoid the co-extraction of chlorophyll and related substances by using water.

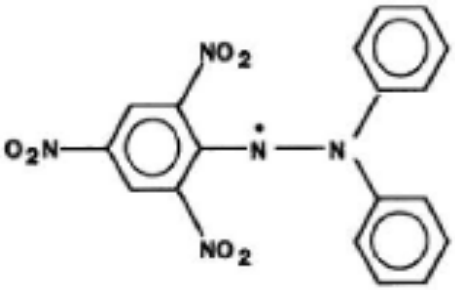
The photometric determination of flavonoids has many advantages, although the absolute values are actually about 20–30% higher. The absolute content of flavonoids ranged between 1.0 and 2.5% in a study of 102 commercially available plants and determination according to References (Franke and Schilcher, 2005) and (Christ and Müller, 1960). Twelve samples of material of different origin cultivated by Schilcher showed values between 0.3 and 2.96% (Schilcher, 1987).

III.3. Antioxidant activity:

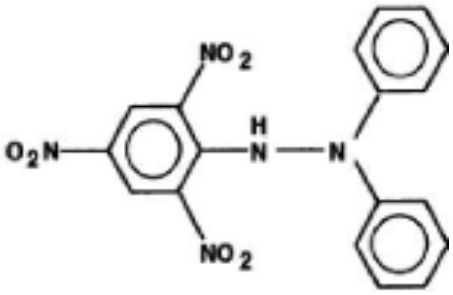
III.3.1. Test of DPPH:

III.3.1.1. Basis of the Method:

DPPH - free radical and reduced form:



1: Diphenylpicrylhydrazyl (free radical)



2: Diphenylpicrylhydrazine (nonradical)

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH: **1**) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in methanol solution centred at about 517 nm.

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (**2**) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is:

$$Z\bullet + AH = ZH + A\bullet \qquad [1]$$

where ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the

number of molecules of DPPH reduced (decolorised) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z• is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH.

The parameter EC_{50} ("efficient concentration" value)

One parameter that has been introduced recently for the interpretation of the results from the DPPH method, is the "efficient concentration" or EC_{50} value (otherwise called the IC_{50} value). This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour).

III.3.1.2. DPPH scavenging of extracts of *Mentha pulegium* L:

The antioxidant activity profiles obtained show that extracts of plants had a dose-dependent antioxidant activity, the IC_{50} of each of the different extracts were determined (table6).

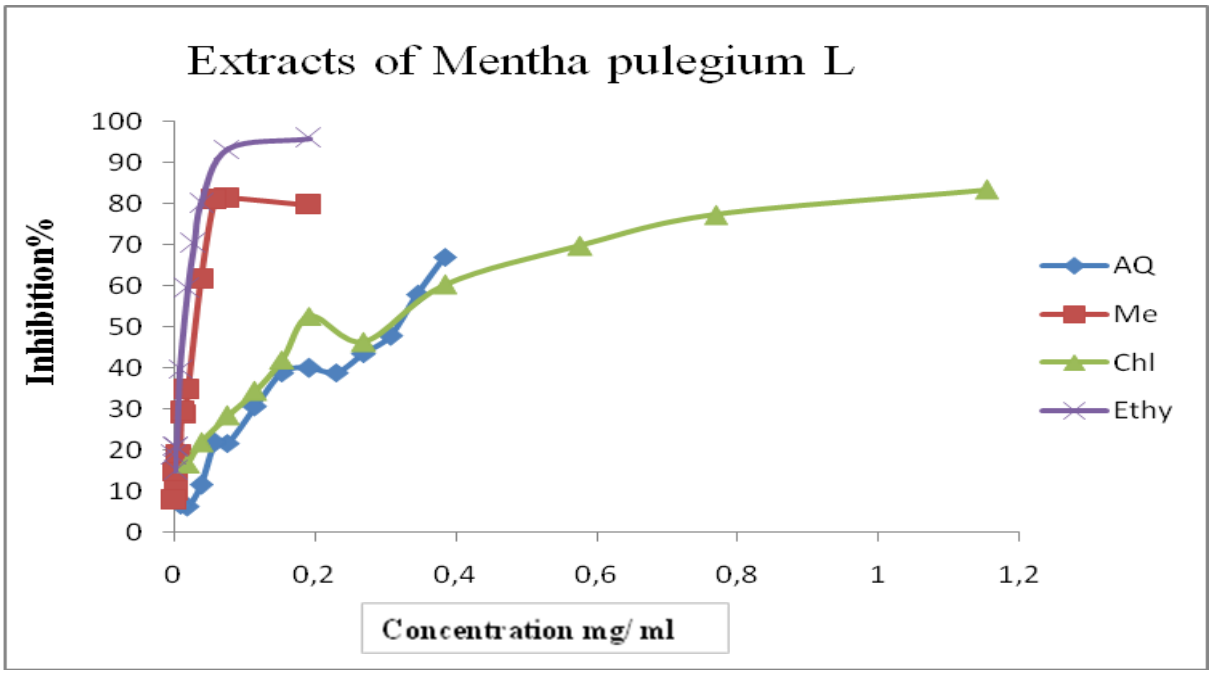
The DPPH free radical method determined the antiradical power of antioxidants. Regarding the IC_{50} values, all the extracts and the commercial standards (BHT, Gallic acid, Quercetin, Rutin) depleted the initial DPPH concentration by 50% within 1h. The lower of IC_{50} value is the higher of free radical scavenging activity of a sample. The free radical scavenging activities of all extracts of *Mentha pulegium* L. were in this order: ethyl acetate > methanolic > chloroform > aqueous (Table 6). The ethyl acetate extract, which contained the most tannin, had the highest free radical scavenging activity. All of the extracts had higher IC_{50} values compared to Gallic acid, Quercetin, Rutin and BHT. When we compared to BHT, the ethyl acetate extract and methanolic extract, Rutin, GA, Quercetin did not show any significant differences (P>0,005). While the chloroformic extract and aqueous extract were significant

