الجمهورية الجزائرية الديمقراطية الشعبية

People's Democratic Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة العربي بن مهيدي – أم البواقي

Larbi Ben M'hidi University – Oum El Bouaghi

Faculty of Exact Science and Life Science and Nature

Department of Life and Natural Sciences

THESIS

Presented by:

CHEMSA AHMED ELKHALIFA

SUBMITTED FOR THE DEGREE OF:
DOCTORAT OF SCIENCES
In Biology
Option: Bioactive substances

SECONDARY METABOLITES PRODUCTS AND BIOLOGICAL ACTIVITIES OF SOME SAHARIAN MEDICINAL PLANTS

Discussed on: July 10th, 2016 **Members of jury:**

Chairman:	Noureddine GHERRAF	Prof. Larbi Ben M'hidi University, Oum El Bouaghi
Supervisor:	Amar ZELLAGUI	Prof. Larbi Ben M'hidi University, Oum El Bouaghi
Examiner:	Mehmet ÖZTÜRK	Prof. Mugla sitki Kocman university, Turkey
Examiner:	Zahia KABOUCHE	Prof. Mentouri Brothers University, Constantine
Examiner:	Abderrahmane BAGHIANI	Prof. Ferhat Abbas University, Setif

بسم الله الرحمن الرحيم

To my parents

To all my teachers

To my sisters and brothers

To all my family

To my wife and her family

To my daughters

To all my

Friends and colleagues

Content

Acknowledgment

First of all, thanks to almighty **ALLAH** Who Enabled me to accomplish this work successfully.

I want to acknowledge:

My sincere deepest gratitude is due to my supervisor Professor Amar ZELLAGUI for the guidance, motivation, and advices during these years not only in the scientific matter but also in the everyday life.

The reviewers of my thesis, Professor Noureddine GHERRAF from University of larbi Ben Mhidi, Oum El Bouaghi, Professor Zahia KABOUCHE from University of Mentouri, constantine, and Professor Abderrahmane BAGHIANI from University of Ferhat Abbas, Setif, for critical reading of the thesis, and for their valuable comments, discussions and suggestions.

Special thanks to:

Prof. Mehmet ÖZTÜRK for his benevolence, kindness and encouragement, advice and also his humor were very helpful throughout my work. Thank you very much.

Special thanks to:

Prof. Mehmet Emin DURU for his continuous support, assistance, advices and guidance.

Special thanks to:

Ebru Erol for her assistance and support during and after my training.

Special thanks to:

Dr. Ozgür Ceylan for his help and continuous support during working in microbiology lab.

I’m very grateful to:

The Algerian Ministry of Higher Education and Scientific Research (MESRS) for financial support during the course of the project in Turkey.

This work was carried out at the Laboratory of Chemistry, Mugla sitki Kocman university, Turkey. I feel very fortunate for having the opportunity to work in this Laboratory with excellent research facilities and surrounded by supporting people. I wish to thank all of them.

Many thanks to my friends: Dredi Lakhdar, Dida Laid, Abdelhamid Deghaa, Oucif Khaled Mohamed Tayeb for their help to collect the plants from different places.

special thanks to Dr. Youcef Halis for identifying the plant sample.

Life in a new place far from the family is very difficult. Thanks to all my friends in Turkey with whom the life has become easier and smoother.

Last but not least, special thanks are due to the people who are the constant sources of my strength and happiness i.e. my mother, my father, my wife, my daughters, my sisters, my brothers and my friends for all the emotional support and encouragement throughout my stay in the Turkey.

I am very much appreciate the understanding, encouragement and support of my wife during my stay in the Turkey. I did not take care of my mother, my father, my wife and my daughters since I was so far from them, I appreciate very much the taking care of them by my brothers Mohammad and Youcef, many thanks to all of them.

I will never forget your help and support.

Summary

Four medicinal plants from Algerian Sahara have been investigated in this study to determine chemical composition and biological activity of these plants. Three of the studied plants belong to Asteraceae family namely: *Anthemis stiparum* subsp. *sabulicola*, *Rhanterium suaveolens* and *Centaurea furfuracea* and the fourth belongs to Lamiaceae family: *Marrubium deserti*.

Chemical analysis of the four essential oils showed that the fraction of monoterpenes compounds is low in all oils. Apart of that, other chemical groups fractions were different from a plant to another. Sesquiterpenes ranged between 7.40 % and 33.85 % in *R. suaveolens* and *M. deserti*, respectively. The highest fraction of Monoterpenoids was 48.25% in *R. suaveolens* mainly represented by Perillaldehyde compound.

Antioxidant activity showed that methanolic extracts higher than essential oils for all plants. The methanol extract of *M. deserti* showed the highest activity than the rest of extracts and nearly similar to standards. Its inhibitory concentration IC_{50} of $DPPH^{\bullet}$ was 12.87 µg/mL, and 2.51 µg/mL for *β*-caroten/linoleic acid assay. In CUPRAC assay methanol extract showed higher absorbance than α-Tocopherol and BHA at 800 µg/mL concertation with Abs_{450nm} = 3.81. whereas, essential oils did not show high antioxidant activity except essential oil of *M. deserti* which showed IC_{50} = 21.42 µg/mL in *β*-caroten/linoleic acid assay.

Methanolic extracts and essential oils were tested for inhibition of acetylcholinesterase enzyme (AChE) and butyrylcholinesterase enzyme (BChE). IC_{50} of BChE inhibitory ranged between 40.94 µg/mL and 142.07 µg/mL. However, essential oils did not record any inhibation except *A. stiparum* subsp. *sabulicola* essential oil with IC_{50} = 212.14 µg/mL. Concerning AChE inhibition, MeOH extract of *C. furfuracea* demonstrated higher AChE activity than other extracts (IC_{50} = 164.4 µg/mL). The essential oils were not active against AChE.

In the study of antimicrobial and antibiofilm formation, the MeOH extract of *A. stiparum* subsp. *sabulicola* showed the best minimum inhibitory concentration against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 with 1.56 mg/mL. The same MIC was obtained with MeOH extract of *R. suaveolens* against *B. subtilis* ATCC 6633. Concerning antibiofilm formation activity, MeOH extract of *C. furfuracea* exibited highest percentage of biofilm formation of *Staphylococcus aureus* ATCC 6538 P (87.90%) at 50 mg/mL.

Summary in Arabic (ملخص)

أجريت هذه الدراسة على أربع نباتات طبية من الصحراء الجزائرية وذلك قصد معرفة التركيب الكيميائي والفعالية البيولوجية لها، ثلاثة من النباتات قيد الدراسة تنتمي للعائلة المركبة (Asteraceae) وتتمثل في: *Anthemis stiparum* subsp. *sabulicola* و *Rhanterium suaveolens* و *Centaurea furfuracea* . ونبات واحد من العائلة الشفوية (Lamiaceae) وهو نبات *Marrubium deserti*.

أظهرت التحاليل الكيميائية للزيوت الأساسية الأربعة أن نسبة المركبات التربينية الأحادية (Monoterpenes) ضعيفة في الزيوت الأربعة أما باقي المركبات تختلف من نبات إلى آخر حيث تراوحت نسبة المركبات السيسكوتربينية بين 7.40 و 33.85 % عند الزيت الأساسي لنبات *R. suaveolens* و *M. deserti* على الترتيب. أما أعلى نسبة للمركبات الأوكسيجينة التربينية الأحادية فقد بلغت 48.25 % عند *R. suaveolens* ممثلة أساسا في مركب Perillaldehyde.

أجريت عدة تجارب على الزيوت الأساسية والمستخلصات الميثانولية لدراسة النشاطية المضادة للأكسدة، حيث أظهرت المستخلصات الميثانولية نشاطية أكبر من تلك المسجلة عند الزيوت الأساسية للنباتات الأربعة. حيث لوحظ أن المستخلص الميثانولي لـ *M. deserti* سجل أكبر نشاطية من باقي المستخلصات ومقاربة جدا للمركبات المرجعية. فقد بلغ التركيز المثبط لـ 50 % من الجذور الحرة $DPPH^\bullet$ حوالي $IC_{50} = 12.87$ µg/mL، بينما بلغ $IC_{50} = 2.51$ µg/mL عند اختبار *β*-caroten/linoleic acid. أما في اختبار CUPRAC فقد سجل المستخلص الميثانولي امتصاصية أكبر من تلك المسجلة عند المركبات المرجعية α-Tocopherol و BHA عند التركيز 800 µg/mL بلغت $Abs_{450nm} = 3.81$. بينما لم تبدي الزيوت الأساسية نشاطية كبيرة مضادة للأكسدة عدا الزيت الأساسي لـ *M. deserti* الذي أظهر $IC_{50} = 21.42$ µg/mL عند اختبار *β*-caroten/linoleic acid.

أما بخصوص دراسة تثبيط المستخلصات الميثانولية والزيوت الأساسية للإنزيم acetylcholinesterase (AChE) و إنزيم butyrylcholinesterase (BChE)، فقد تراوحت قيمة IC_{50} لتثبيط BChE ما بين 40.94 µg/mL و 142.07 µg/mL عند المستخلص الميثانولي لـ *R. suaveolens* و*A. stiparum* subsp. *sabulicola* على التوالي. فيما لم تسجل الزيوت الأساسية أي نشاطية تذكر عدا تلك المسجلة عن الزيت الأساسي لـ *A. stiparum* subsp. *sabulicola* والمقدرة بـ $IC_{50} = 212.14$ µg/mL. أما فيما يخص تثبيط انزيم AChE فقد المستخلص الميثانولي لـ *C. furfuracea* أفضل نشاطية بين باقي المستخلصات قدرت بـ $IC_{50} = 164.4$ µg/mL. أما الزيوت الأساسية فلم تظهر أي نشاطية اتجاه تثبيط الانزيم.

وفي الدراسة التي أجريت لمعرفة النشاطية المضادة للأحياء الدقيقة للمستخلصات الميثانولية وأيضا النشاطية المضادة لتكوين البيوفيلم فقد سجل المستخلص الميثانولي لـ *A. stiparum* subsp. *sabulicola* أفضل تركيز أدنى مثبط لنمو البكتيريا (MIC) قدر بـ 1.56 mg/mL اتجاه كل من *Staphylococcus aureus* ATCC 25923 و *Bacillus subtilis* ATCC 6633. ونفس التركيز أيضا سجل عند المستخلص الميثانولي لـ *R. suaveolens* اتجاه نفس البكتيريا *B. subtilis* ATCC 6633. أما فيما يخص النشاطية المضادة لتكوين البيوفيلم فقد أظهر المستخلص الميثانولي لـ *C. furfuracea* أعلى نسبة في تثبيط تكوين البيوفيلم لبكتيريا *Staphylococcus aureus* ATCC 6538 P بنسبة 87.90 % عند تركيز 50 mg/mL.

Summary in French (Résumé)

Cette étude est faite sur quatre plantes médicinales endémiques au désert algérien dans le but de déterminer leurs compositions chimiques ainsi que leurs activités biologiques. Trois de ces plantes appartiennent à la famille des Asteraceae ou Compositae (Astéracées ou Composées) qui sont: *Anthemis stiparum* subsp. *sabulicola, Rhanterium suaveolens* et *Centaurea furfuracea.* La quatrième plante appartient à la famille des Lamiaceae ou Labiatae: *Marrubium deserti.*

L’analyse chimique des huiles essentielles des plantes montre que le rapport des monoterpenes est faible. Ce taux varie de 4.32 % avec *R. suaveolens* et 5.88 % avec *A. stiparum* subsp. *sabulicola.* Pour les autres composants, les proportions varient d’une plante à l’autre. Le taux des sesquiterpenes fluctue de 7.40 % pour les *R. suaveolens* à 33.85% pour les *M. deserti* dans les huiles essentielles. Le pourcentage des composants monoterpènoïdes atteint les 48.25% pour les *R. suaveolens,* essentiellement représenté par le composé perillaldéhyde.

Les extraits méthanoliques ont montré une activité antioxydante plus élevée que les huiles essentielles et ce pour toutes les plantes. L’extrait de méthanol de *M. deserti* a montré la plus forte activité par rapport aux autres extraits. La concentration inhibitrice de 50 % des radicaux libres, $DPPH^{\bullet}$ a été IC_{50} = 12.87 µg/mL et IC_{50} = 2.51 µg/mL dans le test de *β*-carotène/l'acide linoléique. Avec le test du CUPRAC, l’extrait de méthanol a montré une plus grande absorption que les standards α-tocophérol et BHA à une concentration 800 µg/mL avec Abs_{450nm} = 3.81. Les huiles essentielles ne montrent pas une forte activité antioxydante, sauf pour l'huile essentielle de *M. deserti* qui a montré IC_{50} = 21.42 µg/mL dans le test de *β*-carotène/l'acide linoléique.

Les extraits méthanoliques et les huiles essentielles ont été testés pour l'inhibition de l’enzyme acétylcholinestérase (AChE) et l’enzyme butyrylcholinestérase (BChE). La valeur IC_{50} inhibitrice de BChE se situe entre 40.94 µg/mL et 142.07 µg/mL. dans les extraits méthanoliques de *R. suaveolens* et *Anthemis stiparum* subsp. *sabulicola*, respectivement. Les huiles essentielles n'ont pas enregistré d’inhibition sauf pour l’huile essentielle *Anthemis stiparum* subsp. *sabulicola,* avec IC_{50} = 212.14 µg/mL. L’extrait méthanolique de *C. furfuracea* a montré une activité inhibitrice de AChE plus élevé que les autres extraits (IC_{50} = 164.4 µg/mL). Les huiles essentielles ne sont pas actives contre l’enzyme AChE.

Dans l'étude des activités antimicrobiennes et antibiofilms pour six souches bactériennes et une levure *(Candida albicans)*, l'extrait méthanolique de *Anthemis stiparum* subsp. *sabulicola* a montré la meilleure concentration minimale inhibitrice (CMI) contre *Staphylococcus aureus* ATCC 25923 et *Bacillus subtilis* ATCC 6633 avec 1.56 mg/mL. La CMI a été observée avec l'extrait méthanolique de *R. suaveolens* contre *B. subtilis* ATCC 6633. En ce qui concerne l'activité de formation des antibiofilms, l’extrait méthanolique de *C. furfuracea* a manifesté le pourcentage le plus élevé de la formation de biofilm de *Staphylococcus aureus* ATCC 6538 P (87.90 %) à 50 mg/mL.

Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
BChE	Butyrylcholinesterase
BHA	Butylhydroxyanisole
BHT	Butylated hydroxytoluene
CFU	Colony-forming units
CLSI	Clinical and Laboratory Standards Institute
CUPRAC	Cupric reducing antioxidant capacity
C.V.Ds	Cardiovascular diseases
DPPH	2,2'-diphenyl-1-picrylhydrazyl
DTNB	5-5'-dithiobis2-nitrobenzoique
EI	Electron ionization
EO	Essential oil
FCR	Folin–Ciocalteu reagent
FID	Flame ionization detector
GAE	Gallic acid Equivalent
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
H_2O_2	Hydrogen peroxide
MeOH	Methanol
MIC	Minimal inhibitory concentration
NB	Nutrient broth
OD	Optical density
R	Bleaching rate
RE	Rutin Equivalent
RI	Retention index
ROS	Reactive oxygen species
SD	Standard deviation
SDB	Sabouraud dextrose broth
TSB	Tryptose-Soy Broth

List of figures

List of tables

Introduction

Introduction

Medicinal plants have been added to foods since ancient times, as flavoring agents, folk medicine and food preservatives. In addition to imparting characteristic flavors, certain medicinal plants prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity (Shan *et al.*, 2007). Medicinal plants and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Smid and Gorris, 1999). Being natural foodstuffs, medicinal plants appeal to many consumers who question the safety of synthetic food additives. Restrictions imposed by competent authorities on the use of some synthetic food additives have led to renewed interest in searching for alternatives, like natural antimicrobial compounds. The antimicrobial and preservative activities of spices and essential oils is well documented in many studies especially after the emergence of microbial resistance to antibiotics (Dorman and Deans, 2000). Some medicinal plants used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (Lis-Balchin and Deans, 1997).

Natural products obtained from medicinal plants are generally practiced and progressively used in management of many diseases. It has produced significant drugs both in their natural form as well as templates for synthetic modifications.

Many secondary metabolites have been reported from higher plants. Out of the various classes of natural products reported. phenyl propanoids, polyketides, terpenoids and alkaloids are the most important (Li *et al.*, 2006).

Numerous plants are capable of producing bioactive compounds, perhaps as a chemical defense mechanism against predation or infection. The recent interest in larvicidal compounds from higher plants is largely a result of the many problems associated with the extensive use of synthetic insecticides which are an increasing hazard to human health and the environment.

Reactive oxygen species (ROS) are derived from the metabolism of molecular oxygen (Halliwell, 1999). ROS normally exist in all aerobic cells in balance with biochemical antioxidants. Oxidative stress occurs when this critical balance is disrupted because of excess ROS, antioxidants depletion, or both. Because of its high metabolic rate and relatively reduced capacity for cellular regeneration, the brain is believed to be particularly susceptible to the damaging effects of ROS (Klein and Ackerman, 2003; Scandalios, 2002). Oxidative stress has been implicated as one of the earliest events in Alzheimer's disease (AD) (Barja, 2004). In neurodegenerative diseases like Parkinson's, Alzheimer's and amyotrophic lateral sclerosis (ALS), ROS damage has been reported within the specific brain region that undergo selective neurodegeneration. Protein oxidation has been reported in the hippocampus and neocortex of patients with AD, Lewy bodies in Parkinson's disease and within the motor neurons in ALS (Barja, 2004; Waris and Ahsan, 2006).

Acetylcholinesterase (AChE) is the principal enzyme involved in the hydrolysis of acetylcholine (ACh). The great reduction of this neurotransmissor in the cerebral cortex is a significant factor in AD. Since ancient times phytochemicals have been used in the Chinese and Ayurvedic cultures to restore and declining cognitive functions lost with progression of AD. Many essential oils and their monoterpenes have been also investigated for their capacity of inhibiting AChE such as eugenol (Dohi *et al.*, 2009).

Due to its variety of geographical locations, Algeria has a rich flora of medicinal plants. There are about 3500 plant species reported in Algeria among which 500 are regarded of medicinal values (Quézel and Santa, 1963).

One of the motives that stimulate the interest in this subject, especially in the desert area, is the diversity of vegetation and their content in natural products that can have therapeutic benefit.

In the study in hand, four medicinal desertic plants are highlighted with their biological effect through the study of antioxidant, antibiofilm and acetylcholineseterase activities of their essential oils and methanol extracts. Moreover, polyphenols and falvonoids content and GC/MS analysis of essential oils were carried out. Three of those plants which belong to Asteraceae or Compositae family are: *Centaurea furfuracea* Coss. & Dur., *Rhanterium suaveolens* Desf. and *Anthemis stiparum* subsp. *sabulicola* (Pomel) Oberpr. The later is endemic only in north fringe of Algerian Sahara. The last plant which belongs to Lamiaceae or Labiatae family, it is scientific name is: *Marrubium deserti* De Noé. Which is endemic in central and north Algerian Sahara.

The main objectives of this study were:

- ✓ Extraction of the essential oils and methanol extracts
- ✓ Determination of polyphenols and flavonoids content of methanol extracts.
- ✓ Determination the chemical composition of essential oils by GC/MS analysis.
- ✓ Antioxidant activity using three methods (DPPH, CURAC and β-caroten/linoleic acid).
- ✓ Acetylcholinesterase activity.
- ✓ Antimicrobial activity and antibiofilm formation.

Chapter 1

Essential oils

I. ESSENTIAL OILS

Essential oils are volatile, natural and complex compounds characterized by a strong odour and formed by aromatic plants as secondary metabolites. They are usually obtained by steam or hydro-distillation (Bakkali *et al.,* 2008). They have a low solubility in water but are soluble in fats, alcohol, organic solvents and other hydrophobic substances and are generally liquid at room temperature (Thormar, 2010).

Some plant families are particularly well known for their oil-bearing species. These include Apiaceae (Umbelliferae), Asteraceae (Compositae), Cupressaceae, Hypericaceae (sometimes included as a subfamily of the Guttiferae/Clusiaceae), Lamiaceae (Labiatae), Lauraceae, Fabaceae (Leguminosae), Liliaceae, Myrtaceae, Pinaceae, Piperaceae, Rosaceae, Rutaceae, Santalaceae, Zingiberaceae and Zygophyllaceae (Thormar, 2010).

Essential oils can be located in all plant organs: flowers (*Ferulago angulata*) (Akhlaghi, 2008), leaves (*Torilis arvensis*) (Saad *et al.*, 1995), and although this seems unusual, in: roots (*Heracleum persicum*) (Mojab and Nickavar, 2010), rhizomes (*Zingiber officinale*) (Geiger, 2005), wood (*Santalum album*) (Howes *et al.*, 2004), bark (*Cinnamomum verum*) (Jham *et al.*, 2005), fruits (*Daucus carota*) (Glišić *et al.*, 2007), or seeds (*Daucus carota*) (Musa Özcan and Chalchat, 2007).

1. Chemistry of Essential Oils

In general, pure essential oils can be subdivided into two distinct groups of chemical constituents; the hydrocarbons which are made up almost exclusively of *terpenes* (monoterpenes, sesquiterpenes, and diterpenes), and the oxygenated compounds which are mainly esters, aldehydes, ketones, alcohols, phenols, and oxides.

Essential oils may comprise volatile compounds of terpenoid or non-terpenoid origin. All of them are hydrocarbons and their oxygenated derivatives. Some may also contain nitrogen or sulphur derivatives. They may exist in the form of alcohols, acids, esters, epoxides, aldehydes, ketones, amines, sulphides, *etc.* Monoterpenes, sesquiterpenes and even diterpenes constitute the composition of many essential oils. In addition, phenylpropanoids, fatty acids and their esters, or their decomposition products are also encountered as volatiles (Berger, 2007).

There are two main types of component in essential oils: hydrocarbons (carbon and hydrogen only) and oxygenated hydrocarbons, which also contain oxygen. These are subdivided into groups based on their structures.

1.1. Terpenes Hydrocarbons

Terpenes are a very large group of plant hydrocarbons formed by polymerization of five carbon atom units (isoprenes) that form in both chains and rings. They may be reduced and oxidized into a vast array of other compounds including alcohols, lactones, acids and aldehydes, thus the starting point of synthesis of the majority of aromatic compounds. Terpenes are present in the resinous foliage of leaves. Terpene compounds heavier than diterpene do not contribute to the odour of essential oils, although they may be present (Hunter, 2009).

The majority of essential oils fall into this category; these contain molecules of hydrogen and carbon only and are classified into terpenes (monoterpenes: C10, sesquiterpenes: C15, and diterpenes: C20). These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic or tricyclic) or aromatic. Limonene, p-menthane, α-pinene, *β*-pinene, α-sabinene, p-cymene, myrcene, α-phellandrene, thujane, fenchane, farnesene, azulene, cadinene and sabinene are some examples of this family of products. These compounds have been associated with various therapeutic activities (Djilani and Dicko, 2012).

1.1.1. Monoterpenes

Monoterpene compounds are found in nearly all essential oils and have a structure of ten carbon atomes (two isoprene units) (Hunter, 2009) with the molecular formula $C_{10}H_{16}$ (Pengelly, 2004). Approximately 1500 monoterpenoids have been described (Breitmaier, 2006), Monoterpenes may be cyclic (that is, ring-forming) or acyclic (also known as linear), regular or irregular, and their derivatives include alcohols, esters, phenols, ketones, lactones, aldehydes and oxides (Keszei *et al.*, 2008; Thormar, 2010). Monoterpenes have many different activities. Some are antiseptic, anti-bacterial, stimulating analgesic (weak), and expectorant, while other specific terpenes have anti-fungal, anti-viral, antihistaminic, anti-rheumatic, anti-tumor (antiblastic, anti-carcinogenic), hypotensive, insecticidal, purgative, and pheromonal properties. some monoterpenes include the following:

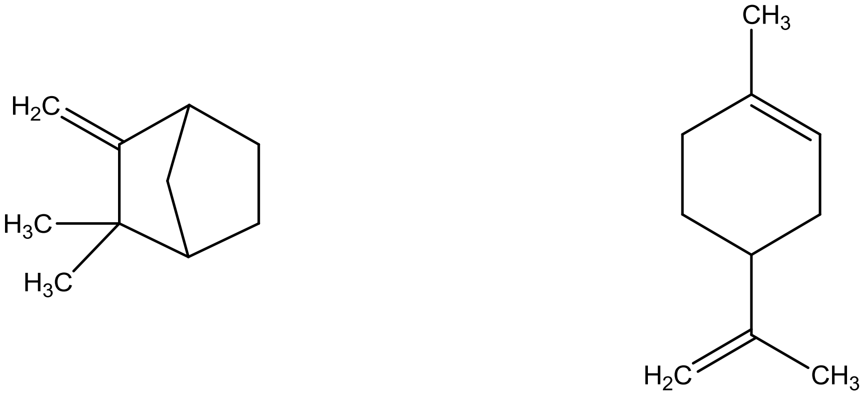
Pinenes (α- & *β*-) have strong antiseptic, anti-bacterial, anti-fungal, and expectorant properties.

Camphene is an insect repellant and according to the Phytochemical Dictionary, it is “used to reduce cholesterol saturation index in the treatment of gallstones”.(Baxter *et al.*, 1998)

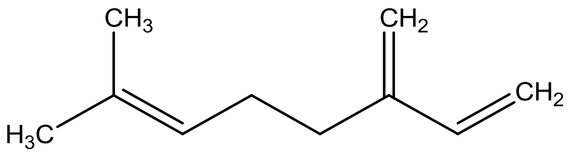
***β*-Myrcene** is a cancer-preventative (Higley and Higley, 1998).

d-limonene is anti-cancerous, anti-bacterial, anti-fungal, antiseptic (5x phenol), and highly anti-viral. It can be found in 90% of the Citrus oils (Higley and Higley, 1998).

α-Pinene *β*-Pinene



Camphene d-limonene



β-Myrcene

Figure 1. Chemical structure of some monoterpenes

Note: all chemical structures from (ChemSpider)

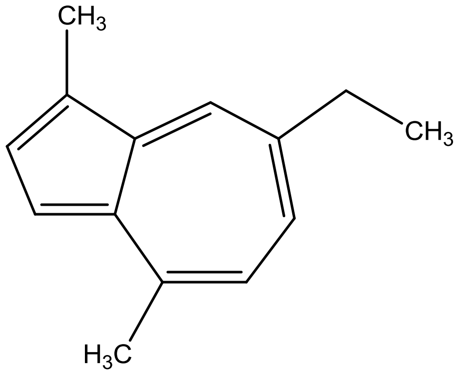
1.1.2. Sesquiterpenes

Sesquiterpenes are found in great abundance in essential oils and consist of 15 carbon atoms or three isoprene units linked to each other, head to tail, giving them the molecular formula $C_{15}H_{24}$. This formation can produce more than 300 different hydrocarbon sesquiterpenes (Hunter, 2009). Sesquiterpenes may be linear, branched or cyclic (Thormar, 2010)**.** They are antibacterial, strongly anti-inflammatory, slightly antiseptic and hypotensive and sedative. Some have analgesic properties, while others are highly antispasmodic. some sesquiterpenes include the following:

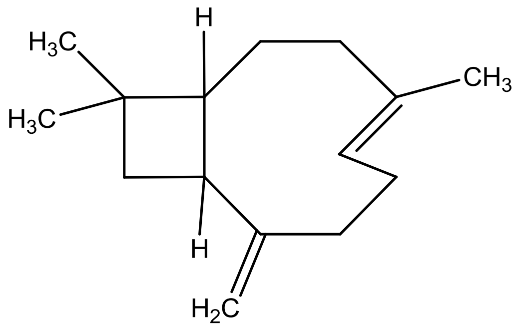
***β*-Caryophyllene** is anti-edemic, anti-inflammatory, anti-spasmodic, and an insect and termite repellant. Found in high proportions in plants from the Labiatae family

Chamazulene is very high in anti-inflammatory and anti-bacterial activity.