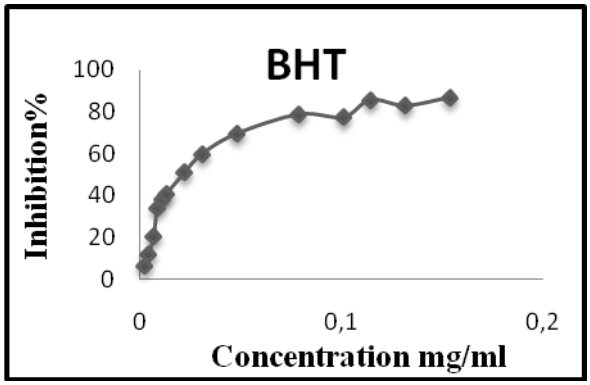
(P□ 0,001) The effect of extracts MeE and AcE is very probably attributed to their high phenolic componuds and flavonoids.

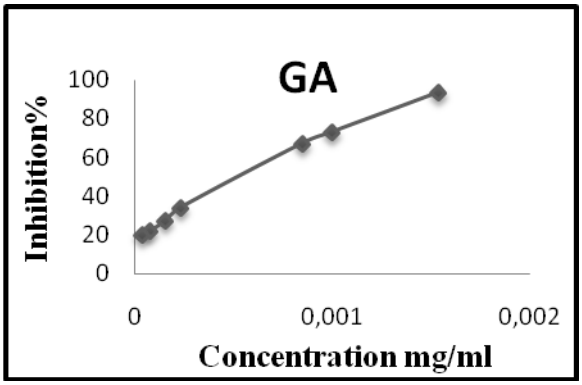
Table 6: DPPH scavenging of extracts of *Mentha puleguim* L.

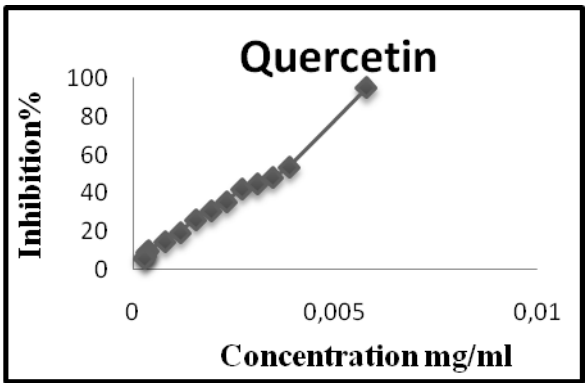
Extract	**DPPH (IC_{50} mg/ml)**
Methanolic	0,031±0,00064
Chloroform	0,223±0,0497
Ethyl acetate	**0,017±0,00043**
Aqueous	0,292±0,0295
Gallic acid	0,00058±1,0076
BHT	0,0318±0,00064
Quercetin	0,0034±5,38648
Rutin	0,0040±0,00080

The values present the mean of three measurements ± SD.









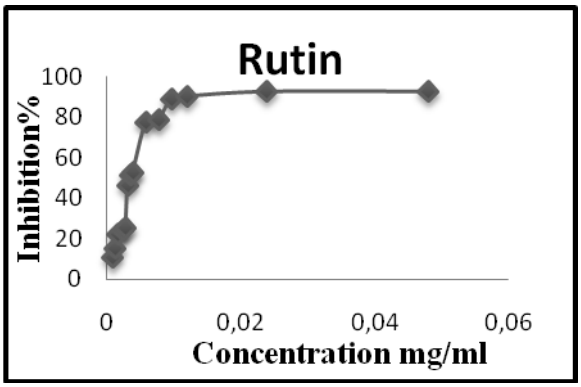


Fig 9. The DPPH scavenging of extracts of *Mentha pulegium* L.