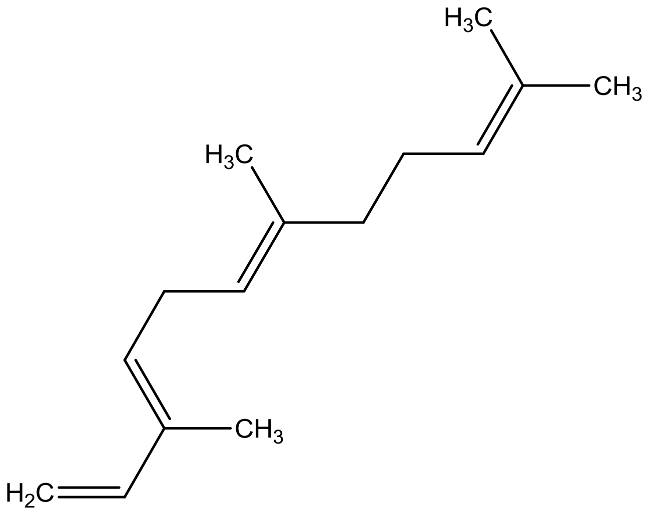
Farnesene is anti-viral in action (Higley and Higley, 1998).



Chamazulene



β-Caryophyllene



Farnesene

Figure 2. Chemical structure of some sesquiterpenes

1.2. Oxygenated Compounds

Oxygenated compounds contain oxygen molecules within their structures. These include alcohols, aldehydes, amides, carboxylic acids, esters, ketones, nitro compounds and oxides (Hunter, 2009).

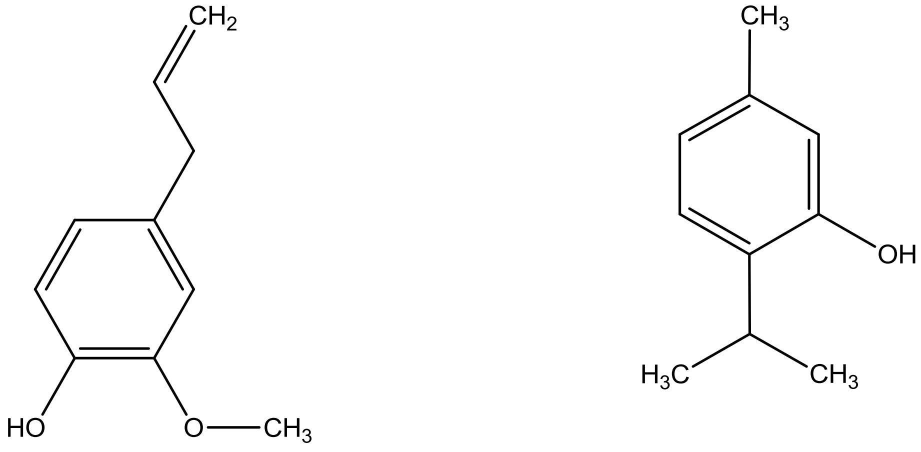
1.2.1. Phenols

Phenols are compounds that have one or more hydroxyl groups and their names usually end in –ol. (-OH) attached (Vermerris and Nicholson, 2007). However, in phenols, the -OH is attached directly to an aromatic ring, which makes the -OH group very weakly acidic and fairly reactive. Consequently, phenols may be irritating to the skin and mucous membranes (Hunter, 2009; Tisserand and Young, 2014). They are antiseptic, anti-bacterial, and strongly stimulating but can also be quite caustic to the skin. They contain high levels of oxygenating molecules and have anioxidant properties.

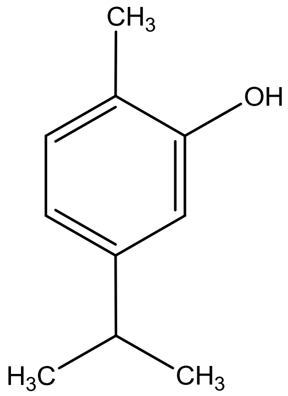
Eugenol may be found in clove and cinnamon oil.

Thymol is found in thyme and may not be as caustic as other phenols.

Carvacrol may be found in oregano and savory. Researchers believe it may possibly contain some anti-cancerous properties.(Higley and Higley, 1998).



Eugenol Thymol

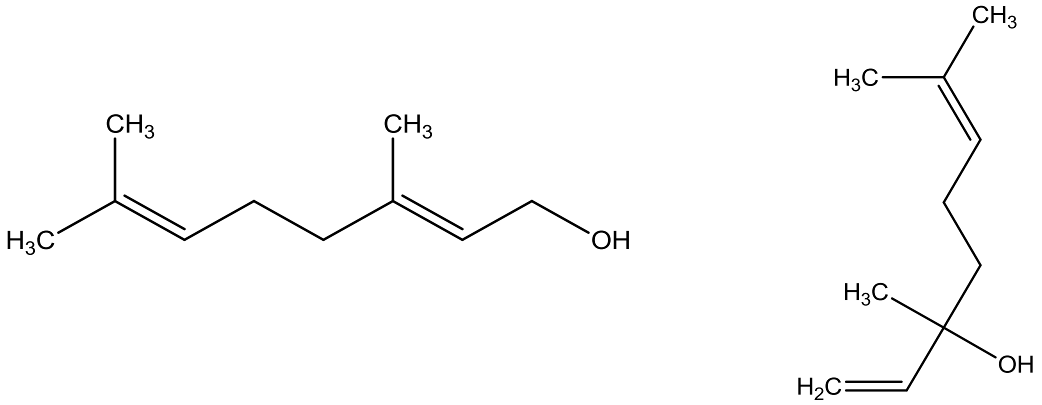


Carvacrol

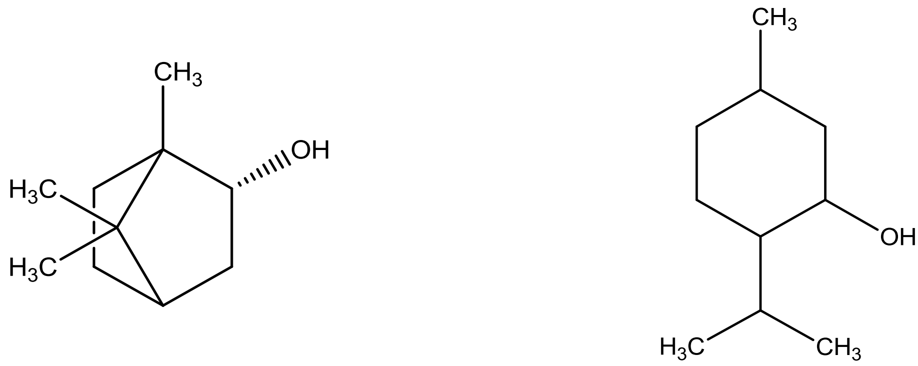
Figure 3. Chemical structure of some phenols

1.2.2. Alcohols

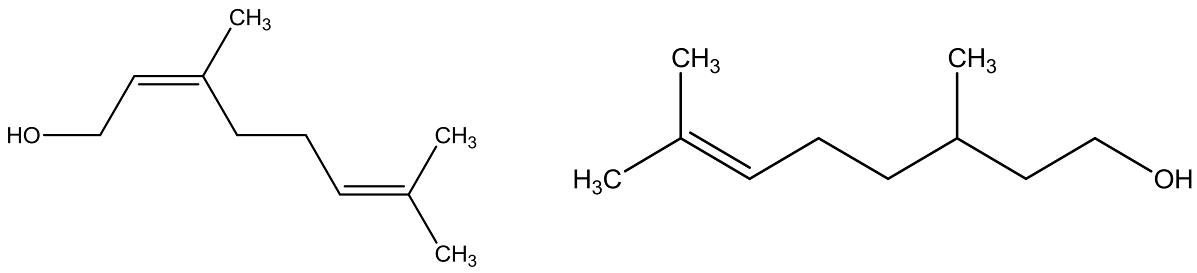
Alcohols are very similar to phenols and aldehydes in structure. Alcohols contain the hydroxyl functional group, and are perhaps the most varied group of terpene derivatives found in essential oils. The names of all alcohols end in –ol. Monoterpene alcohols are not large in number, but occur in a great many number of essential oils. There are many sesquiterpene alcohols, but most of them are found in few essential oils. They are antimicrobial, antiseptic, tonifying, balancing and spasmolytic. Examples of essential oil alcohols are linalool, menthol, borneol, santalol, nerol, citronellol and geraniol (Djilani and Dicko, 2012; Hunter, 2009; Pengelly, 2004; Tisserand and Young, 2014).



geraniol linalool



borneol menthol

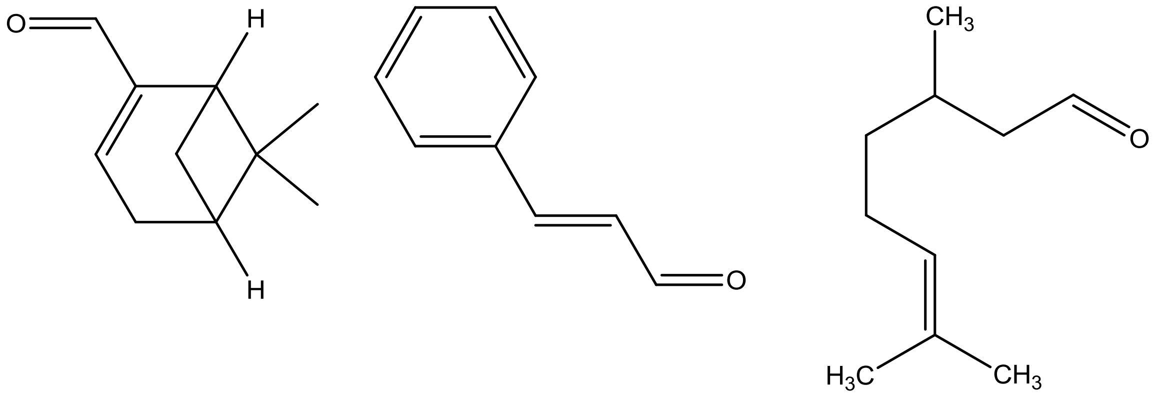


nerol citronellol

Figure 4. Chemical structure of some Alcohols

1.2.3. Aldehydes

These compounds contain the –CHO functional group, which one hydrogen atom is bonded to a carbonyl group at the end of a hydrocarbon chain. The names of aldehydes end in -al or –aldehyde. Therapeutically, certain aldehydes have been described as: antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic and spasmolytic. Common examples of aldehydes in essential oils include citral (geranial and neral), myrtenal, cuminaldehyde, citronellal, cinnamaldehyde and benzaldehyde (Djilani and Dicko, 2012; Pengelly, 2004; Tisserand and Young, 2014).

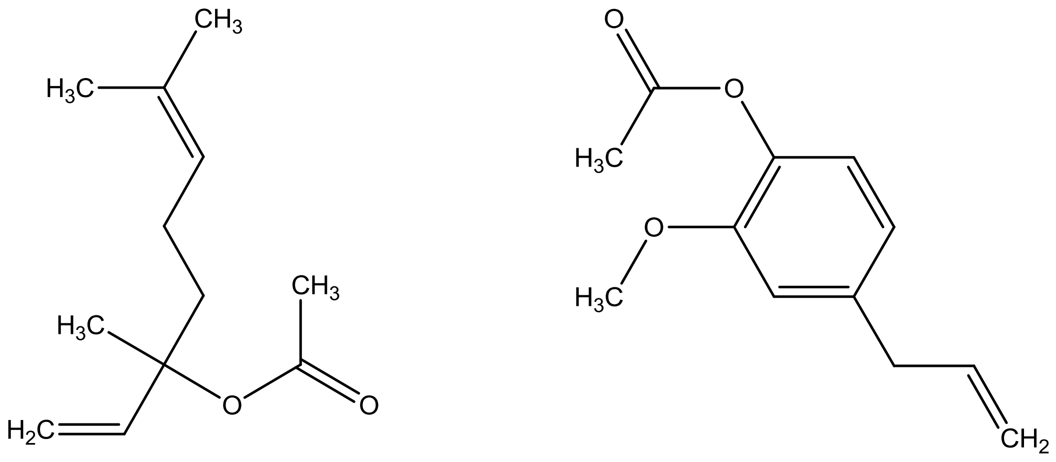


myrtenal cinnamaldehyde citronellal

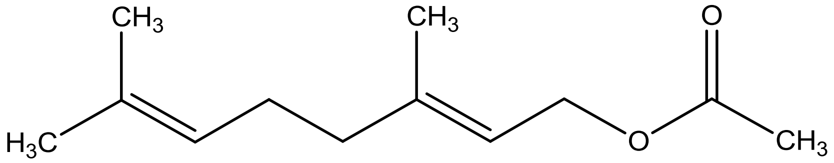
Figure 5. Chemical structure of some Aldehydes

1.2.4. Esters

Esters (R-CO-O-R') are formed from acids and alcohols, their names generally contain the roots: -yl and –ate. The esters found in essential oils are normally very fragrant and tend to be fruity and their therapeutic effects include being sedative and antispasmodic. Some esters also have anti-fungal and anti-microbial properties. They are generally present in small amounts. They include for example, linalyl acetate, geraniol acetate, eugenol acetate and bornyl acetate (Djilani and Dicko, 2012; Hunter, 2009; Pengelly, 2004; Tisserand and Young, 2014).



linalyl acetate eugenol acetate

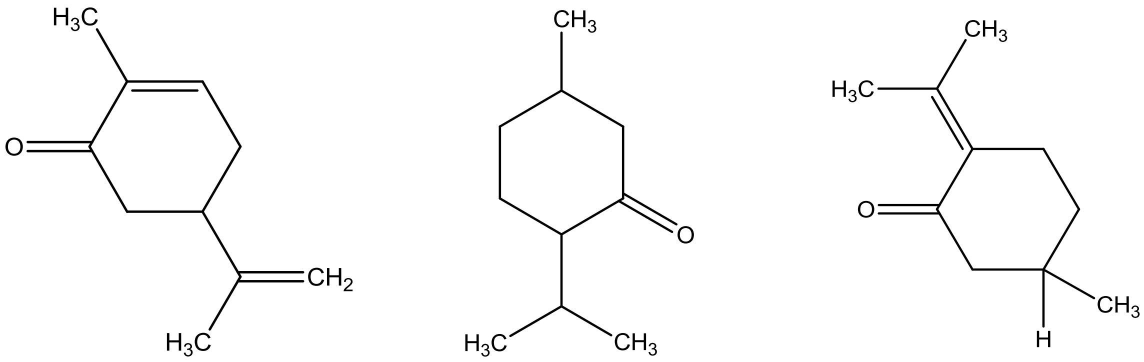


geraniol acetate

Figure 6. Chemical structure of some Esters

1.2.5. Ketones

Ketones are structurally similar to aldehydes and also possess a carbonyl group. Their names generally end in –one. Ketones are often present in small quantities in plants and provide fruity flavours in fruits. In some cases, ketones are neurotoxic and abortifacients such as camphor and thujone, but have some therapeutic effects. They may be mucolytic, cell regenerating; sedative, antiviral, analgesic and digestive. Common examples of ketones found in essential oils include carvone, menthone, pulegone, fenchone, camphor, thujone and verbenone (Djilani and Dicko, 2012; Gali-Muhtasib *et al.*, 2000; Hunter, 2009; Tisserand and Young, 2014).



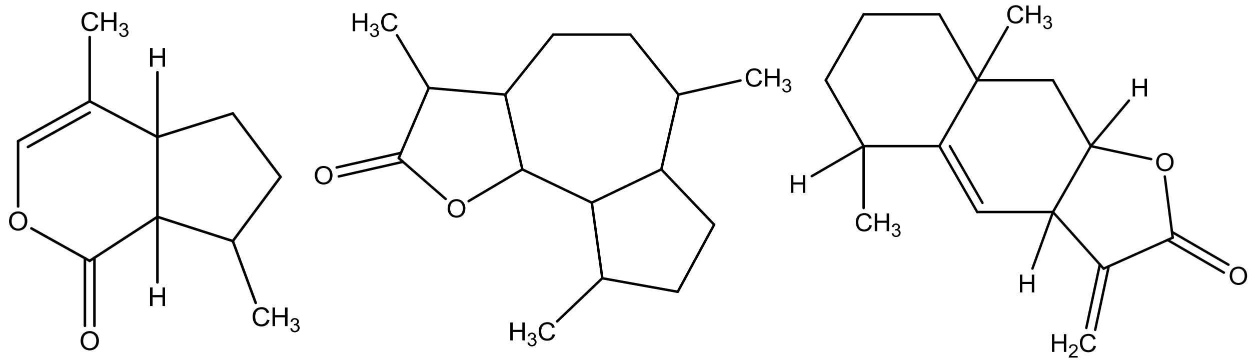
carvone menthone pulegone

Figure 7. Chemical structure of some Ketones

1.2.6. Lactones

Lactones are cyclic esters, contain an ester functional group in the cyclic part of the molecule. The names of lactones are rather variable, although the suffixes -olide and -lactone are fairly common. Some examples of lactones are nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone, epinepetalactone, aesculatine, citroptene, and psoralen. They may be used for antipyretic, sedative and hypotensive purposes, but their contraindication is allergy, especially such involving the skin (Djilani and Dicko, 2012; Hüsnü *et al.*, 2007; Tisserand and Young, 2014).

Coumarin is a benzenoid lactone that is found in several essential oils, as well as in the form of derivatives. Consisting of two fused rings, it might be considered as a structural moiety as well as a functional group. Coumarin has a vanilla-like odor, and is responsible for the smell of new-mown hay. Citropten, the 5,7-dimethoxy derivative of coumarin, is phototoxic (Djilani and Dicko, 2012).

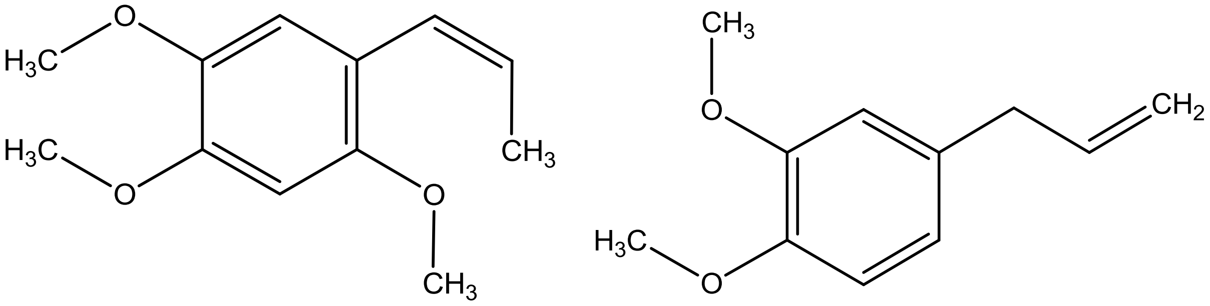


nepetalactone costuslactone alantrolactone

Figure 8. Chemical structure of some Lactones

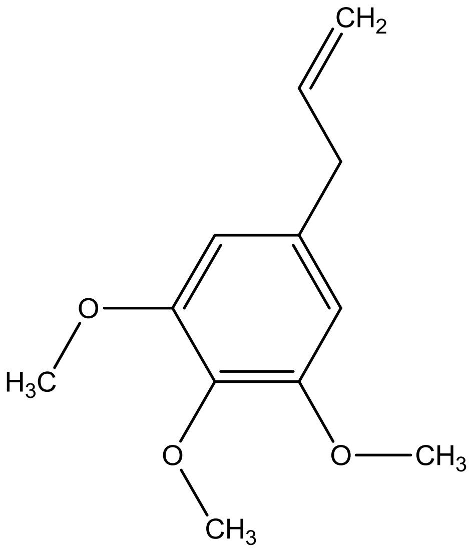
1.2.7. Ethers

Ethers are compounds in which an oxygen atom in the molecule is bonded to two carbon atoms. Common examples of Ethers in essential oils include *β*-Asarone, Elemicin, Estragole, Methyleugenol, Phenylethyl methyl ether, Myristicin and Safrole. Ethers also exist in cyclic forms, where an oxygen atom forms part of a ring. These ethers are also known as oxides. The most important oxide found in essential oils is cineole, which exists in two forms (1,8-cineole and 1,4-cineole). Other examples of oxides are bisabolone oxide, linalool oxide, sclareol oxide and ascaridole. Their therapeutic benefits are expectorant and stimulant of nervous system. Another type of cyclic ether is the epoxide. In this case, the oxygen atom forms part of a 3-membered ring with two carbon atoms, e.g., limonene 1,2-epoxide (Buckle, 2003; Djilani and Dicko, 2012).



β-Asarone

Methyleugenol

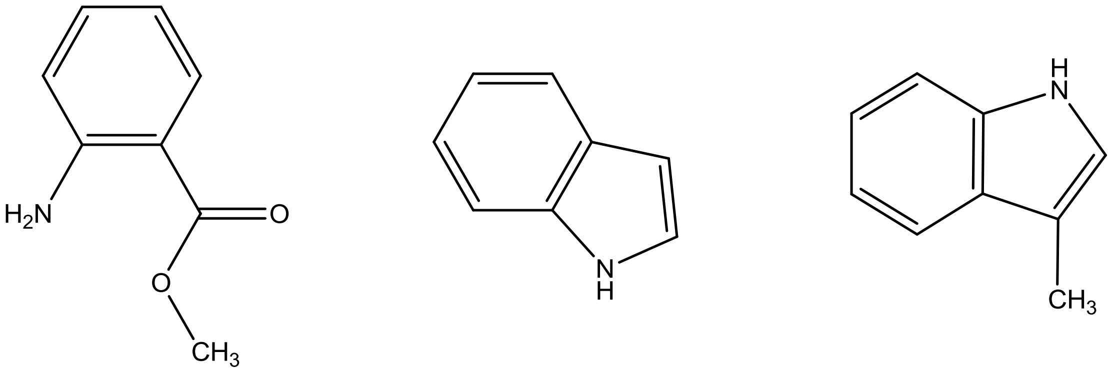


Elemicin

Figure 9. Chemical structure of some Ethers

1.2.8. Nitrogen Compounds

Methyl anthranilate is a very freshly scented citrus-floral odour compound. As secondary metabolites, amines maybe generally involved in growth regulation of plants and along with other aromatic chemicals, methyl anthranilate has been found to be both a bird attractant and repellent. Amines are very reactive to air and darken on exposure, as well as being photosensitive. They can also react with aldehydes to form aldimines. Amines are produced through a degradation pathway controlled by amine oxidase enzymes, within the amino acid pathway. Indole and skatole are two other nitro compounds aromatic compounds that are found in plants. They are heterocyclic compounds and act as hormones in plants (Hunter, 2009).

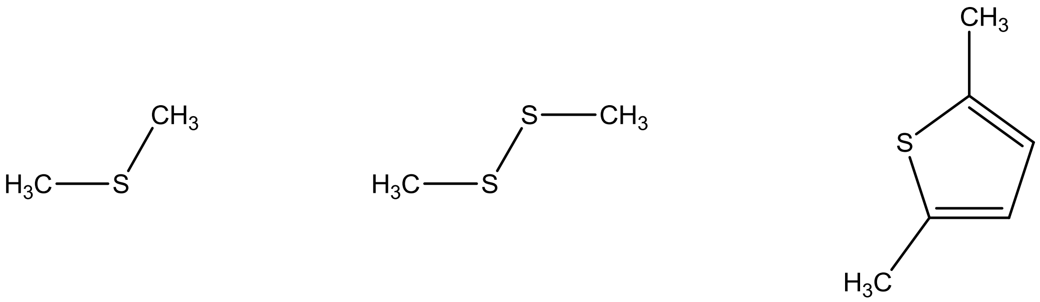


Methyl anthranilate Indole skatole

Figure 10. Chemical structure of some Nitrogen Compounds

1.2.9. Sulphur Compounds

A few plants are known to contain volatile sulphur compounds such as dimethyl sulphide, dimethyl disulphide and dimethylthiophene in garlic, onion, leek and shallots. Little is known about the purpose of sulphur compounds in plants except they play some role against pathogens and nitrogen detoxication of plants. Sulphur compounds are believed to be derived through a sulphur reduction pathway (Hunter, 2009).



dimethyl sulphide dimethyl disulphide 2,5-Dimethylthiophene

Figure 11. Chemical structure of some Sulphur Compounds

2. Biosynthesis of essential oils

The chemicals present in essential oils are synthesized during normal development of the herbs and may be classified as hydrocarbons built from multiple of 5-carbon hemiterpenoid units, oxygenated derivatives of these hydrocarbons, aromatic compounds having a benzoid structure and compounds containing sulphur or nitrogen (Makang’a, 2012). Chemically, the essential oils are composed of terpenoids and aromatic polypropanoids synthesized via the melvalonic acid pathway for terpenes and the shikimic acid pathway for aromatic polypropanoids (Simon *et al.*, 1990). These pathways are schematically represented in Figure 12 .

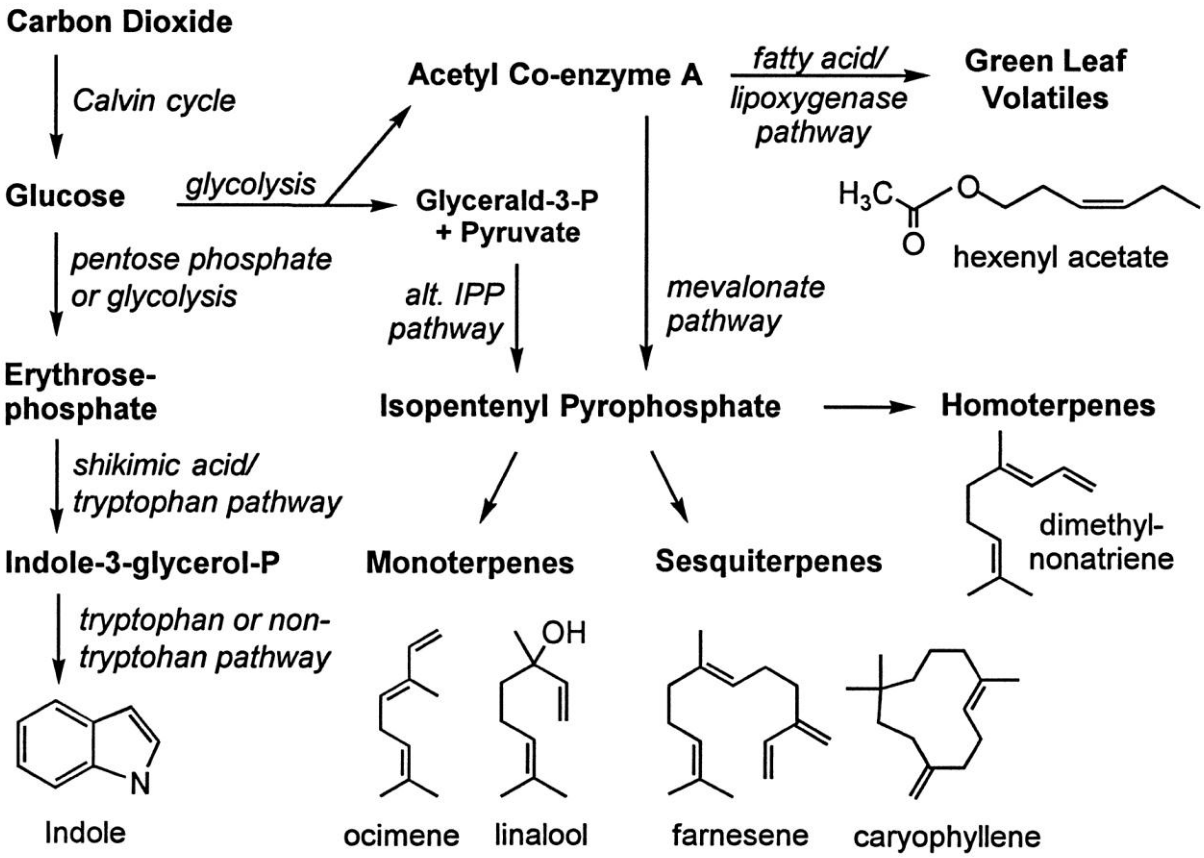


Figure 12. Biosynthetic pathways leading to the release of plant volatiles (Paré and Tumlinson, 1999)

3. Extraction of essential oils

The choice of extraction method for the recovery of essential oils and other volatile materials from plants has great bearing on the composition and quality of the crude product and the viability of the whole enterprise. There are a variety of methods available for extracting volatile constituents from plants. The chemical composition and yield of the extract will vary greatly according to the chosen method of extraction. True essential oils are extracted through various distillation methods. However a number of volatile constituents of plants are very fragile and susceptible to heat, reactive to moisture during extraction and are difficult to liberate from the surrounding plant material, thus other more situational effective methods of extraction are also utilized (Hunter, 2009).

Methods to isolate essential oils may be categorized into enfleurage, steam distillation, solvent extraction, hydrodistilation, and supercritical fluid extraction. Hydrodistillation or steam distillation is the most widely utilized physical method for isolating essential oils from the botanical material (Hussain, 2009; Masango, 2005; Whish, 1996)**.**

3.1. Distillation

This is the most popular. widely used and cost-effective method for producing essential oils throughout the world. Distillation simply implies vaporizing or liberating the volatile compounds from the oil glands in the plant tissue in the presence of moisture, by applying high temperature and then cooling the vapour mixture to separate the oil from the water on the basis of the immiscibility and density of the essential oil with respect to water. There are different techniques of distillation: water distillation (only water is used), water and steam ditillation (both water and steam are used) and direct steam distillation (only steam is used) (Bialecki and Smadja, 2014; Handa, 2008).

3.1.1. Hydro-distillation

In water distillation, the plant material is placed inside a still compartment and immersed in water that is then brought to boil by heating. This process frees plant biochemicals, particularly volatile compounds (essential oils), from the plain tissue. The vapor is then condensed by indirect cooling with water. The distillate is then filtered through a separator to separate oil from distillate water. It is mostly applicable to such plant material which is dried initially in air and the constituents are not degraded by boiling upto 100 °C (Handa, 2008; Kar, 2003).

3.1.2. water and steam distillation

The equipment used for water and steam distillation is similar to that used for water distillation. The difference is that the plant material is placed above the boiling water on a perforated grid in water and steam distillation. When compared with water distillation. this method produces a higher yield of oil with less hydrolysis and polymerization while being faster and more energy efficient. It is often suitable for such plant material, whether fresh or dried, the constituents of which undergo degradation by direct boiling (Handa, 2008; Kar, 2003).

3.1.3. Direct Steam distillation

Direct Steam distillation is a method for distilling compounds which are heat-sensitive, the steam used to distil plant materials is generated from a boiler placed outside the still. This distillation method is advantageous because the released steam can easily be controlled and the temperature generated will not exceed 100 °C so thermal degradation of phytochemicals is less likely to occur. It is invariably applicable to fresh drugs that is loaded with sufficient natural moisture and hence no maceration is required (Handa, 2008; Kar, 2003).

3.2. Expression

Expression or cold pressing is a process in which the oil glands within the peels are mechanically crushed to release their content. Expression is used almost exclusively for citrus family (Clarke, 2008; Kubeczka, 2010).

3.3. Enfleurage

Some flowers do not respond well to heat, so the essential oil must be extracted using some other method. In enfleurage, thin layers of cold, odourless fat are coated onto glass plates called *chassis* and the plant material is spread in layers onto the top of the fat. Other chassis with fat and plant material are stacked onto each other and the essential oil is absorbed into the fat. The resulting oil/fat mixture is called a *pomade*. The pomade is washed with hexane to dissolve the essential oil. After removal of hexane, the residue is washed with alcohol and the resultant solution is evaporated to give purer essential oil (Buckle, 2003; Clarke, 2008).

3.4. CO_2 extraction (Supercritical Carbon Dioxide gas)

CO_2 becomes hypercritical at 33 °C, which is a state in which it is not really gas or liquid, but has qualities of both, and is an excellent solvent to use in the extraction of essential oils since the low temperature required and the fact that the process is near to instantaneous. CO_2 is furthermore inert and therefore does not chemically interact with the essence that is being extracted. To remove the carbon dioxide solvent, you simply need to remove the pressure under which it is kept. This process has to take place in a closed chamber for the hypercritical pressure required for carbon dioxide is 200 atmospheres - that is 200 times the pressure of normal atmosphere (Buckle, 2003; Clarke, 2008; Hunter, 2009).

3.5. Solvent extraction

An organic solvent such as propanone, petroleum ether or hexane is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing a combination of wax and essential oil. From the concentrate, pure alcohol is used to extract the oil. When the alcohol evaporates, the oil is left behind. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system (Clarke, 2008).

3.6. Microwave-Assisted Extraction

Microwave is a non-contact heat source which can achieve a more effective and selective heating. With the help of microwave. In this method, plant materials are extracted in a microwave reactor with or without organic solvents or water under different conditions depending on the experimental protocol. The first Microwave-Assisted Extraction of essential oils was proposed as compressed air microwave distillation. Based on the principle of steam distillation, the compressed air is continuously injected into the extractor where vegetable matrices are immersed in water and heated by microwave. The water and essential oils are condensed and separated outside the microwave reactor (Li *et al.*, 2014).

Table 1. Advantages and disadvantages of various extraction processes (Buckle, 2015)

Extraction process	**Advantages**	**Disadvantages**
Distillation	Economical large quantities can be processed Little labor needed	Changing constituents Depending on time/temp
Expression	No heat required Simple apparatus	Some flavoring left Only citrus peel oils Oxidize quickly
Enfleurage	Low temperature needed	Time consuming Labor intensive expensive
CO_2 extraction	Constant product No heat used	Expensive Different chemistry to essential oil
Solvent extraction	Constant product	Solvent residues Different chemistry to essential oil

4. Biological activities of Essential oils

4.1. Antioxidant Activity

Essential oils and extracts from botanical materials are known to have varying degrees of antioxidant activities. Some recent publications showed antioxidative activities of essential oils. Some of these essentials oils and extracts have been reported to be more effective than some synthetic antioxidants. Literature reported the antioxidant activities of the *Mentha* essential oils. Recently, many studies have focused on the biological and antioxidant activities of the Origanum and Rosemary essential oils. Rosemary’s antioxidant extracts are still used to extend the shelf-life of prepared foods. *Melissa officinalis* essential oils and extracts also exhibited good antioxidant potential. The antioxidant effects of plant essential oils and extracts are mainly due to the presence of hydroxyl groups in their chemical structure (Bakkali *et al.*, 2008; Djilani and Dicko, 2012; Hussain, 2009).

The antioxidant activity that some essential oils possess is not surprising in view of the presence of phenol groups. It is well known that almost all phenols can function as antioxidants of lipid peroxidation because they trap the chain-carrying lipid peroxyl radicals. Plant phenolics are multifunctional and can act as reducing agents, hydrogen-donating antioxidants and singlet-oxygen quenchers; therefore, dietary antioxidants are needed for diminishing the cumulative effects of oxidative damage (Koroch *et al.,* 2007; Ruberto and Baratta, 2000).

Many EOs also exhibit antioxidant activity and therefore several studies have been carried out in order to elucidate the activity of the components. For instance, *γ*-terpinene retarded the peroxidation of linoleic acid, sabinene showed strong radical-scavenging capacity, *α*-pinene and limonene showed low antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, while terpinene and terpinolene showed high hydrogen-donating capacity against the DPPH radical (Koroch *et al.,* 2007).

4.2. Antimicrobial Activity

A number of methods used for evaluation of Antimicrobial activity of essential oils have been reported in literature. Different assays like disc diffusion assay, well diffusion assay, microdilution assay, measurement of minimum inhibitory concentration are often used for measuring the antimicrobial activity of essential oils and plants based constituents (Burt, 2004).

A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active constituents, mainly attributable to isoprenes such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols (Burt, 2004; Kalemba and Kunicka, 2003; Koroch *et al.*, 2007).

In the antimicrobial action of essential oil components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of EO components. Therefore, a rank of activity has been proposed as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons (Kalemba and Kunicka, 2003).

4.3. Cytotoxic activity

Due to a number of chemical constituents, essential oils apparently have no specific cellular targets. Cytoplasmic membrane is passed through easily by essential oils which disrupt its structure and make it permeabilized. Cytotoxicity can thus cause such damage to cell-membrane. Essential oils have ability to coagulate the cytoplasm, hence damaging lipids and proteins. Injury to the cell wall and cell membrane can lead to lysis and to the escape of macromolecules (Bakkali *et al.*, 2008; Burt, 2004).

Various assays are reported in the literature to evaluate the cytotoxic activities of essential oils or their main constituents using fluorescent dyes or specific cell staining including MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) test. MTT assay has been extensively reported because of its simplicity and reliability to measure cell viability for screening of antiproliferative agents (Hussain, 2009).

Mostly alcohols, phenols and aldehydes are responsible for the cytotoxicity of essential oils. This cytotoxic property is of immense worth with regard to the applications of essential oils, not only for the preservation of marine or agricultural products but also against certain animal or human parasites or pathogens (Bruni *et al.*, 2004; Hussain, 2009).

Essential oils along with some of their constituents are certainly effective against a variety of organisms including virus, bacteria and fungi. Carvacrol, a main constituent of *Origanum majorana* and *Melissa officinalis* essential oils, is reported to reduce the fluidity of the membrane by changing its fatty acid profile (Hussain, 2009).

4.4. Other activities

Potential activities for the treatment of AD were demonstrated in a pilot open-label study involving oral administration of the essential oil of *Salvia lavandulaefolia* (Perry *et al.*, 2003). Also, essential oil have been showed other activities such as antitumor, anti-nociceptive, anticancer and antiphlogistic (Adorjan and Buchbauer, 2010).

Chapter 2

Phenolic compounds

II. PHENOLIC COMPOUNDS

Phenolic or polyphenolic compounds are bioactive non-nutritional substances widely distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010; Huang *et al.*, 2009; Yordi *et al.*, 2012). They act as natural antioxidants and their presence contributes to the color, flavor and aroma of food (Yordi *et al.*, 2012). Chemically, they are compounds possessing one or more aromatic rings with one or more hydroxyl groups (Dai and Mumper, 2010).

1. Classification

According to number and arrangement of their carbon atoms, phenolic compounds can be classified in a number of ways (Vermerris and Nicholson, 2006). Harborne classified these compounds into groups based on the number of carbons in the molecule (Table 1) (Harborne, 1989), the most common groups of phenolic compounds are phenolic acids and flavonoids (Bravo, 1998; Harborne, 1989).

Table 2. The major classes of phenolics in plants

Number of carbon atoms	Basic skeleton	Number of phenolic cycles	Class	Examples
6	C6	1	Simple phenols, Benzoquinones	Catechol, Hydroquinone, 2,6-Dimethoxybenzoquinone
7	C6-C1	1	Phenolic acids, Phenolic aldehydes	Gallic, salicylic acids
8	C6-C2	1	Acetophenones, Tyrosine derivatives, Phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde, Tyrosol, p-Hydroxyphenylacetic acid, Homogentisic acid
9	C6-C3	1	Hydroxycinnamic acids, Phenylpropenes,	Caffeic, ferulic acids, Myristicin, Eugenol, Umbelliferone, aesculetin, Bergenon, Eugenin

			Coumarins, Isocoumarins, Chromones	
10	C6-C4	1	Naphthoquinones	Juglone, Plumbagin
13	C6-C1-C6	2	Xanthonoids	Mangiferin
14	C6-C2-C6	2	Stilbenoids, Anthraquinones	Resveratrol, Emodin
15	C6-C3-C6	2	Chalconoids, Flavonoids, Isoflavonoids, Neoflavonoids	Quercetin, cyanidin, Genistein
16	C6-C4-C6	2	Halogenated algal phenolic compounds	Kaviol A, colpol
18	(C6-C3)2	2	Lignans, Neolignans	Pinoresinol, Eusiderin
30	(C6-C3-C6)2	4	Biflavonoids	Amentoflavone
many	(C6-C3)n, (C6)n, (C6-C3-C6)n	n > 12	Lignins, Catechol melanins, Flavolans (Condensed tannins), Polyphenolic proteins, Polyphenols	Raspberry ellagitannin, Tannic acid

2. The main classes of phenolic compounds

2.1. Flavonoids

Flavonoids are polyphenolic compounds comprising 15 carbons, widely distributed in the plant kingdom (Crozier *et al.*, 2009). These compounds commonly have the basic skeleton of phenylbenzopyrone structure (C6-C3-C6) (Huang *et al.*, 2009), consists of two aromatic rings (A and B) linked through three carbons that usually form an oxygenated heterocycle (C) (Figure 14) (Bravo, 1998).

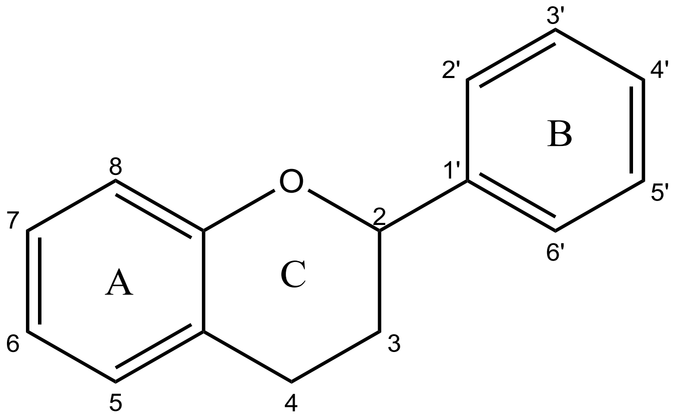


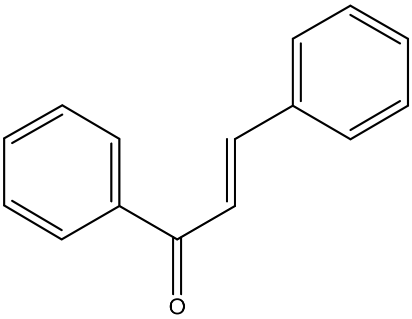
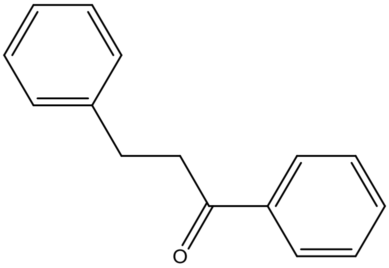
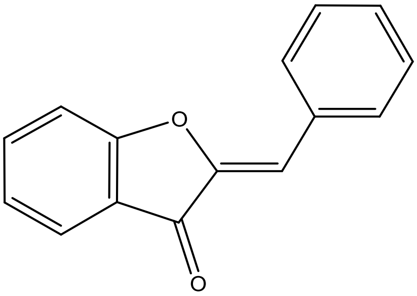
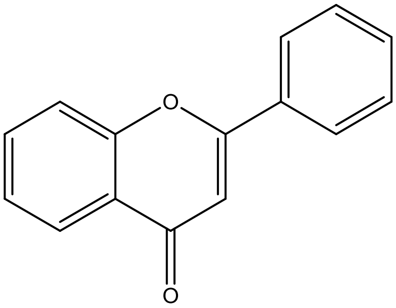
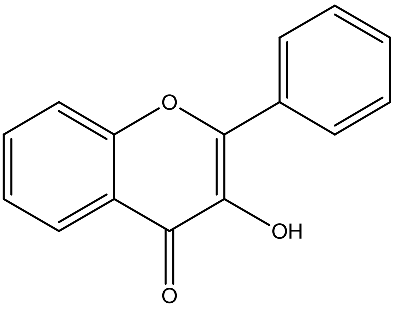
Figure 13. Basic Structure and numbering system of Flavonoids

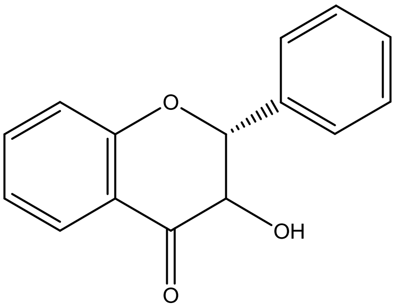
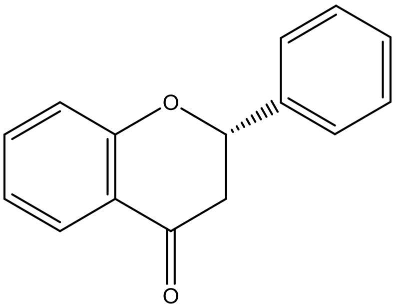
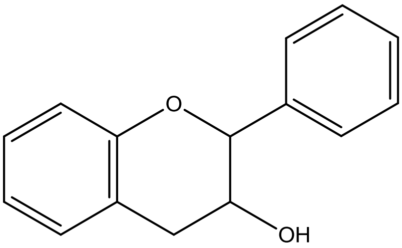
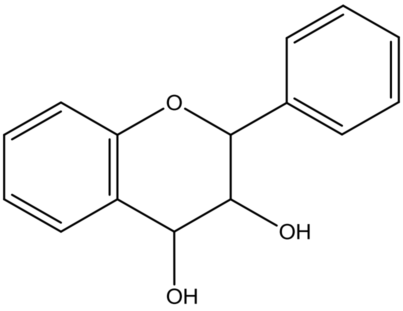
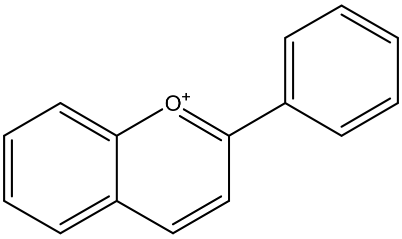
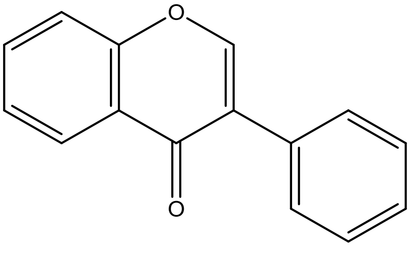
In nature, flavonoids can occur either in the free or conjugated forms, and often in plants they are mainly present as glycosides with a sugar moiety or more sugar moieties linked through an OH group (*O*-glycosides) or through carbon-carbon bonds (*C*-glycosides); but some flavonoids are present as aglycones. More than 80 different sugars have been discovered bound to flavonoids, and common glycosides include glucoside, glucuronide, galactoside, arabinoside, rhamnoside, apiosylglucoside, and malonyl (Huang *et al.*, 2009).

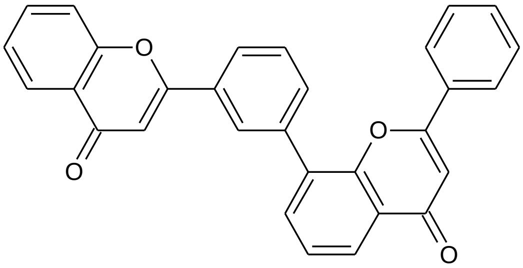
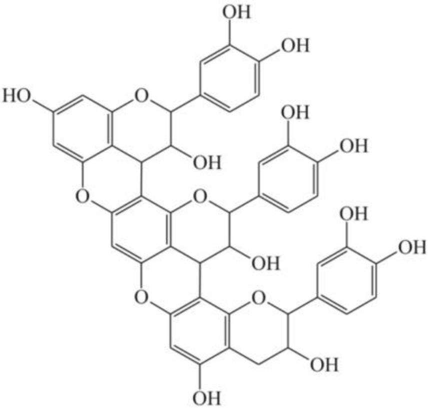
Flavonoids also occur as sulfated and methylated derivatives, conjugated with monosaccharides and disaccharides and forming complexes with oligosaccharides, lipids, amines, carboxylic acids and organic acids (Giada, 2013). Hydroxyl groups are usually present at the 4-, 5- and 7-positions. Sugars are very common, with the majority of flavonoids existing naturally as glycosides. Whereas both sugars and hydroxyl groups increase the water solubility of flavonoids, other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic (Crozier *et al.*, 2009).

According to the degree of hydroxylation and the presence of a C2-C3 double bond in the heterocycling pyrone ring (Giada, 2013), flavonoids can be divided into 13 classes (Table 2).

Table 2. Classification of flavonoids (Bravo, 1998)

Flavonoid	Basic structure
Chalcones	
Dihydrochalcone	
Aurones	
Flavones	
Flavonols	

dihydroflavonols	
Flavanones	
Flavanols	
Flavandiol or Leucoanthocyanidin	
Anthocyanidins	
Isoflavonoids	

Biflavonoid	
Proanthocyanidins or Condensed tannins	

2.2. Phenolic acids

Phenolic acids can be divided into two groups: Hydroxybenzoic acids and Hydroxycinnamic acids which are derivatives of benzoic and cinnamic acid, respectively.

Four Hydroxybenzoic acids occur commonly: phydroxybenzoic acid, vanillic acid, syringic acid, and protocatechuic acid. A common hydroxybenzoic acid is also salicylic acid (2-hydroxybenzoate). Gallic acid (Figure 14) is a trihydroxyl derivative which participates in the formation of hydrolysable gallotannins (Strack, 1997). It is commonly used in the pharmaceutical industry. Also, it is used as a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay; results are reported in *gallic acid equivalents* (Fiuza *et al.*, 2004; Waterhouse, 2001).

The hydroxycinnamic acids and their derivatives are more common than are the hydroxybenzoic acids in foods The four most widely distributed hydroxycinnamic acids in plants are *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid (Shahidi and Chi-Tang, 2005).

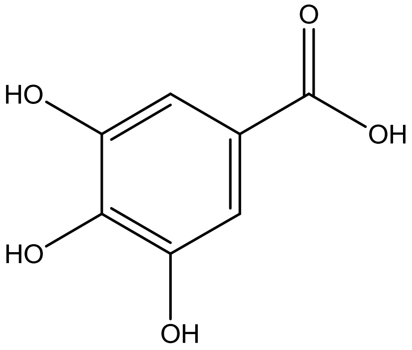


Figure 14. Structure of Gallic acid

2.3. Tannins

Tannins are complex polyphenolics of intermediate to high molecular weight ranging from 500 to > 20.000 Da. Found widely in the plant kingdom, they are found in leaves, twigs, flowers, fruits and tree bark. (Alokan and Aletor; Hagerman and Butler, 1978; Santos-Buelga and Scalbert, 2000). They are thought by some to constitute one of the most important groups of higher plant defensive secondary metabolites (Nascimento *et al.*, 2013).

Tannins can be classified into two major groups: hydrolysable tannins and non-hydrolysable or condensed tannins (Chung *et al.*, 1998). There is a third group of tannins, phlorotannins, which are only found in brown seaweeds and are not commonly consumed by humans (Giada, 2013).

2.3.1. The hydrolysable tannins

Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid, also called gallotannins, or with hexahydroxydiphenic acid, also called ellagitannins (Dai and Mumper, 2010). The best-known hydrolyzable tannin is tannic acid (Figure 15), which is a gallotannin consisting of a pentagalloyl glucose molecule that can further esterify with another five gallic acid units (Bravo, 1998).

Hydrolysable tannins are usually present in low amounts in plants Phenolic Extractives and Natural Resistance of Wood and can be broken down to their hydrolysis upon heating with weak acids or enzymatically (Alokan and Aletor).

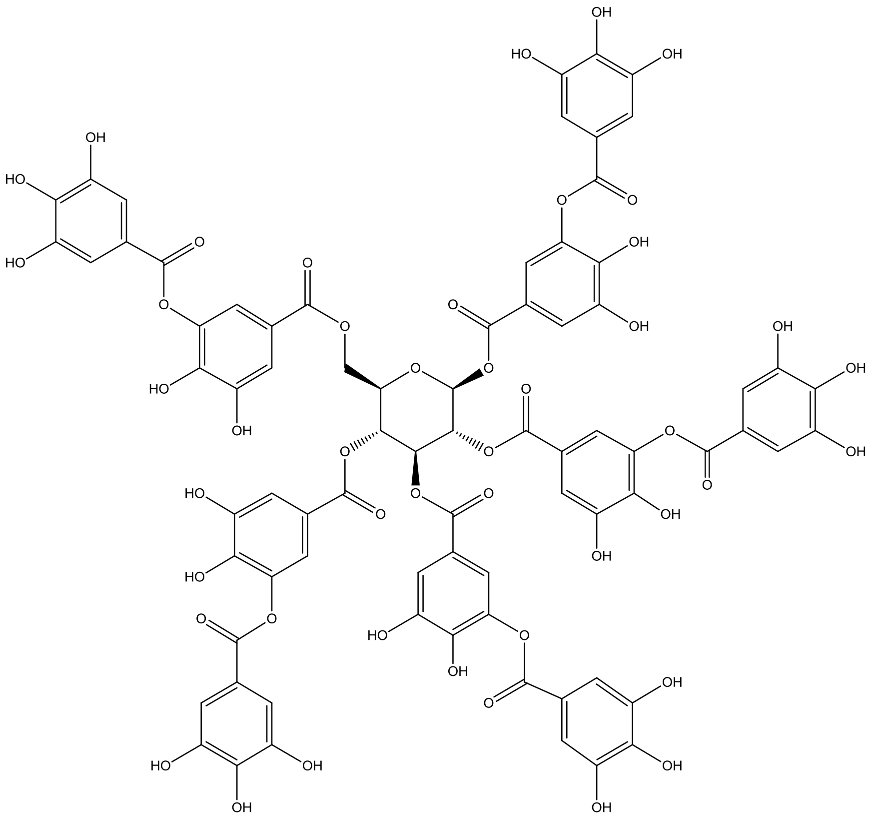


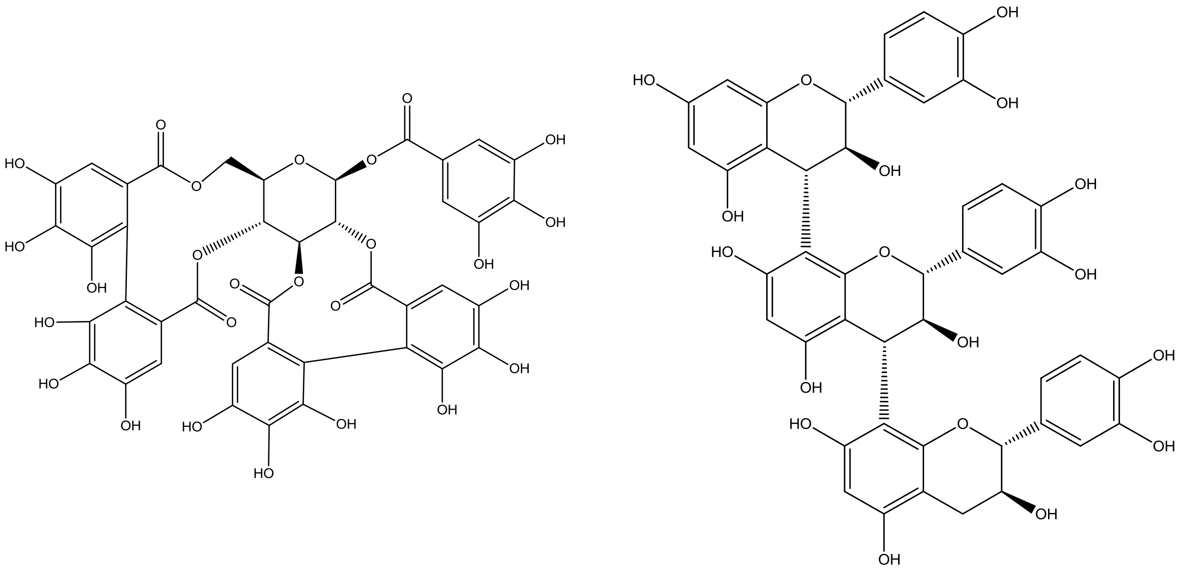
Figure 15. Structure of tannic acid

2.3.2. The condensed tannins

Condensed tannins (also called proanthocyanidins) are oligomers or polymers of flavonoid units (i.e. flavan-3-ol) linked by carbon-carbon bonds not susceptible to cleavage by hydrolysis (Nascimento *et al.*, 2013). Condensed tannins are natural preservatives and antifungal agents, found in high concentrations in the bark and wood of some tree species (Nascimento *et al.*, 2013; Zucker, 1983).

The ability of tannins to precipitate proteins depends on their molecular weight, water solubility, conformation and compatibility of binding sites. It also depends on the properties of the solvent especially its pH. Reed *et al.* (1985) showed that condensed tannins seem to be more important in forming complexes in feed than hydrolysable tannins (Alokan and Aletor).

The chemical structures of casuarictin (hydrolysable tannin) and Procyanidin C2 (condensed tannins) are shown in Figure 16.



casuarictin procyanidin C2

Figure 16. chemical structures of casuarictin and procyanidin C2

3. Potential Medicinal Benefits of Phenolic Compounds

Phenolic compounds are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens and may also contribute to the bitterness, astringency of the food. Researchers have explored that these molecules are very good antioxidants and may neutralize the destructive reactivity of undesired reactive oxygen/nitrogen species produced as byproduct during metabolic processes in the body. Epidemiological studies have revealed that polyphenols provide a significant protection against development of several chronic diseases such as cardiovascular diseases (CVDs), cancer, diabetes, infections, aging, asthma etc (Figure 17) (Pandey and Rizvi, 2009).