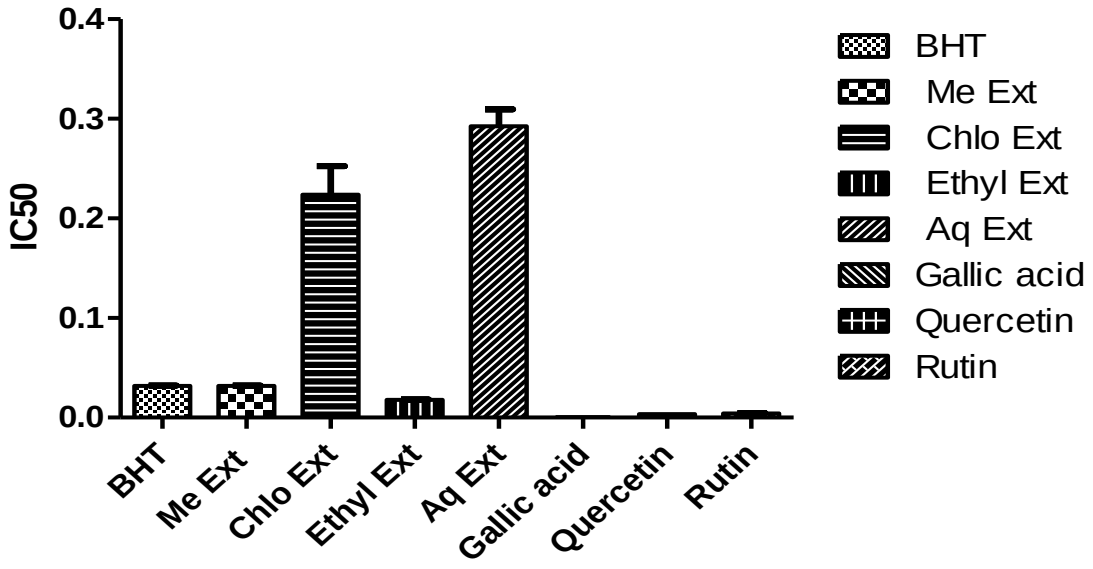


Fig 10. IC_{50} values of extracts of *Mentha pulegium* L. determined by DPPH assay. Bares are mean± SEM.

According to the method of Burits and Bucar (2000) which shows IC_{50} values for both extracts (water extract and methanol extract) and essential oil in DPPH assay of *M. pulegium* L . Results showed that an increase in extracts concentration resulted an increase in free radical-scavenging activity. For example, water extract had 5.5 ± 0.3 µg/ ml, methanol extract had 6.1 ± 0.1 µg/ml and BHT 4.9 ± 0.2 µg/ml. So, free radical-scavenging activities of the extracts were comparable to the BHT.

At present study, decrease in DPPH radical-scavenging activity due to the water (IC_{50}: 5.5 l g/mL) and methanol (IC_{50}: 6.1 l g/ml) extracts of *M. pulegium* L. was higher than those reported by Nickavar *et al* (2008) on the ethanol extract (17.92 l g/ml) and Mata *et al* (2007) on the ethanol (24.9 l g/ml) and water extract (8.9 l g/ml) of *M. pulegium* L.

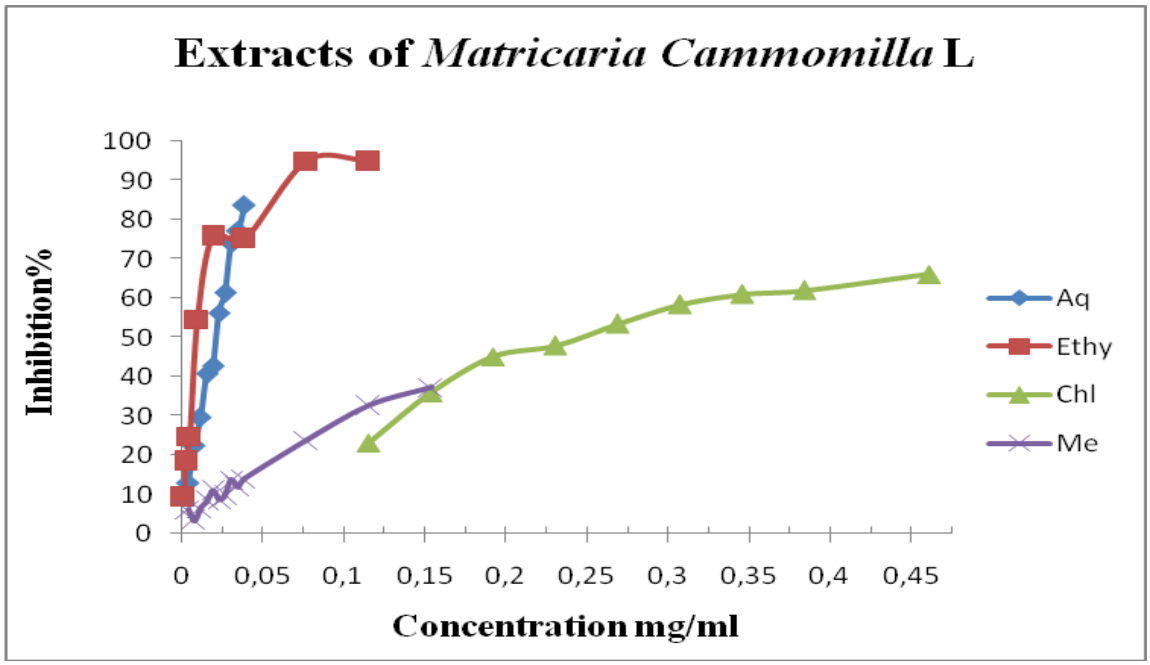
III.3.1.3. DPPH scavenging of extracts of *Matricaria chamomilla* L.:

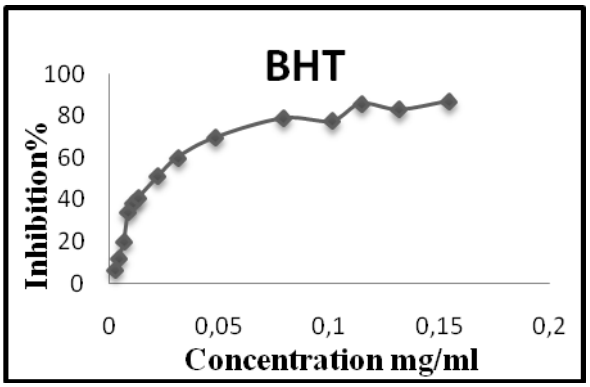
Results showes an increase in ethyl acetate extract with: IC_{50}= 0,0111±0,00091 mg/ml resulted in increase in free radical-scavenging activity. Interestingly, free radical-scavenging activities of the aqueous extract with 0,021±0,0017 mg/ml then methanolic extract were 0,069±0 ,0009 mg/ml and the chloroformic extract with 0,238±0,0228 mg/ml (table7) . So it is found that the effect of DPPH radical scavenging extracts of the plant is inferior compared to the levels of standards: BHT, Gallic acid (AG), Quercetin and Rutin. BHT was more potent than the chloroformic extract (P□ 0,001) Whereas, the other extracts: MeE, AcE, AqE, AG, Quercetin and rutin did not show any significant differences (P>0,05).

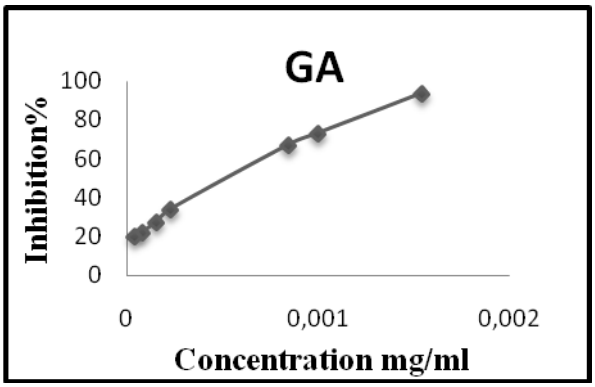
Table 7: DPPH scavenging of extracts of *Matricaria chamomilla* L**.**

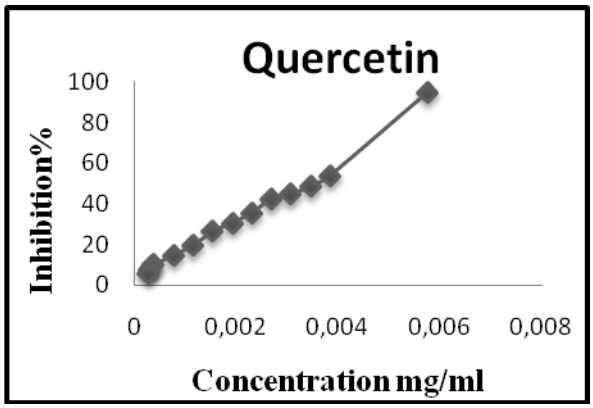
Extract	**DPPH (IC_{50} mg/ml)**
Methanolic	0,069±0 ,0009
Chloroform	0,238±0,0228
Ethyl acetate	**0,0111±0,00091**
Aqueous	0,021±0,0017
Gallic acid	0,00058±1,0076
BHT	0,0318±0,00064
Quercetin	0,0034±5,38648
Rutin	0,0040±0,00080

The values present the mean of three measurements ± SD.









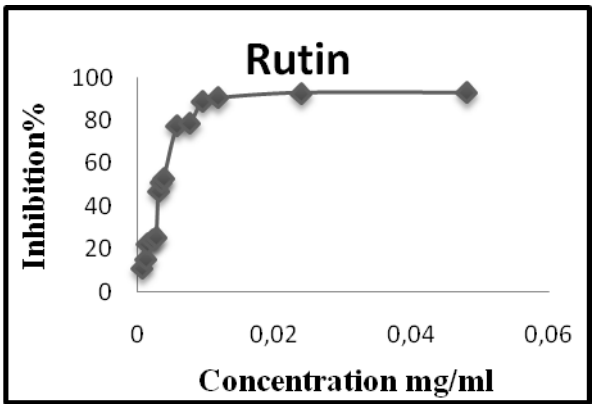


Fig 11. The DPPH scavenging of extracts of *Matricaria chamomilla* L.