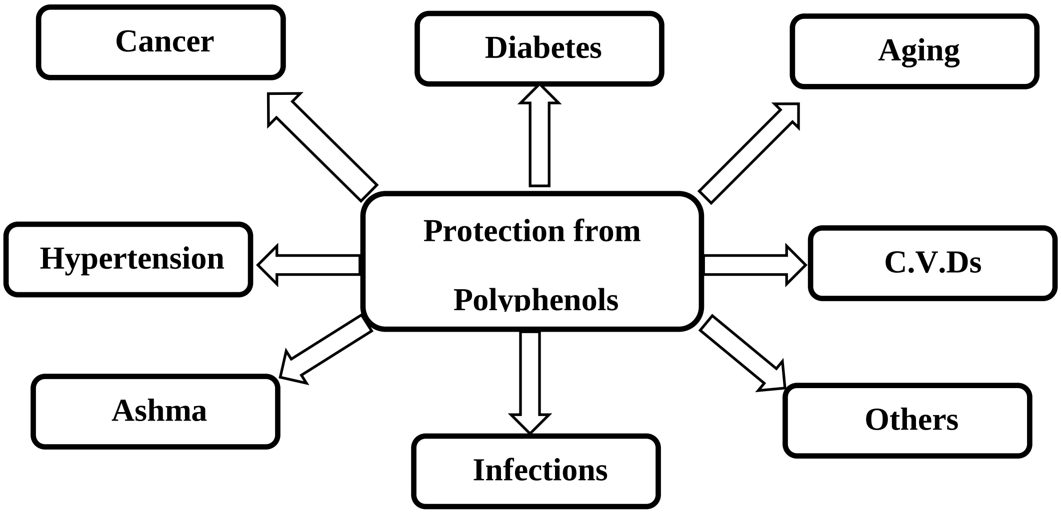


Figure 17. Polyphenols protection against development of several diseases

There are numerous in *vitro* and *in vivo* studies that have indicated the potential medicinal benefits of phenolic compounds. The anthocyanins extracted from bilberry (*Vaccinium myrtillus*) juice have been reported to protect the vascular system *in vivo* by increasing the permeability of capillary blood vessels (Azar *et al.*, 1987). Tea phenolic compounds have been reported to exhibit a very broad spectrum of medicinal activities. Green tea phenolic components inhibit intestinal uptake of glucose through rabbit intestinal epithelial cells and thus may contribute to the reduction of blood glucose levels (Kobayashi *et al.*, 2000). Epigallocatechin gallate, a phenolic component of green tea, has been found to reduce the incidence of chemically induced tumours in the esophagus, liver, lungs, skin and stomach of experimental animals (Shahidi and Naczk, 2003). Epigallocatechin gallate was also reported to possess effective antioxidant properties and can provide protection *in vitro* against both peroxyl radical and hydroxyl radical-induced oxidation of DNA (Green, 2007; Hu and Kitts, 2001).

Tannins from plants have also been reported to play a vital role in the treatment of type 2 diabetes. Chebulanin, chebulagic acid and chebulinic acid from fruit of *Terminalia chebula* were reported that to inhibit α-glucosidase (Ali Asgar, 2013; Gao *et al.*, 2007).

Flavonoids have also been reported to exert positive effects on the cardiovascular system through modulation of the nitric oxide synthase system (Fitzpatrick *et al.*, 1998; Park *et al.*, 2000; Visioli *et al.*, 1998). However, not all epidemiological studies have found a protective effect of dietary phenolic compounds against heart disease (Hertog *et al.*, 1997; Rimm *et al.*, 1996).

Hibasami *et al.* (1995) reported that catechin-rich persimmon extract induced programmed cell death (apoptosis) in human lymphoid leukemia cells. Citrus flavonoids, in particular, flavanones have been shown to display anticarcinogenic and antiallergenic (Noguchi *et al.*, 1999) activity *in vitro*.

Other studies have suggested that caffeic acid and some of its esters possess antitumour activity against colon carcinogenesis (Olthof *et al.*, 2001; Rao *et al.*, 1993). Caffeic acid derivatives have also been shown to be potent and selective inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase 77 which catalyzes the integration of the viral DNA into the host cell. Caffeic and ferulic acid derivatives have also been suggested to be potential protective agents against photooxidative skin damage (Saija *et al.*, 1999). Quercetin has been reported to be a potent inhibitor of human immunodeficiency virus (HIV)-1 protease (Xu *et al.*, 1994). This compound has also been shown to decrease the infectivity of herpes simplex virus type I, poliovirus type I and parainfluenza virus *in vitro* (Green, 2007; Kaul *et al.*, 1985).

Chapter 3

Studied plants

III. STUDIED PLANTS

1. *Marrubium deserti* De Noé.

1.1. Classification

Domain: Eukarya

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida (Dicotyledons)

Order: Lamiales

Family: Lamiaceae

Genus: *Marrubium*

Species: *Marrubium deserti* De Noé.

Vernacular names: Djaîdi, Djaida, Djaada ou marrube du desert (Quezel and Santa, 1963).

Synonyme: *Ballota deserti (de Noé)*

Maropsis deserti (de Noé) Pomel

Sideritis deserti de Noé

Maropsis deserti Pomel.

1.2. Morphological description

The genus *Marrubium* includes six species and one hybrid in Algeria : *Marrubium vulgare* L., *M. spinum* L., *M. peregrinum* L., *M. alysson* L., *M. alyssoides* Pomel, *M. willkommu* Magn. (*M. supinum* X *vulgare*) and *M. deserti* de Noé. (Quézel and Santa, 1963).

M. deserti is a small perennial shrub (20-30 cm high), very branched, with woolly leaves and stems. the flowers are pale violet or pink. The calyx is bright green and evergreen around the fruit, which is a tetra-achene typical of the Labiatae. The leaves are velvety and opposite, and are generally terminated by three large teeth of variable form (Benhouhou, 2005; Quézel and Santa, 1963).

Figure 18. Different parts of *M. deserti* De Noé.

1.3. Geographic distribution

The species is endemic of central and north Algerian Sahara, growing wild on desert pastures and flowering in spring (March-April) (Benhouhou, 2005; Ozenda, 2004; Quézel and Santa, 1963).

The plant grows in an arid climate, with an annual rainfall of 100 mm. It is usually found in non-saline wadis on gravelly-sandy soils (Ozenda, 2004).

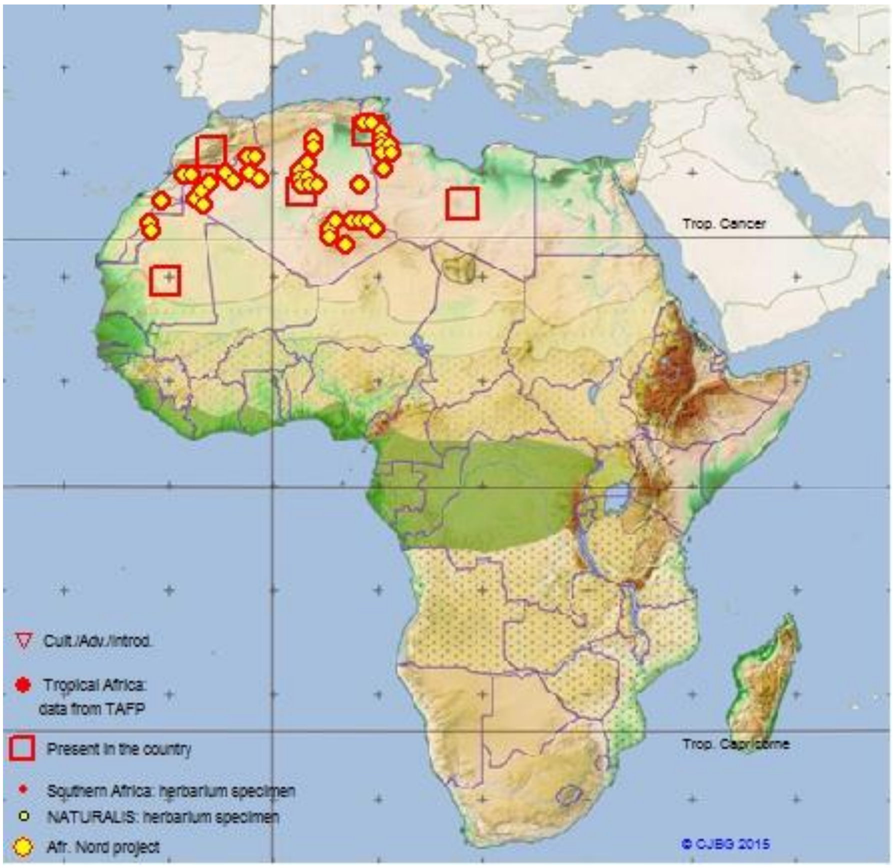


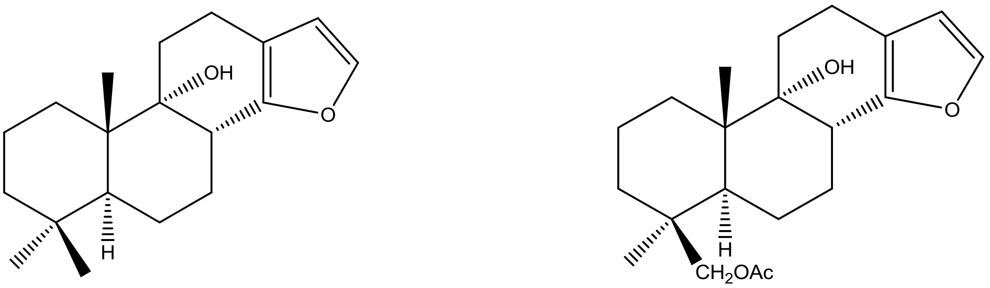
Figure 19. Geographic distribution of *M. deserti* De Noé. (African Plants Database)

1.4. Previous studies

The *Marrubium* species are reported to be rich in phenolic compounds, flavonoids, phenyl ethanoid and diterpenoids such as marrubiin which is designed for the chemical, therapeutic (anti-tumor) and pharmacological (El Bardai *et al.*, 2003; Hatam *et al.*, 1995; Hennebelle *et al.*, 2007; Karioti *et al.*, 2005; Sarikurkcu *et al.*, 2008). *M. deserti* is widely used in traditional medicine in Algeria. In other words, leaves, twigs and young buds are used in decoction, infusion and maceration against intestinal disorders, respiratory diseases, fever, cough, dysmenorrhea, scorpion sting and Allergies (Hammiche and Maiza, 2006; Ould El Hadj *et al.*, 2003).

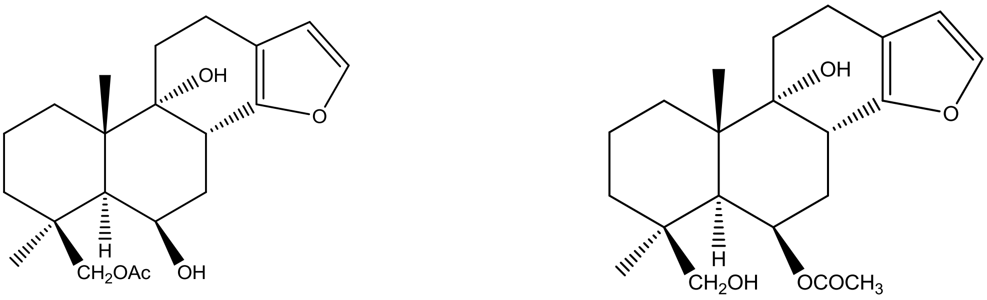
Other authors reported antioxidant effect, antiviral, antibacterial and antigenotoxic activites of methanolic and aqueous extracts of *M. deserti* (Benhammou *et al.*, 2009; Djeridane *et al.*, 2010; Edziri *et al.*, 2012; Zaabat *et al.*, 2010; Zaabat *et al.*, 2011).

In phytochemical study by Dendougui and his team, they isolated four labdane diterpenes (Figure 20) (6-dehydroxy-19-acetyl-marrubenol, 19-acetyl-marrubenol, 6-acetyl-marrubenol and 15 and 16-epoxy-9-hydroxy-labda-13(16),14- diene) and three sterols (*β*-sitosterol, stigmasterol and *β*-sitosterol 3-*O*-glucoside) and Phytol (Dendougui *et al.*, 2011).



16-epoxy-9-hydroxylabda-13(16), 14- diene

6-dehydroxy-19-acetyl-marrubenol

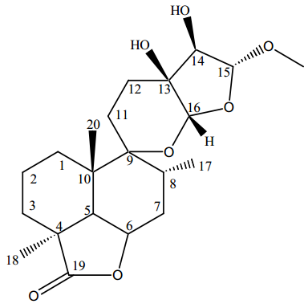


19-acetyl-marrubenol

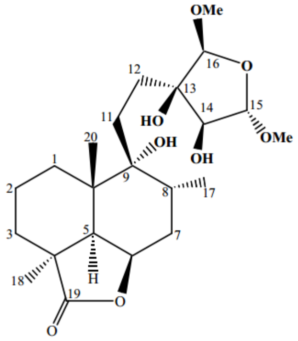
6-acetyl-marrubenol

Figure 20. Labdane diterpenes isolated from *M. deserti*

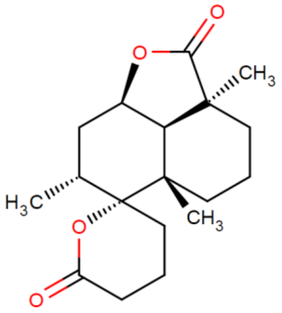
In other phytochemical study by Zaabat and her team, they isolated and identified Fifteen compounds (Figure 21) . Eight of them were terpenoid derivatives among which two were new labdane diterpenes named marrulibacetal A and desertine, respectively. Six of them were known compounds (a mixture of the isomers cyllenin A and 15-epi-cyllenin A, marrubiin, marrulactone, marrulibacetal and *β*-stigmasterol) and seven known phenolic compounds were also isolated: apigenin and several 7-*O*-substituted derivatives (apigenin-7-*O*-*β*-neohesperidoside, apigenin-7-*O*-glucoside, terniflorin and apigenin-7-*O*-glucuronide) together with two phenylethanoid glucosides (acteoside and forsythoside B) (Zaabat *et al.*, 2011).



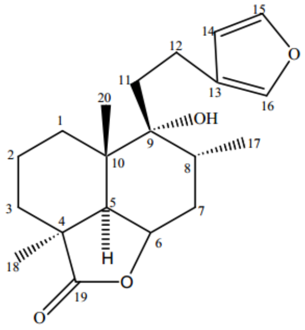
Marrulibacetal A



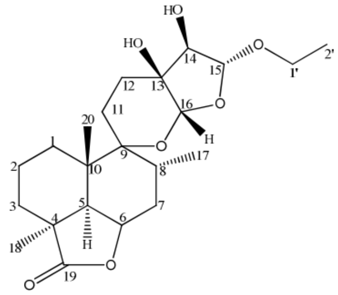
Desertine



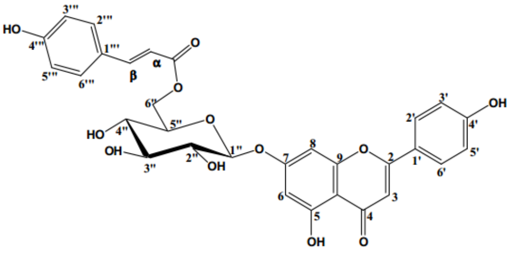
Marrulactone



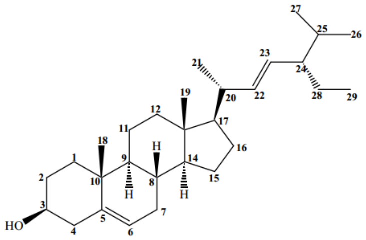
Marrubiin



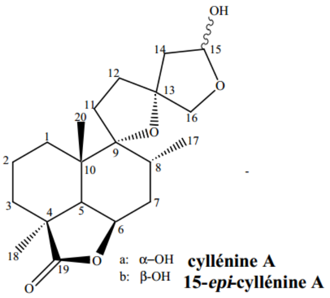
Marrulibacetal



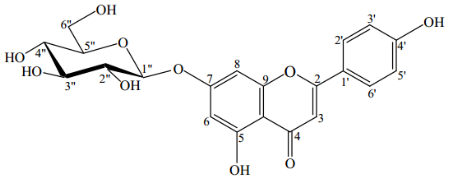
Terniflorin



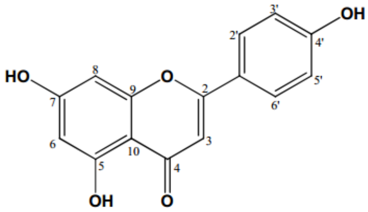
β-stigmasterol



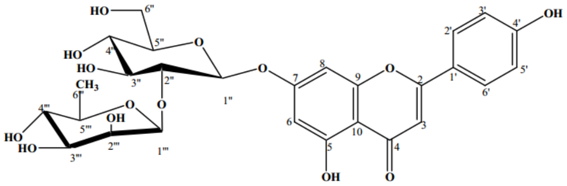
Cyllenin A and 15-epi-cyllenin A



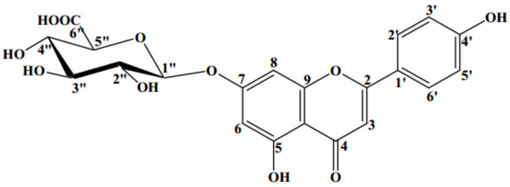
Apigenin-7-*O*-glucoside



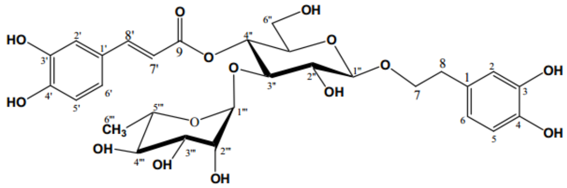
Apigenin



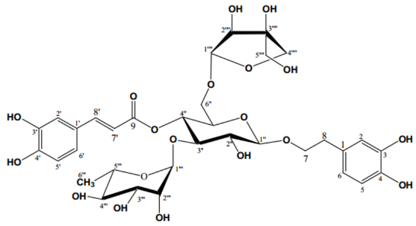
Apigenin-7-*O*-*β*-neohesperidoside



Apigenin-7-*O*-glucuronide



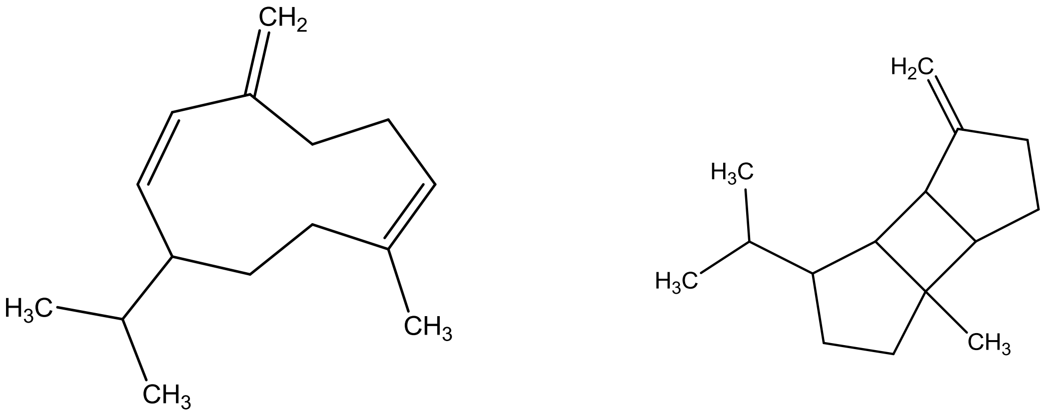
Acteoside



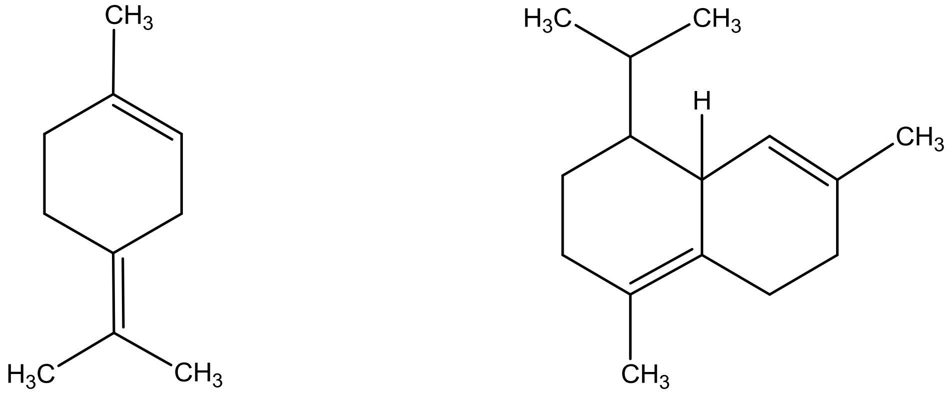
Forsythoside B

Figure 21. Chemical structures of some compounds isolated from *M. deserti*

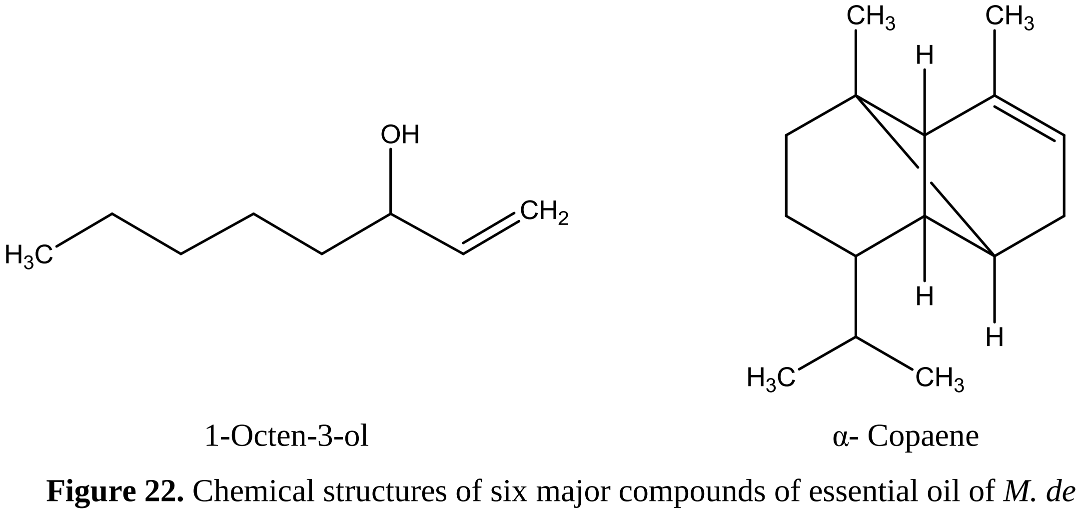
The chemical composition of essential oil of *M. deseti* showed six major compounds: germacrene D (45.7 %), *β*-Bourbonene (4.0 %), α- terpinolene (3.9 %), Δ-cadinene (3.8 %), 1-octen-3-ol (3.7 %) and α- copaene (3.5 %) (Laouer *et al.*, 2009).



Germacrene D *β*-Bourbonene



α-Terpinolene Δ-Cadinene



1-Octen-3-ol α- Copaene

Figure 22. Chemical structures of six major compounds of essential oil of *M. desert*

2. *Anthemis stiparum* subsp. *sabulicola* (Pomel) Oberpr.

2.1. Classification

Domain: Eukarya

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida (Dicotyledons)

Order: Asterales

Family: Asteraceae

Genus: *Anthemis*

Species: *Anthemis stiparum* subsp. *sabulicola* (Pomel) Oberpr.

Vernacular names: Arbian, Itima

Synonyme: *Anthemis monilicostata* subsp. *stiparum* (Pomel) Maire

2.2. Morphological description

Annul, 15-25 cm tall, branched from immediately above the ground. Stems (3-) 6-14 (-20) cm long, basally (0.6-) 0.8-1.4 (-1.8) mm in diameter, densely hairy, procumbent to ascending-erect. Basal and lower cauline leaves (6-) 10-21 (-28) mm long and (3-) 4-8 (-10) mm wide; petiole (3-) 5-11 (-15) mm long; blade 2-3-pinnatipartite; ultimate segments broadly elliptical to narrowly elliptical, (1.0-) 1.2-1.8 (-2.3) mm long and 0.5-1.1 mm wide, densely hairy. Upper cauline leaves (5-) 7-16 (-25) mm long and 1-9(-11) mm wide; petiole 2-9(-16) m long; blade 1-2(-3)-pinnatipartite; ultimate segments (1.3-) 1.5-2.2 (-3.0) mm long and 0.5-1.1 mm wide, linear or elliptical, sometimes triangular. Peduncles (5-) 15-45 (-80) mm long and 0.7-1.4 mm in diameter, densely hairy. It's comparatively short ray florets with usually circular to elliptical limbs. Capitula (12-) 15-20 (-25) mm in diameter. Ray florets white 7-12 per capitulum, (6.0-) 6.5-8.3 (-9.7) mm long; limb elliptical to obovate. Disc florets yellow 2.1-3.0 mm long; the basal part 1.0-1.5 mm long and 0.6-1.0 mm wide (Oberprieler, 1998).

Figure 23. Different parts of *A .stiparum* subsp. *sabulicola* (Pomel) Oberpr.

2.3. Geographic distribution

Endemic to the North fringe of the Algerian Sahara. Not found neither in the southern regions of Erq (dune sea) and saline areas (Halis, 2007; Oberprieler, 1998).

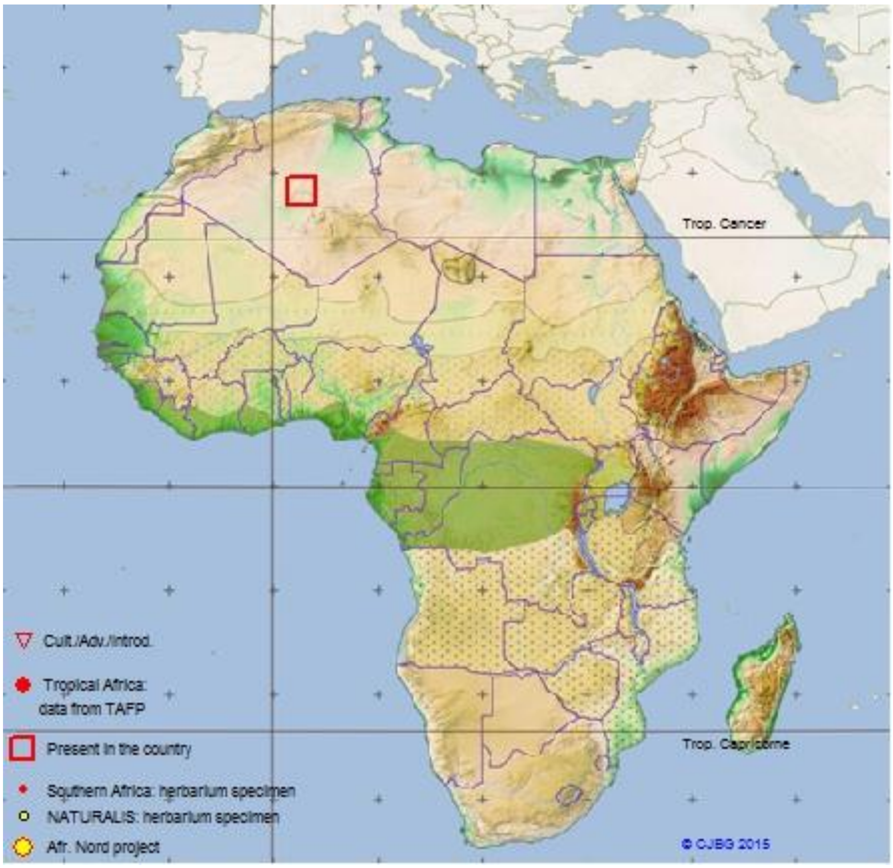


Figure 24. Geographic distribution of *A. stiparum* subsp. *sabulicola* (African Plants Database)

2.4. Previous studies

There is no study in the literature concerning phytochemical or biological activities about this plant.

3. *Rhanterium suaveolens* Desf.

3.1. Classification

Domain: Eukarya

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida (Dicotyledons)

Order: Asterales

Family: Asteraceae

Genus: *Rhantherium*

Species: *Rhanterium suaveolens* Desf.

Vernacular names: Arfadja, Arfej

Synonyme: *Rhanterium suaveolens* Desf. subsp. *Suaveolens*

3.2. Morphological description

Rhanterium suaveolens Desf. found by Desfontaines on the sandy sea-coast, near Sfax, in the kingdom of Tunis. Root perennial. Stem erect, much branched, lower branches stiff, The modern upper branches are white flexible and carry soft bristles dense. Leaves small, slightly toothed , somewhat elongated oval, with serrated edges which also carry soft bristles. Flowers terminal solitary, yellow, almost shiny, small does not exceed 1.5 cm in length and is surrounded by leaves (Bracts) pointed which are compose smooth involucre. This plant flowers in summer. The bruised leaves have a fragrant smell (Halis, 2007; Quézel and Santa, 1963; Wilkes, 1827).