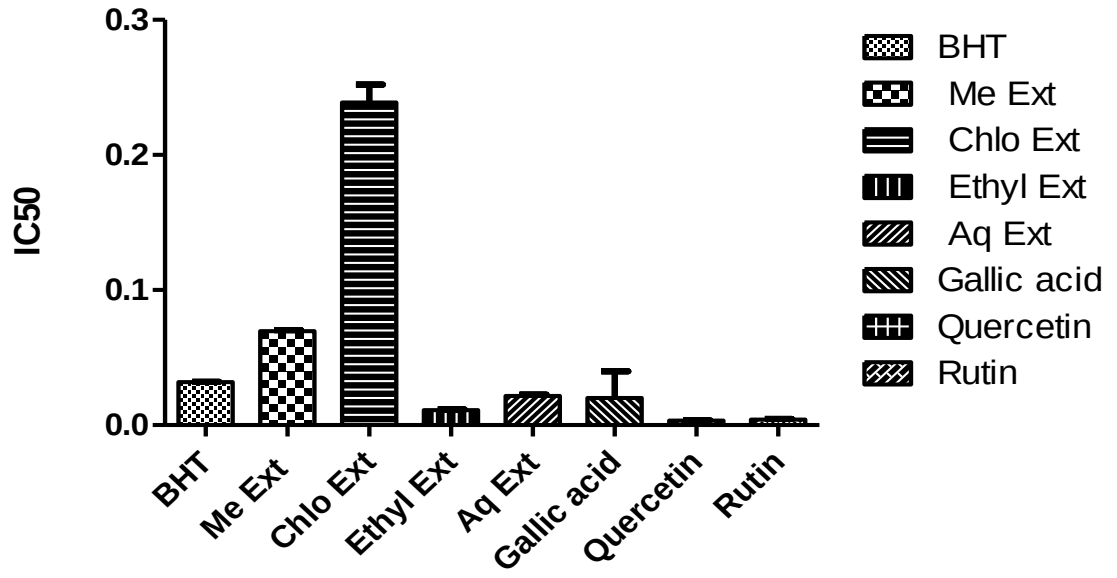


Fig 12. IC_{50} values of extracts of *Matricaria chamomilla* L. determined by DPPH assay. Bares are mean± SEM.

The results of DPPH radical scavenging activities showed that chamomile exhibited the greatest free radical scavenging activity (91%), Based on these results, we concluded that the effect increases with increasing scavenger concentration of polyphenols in the extract which leads to suggest that the antioxidant effect of plant extract is related to the amount of polyphenols are present. This hypothesis is demonstrated by several researchers such as (Jayaprakash *et al.,* 2007; Agbor *et al,* 2007 and Hodzic *et al*., 2009). The antioxidant effect of an extract may also differ depending on the quality of polyphenols such as flavonoids are present that have shown an antioxidant activity (Wang and Mazza, 2002). The mechanism of the reaction between the antioxidant and DPPH depends on the structural conformation of the antioxidant (Kouri *et al.,* 2007). Some compounds react rapidly with the DPPH reducing the number of DPPH equal to that of the hydroxyl groups of the antioxidant

(Bondet *et al*., 1997). Spatial configuration and number of OH group of flavonoid structures can influence the different antioxidant mechanisms (Hein *et al*., 2002).

III.3.2. β-carotene/ linoleic acid:

III.3.2.1. Antioxidant activity of *Mentha pulegium* L. extracts*:*

The antioxidant activities of the extracts determined by the β-carotene/ linoleic acid system assay were also presented in Table 8. The antioxidant activity of samples was reflected in their ability to inhibit the bleaching of β-carotene. In this assay, the chloroformic extract possessed better antioxidant activity (61,07±0,017%) than other extracts and Rutin, Gallic acid, but it was not as good as BHT (85,78±0,033%). Other extracts were also effective in inhibiting lipid peroxidation in this order: ethyl acetate with 59, 36±0,084%, methanolic extract with 30,19±0,080% and aqueous extract with 44,29±0,069%. Comparison made between to BHT and extracts show significant differences (P□ 0,05) except one; the AcE while the ChE show a slightly difference 4,448 to 57,57(P□ 0,05) These resultants are due to the polyphenol compounds in the extracts. The MeE, AqE, GA, Rutin, MeOH and H2O are all significant (P□ 0,001).