Figure 25. Different parts of *R. suaveolens* Desf.

3.3. Geographic distribution

The genus *Rhanterium* is distributed over western North Africa, the Arabian Peninsula, Iraq and Iran. Three species; namely, *R. adpressum* Coss. & Durieu, *R. epapposum* Oliver and *R. suaveolens* Desf. of this genus have been reported in literature. *R. intermedium* Coss. & Durieu ex Pomel is another species, but it is a hybrid between *R. suaveolens* and *R. adpressum*. *R. suaveolens* Desf., a member of the Asteraceae (Compositae), is locally known as “Arfadja”. Quézel and Santa reported that it is an endemic desert plant growing in North Africa (Quézel and Santa, 1963; Wiklund, 1986). Some of the *Rhanterium* species are used in folk medicine as an antidiuretic (Hamia *et al.*, 2013).

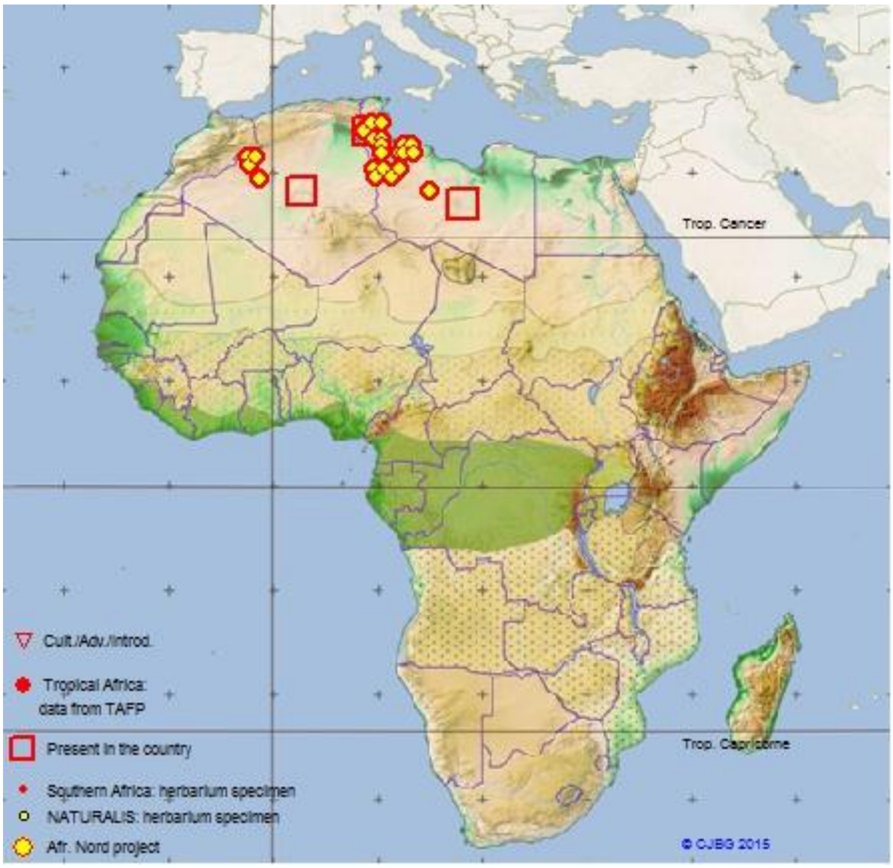


Figure 26. Geographic distribution of *R. suaveolens* (African Plants Database)

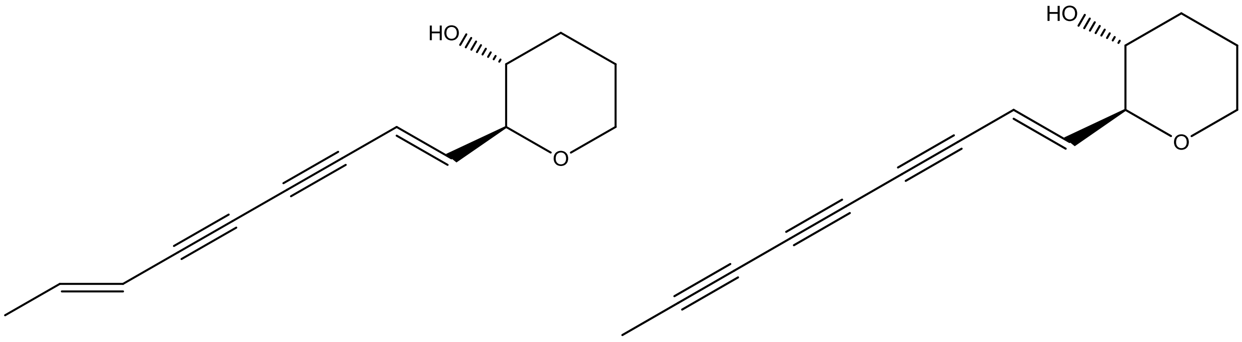
3.4. Previous studies

There are some studies on the *Rhanterium* species in the literature. In one study, β-eudesmol, 16β-hydroxylupeolyl-3-hexadecanoate, stigmasterol, (+)-3-[3′-(nona-1″-en-3″,5″,7″-trynyl) oxiran-2′-yl] propan-2-ol were isolated from *R. adpressum* (Bouheroum *et al.*, 2007). In another study, scopoletin was isolated from *R. epapposum* (Miana and Al-Hazimi, 1983). The chemical composition the essential oil of *R. adpressum* and *R. epapposum* were also studied (Al-Easa, 2004; Al-Mazroa *et al.*, 2006; Kala *et al.*, 2009; Yaghmai and Kolbadipour, 1987).

Habib Oueslati and his team isolated and identified six compounds: Ranthenone glucoside as a new compound, 9-hydroxylinaloyl glucoside, scopolin, fraxetin, scopoletin and sitosterol-3β-O-[6'-palmitoyl-β-D-glucopyranoside] (Oueslati *et al.*, 2007), and antimicrobial polyacetyleneic alcohols in other study (Oueslati *et al.*, 2004).

(2S, 3R, 1’E, 7’E)-tetrahydro-2-(nona-1', 7'- diène-3',5'-diynyl)pyran-3-ol (Figure 28 A)

(2S, 3R, 1’E)-tetrahydro-2-(nona-1'-ène-3',5',7'-triynyl)pyran-3-ol (Figure 28 B)



A B

Figure 27. Chemical structures of polyacetyleneic alcohols isolated from *R. suaveolens*

and new ceramides (Figure 29) were isolated from the *R. suaveolens* (Oueslati *et al.*, 2005).

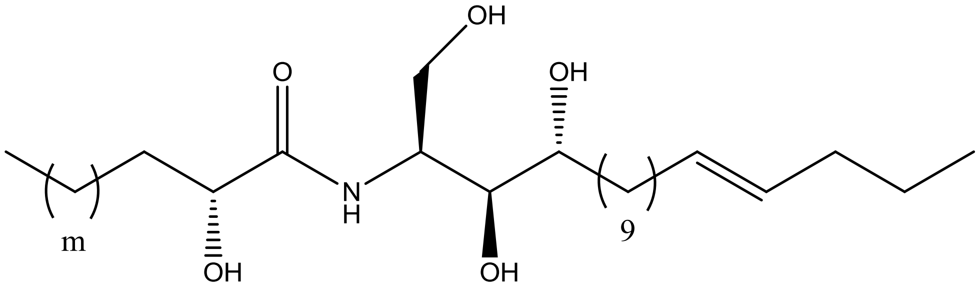
(2S, 3S, 4R, 2'R, 14 E)-2-(2'-hydroxydocosanoylamino)-14-octadecene-1, 3, 4-triol

(2S, 3S, 4R, 2'R, 14 E)-2-(2'-hydroxytricosanoylamino)-14-octadecene-1, 3, 4-triol

(2S, 3S, 4R, 2'R, 14 E)-2-(2'-hydroxytetracosanoylamino)-14-octadecene-1, 3, 4-triol

(2S, 3S, 4R, 2'R, 14 E)-2-(2'-hydroxypentacosanoylamino)-14-octadecene-1, 3, 4-triol

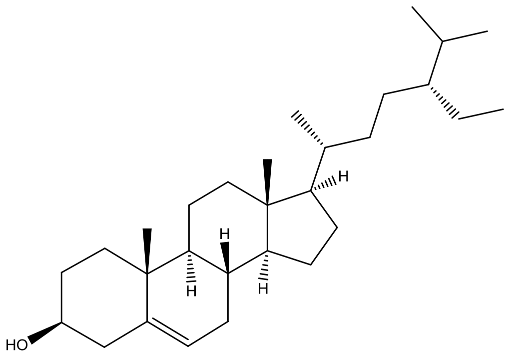
(2S, 3S, 4R, 2'R, 14 E)-2-(2'-hydroxyhexacosanoylamino)-14-octadecene-1, 3, 4-triol



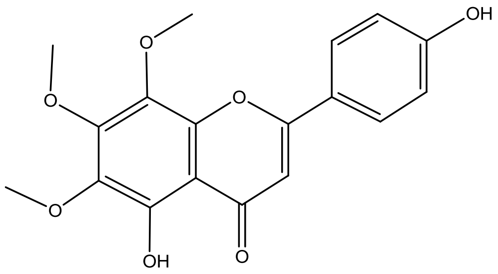
m = 18 to 25

Figure 28. Chemical structures of ceramides isolated from *R. suaveolens*

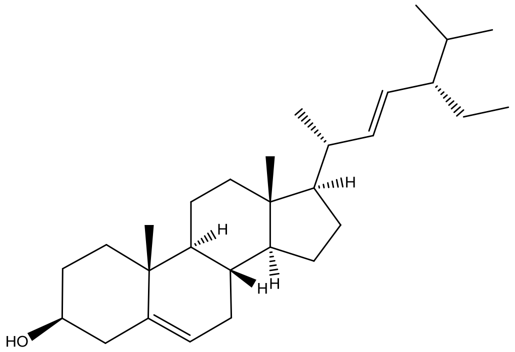
In other phytochemical study, Benaissa and her team isolated and identified eight products from *Rhanterium Suaveolens* (Figure 29). which six are new to the genus and species (Benaissa, 2011), These products are:



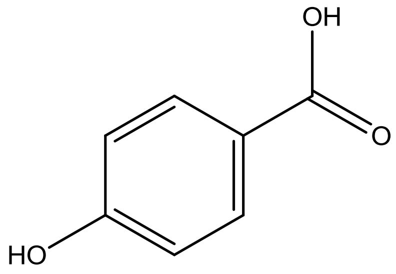
β-Sitosterol



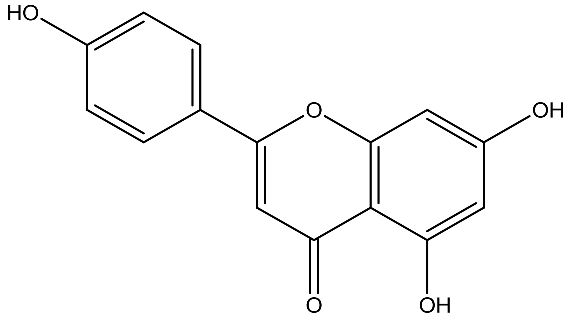
Xanthomicrol



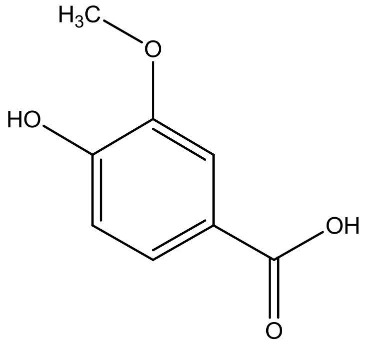
Stigmasterol



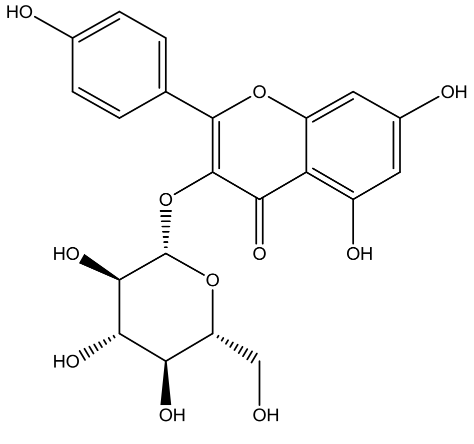
4-hydroxybenzoic acid



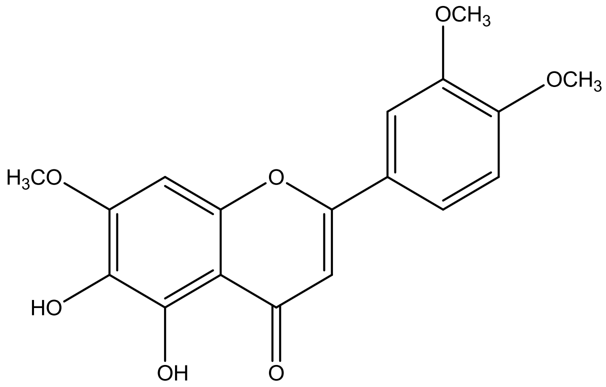
Apigenin



4-hydroxy-3-methoxybenzoic acid



Astragalin



5,6-dihydroxy-7,3',4'-trimethoxyflavone

Figure 29. Chemical structures of some compounds isolated from *R. suaveolens*

4. *Centaurea furfuracea* Coss. & Dur.

4.1. Classification

Domain: Eukarya

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida (Dicotyledons)

Order: Asterales

Family: Asteraceae

Genus: *Centaurea*

Species: *Centaurea furfuracea* Coss. & Dur.

Vernacular names: Merioua, Merir

Synonyme: *Calcitrapa furfuracea* (Coss. & Durieu) Holub

4.2. Morphological description

Centaurea furfuracea is an Annual or perennial herb, with short main stem and ending with a head (capitulum) in which the branches are born long branches and ending with heads (less than 15 mm diameter), lobed leaves, Bracts with short woolly hairs, Yellowish-white achene (Ozenda, 1977; Quézel and Santa, 1963).

4.3. Geographic distribution

The genus *Centaurea* (Asteracea) comprises about 500 species, which are predominately distributed around the Mediteranean area and in West Asia (Djeddi *et al.*, 2008). The genus *Centaurea* is represented by 42 species in the Algerian flora, which seven are localised in the Sahara (Quézel and Santa, 1963).

Figure 30. Different parts of *C. furfuracea* Coss. & Dur.

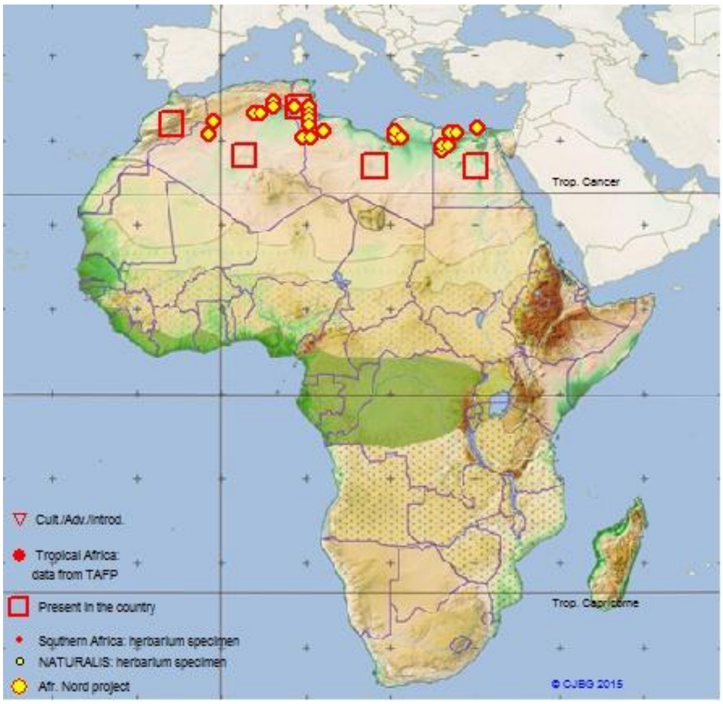


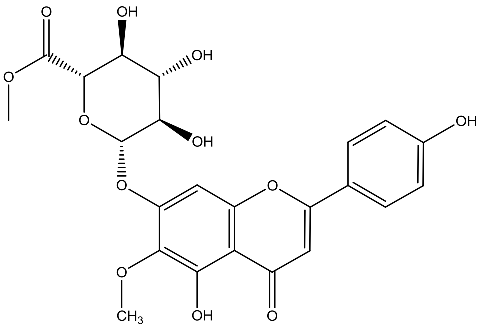
Figure 31. Geographic distribution of *C. furfuracea* (African Plants Database)

4.4. Previous studies

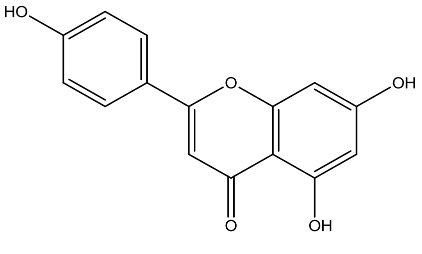
Chemical investigations of various *Centaurea* species have revealed mainly flavanoids, alkaloids and lignans (Karamenderes *et al.*, 2007; Shoeb *et al.*, 2005) and sesquiterpene lactones lactones, which are guaiane, germacrane, elemane and eudesmane skeletons (Gonzalez *et al.*, 1978; Gürbüz and Yesilada, 2007; Koca *et al.*, 2009). Many *Centaurea* species are reported in the literature to be used in folk medicine such us antidandruff, antidiarrhoic, antirhevmatic, anti-inflammatory, and antibacterial (Bülent Köse *et al.*, 2007; Csupor *et al.*, 2010; Zengin *et al.*, 2010).

Centaurea furfuracea was found to contain 13 flavonoid compounds. such as: hispidulin-7-*O*-methylglucuronide (Akkal *et al.*, 1999), apigenin, hispidulin, cirsimaritin, 5,7,4'-trihydroxy-3-methoxyflavones, apigenin-7-*O*-glucoside, apigenin-7-*O*-methylglucuronide, hispidulin-7-*O*-glucoside, patelutin-7-*O*-glucoside (Akkal *et al.*, 2003), acacetin, jaceosidin (Fakhfakh *et al.*, 2005), isokaempferide-7-*O*-methylglucuronide, isokaempferide-7-*O*-glucuronide . The last two compounds have showed cytotoxic and antiparasitic activities (Akkal *et al.*, 2007).

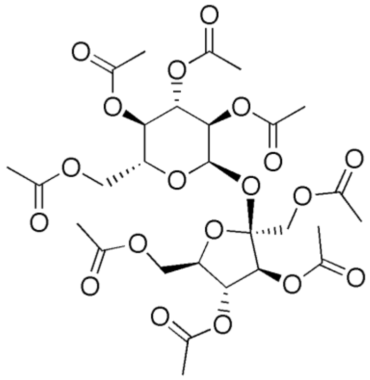
It was also reported that *Centaurea furfuracea* contains lignans: (-)trachelogenin and tracheloside and sucrose acetat (Fakhfakh *et al.*, 2005). Akkal and his team reported that two new sesquineolignans, furfuraceol A and furfuraceol B, have been isolated from the flowers of *Centaurea furfuracea* as a mixture of two isomers (Figure 33) (Fakhfakh and Damak, 2007).



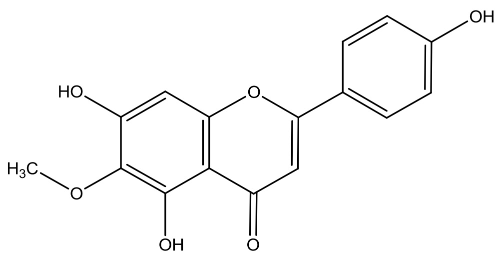
Hispidulin 7-*O*-methylglucuronide



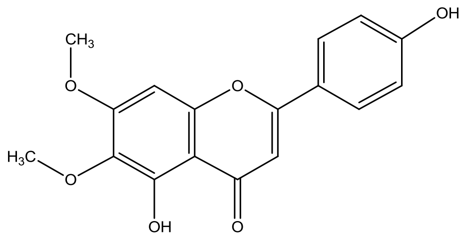
Apigenin



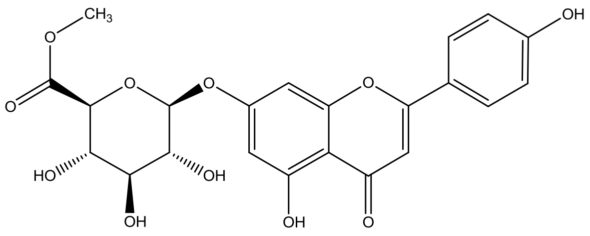
Sucrose acetat



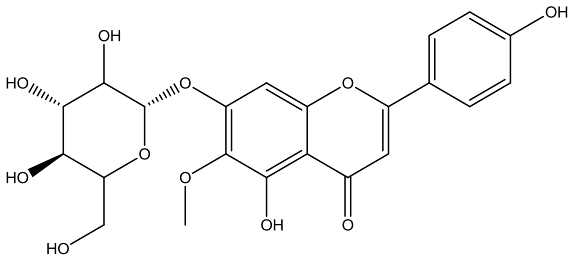
Hispidulin



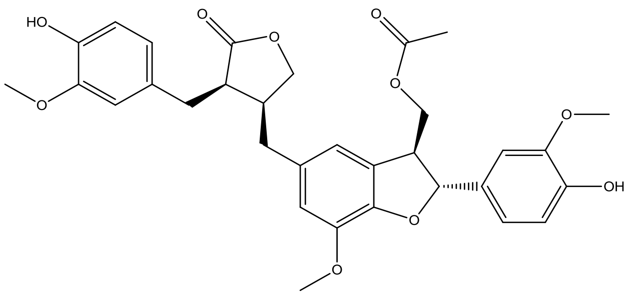
Cirsimaritin



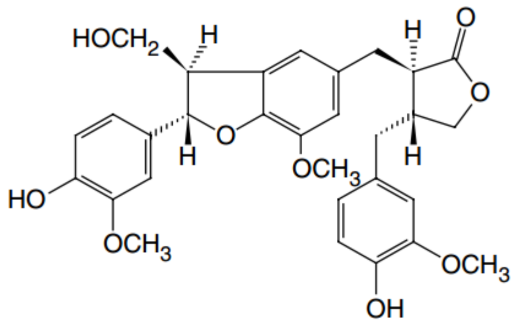
Apigenin-7-*O*-methylglucuronide



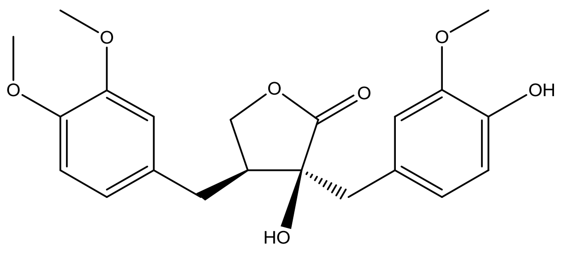
Hispidulin-7-*O*-glucoside



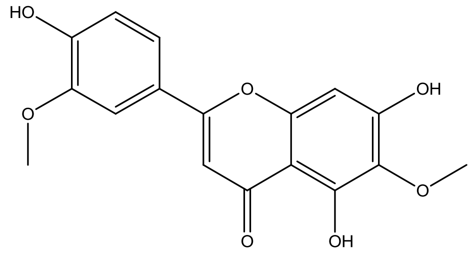
Furfuraceol A



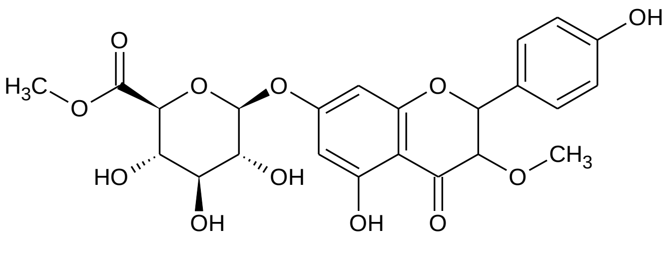
Furfuraceol B



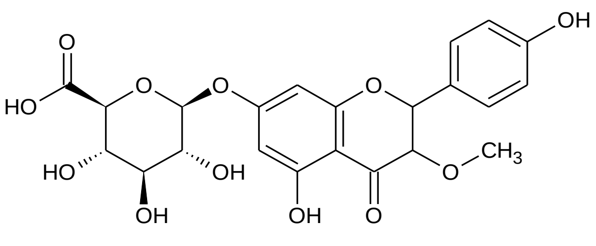
(-)trachelogenin



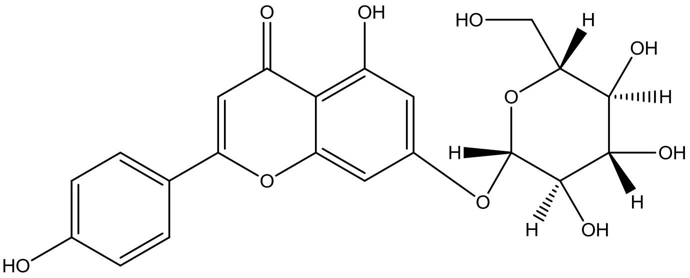
Jaceosidin



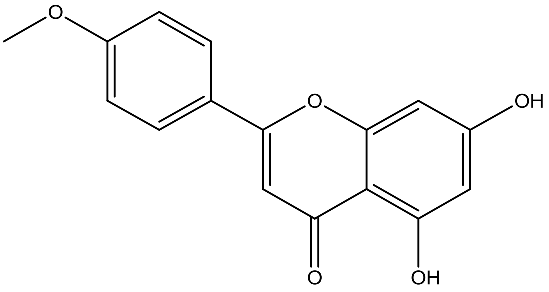
Isokaempferide 7-O-Methylglucuronide



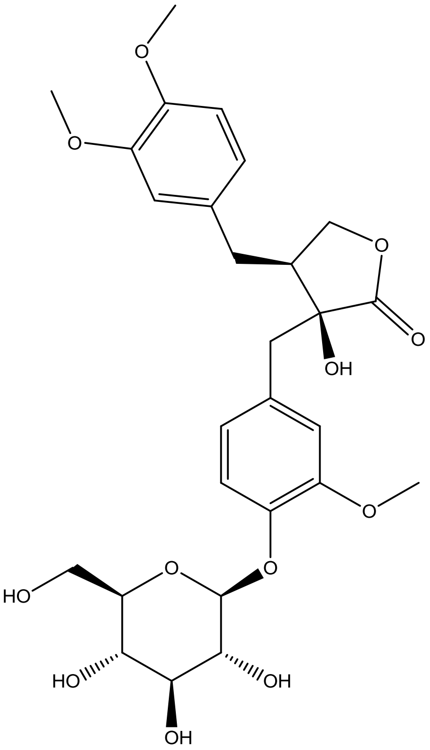
Isokaempferide 7-O-Glucuronide



Apigenin-7-*O*-glucoside



Acacetin



Tracheloside

Figure 32. Chemical structures of some compounds isolated from *C. furfuracea*

Chapter 4

Materials and methods

IV. MATERIALS AND METHODS

1. Materials

1.1. Plant material

The aerial parts of *M. deserti*, *A. stiparum* subsp. *sabulicola, R. suaveolens* and *C. furfuracea* were collected in the North fringe of the Algerian Sahara during the flowering period in April 2012 near Stile, El-Oued, Algeria. Taxonomic identification of the plants were confirmed by Dr. Youcef Halis in the Scientific and Technical Research Centre for Arid Areas (CRSTRA). A voucher specimens were deposited in the herbarium of the Faculty of Life and Natural Sciences of El Oued University and the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria (Table 4). The collected plant materials were air-dried in darkness at room temperature for three weeks.

Table 4. Location and altitude of studied plants

plants	Site	Altitude	Voucher number
M. deserti	34°16'N, 6°2'E	-22 m	ZA 143 (Oum El Bouaghi University) CAK 3 (El Oued University)
A. stiparum subsp. *sabulicola*	34°14'N, 6°1'E	-11 m	CAK 2 (El Oued University)
R. suaveolens	34°18'N, 5°54'E	33 m	CAK 4 (El Oued University)
C. furfuracea	34°18'N, 5°54'E	33 m	CAK 5 (El Oued University)

1.2. Strains and growth conditions

In the present study, the microorganisms used in the experiments were: Gram positive bacteria **(***Staphylococcus aureus* (ATCC 25923, ATCC 6538-P), *Staphylococcus epidermidis* MU 30, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* RSKK 863, *Streptococcus mutans* CNCTC 8/77 and, *Micrococcus luteus* NRRL B-4375) and the yeast (*Candida albicans* ATCC 10239). The above-mentioned microorganisms except *C. albicans* were grown in nutrient broth (NB, Difco); *C. albicans* was grown in sabouraud dextrose broth (SDB, Difco).

2. Methods

2.1. Preparation of the extracts

2.1.1. Extraction of the essential oils

The essential oils of dried aerial parts (400 g) of studied plants were obtained via hydrodistillation by using a Clevenger type apparatus (Figure 33) for 4 hours. The oils were dried over anhydrous sodium sulphate and stored under nitrogen until required.

Figure 33. Clevenger apparatus for essential oil extraction

2.1.2. Preparation of the methanol extract

Total methanol extract of studied plants were prepared by maceration technique, the dried and powdered aerial parts of the plants (150 g) were macerated with 300 mL of methanol at room temperature (25 °C) 3 times (72 hours × 3). After filtration, The extracts were concentrated using a rotary evaporator (Buchi Rotavapor R-200, Flawil, Switzerland) at a maximum temperature of 45 °C, The residuals obtained were stored in a freezer at −20 °C until further study.

2.2. Gas chromatography

GC analysis of the oils were performed using a Shimadzu GC-17 AAF, V3, 230V LV Series (Kyoto, Japan) gas chromatography, equipped with a FID and a DB-1 fused silica column [30 m x 0.25 mm (i.d.), film thickness 0.25 µm]; the oven temperature was held at 60 °C for 5 minutes., then programmed to 240 °C at 4°C/minute and held isothermal for 10 minutes; injector and detector temperatures were 250°C and 270 °C respectively; carrier gas was helium at a flow rate of 1.3 mL/minute; Sample size, 1.0 µL; split ratio, 50:1. The percentage composition of the essential oils were determined with a Class-GC 10 computer program.

Figure 34. Shimadzu GC-17 Gas chromatography

2.3. Gas chromatography–mass spectrometry (GC–MS)

The analysis of the essential oils were performed using a Varian Saturn 2100 (Old York Rd., Ringoes, NJ, USA), ion trap machine, equipped with a DB-1 MS fused silica non-polar capillary column [30 m x 0.25 mm (i.d.), film thickness 0.25 µm]. Helium was used as a carrier gas at a flow rate of 1.4 mL/minute. The oven temperature was held at 60 °C for 5 minutes, then increased up to 240 °C with 4 °C/minute increments and held at this temperature for 10 minutes. Injector and transfer line temperatures were set at 250 and 180 °C, respectively.

Ion trap temperature was 200 °C. The injection volume was 0.2 µL and split ratio was 1:30. EI-MS measurements were taken at 70 eV ionisation energy. Mass range was from *m/z* 28 to 650 amu. Scan time was 0.5 s with 0.1 s inter scan delays.

Identification of components of the essential oils were based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams, 2007), and whenever possible, by co-injection with authentic compounds.

GC and GC-MS spectra were performed at the Department of Chemistry, Faculty of Sciences, Muğla Sıtkı Koçman University in Turkey.

Figure 35. Varian Saturn 2100 Gas Chromatography /Mass Spectrometry

2.4. Determination of total phenolic compounds

The concentration of phenolic content in Methanol extracts was expressed as microgrammes of pyrocatechol equivalents, determined with Folin–Ciocalteu reagent (FCR), according to the method of Slinkard and Singleton (1977). 1 mL of the solution containing 1 mg of the tested extracts in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed thoroughly. After 3 minutes, 3 mL of sodium carbonate (2 %) were added to the mixture and shaken intermittently for 2 hours at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard pyrocatechol graph (Figure 36).

$$Absorbance = 0.006\, \mu g\ pyrocatechol + 0.035 \quad (\mathrm{r}^2 = 0.978)$$

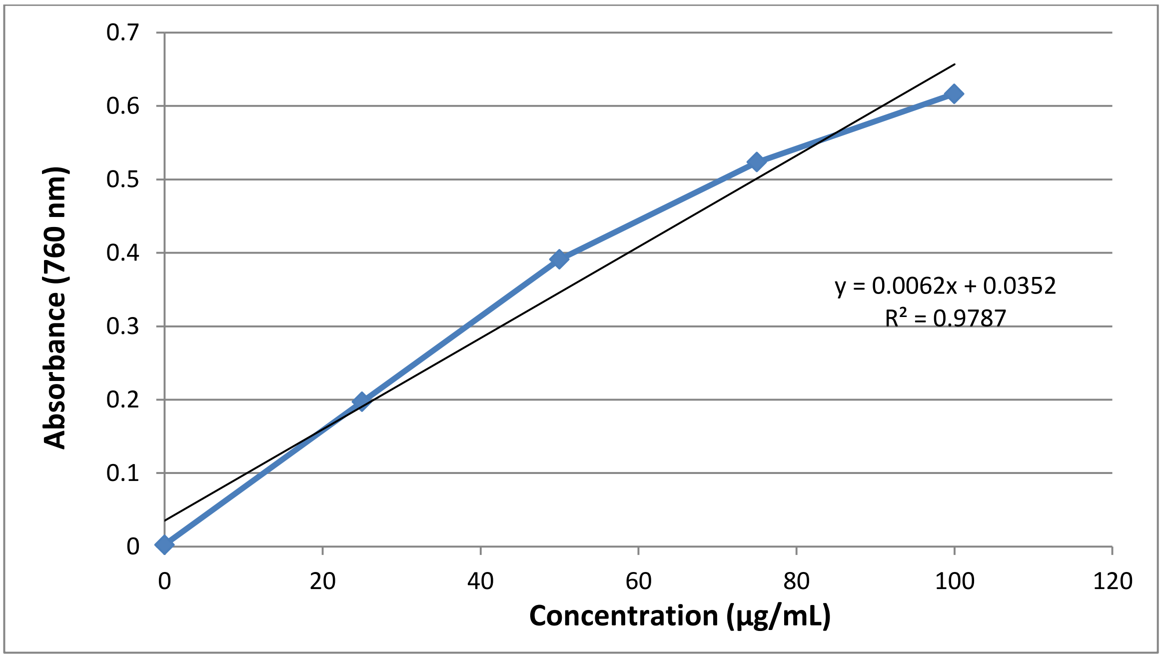


Figure 36. Standard curve of pyrocatechol for determination of total polyphenols in studied plants.

Each value represent mean ± SD

2.5. Determination of total flavonoid compounds

Measurement of flavonoid concentration of the extracts was based on the method described by Park et al. in 1997 with a slight modification (Park *et al.*, 1997), and results were expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg of extract in methanol) was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of ethanol. After 40 minutes at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard. The concentration of flavonoid compounds was calculated according to following equation that was obtained from the standard quercetin graph (figure 37).

$$Absorbance = 0.051\, \mu g\ quercetin + 0.001 \quad (\mathrm{r}^2 = 0.999)$$

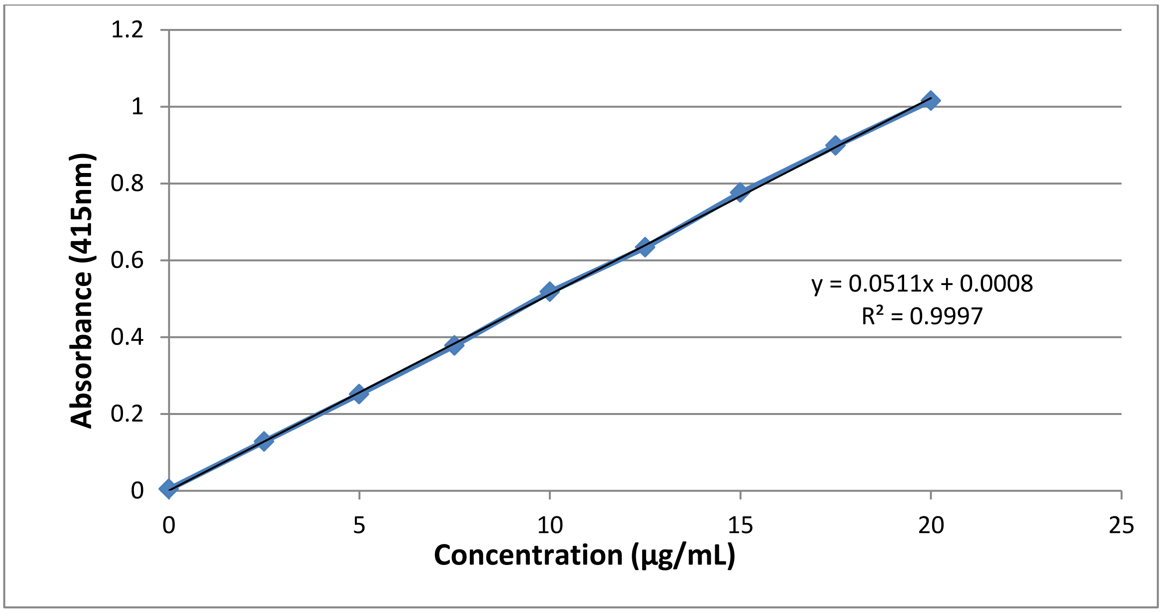


Figure 37. Standards curve of quercetin for determination of total flavonoids in studied plants.

Each value represent mean ± SD

2.6. Antioxidant activity

2.6.1. Free radical-scavenging activity (DPPH assay)

The free radical scavenging activity of the essential oils and methanol extracts were determined by the DPPH assay described by Blois with a slight modification (Blois, 1958; Öztürk *et al.*, 2011). In its radical form DPPH absorbs at 517 nm, but on reduction by an antioxidant or a radical species its absorption decreases. Briefly, a 0.1 mmol L−1 solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of samples solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

The sample concentration providing 50 % free radical scavenging activity (IC_{50}) was calculated from the graph of DPPH Scavenging effect percentage against sample concentration. BHA and α-tocopherol were used as antioxidant standards for comparison of the activity.

2.6.2. *β*-Carotene-linoleic acid assay

The antioxidant activity of the essential oils and methanol extracts of studied plants were evaluated using the *β*-Carotene-linoleic acid assay (Miller, 1971; Öztürk *et al.*, 2011). with slight modifications. *β*-Carotene (0.5 mg) in 1 mL of chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween-40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, was added by vigorous shaking. 4 mL of this mixture were transferred into different test tubes containing different concentrations of the essential oil and MeOH extract. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a 96-well microplate reader (SpectraMax 340PC, Molecular Devices, USA). The emulsion system was incubated for 2 hours at 50 °C. A blank, devoid of *β*-carotene, was prepared for back ground subtraction. BHA and α-tocopherol were used as standards.

The bleaching rate (R) of *β*-Carotene was calculated according to the following equation:

$$R = \frac{ln\frac{a}{b}}{t}$$

Where: ln=natural logarithm, a=absorbance at time zero, b=absorbance at time t (120 minutes).

The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using the following equation:

$$AA\ (inhibition\%) = \frac{R_{Control} - R_{Sample}}{R_{Control}} \times 100$$

2.6.3. Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the method of Apak et al., with a slight modifications (Apak *et al.*, 2004; Öztürk *et al.*, 2011). To each well, in a 96 well plate, 50 µL 10 mM Cu (II), 50 µL 7.5 mM neocuproine, and 60 µL NH_4Ac buffer (1 M, pH 7.0) solutions were added. 40 µL essential oils and methanol extracts at different concentrations were added to the initial mixture so as to make the final volume 200 µL. After 1 hour, the absorbance at 450 nm was recorded against a reagent blank by using a 96-well microplate reader. BHT and α-tocopherol were used as antioxidant standards for comparison of the activity.

2.7. Anticholinesterase activity

The inhibition activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were measured by the method developed by Ellman *et al.* (1961), with a slight modification (Öztürk *et al.*, 2011), using 96-well microplate reader (SpectraMax PC340, Molecular Devices, USA). The substrates of the reaction of both enzymes were acetylthiocholine iodide (0.71 mM) and butyrylthiocholine chloride (0.2 mM). In a 96 well plate, 10 µL of samples (MeOH extracts and essential oils) were mixed with 150 µL sodium phosphate buffer 100 mM (pH = 8) and 20 µL of enzymes solution [AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U)]. After 15 minutes incubation at 25 °C, 10 µL of Ellman's Reagent (DTNB 0.5 mM) and 10 µL of substrates were added, so as to make the final volume 200 µL. The absorbance was measured at 412 nm. Percentage of inhibition of AChE or BChE was determined by comparison of reaction rates of samples relative to control using the formula: $(E - S)/E \times 100$

Where:

E: activity of enzyme with control.

S: activity of enzyme with sample.

The experiments were carried out in triplicate. Galantamine was used as standard.

Methanol and Ethanol were used as solvents to dissolve MeOH extracts and essential oils and controls.

2.8. Determination of minimum inhibitory concentrations and antibiofilm activity

2.8.1. Minimal inhibitory concentration (MIC) assay

MICs were determined by a microtitre broth dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (2006). The MIC was defined as the lowest essential oil/extract concentration that yielded no visible growth. The test medium was Mueller-Hinton broth and the density of bacteria was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (100 µL) were inoculated into the wells of 96-well microtitre plates in the presence of essential oils with different final concentrations (5, 10, 20, 40, 60, 80, 100 µL/mL) and in the presence of methanol extracts with different final concentrations (1.56, 3.125, 6.25, 12.5, 25, 50 mg/mL). The inoculated microplates were incubated at 37ºC for 24 hours. The absorbance was measured at 630 nm.

2.8.2. Effect of essential oil and methanol extract on bacterial biofilm formation

The effect of essential oils and Methanol extracts at concentrations including 1, ½, ¼, 1/8 and 1/16 MIC on biofilm-forming ability of test microorganisms were tested with a microplate biofilm assay (Merritt *et al.*, 2005). Briefly, 1% of overnight cultures of isolates were added into 200 µL of fresh Tryptose-Soy Broth (TSB) supplemented with 0.25% glucose and cultivated in the presence and absence of essential oils/extracts without agitation for 48 hours at 37 ºC. The wells containing TSB+cells served as control. After incubation, the wells were washed with water to remove planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 minutes at room temperature. Wells were washed once again to remove the crystal violet solution. A volume of 200 µL of 33% glacial acetic acid were poured in wells. After shaking and pipetting of wells, 125 µL of the solution from each well were transferred to a sterile tube and volume was adjusted to 1 mL with distilled water. Finally optical density (OD) of each well was measured at 550 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). Percentage of inhibition of the tested extracts was calculated using the formula:

$$Biofilm\ inhibition\ (\%) = \frac{OD_{550Control} - OD_{550Sample}}{OD_{550Control}} \times 100$$

2.9. Statistical analysis

The antioxidant and the anticholinesterase activity assays were performed in triplicate analyses. The data were recorded as means ± standard error meaning. Student's *t*-test were used to determine the significant differences between means; $p < 0.05$ were regarded as significant. Pearson correlation coefficient (R^2) was used to show correlations and significance.

Chapter 5

Results and discusion

V. RESULTS AND DISCUSSION

1. Results of *Marrubium deserti*

1.1. GC/MS of the essential oil of *M. deserti*

The essential oil having yellow color was obtained from aerial parts using hydrodistillation (0.15 %, v/w) method. Totally, 38 components were identified representing 99.70 % of the oil (Table 5). *β*-caryophyllene is omnipresent in the essential oils of most *Marrubium* species, followed by germacrene D and bicyclogermacrene (Laouer *et al.*, 2009). Whereas, the major compounds of essential oil of *M. deserti* were tetracosane (31.11 %,), germacrene D (7.91 %), Δ-Cadinene (6.51 %), α-cadinol (6.26 %), and t-cadinol (5.81 %). bicyclogermacrene (2.84 %) and *β*-Caryophyllene (1.32 %) were also determined as constituent of the essential oil (Table 4). The monoterpenoids represented 9.26 % while sesquiterpenoids 16.45 % of the total oil. The monoterpenes and sesquiterpenes were in the ratio of 4.32 % and 33.85 %, respectively (Figure 39).

Table 5. Chemical composition (%) of the essential oil of *M. deserti*

N°	Compounds	Composition (%)	RI[a]
1	Cyclofenchene	1.52	890
2	*α*-Thujene	0.52	932
3	*α* -Pinene	2.15	936
4	*β*-Pinene	0.82	978
5	*D*-Limonene	1.05	1025
6	Camphor	1.94	1123
7	Citronellal	0.88	1131
8	*n*-Nonanol	1.06	1149
9	Terpinen-4-ol	0.62	1164
10	Myrtenal	0.72	1172
11	*α*-Terpineol	0.66	1176
12	Myrtenol	0.28	1178

13	*p*-Cumic aldehyde (Cuminal)	0.27	1241
14	Carvone	1.54	1243
15	Perillaldehyde	1.70	1271
16	Thymol	1.15	1290
17	*α*-Cubebene	0.74	1351
18	*β*-Bourbonene	0.56	1388
19	*β*-Elemene	3.84	1390
20	*β*-Caryophyllene	1.32	1408
21	Alloaromadendrene	2.54	1460
22	*γ*-Gurjunene	0.84	1477
23	**Germacrene D**	**7.91**	1481
24	Eremophilene	1.44	1486
25	Bicyclogermacrene	2.84	1494
26	*α*-Muurolene	0.69	1500
27	*γ*-Cadinene	1.95	1513
28	**_Δ_-Cadinene**	**6.51**	1523
29	*α*-Calacorene	0.49	1545
30	*E*-Nerolidol	0.86	1553
31	Spathulenol	0.96	1572
32	Caryophyllene oxide	0.90	1578
33	**_t_-Cadinol**	**5.81**	1633
34	*α*-Muurolol	1.07	1635
35	**_α_-Cadinol**	**6.26**	1643
36	*allo*-Himachalol	2.43	1648
37	7-*epi*-*α*-Eudesmol	1.57	1653
38	**Tetracosane**	**31.11**	2400

39	Unidentified	0.11	2406
40	Unidentified	0.13	2418
41	Unidentified	0.05	2422
Total identified:		**99.70**	
Monoterpenes:		**4,32**	
Monoterpenoids:		**9,26**	
Sesquiterpenes:		**33.85**	
Sesquiterpenoids:		**16,45**	
Others:		**35.84**	

[a]Kovats index on DB-1 fused silica column.

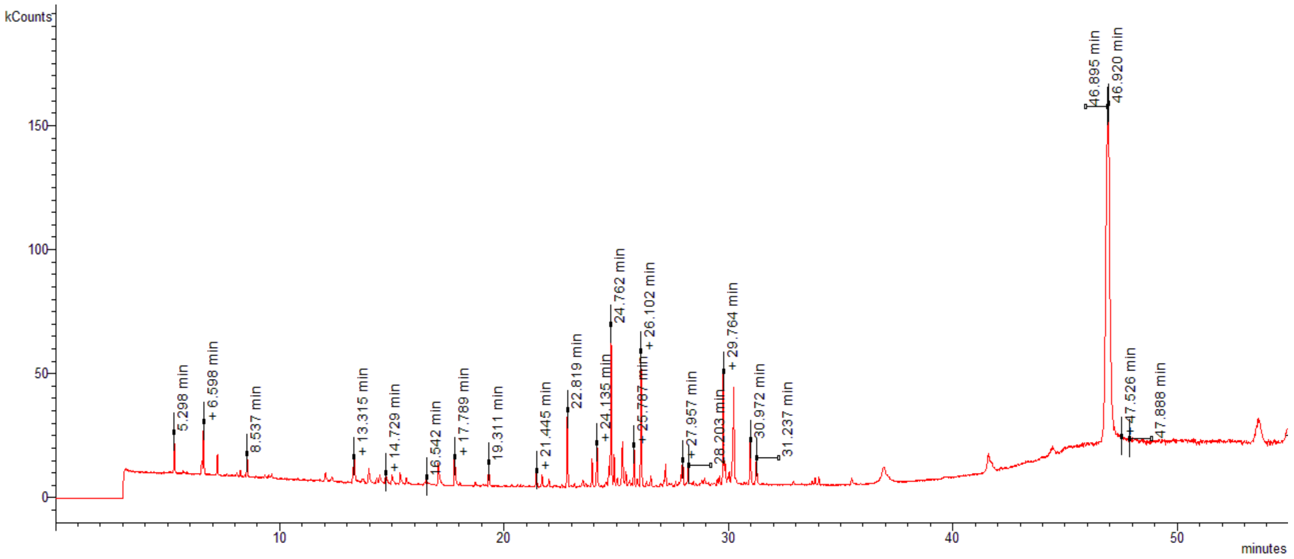


Figure 38. GC/MS chromatogram of *M. deserti* essential oil showing the detection of chemical components using DB-1 column

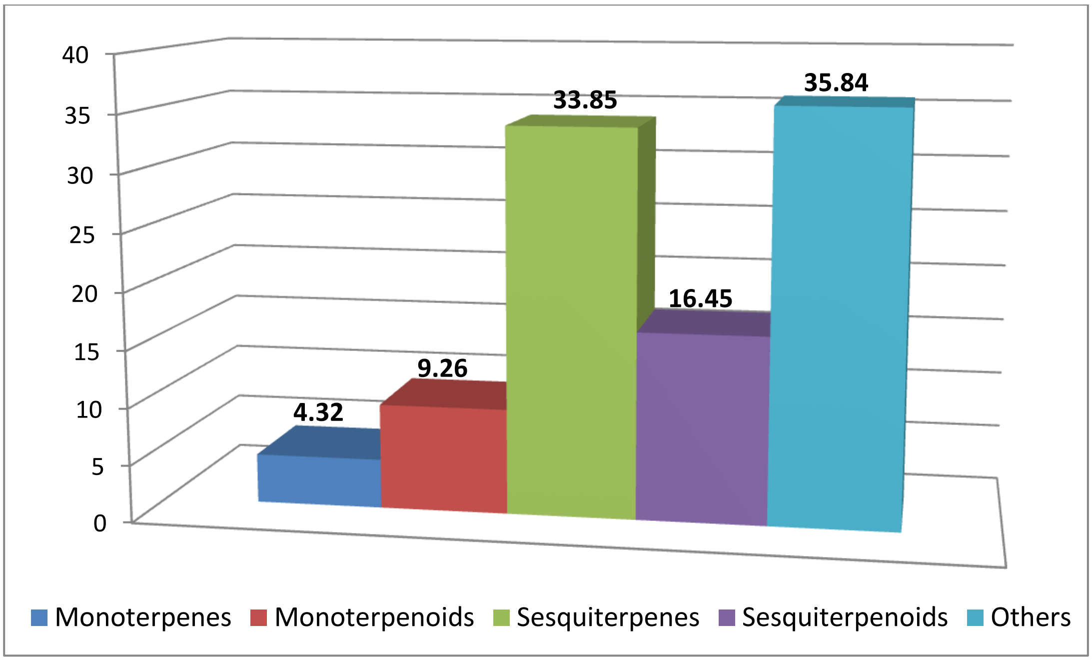
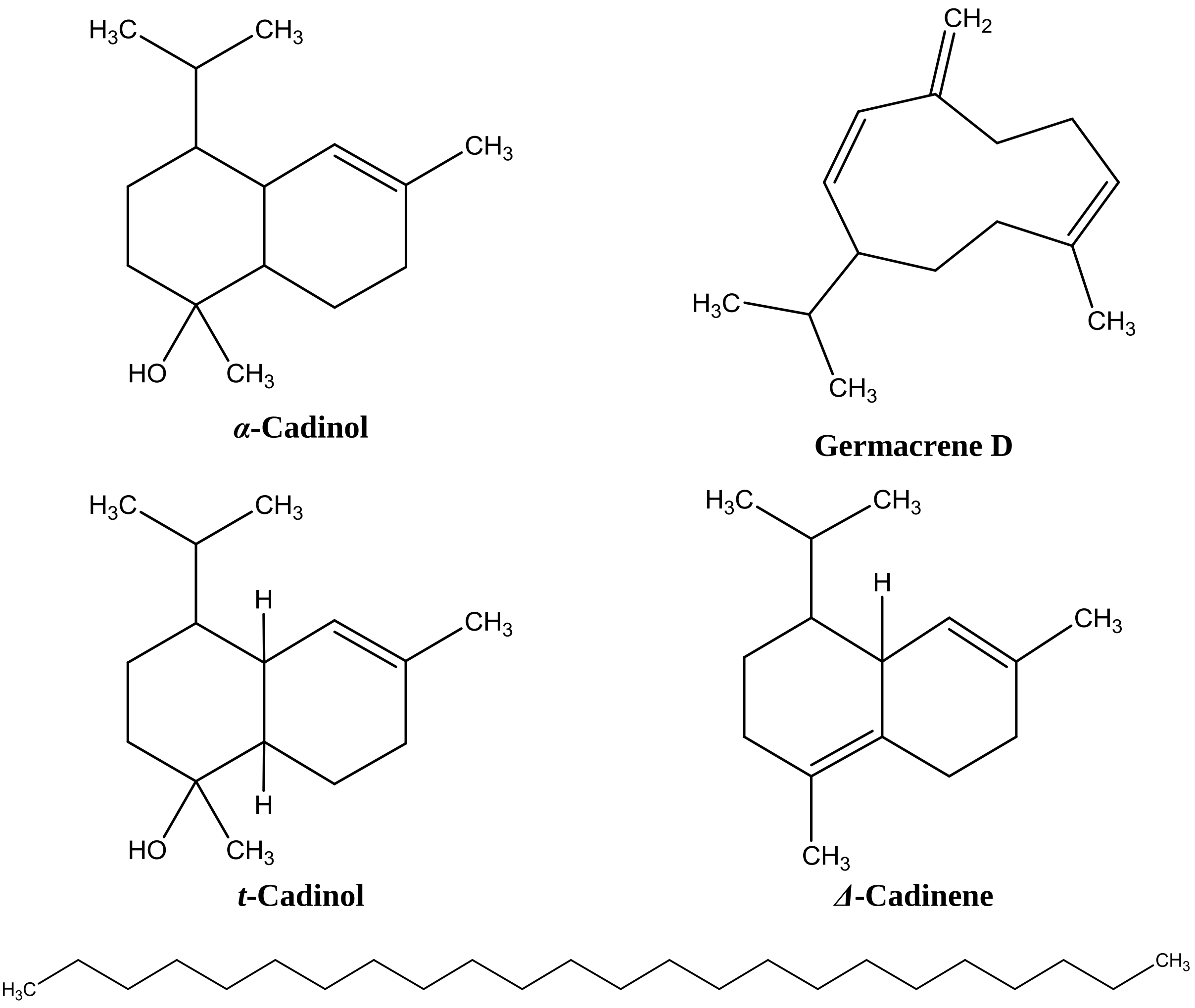


Figure 39. Histogram of *M. deserti* essential oil showing the chemical groups



***α*-Cadinol**

Germacrene D

***t*-Cadinol**

***Δ*-Cadinene**

Tetracosane

Figure 40. Chemical structures of major compounds of *M. deserti* essential oil

1.2. Total phenolic and total flavonoid contents and Antioxidant activity of *M. deserti*

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity and phenolic compounds are also known as powerful chain breaking antioxidants (Shahidi *et al.*, 1992). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily is ingested from a diet rich in stems and vegetables (Tanaka *et al.*, 1988). The concentration of phenolics and flavonoids in the extract was expressed as micrograms of pyrocatechol and micrograms of quercetin equivalents per milligrams of the extract, respectively. The methanol extract had 58.17 ± 0.03 µg pyrocatechol equivalents as its phenolic content, and demonstrated 5.2 ± 0.03 µg quercetin equivalents as its flavonoid content.

In lipid peroxidation inhibition assay (Table 6), the essential oil exhibited a high inhibition (IC_{50} = 21.42 ± 4.8 µg/mL) against lipid peroxidation, while in DPPH assay, the essential oil demonstrated a weak $DPPH^{\bullet}$ scavenging activity. As seen it the essential oil composition (Table 5), there are no phenolic compounds (only Thymol) to scavenge the DPPH radicals. However, the compounds particularly the bicyclic compounds as well as the conjugated mono and sesquiterpenoids are responsible for the lipid peroxidation activity. These compounds can scavenge the singlet oxygen. Therefore, they protect the *β*-carotene colour against bleaching, indirectly. Whereas, the methanol extract showed highest antioxidant activity both in DPPH assay and against Lipid peroxidation better than essential oil. These results are supported the previously studies, where the polar extracts showed higher antioxidant activity than no polar extracts (Edziri *et al.,* 2012).