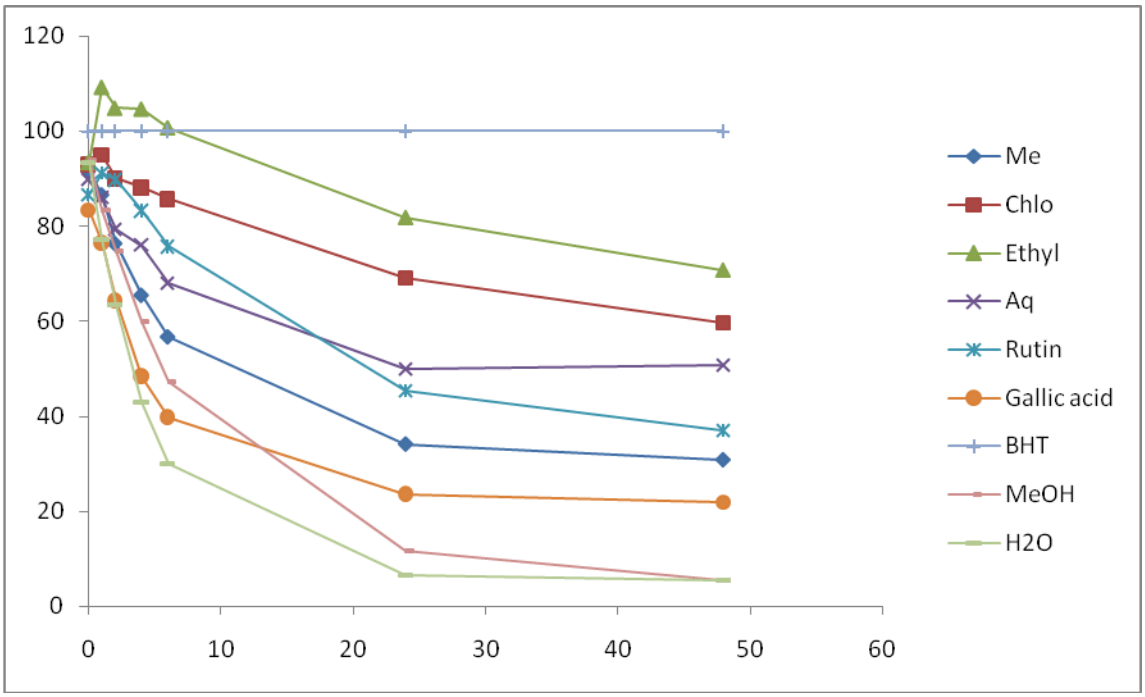


Fig 13. Antioxidant activity of *Mentha pulegium* L. extracts by β-carotene/ linoleic acid assay for 48h (using MeOH, H2O, BHT as standards).

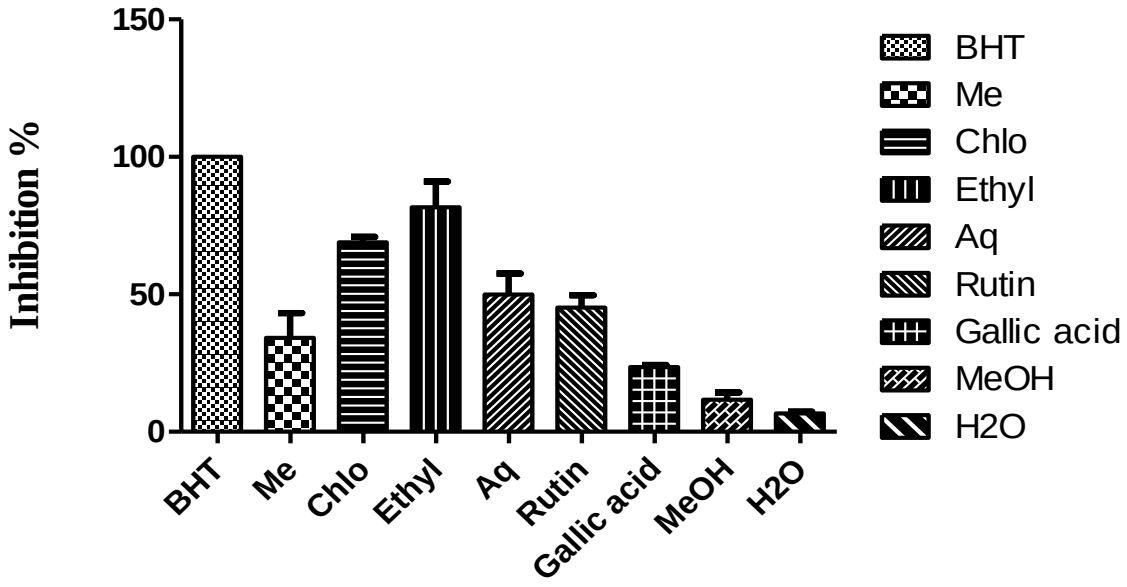


Fig 14. Inhibition percentage of divers extracts of *Mentha pulegium* L. in β-carotene/ linoleic acid assay after 24h (using MeOH, H2O, BHT as standards).