The CUPRAC assay utilised copper(II)-neocuproine (CU(II)-Nc) reagent as the chromogenic oxidizing agent. It is based on the measurement of absorbance at 450 nm by the formation of a stable complex between neocuproine and copper (I). CUPRAC of the methanol extract and essential oil of *M. deserti* were assessed and compared to that of the positive controls BHA and *α*-tocopherol. As shown in Figure 41, the methanol extract and α-tocopherol had similar activities. Only at 800 µg/mL concentration, the methanol extract (3.81 ± 0.00) exhibited higher reducing power activity than *α*-tocopherol (2.9 ± 0.00) and nearly the activity of BHA (3.8 ± 0.00) absorbances at 450 nm. However, the activity of essential oil was less than the positive controls.

Table 6. Antioxidant activity (%) of the essential oil and methanol extract of *M. deserti* by the DPPH and β-carotene/linoleic acid assays[a]

	Concentration ug/mL	**Inhibition %**			
		MeOH	**EO**	**BHA**	**α-Tocopherol**
DPPH$^\bullet$	12.5	41.71±0.32	*NA*	31.15±0.65	90.7±0.23
	25	72.02±0.15	*NA*	38.56±0.81	91.16±0.17
	50	76.77±0.17	1.34±0.54	43.78±0.21	92.03±0.55
	100	77.85±0.26	3.05±0.58	59.9±0.35	93.77±0,07
	200	78.35±0.10	5.47±0.52	79.83±0.51	95.9±0.05
	400	78.96±0.03	10.04±0.46	90.58±0.24	96.1±0.9
	800	79.58±0.05	18.09±0.12	94.16±0.15	96.7±0.21
	IC$_{50}$	**12.87±0.25**	**559.78±9.08**	**45.4 ± 0.47**	**7.31 ± 0.17**
β-carotene/ linoleic acid	12.5	67.13±2.58	34.58±9.21	90.1±0.2	89.15±0.1
	25	77.62±0.9	62.29±4	91.56±0.22	90.6±0.3
	50	81.9±0.45	72.96±1.37	92.68±0.3	91.89±0.27
	100	85.97±0.11	75.09±0.08	93.6±0.16	92.1±0.51
	200	86.33±0.27	76.81±0.59	94.8±0.21	93.32±0.33
	400	89.35±1.29	84.68±0.17	95.8±0.15	94.22±0,28
	800	91.72±0.45	83.22±0.36	97.7±0.16	96.02±0,30
	IC$_{50}$	**2.15±1.83**	**21.42±4.8**	**1.34 ± 0.04**	**2.10 ± 0.08**

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

NA: not active

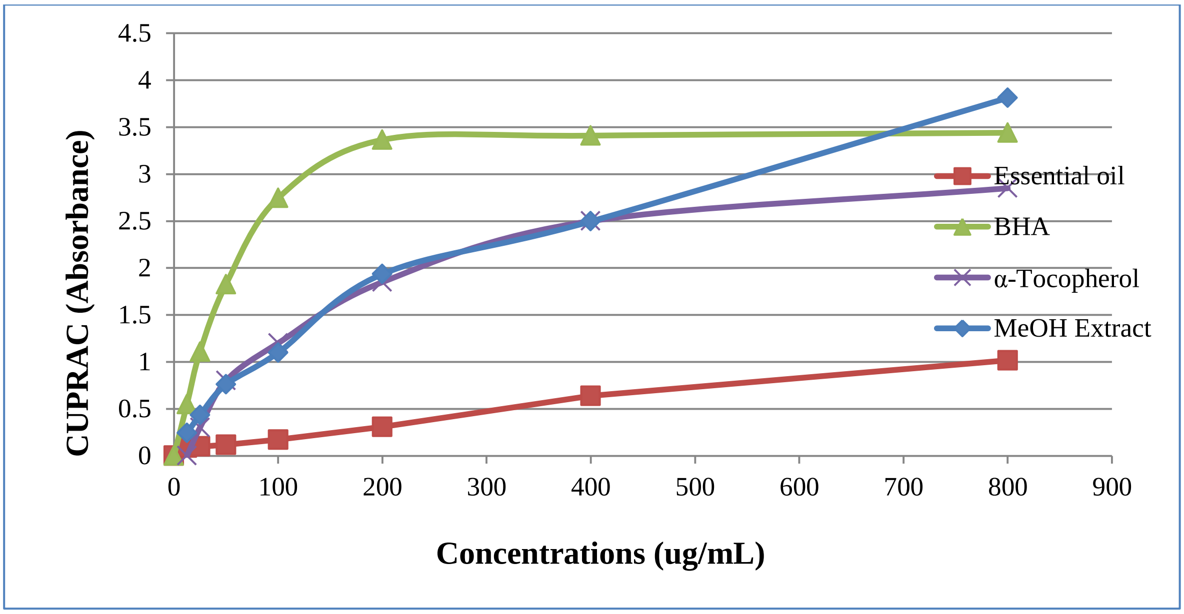


Figure 41. Cupric reducing antioxidant capacity of the methanol extract and essential oil of *M. deserti*

Values expressed as absorbance at 450 nm are means ± standard deviation of three parallel measurements. ($p < 0.05$).

1.3. Anticholinesterase activity of *M. deserti*

The anticholinesterase activity of the essential oil and methanol extract of *M. deserti*, against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes was given in Table 7. Galantamine was the standard drug for comparison. The methanol extract exhibited a weak inhibitory activity against AChE and BChE enzymes. The IC_{50} values were 277.39 ± 13.55, and 93.3 ± 0.67 µg/mL, respectively. whereas, the essential oil was inactive against both AChE and BChE.

Anticholinesterase activity of *Marrubium* species have been reported earlier. Orhan *et al.* (2010) found that the highest inhibition against AChE (76.30 ± 0.18 % at 100 µg/mL) and BChE (83.51 ± 1.27 % at 50 µg/mL) was caused by the acetone extract of aerial parts of *M. vulgare* collected from Algeria. Whereas, Ethanol (EtOH) extract of the same plant and acetone extract of *M. astranicum* growing in Turkey were almost not active against both enzymes. MeOH extract of leaves of *M. vulgare* grown in Algeria showed a weak AChE activity (IC_{50} = 0.52 ± 0.03 mg/mL) (Amessis-Ouchemoukh *et al.*, 2014). In another study, the EtOH extract (aerial parts and flowers) of *M. incanum* did not achieve 50% inhibition of enzyme activity at 100 µg/mL concerntration (Vladimir-Knežević *et al.*, 2014). Our anticholinesterase activity of essential oil and methanol extract of *M. deserti* are nearly similar with other *Marrubium* species.

1.4. Minimum inhibitory concentrations and antibiofilm activity of *M. deserti*

The antimicrobial activity of the essential oil and methanol extract against 6 bacteria and *Candida albicans* ATCC 10239 are given in Table 8. The essential oil inhibited the growth of all microorganisms between 25 and 80 µL/mL concentrations. The methanol extract of *M. deserti* had MICs in the range of 3.25-25 mg/mL. According to the results, *B. subtilis* ATCC 6633 was found as the most susceptible strain against methanol extract of *M. deserti*. The extract has low activity on the growth of *M. luteus* NRRL B-4375, *S. mutans* CNCTC 8/77 and *S. epidermidis* MU 30 which were only inhibited at high concentration (25 mg/mL). Essential oil at the MIC's inhibited biofilm formations of all microorganisms tested in various percentages. The essential oil exhibited the highest antibiofilm activity against *C. albicans* ATCC 10239 at 25 µg/mL (MIC) concentration with 36.62 %.

In the presence of 25 mg/mL extract (MIC), the mean biofilm formation values were equal to 69.31 % for *M. luteus* NRRL B-4375, 29.27 % for *S. mutans* CNCTC 8/77 and 56.28 % for

S. epidermidis MU 30. The antibiofilm activity of essential oil on tested strains was lower than methanol extract.

Table 7. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil and methanol extract of *M. deserti*[a]

		Inhibition %		
	Concentration µg/mL	**MeOH**	**EO**	**Galantamine**
AChE	3.125	*NA*	*NA*	41.75±0.65
	6.25	*NA*	*NA*	52.32±1.20
	12.5	*NA*	*NA*	62.21±0.32
	25	*NA*	*NA*	68.36±1.10
	50	7.22±1.32	*NA*	74.38±0.65
	100	19.89±3.89	*NA*	78.59±0.47
	200	35.41±1.92	*NA*	80.4±0.9
	IC_{50}	**277.39±13.55**	*NA*	**5.01±0.09**
BChE	3.125	*NA*	*NA*	17.44±1.08
	6.25	*NA*	*NA*	21.35±0.66
	12.5	*NA*	*NA*	29.62±1.30
	25	14.15±1.82	*NA*	40.59±2.88
	50	28.56±2.01	*NA*	48.73±0.90
	100	**53.16±0.13**	*NA*	65.02±0.44
	200	-[b]	*NA*	82.2±1.6
	IC_{50}	**93.3±0.67**	*NA*	**53.9±0.56**

NA: not active

[a] IC_{50} values represent the means ± SD. of three parallel measurements ($p < 0.05$).

[b] Could not be determined due to turbidity in the well.

Table 8. MIC and antibiofilm activity results of the essential oil and methanol extract of *M. deserti*

	Essential oil						**Methanol extract**					
	Planktonic	% inhibition on biofilms					Planktonic	% inhibition on biofilms				
Microorganism	MIC µL/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16	MIC mg/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16
Staphylococcus aureus ATCC 25923	50	19.21	*NI*	*NI*	*NI*	*NI*	6.25	28.90	11.65	*NI*	*NI*	*NI*
Staphylococcus aureus ATCC 6538 P	80	4.96	*NI*	*NI*	*NI*	*NI*	12.5	27.25	7.37	*NI*	*NI*	*NI*
Staphylococcus epidermidis MU 30	25	29.81	20.33	*NI*	*NI*	*NI*	25	56.28	28.51	10.78	5.31	*NI*
Bacillus subtilis ATCC 6633	50	27.98	11.25	*NI*	*NI*	*NI*	3.25	7.57	*NI*	*NI*	*NI*	*NI*
Bacillus cereus RSKK 863	25	26.66	18.25	4.62	*NI*	*NI*	12.5	49.66	25.68	10.85	*NI*	*NI*
Micrococcus luteus NRRL B-4375	25	23.75	10.70	*NI*	*NI*	*NI*	25	69.31	45.46	27.45	3.32	*NI*
Streptococcus mutans CNCTC 8/77	25	20.36	*NI*	*NI*	*NI*	*NI*	25	29.27	10.88	*NI*	*NI*	*NI*
Candida albicans ATCC 10239	25	36.62	17.69	*NI*	*NI*	*NI*	12.5	34.32	18.47	5.51	*NI*	*NI*

NI : no inhinition

2. Results of *Anthemis stiparum* subsp. *sabulicola*

2.1. GC/MS of the essential oil of *A. stiparum* subsp. *sabulicola*

The essential oil having yellow color was obtained from aerial parts using hydrodistillation (0.30 %, v/w) method. Totally, 72 components were identified representing 99.02% of the oil (Table 9). The major compounds of essential oil of *A. stiparum* subsp. *sabulicola* were Germacrene D (11.13 %), t-cadinol (11.01 %), Camphor (6.73 %), Spathulenol (6.50 %), and Isoamyl salicylate (6.45 %).

Germacrene D has been identified as the major fraction (8.9%) of the oil of aerial part of *A. cotula* L. (Saroglou *et al.*, 2006). Aslo, presente in many *Anthemis* essential oils with different percentages. Such as: Aerial parts of *A. segetalis* Ten. (12.6%) (Radulović *et al.*, 2009); *A. auriculata* Boiss. (0.8-9.5%) and *A. chia* L. (0.6-4.6%) from Greece (Saroglou *et al.*, 2006); *A. hyaline* (5.1%) (Sajjadi and Mehregan, 2006) and in aerial parts of essential oil of *A. altissima* L. (6.9%) (Javidnia *et al.*, 2004) grown in Iran.

Also, t-cadinol was detected as a second major compound with 11.01 %. Many reports on essential oil of *Anthemis* species have been detected t-cadinol compound with a high percentages. For exemple; aerial parts of *A. tinctoria* L. var. *parnassica* (11.5%) (Saroglou *et al.*, 2006) and *A. segetalis* Ten (4.2%) growing in Montenegro (Radulović *et al.*, 2009).

Other studies concerning the chemical composition of *Anthemis* essential oils, Hanbali *et al.* (2007) found that camphor (17.5%) was among the major compounds in *A. tenuisecta* growing in Morocco and in *A. cretica* L. subsp. *Leucanthemoides* from Turkey with 19.4% (Baser *et al.*, 2002). Camphor was determined as a constituent of the essential oil 6.73% (Table 9). Borneol and 1,8 Cineole were identified as dominant compounds in essential oils of *Anthemis* species (Radulović *et al.*, 2009). Whereas, they were present in lower amounts in our sample with 0.28 % and 0.26%, respectively. Interestingly, Isoamyl salicylate was found to be one of the major compounds in essential oil of *A. stiparum* subsp. *sabulicola* with 6.45%. However, this compound was not reported from any *Anthemis* essential oils in the literature. This compound is used in perfumes and fragrances (Khokhar *et al.*, 2011). Changes in the composition of essential oil might have arisen from several different factors such as climatical, seasonal and geographical (Zengin *et al.*, 2012).

Table 9. Chemical composition (%) of the essential oil of *A. stiparum* subsp. *sabulicola*

N°	**Compounds**	**Composition (%)**	**RI**
1	Cumene	0.49	906
2	α-Pinene	0.49	936
3	Camphene	0.24	950
4	6-Methyl-5-heptene-2-one	4.34	976
5	*β*-Pinene	0.05	978
6	o-Cymene	0.05	1009
7	Eucalyptol (1,8 Cineol)	0.26	1024
8	Limonene	0.06	1025
9	γ-Terpinen	0.15	1051
10	**Camphor**	**6.73**	1123
11	Borneol	0.28	1150
12	Isogeranial	0.99	1156
13	α-Terpineol	0.10	1176
14	2-Decanone or 3-Decanone	0.13	1178
15	n-Dodecane	0.10	1200
16	δ-Elemene	0.49	1340
17	Neryl acetate	0.17	1342
18	*β*-Damascenone	0.18	1348
19	Decanoic acid	0.29	1349
20	α-Copaene	0.62	1379
21	Methyleugenol	0.12	1385
22	*β*-Bourbonene	0.12	1388
23	*β*-Elemene	0.28	1390
24	*β*-Caryophyllene	1.97	1408
25	*β*-Gurjunene	0.15	1431
26	Seychellene	0.64	1447

27	α-Humulene	0.33	1455
28	Alloaromadendren	0.57	1460
29	Aromadendrane <dehydro->	0.41	1462
30	Sesquicineole <dehydro->	4.46	1469
31	Sesquicineole <7-epi-1,2-dehydro->	0.16	1471
32	**Germacrene D**	**11.13**	1481
33	δ-Selinene	0.34	1492
34	*β*-Himachalene	5.19	1500
35	(E,Z)-α-Farnesene	0.50	1501
36	1,5-Cycloundecadiene, 8,8-dimethyl-9-methylene-	0.84	1504
37	*β*-Bisabolene	0.18	1505
38	γ-Cadinene	1.60	1510
39	δ-Cadinene	1.15	1520
40	**Isoamyl salicylate**	**6.45**	1523
41	cis-3-Hexenyl Benzoate	0.74	1545
42	γ-Elemene	0.05	1570
43	**Spathulenol**	**6.50**	1572
44	Caryophyllene oxide	2.56	1578
45	Globulol	0.44	1589
46	Isoaromadendrene epoxide	1.22	1592
47	Ledene oxide-(II)	0.65	1604
48	Cubenol	0.56	1630
49	Cedren-3-one <2-epi-α>	2.22	1631
50	Hexenyl Phenyl acetate <(3Z)->	1.50	1635
51	**t-Cadinol**	**11.01**	1640
52	*β*-Eudesmol	0.53	1641
53	α-Cadinol	2.56	1643

54	Edusmol <7-epi-α>	1.41	1653
55	Khusinol	0.88	1658
56	α-Bisabolol	1.39	1673
57	cis-Z-α-Bisabolene epoxide	3.80	1704
58	Nuciferol <(Z)->	0.32	1713
59	Farnesol <2Z,6E)->	0.42	1720
60	γ-Costol	0.26	1746
61	Cedryl acetate	0.26	1769
62	Farnesyl acetate <(2Z,6E)->	0.28	1812
63	E-10-Pentadecenol	0.13	1908
64	Pimaradiene	0.08	1962
65	Hexadecanoic acid	0.43	1981
66	2-Heptanone, 6-methyl-5-meth	0.35	2002
67	Hexadecanoic acid, trimethylsilyl ester	0.30	2040
68	Heneicosane	0.67	2100
69	1,18-Nonadecadien-7,10-dione	0.10	2168
70	Labda-8(20),14-dien-6α,13-ol, (13S)-	0.55	2265
71	Tricosane	3.29	2306
72	Pentacosane	0.78	2500
	Total identified:	99.02	
	Monoterpenes:	5.88	
	Monoterpenoids:	8.36	
	Sesquiterpenes:	26.54	
	Sesquiterpenoids:	28.61	
	others	29.64	

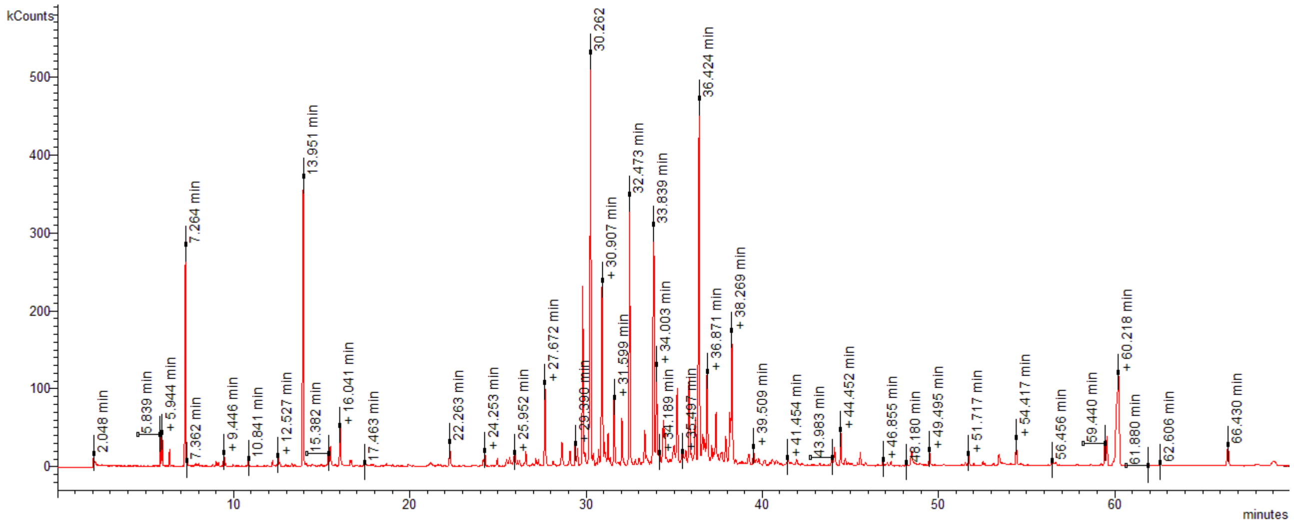


Figure 42. GC/MS chromatogram of *A. stiparum* subsp. *sabulicola* essential oil showing the detection of chemical components using DB-1 column

The monoterpenoids represented 8.36 % while sesquiterpenoids 28.61 % of the total oil. The monoterpenes and sesquiterpenes were in the ratio of 5.88 % and 26.54 %, respectively (figure 43). These results are nearly similar to other studies about essential oils of *Anthemis* species, such as, essential oil of *A. odontostephana* Boiss. var. *odontostephana* growin in Iran (Sajjadi *et al.*, 2013) and Italian *A. maritima* grown in Pianosa (Ciccarelli *et al.*, 2013) and *A. cotula* from Serbia and Montenegro (Pavlović *et al.*, 2010).

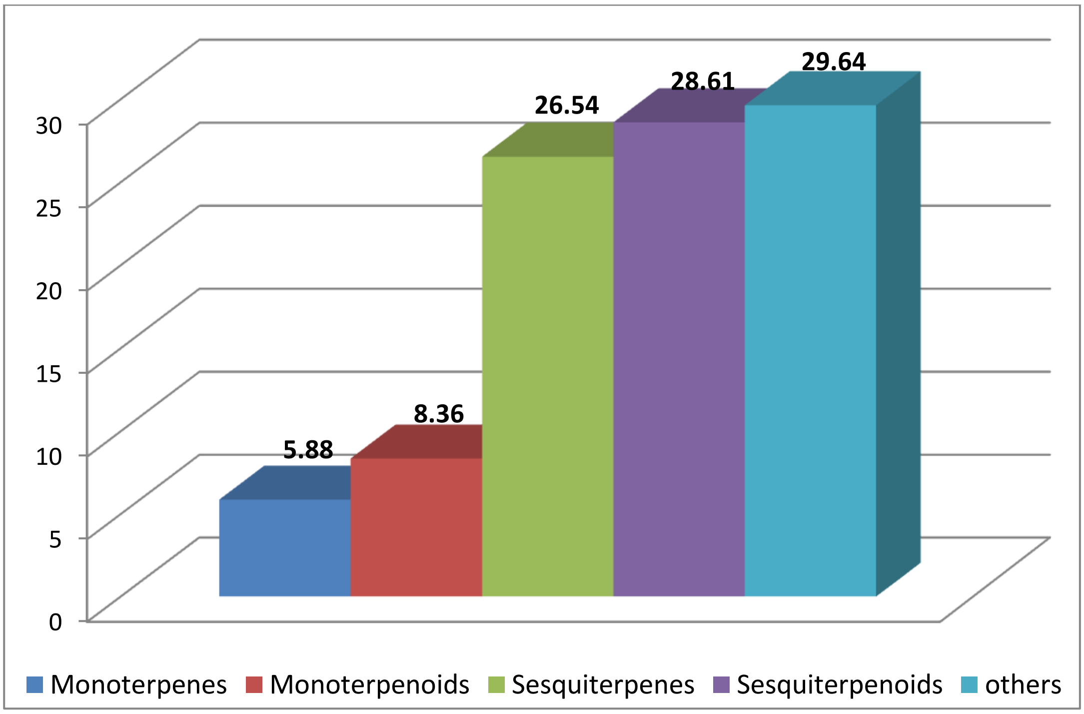
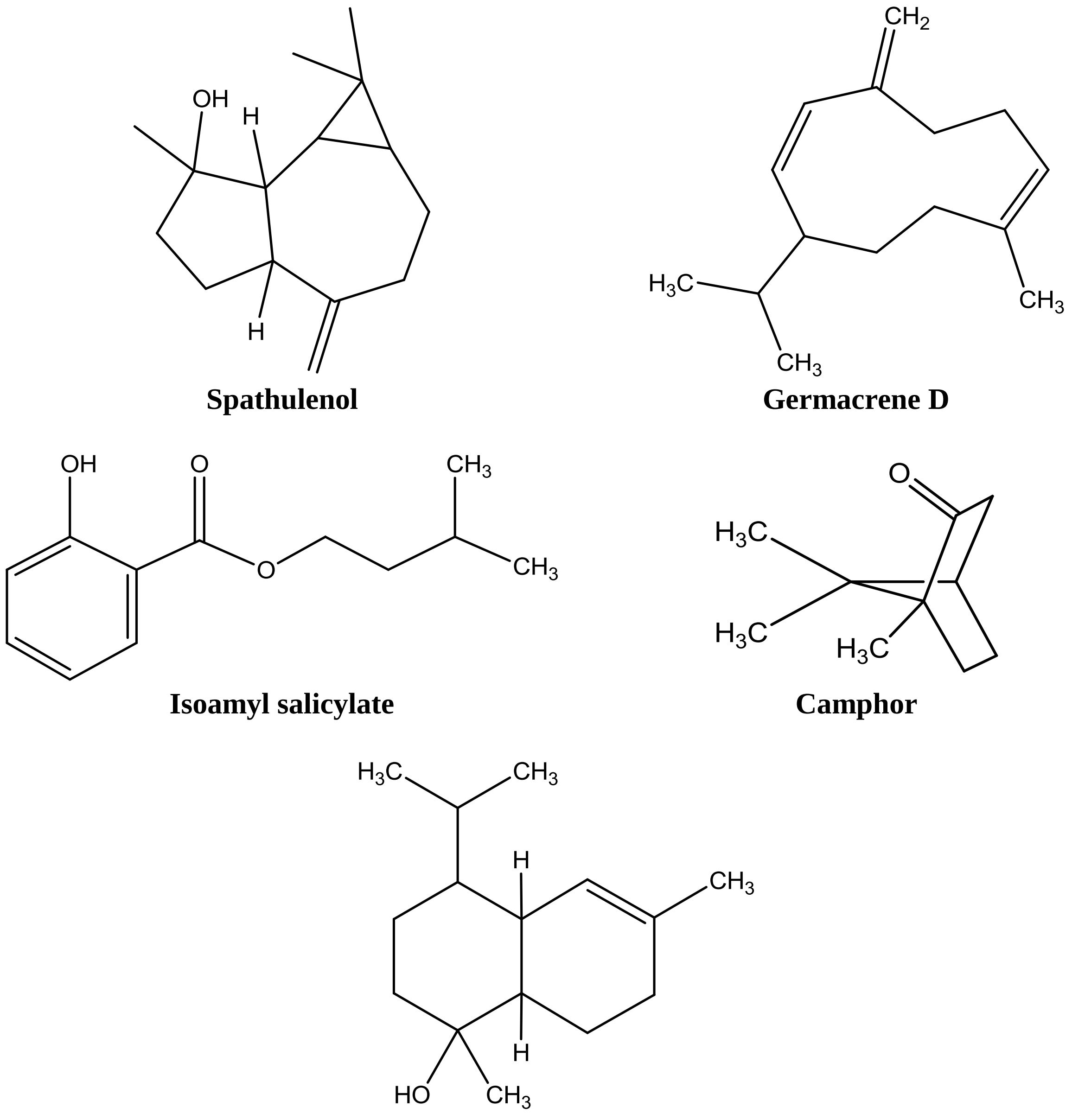


Figure 43. Histogram of *A. stiparum* subsp. *sabulicola* essential oil showing the chemical groups



Spathulenol

Germacrene D

Isoamyl salicylate

Camphor

t-Cadinol

Figure 44. Chemical structures of major compounds of *A. stiparum* subsp. *sabulicola* essential oil

2.2. Total phenolic and total flavonoid contents and Antioxidant activity of *A. stiparum* subsp. *sabulicola*

Results of antioxidant activity of MeOH extract and essential oil of *Athemis stiparum* Subsp. *sabulicola* by three the aforesaid assays are summarized in Table 10 and Figure 45. Antioxidant activity increases with increasing extract/oil concentration in three assays. In DPPH assay, MeOH extract of *A. stiparum* subsp. *sabulicola* scavenged 69.62 ± 1.23 % of $DPPH^{\bullet}$ radical at 800 ug/mL concentration less than BHT and α-tocopherol. Wherase, EO showed week scavenging (12.54 ± 1.40 %) at the same concentration. In *β*- caroten/linoleic acid assay, MeOH extract exhibiting nearly similar activity to the standards antioxidant agent BHT and α-Tocopherol (94.03 ± 0.92% at 800 ug/mL concentration). While, the oil inhibited 52.65 ± 0.75 %. The CUPRAC assay showed moderate activity for MeOH extract compared with standards, while EO showed weak activity.