According to the method of Dapkevicius *et al* (1998) which shows inhibition on lipid peroxidation in response to both extracts and essential oil. Both methanol and water extracts effectively inhibited the linoleic acid oxidation as much as 60.38% and 91.67%, respectively. In addition, at the same concentration water extract showed higher inhibition (91.67%) compared to the BHT (89.35%). In this regard, essential oil was not able to effectively inhibit the oxidation as much as extracts. It made only 26.01% inhibition compared to the control (6.41%).

In spite of our findings about a decrease in the values of β-carotene/ linoleic acid on both water (91.67%) and methanol (60.38%) extracts, Mata *et al* (2007) did not show such decrease. In this respect, they showed that BHT was more potent than the water extract (about 12, 91%) and ethanol extract (about 12,75%) extracts.

These characteristics of the water and methanol extracts of the *M. pulegium* can be attributed to its phenolics, flavonoids and terpenoids constituents. These compounds have been shown in our phytochemical analysis. In this regard, Luximun-Ramma *et al* (2002) showed a linear correlation between antioxidant activity and phenolic contents of the plant extracts, fruits and beverages. Sugihara *et al* (1999) and Spencer (2008) discussed that flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals, also. Moreover, Joshi *et al* (2008) showed a potent antioxidant activity for terpenoids. These discrepancies in the antioxidative properties of menthe subspecies can be due to their ingredients.

In this regard, Duh (1999) discussed that the presence and synergism of different antioxidants in an extract will determine the antioxidative properties of a specific extract (Duh, 1999).

III.3.2.2. Antioxidant activity of *Matricaria chamomilla* L. extracts:

In the β-carotene/linoleic acid assay the antioxidant capacity is determined by measuring the inhibition of the organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Tepe *el al*., 2005).This assay has been used to simulate the oxidation of the membrane lipid components in the presence of antioxidants inside the cell (Mata *el al*., 2007). The results obtained from extracts of *Matricaria chamomilla* L. flowers are all significant ($P< 0.05$) and presented in table 9. Sample Aq shows low inhibition of peroxidation 25,59±0,002 % , while the chloroformic extract shows appreciable inhibiton of 37,15±0,038% with values in some way similar to the methanolic extract 37,04±0,074% , higher than Rutin 31,50±0,034% and Gallic acid with 20,83±0,0075 % . The ethyl acetate extract is with 28,36±0,038% and aqueous extract is with25,59±0,002% and lower than BHT 85,78±0,033%. So compared to BHT as a standard, all extracts are significantly (P 0,001)in this order: H_2O> MeOH> GA> AqE> AcE> MeE> ChE> Rutin.

Given that several studies have shown that the antioxidant effect of natural sources is related to the presence of phenolic compounds (Abdille *et al*., 2005; Velioglu *et al*., 1998), the ChE has demonstrated the highest polyphenol content and the best activity in this test, leaving the conclusion that the high antioxidant activity of aqueous extracts of *Matricaria chamomilla* L., ethyl acetate and methanol is due to their capacity of phenolic compounds.

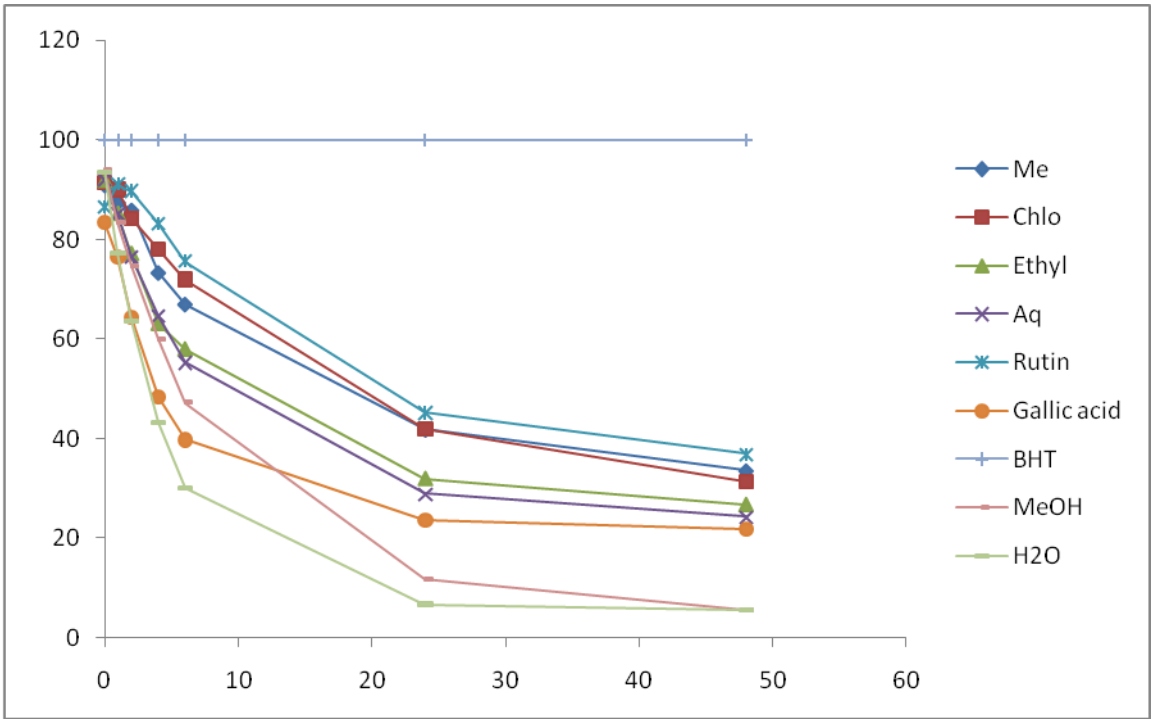


Fig 15. Antioxidant activity of *Matricaria chamomilla* L. extracts in β-carotene/ linoleic acid assay for 48h (using MeOH, H2O, BHT as standards).

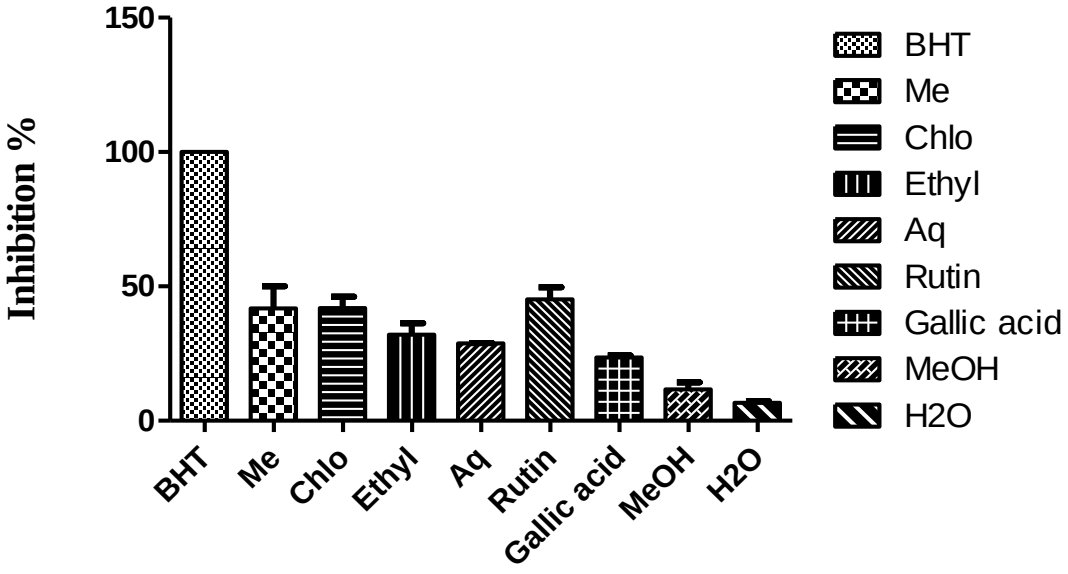


Fig 16. Inhibition percentage of divers extracts of *Matricaria chamomilla* L. in β-carotene/ linoleic acid assay after 24h (using MeOH, H2O, BHT as standards).

CONCLUSION

CONCLUSION:

We have focused on plants belonging to several different families from Algeria (*Lamiaceae*, *Asteraceae*) to understand their therapeutic uses and their potential antioxidant activities. The two plants are widely used in the country as aromatic herbs in cooking, and also for their medicinal properties in folk medicine, may act as important supplements of antioxidants in the diet, especially in the cooking of some dishes of poor nutritional value. But unfortunately, most of the species that are claimed to contain potent antioxidant activity have not been studied *in vivo*. Screening with *in vitro* assays has little meaning if there is no clear evidence of the effectiveness of the extracts *in vivo*. Therefore, further *in vivo* studies of these species are required, and a systematic investigation of these antioxidant rich species is needed before they can be used in the food processing industry and as preventive medicine.

Overall, it could be concluded that plants bear a potent antioxidant activity at a concentration level which is related to constituents. Their constituents scavenge free radicals and exert a protective effect against oxidative damage induced to cellular macromolecules because it has shown the presence of number of polyphenols, which are related to their chemical structure and may be responsible of the antioxidant activities. Further studies on the isolation of these compounds are in progress.

So all plants exhibited moderate to good antioxidant activity, at least in one of the extract tested, being the more active, the ethyl acetate for *Mentha pulegium* L. and chloroformic extract for *Matricaria camomilla* L. In general, as effective as a known standard, BHT, in scavenging free radicals.

Plants showing simultaneous polyphenols contents and antioxidant activity capacity could be considered as food, having some function besides their traditional value, which makes them promising candidates for more detailed *in vitro* and *in vivo* studies because of the different experimental methods used in various studies.

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