Table 10. Antioxidant activity (%) of the essential oil and methanol extract of *A. stiparum* subsp. *sabulicola* by the DPPH and *β*-carotene/linoleic acid assays

	Concentration ug/mL	**Inhibition %**			
		MeOH	**EO**	**BHA**	**α-Tocopherol**
DPPH	12.5	13.14±2.60	*NA*	31.15±0.65	90.7±0.23
	25	17.53±1.91	*NA*	38.56±0.81	91.16±0.17
	50	31.35±2.34	2.52±0.77	43.78±0.21	92.03±0.55
	100	53.09±2.58	3.96±0.34	59.9±0.35	93.77±0,07
	200	58.56±2.03	5.30±1.12	79.83±0.51	95.9±0.05
	400	67.42±1.45	8.45±0.94	90.58±0.24	96.1±0.9
	800	69.62±1.23	12.54±1.40	94.16±0.15	96.7±0.21
	IC_{50}	**92.69±4.48**	**917.69±68.14**	**45.4 ± 0.47**	**7.31 ± 0.17**
β-carotene/ linoleic acid	12.5	58.11±0.28	*NA*	90.1±0.2	89.15±0.1
	25	75.17±2.78	8.46±2.45	91.56±0.22	90.6±0.3
	50	81.85±2.68	18.39±0.39	92.68±0.3	91.89±0.27
	100	88.56±0.39	33.1±6.07	93.6±0.16	92.1±0.51
	200	90.04±2.64	43.29±4.63	94.8±0.21	93.32±0.33
	400	93.23±0.54	44.37±1.45	95.8±0.15	94.22±0,28
	800	94.03±0.92	52.65±0.75	97.7±0.16	96.02±0,30
	IC_{50}	**9.96±3.36**	**619.98±26.35**	**1.34 ± 0.04**	**2.10 ± 0.08**

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

NA: not active

The activity might be related to its phenolic content and flavonoids as reported in a previous study (Mimica-Dukic *et al.*, 1999). The concentration of phenolics and flavonoids in the extract was expressed as micrograms of pyrocatechol and micrograms of quercetin equivalents per milligrams of the extract, respectively. The methanol extract had 13.6 ± 0.03 µg pyrocatechol equivalents as its phenolic content, and demonstrated 5.9 ± 0.04 µg quercetin equivalents as its flavonoid content.

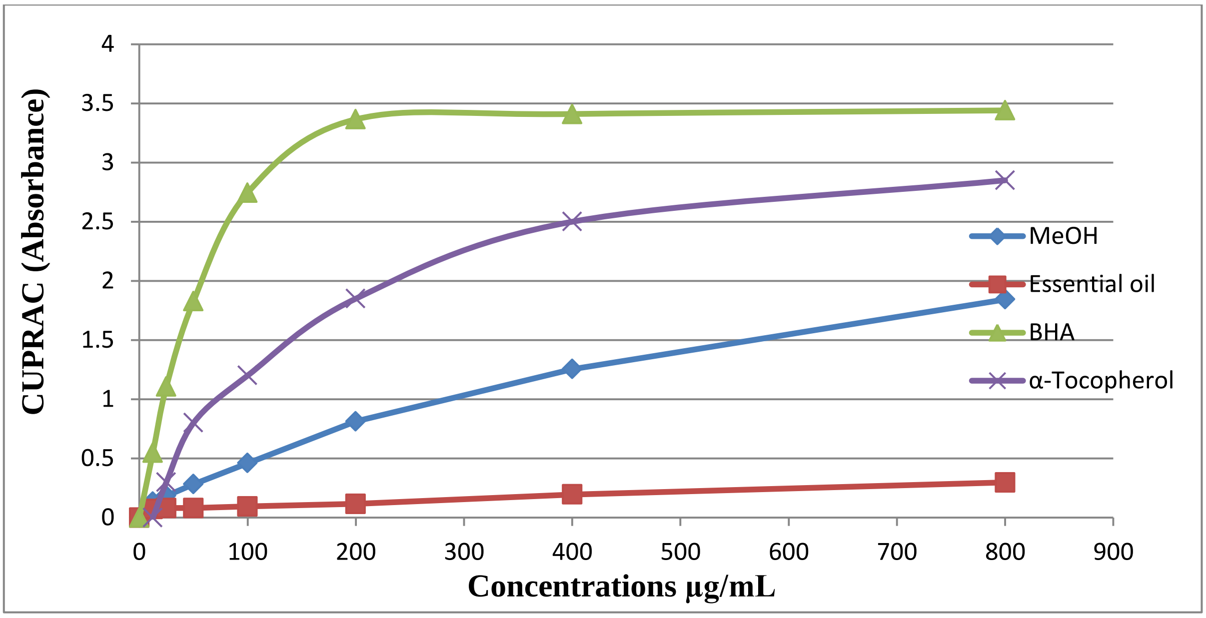


Figure 45. Cupric reducing antioxidant capacity of the methanol extract and essential oil of *A. stiparum* subsp. *sabulicola*

Values expressed as absorbance at 450 nm are means ± standard deviation of three parallel measurements. ($p < 0.05$).

2.3. Anticholinesterase activity of *A. stiparum* subsp. *sabulicola*

The anticholinesterase activity of the essential oil and methanol extract of *A. stiparum subsp. sabulicola*, against AChE and BChE enzymes was given in Table 11. Galantamine was the standard drug for comparison. The methanol extract exhibited a weak inhibitory activity against AChE and BChE enzymes. The IC_{50} values were 490.46 ± 76.53, and 142.07 ± 5.41 µg/mL, respectively. Whereas, the essential oil was inactive against AChE and exhibited a weak inhibitory activity against BChE 212.14 ± 2.31.

Table 11. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil and methanol extract of *A. stiparum* subsp. *sabulicola*[a]

		Inhibition %		
	Concentration µg/mL	**MeOH**	**EO**	**Galantamine**
AChE	3.125	*NA*	*NA*	41.75±0.65
	6.25	*NA*	*NA*	52.32±1.20
	12.5	*NA*	*NA*	62.21±0.32
	25	*NA*	*NA*	68.36±1.10
	50	6.64±1.88	*NA*	74.38±0.65
	100	16.84±2.52	*NA*	78.59±0.47
	200	22.18±3.88	*NA*	80.4±0.9
	IC_{50}	**490.46±76.53**	*NA*	**5.01±0.09**
BChE	3.125	*NA*	*NA*	17.44±1.08
	6.25	*NA*	*NA*	21.35±0.66
	12.5	*NA*	*NA*	29.62±1.30
	25	14.29±3.08	15.42±0.98	40.59±2.88
	50	25.35±0.71	27.1±2.78	48.73±0.90
	100	36.97±2.77	37.11±0.98	65.02±0.44
	200	-[b]	47.69±0.81	82.2±1.6
	IC_{50}	**142.07±5.41**	**212.14±2.31**	**53.9±0.56**

NA: not active

[a] IC_{50} values represent the means ± SD. of three parallel measurements ($p < 0.05$).

[b] Could not be determined due to turbidity in the well.

2.4. Minimum inhibitory concentrations and antibiofilm activity of *A. stiparum* subsp. *sabulicola*

The antimicrobial and anti-biofilm activities of essential oil/MeOH extract of *A. stiparum* subsp. *sabulicola* against 8 microorganisms were examined in the present study and the results are given in Table 12. The essential oil inhibited the growth of all microorganisms between 25 to 100 µL/mL concentrations. MIC of essential oil was 25 µL/mL for *S. aureus* ATCC 25923 and *S. epidermidis* MU 30. These results are consistent with previous studies (Bardaweel *et al.*, 2014; Vakili *et al.*, 2013) demonstrating that *Anthemis* species essential oil possess antibacterial activity on the *Staphylococcus* spp. The essential oil of *A. stiparum* subsp. *sabulicola* exhibited moderate antifungal activity against *C. albicans* ATCC 10239 with MIC value of 50 µL/mL. Essential oil at the MIC's inhibited biofilm formations of all microorganisms tested in various percentages. The oil exhibited the highest antibiofilm activity against *M. luteus* NRRL B-4375 at 100 µL/mL (MIC) concentration with 45.41%. In the presence of 50 µL/mL essential oil (MIC), the mean biofilm formation value was equal to 44.44 % for *B. subtilis* ATCC 6633. At a concentration of 25 µL/mL essential oil, the biofilm formations of *S.epidermidis* MU 30 and *S.aureus* ATCC 25923 were inhibited to 29.17 and 8.25%, respectively.

According to the results, *S.aureus* ATCC 25923 and *B. subtilis* ATCC 6633 were found as the most susceptible strains against methanol extract of *A stiparum* subsp. *sabulicola.*

Using methanol extract, a MIC of 6.25 mg/mL was observed for *C. albicans* ATCC 10239. On the other hand, methanol extract has low activity on the growth of the other strains, which were only inhibited at high concentrations (12.5 and 25 mg/mL). In the presence of 1.56 mg/mL extract (MIC), the mean biofilm formation values were equal to 59.06 % for *S. aureus* ATCC 25923, and 5.09 % for *B. subtilis* ATCC 6633. Methanol extract showed the strongest antibiofilm activity against the *C. albicans* ATCC 10239 and it was induced 80.02 % inhibition of the biofilm when used at MIC.

Our findings indicate that the essential oil/MeOH extract of *A. stiparum* subsp. *sabulicola* may have a potential for the development of new anti-biofilm, as well as antimicrobial agents, and demonstrate the importance of such medicinal plant in pharmaceutical production.

Table 12. MIC and antibiofilm activity results of the essential oil and methanol extract of *A. stiparum* subsp. *sabulicola*

	Essential oil						**Methanol extract**					
	Planktonic	% inhibition on biofilms					Planktonic	% inhibition on biofilms				
Microorganism	MIC µL/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16	MIC mg/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16
Staphylococcus aureus ATCC 25923	25	8.25	*NI*	*NI*	*NI*	*NI*	1.56	59.06	*NI*	*NI*	*NI*	*NI*
Staphylococcus aureus ATCC 6538 P	100	22.47	9.80	*NI*	*NI*	*NI*	12.5	27.84	11.43	*NI*	*NI*	*NI*
Staphylococcus epidermidis MU 30	25	29.17	*NI*	*NI*	*NI*	*NI*	25	30.43	16.07	2.27	*NI*	*NI*
Bacillus subtilis ATCC 6633	50	44.44	27.98	7.40	*NI*	*NI*	1.56	5.09	*NI*	*NI*	*NI*	*NI*
Bacillus cereus RSKK 863	100	8.80	*NI*	*NI*	*NI*	*NI*	12.5	34.33	30.29	20.91	*NI*	*NI*
Micrococcus luteus NRRL B-4375	100	45.41	20.21	8.25	*NI*	*NI*	12.5	57.96	19.49	3.36	*NI*	*NI*
Streptococcus mutans CNCTC 8/77	50	20.21	8.25	*NI*	*NI*	*NI*	12.5	25.80	9.67	*NI*	*NI*	*NI*
Candida albicans ATCC 10239	50	21.93	4.83	*NI*	*NI*	*NI*	6.25	80.02	37.42	30.26	*NI*	*NI*

NI : no inhinition

3. Results of *Rhanterium suaveolens*

3.1. GC/ MS of the essential oil of *R. suaveolens*

The essential oil having yellow color was obtained from aerial parts using hydrodistillation method, the yield was 0.14% (volume/dry-weight). A total of 20 compounds were identified representing 98.01% of the total oil content. The major compounds were perillaldehyde (45.79%), caryophyllene oxide (24.82%), *β*-cadinol (5.61%), *β*-caryophyllene (5.17%), and 8-cedren-13-ol (4.98%). *β*-pinene (3.21%) and α-irone (1.62%) were also determined as a constituent of the essential oil (Table 13). Monoterpenoids accounted for 48.25 % of the total oil content while sesquiterpenoids amounted to 37.97% of the total oil. The sesquiterpenes and monoterpenes were detected in the ratio of 7.40% and 4.39%, respectively. The essential oils of *R. adpressum* and *R. epopposum* were rich of monoterpenoids (Hamia *et al.*, 2013; Yaghmai and Kolbadipour, 1987). The essential oil of *R. suaveolens* also resembled to those oils from classification side.

Table 13. The Chemical composition (%) of essential oil of *R. suaveolens*

N°	Compounds	Composition (%)	RI[a]
1	*β*-Pinene	3.21	980
2	3-Carene	0.33	1015
3	*α*-Terpinene	0.85	1026
4	**Perillaldehyde**	**45.79**	**1270**
5	*γ*-Elemene	*t*	1298
6	Dihydroedulan	0.47	1305
7	*cis*-Isoeugenol	0.32	1375
8	*β*-citrylideneethanol	1.67	1398
9	***β*-Caryophyllene**	**5.17**	**1415**
10	*α*-Caryophyllene	*t*	1448
11	Valencene	0.33	1455
12	*β*-Vatirenene	1.12	1464

13	*α*-Muurolene	*t*	1471
14	*α*-Selinene	*t*	1479
15	*α*-Irone	1.62	1485
16	*cis*-*α*-Bisabolene	0.78	1498
17	**Caryophyllene oxide**	**24.82**	**1572**
18	Isoaromadendrene epoxide	0.94	1579
19	***β*-Cadinol**	**5.61**	**1655**
20	**8-Cedren-13-ol**	**4.98**	**1680**
	Total identified:	**98.01**	
	Monoterpenes:	**4.39**	
	Monoterpenoids:	**48.25**	
	Sesquiterpenes:	**7.40**	
	Sesquiterpenoids:	**37.97**	

[a] Kovats index on DB-1 fused silica column. *t: trace*

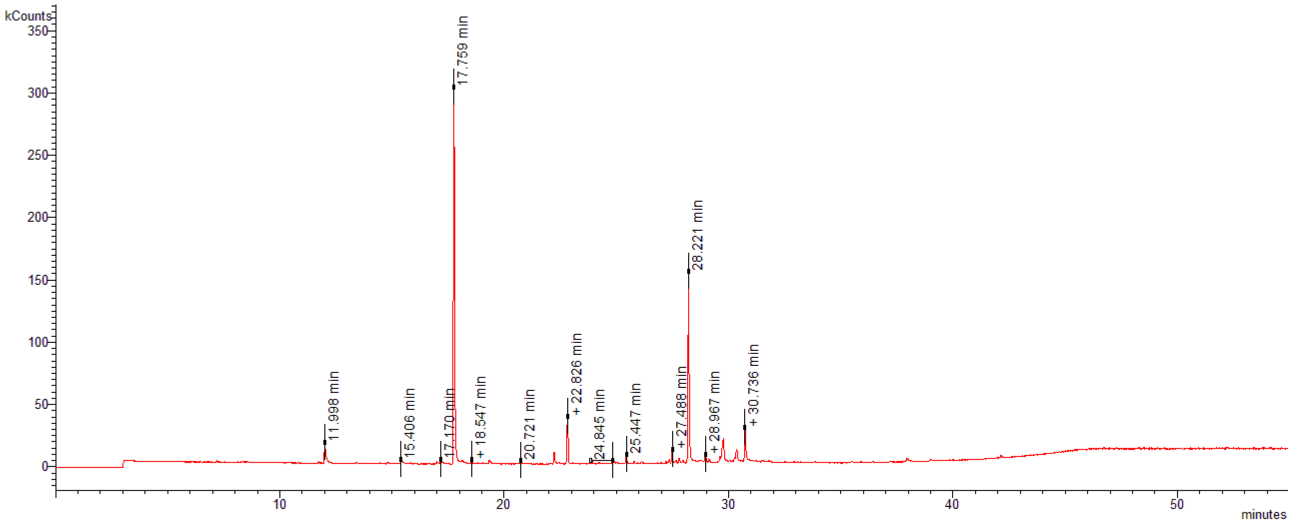


Figure 46. GC/MS chromatogram of *R. suaveolens* essential oil showing the detection of chemical components using DB-1 column

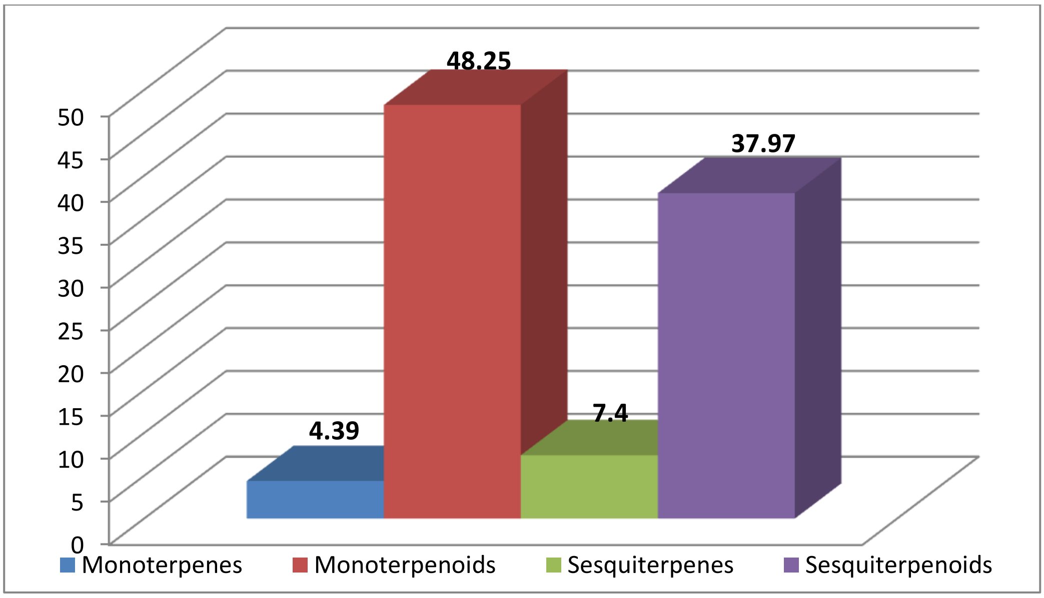
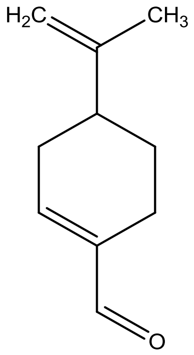
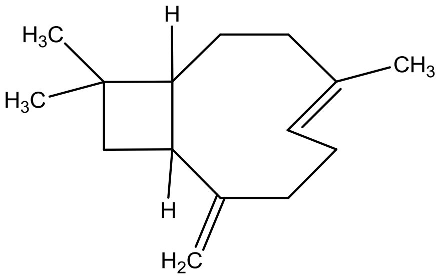


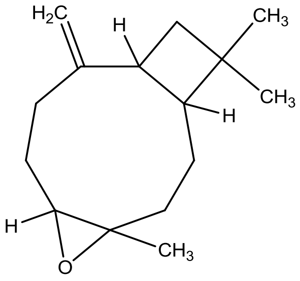
Figure 47. Histogram of *R. suaveolens* essential oil showing the chemical groups



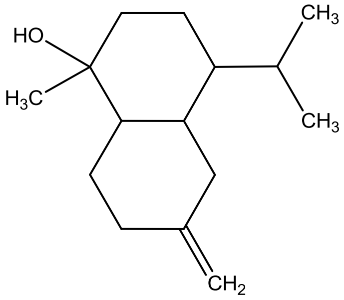
Perillaldehyde



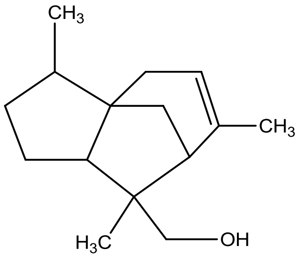
***β*-Caryophyllene**



Caryophyllene oxide



***β*-Cadinol**



8-Cedren-13-ol

Figure 48. Chemical structures of major compounds of *R. suaveolens* essential oil

3.2. Total phenolic and total flavonoid contents and Antioxidant activity of *R. suaveolens*

The total phenolic and total flavonoid contents were performed spectrophotometrically using pyrocatechol and quercetin as standard compounds, respectively. The phenolic content of MeOH extract of *R. suaveolens* was 35.58 ± 0.04 µg pyrocatechol equivalents/mg extract and the flavonoid content was 5.5 ± 0.02 µg quercetin equivalents/mg extract. It can be said that the *R. suaveolens* is poor in flavonoids. Supportively, in a previous study (Bouaziz *et al.*, 2009). 625 ± 75 mg pyrogallol/100 g and trace milligrams of quercetin/100 g were reported for the same plant collected in November of Douz area in Tunisia. The season and area of collection of the plant may lead to the different results.

Table 14 shows the $DPPH^{\bullet}$ scavenging activity and lipid peroxidation inhibitory activity by *β*-carotene/linoleic acid assay of the EO and the MeOH extract of *R. suaveolens*. Antioxidant activity of the extract and oil increased dose dependently in both assays. In DPPH assay, the MeOH extract demonstrated good $DPPH^{\bullet}$ scavenging activity (IC_{50} = 36.59 ± 0.16 µg/mL), while the EO exhibited weak activity. In the previous study the DPPH assay was performed only for MeOH extract, and the IC_{50} value was calculated as 1.09 ± 0.19 µg/mL (Bouaziz *et al.*, 2009). These differences may be due to the collection time and locality of the plant.

Table 14. Antioxidant activity (%) of the essential oil and methanol extract of *R. suaveolens* by the DPPH and *β*-carotene/linoleic acid assays[a]

	Concentration ug/mL	**Inhibition %**			
		MeOH	**EO**	**BHA**	**α-tocopherol**
DPPH	12.5	19.84±1.27	*NA*	31.15±0.65	90.7±0.23
	25	35.38±1.70	*NA*	38.56±0.81	91.16±0.17
	50	66.95±0.27	3.27±0.17	43.78±0.21	92.03±0.55
	100	68.24±0.32	5.85±0.43	59.9±0.35	93.77±0,07
	200	73.52±0.19	10.42±0.31	79.83±0.51	95.9±0.05
	400	74.98±0.16	17.36±0.04	90.58±0.24	96.1±0.9
	800	75.62±0.07	27.21±0.32	94.16±0.15	96.7±0.21
	IC_{50}	**36.59±0.16**	**373.86±3.47**	**45.4 ± 0.47**	**7.31 ± 0.17**
β-carotene /linoleic acid	12.5	58±3.37	35.79±0.76	90.1±0.2	89.15±0.1
	25	74.71±0.21	56.71±1.45	91.56±0.22	90.6±0.3
	50	81.14±0.27	60.07±1.39	92.68±0.3	91.89±0.27
	100	85.04±0.05	64.64±0.11	93.6±0.16	92.1±0.51
	200	87.07±0.27	66.61±2.2	94.8±0.21	93.32±0.33
	400	87.75±1.03	71.08±3.13	95.8±0.15	94.22±0,28
	800	88.76±1.23	68.36±0.15	97.7±0.16	96.02±0,30
	IC_{50}	**11.55±3.39**	**17.97±5.40**	**1.34 ± 0.04**	**2.10 ± 0.08**

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).
NA: not active

In lipid peroxidation inhibition assay, the MeOH extract exhibited higher inhibition (IC_{50} = 11.55 ± 3.39 µg/mL) against lipid peroxidation, while the EO exhibited (IC_{50} = 17.97 ± 5.40 µg/mL). The EO composition was given in Table 13. As seen, there are no phenolic compounds to scavenge the DPPH radicals. However, compounds as well as the conjugated mono and sesquiterpenoids are responsible for the lipid peroxidation activity. These compounds can scavenge the singlet oxygen and therefore protect the *β*-carotene colour against bleaching, indirectly.

Figure 49 shows the results of the CUPRAC (cupric reducing antioxidant capacity) of both the MeOH extract and the EO of *R. suaveolens*. *α*-Tocopherol and BHT were used as positive controls. As shown in figure 49 the activity of the MeOH extract of the *R. suaveolens* was compatible with *α*-tocopherol at all concentrations. As expected the EO indicated a less reducing activity.

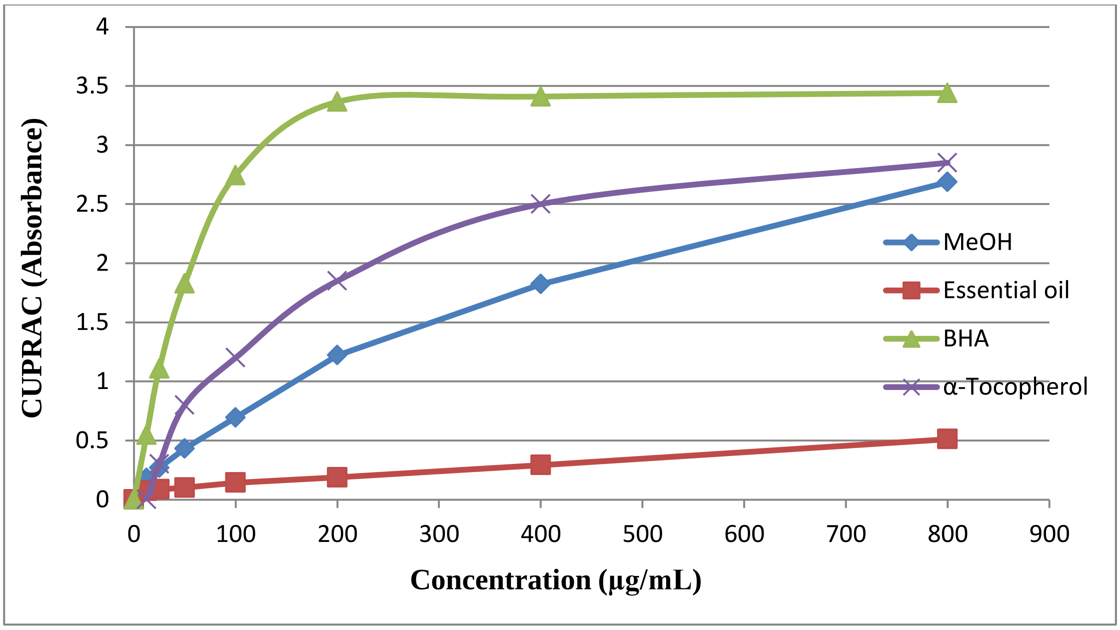


Figure 49. Cupric reducing antioxidant capacity of the essential oil and methanol extract of *R. suaveolens*

Values expressed as absorbance at 450 nm are means ± SD of three parallel measurements. ($p < 0.05$).

3.3. Anticholinesterase activity of *R. suaveolens*

The anticholinesterase activity of the EO and MeOH extract of *R. suaveolens*, against AChE and BChE enzymes was given in Table 15. Galantamine was the standard drug for comparison. The MeOH extract exhibited moderate to good inhibitory activity against AChE and BChE enzymes. The IC_{50} values were 169.29 ± 3.87 and 40.94 ± 0.97 µg/mL, respectively. On the contrary, the EO was inactive against both AChE and BChE.

Table 15. Acetylcholinesterase and butyrylcholinesterase inhibitory activities (%) of the essential oil and methanol extract of *R. suaveolens*.[a]

		Inhibition %		
	Concentration µg/mL	**MeOH**	**EO**	**Galantamine**
AChE	3.125	*NA*	*NA*	41.75±0.65
	6.25	*NA*	*NA*	52.32±1.20
	12.5	*NA*	*NA*	62.21±0.32
	25	*NA*	*NA*	68.36±1.10
	50	14.97±1.72	*NA*	74.38±0.65
	100	34.73±2.7	*NA*	78.59±0.47
	200	57.15±0.85	*NA*	80.4±0.9
	IC_{50}	**169.29±3.87**	*NA*	**5.01±0.09**
BChE	3.125	*NA*	*NA*	17.44±1.08
	6.25	*NA*	*NA*	21.35±0.66
	12.5	18.62±1.16	*NA*	29.62±1.30
	25	35.73±6.98	*NA*	40.59±2.88
	50	58.59±0.75	*NA*	48.73±0.90
	100	69.66±0.06	*NA*	65.02±0.44
	200	-[b]	*NA*	82.2±1.6
	IC_{50}	**40.94±0.97**	*NA*	**53.9±0.56**

NA: not active

[a] IC_{50} values represent the means ± SD. of three parallel measurements ($p < 0.05$).

[b] Could not be determined due to turbidity in the well.

3.4. Minimum inhibitory concentrations and antibiofilm activity of *R. suaveolens*

The antimicrobial activity of the EO and MeOH extract against 6 bacteria species and *Candida albicans* are given in (Table 16). The oil inhibited the growth of all microorganisms between 10 and 80 µg/mL concentrations. EO at the MIC's inhibited biofilm formations of all microorganisms tested in various percentages. The oil exhibited the highest antimicrobial activity against *S. epidermidis* MU 30 at 20 µg/mL (MIC/1) and at 10 µg/mL (MIC/2) concentrations with 50.3 % and 32.96 %, respectively.

According to the results, *B. subtilis* was found as the most susceptible strain against MeOH extract of *R. suaveolens*. The extract has low activity on the growth of *M. luteus* NRRL B-4375 and *S. epidermidis* MU 30 which were only inhibited at high concentration (25 mg/mL).

In the presence of 25 mg/mL extract (MIC), the mean biofilm formation values were equal to 58.34 % for *M. luteus* NRRL B-4375 and 49.06% for *S. epidermidis* MU 30.

In the current investigation, the EO exhibited antimicrobial activity, particularly against *S. epidermidis* MU 30, *M. luteus* NRRL B-4375 and *B. subtilis*ATCC 6633. Perillaldehyde, the major compound of the oil is an antimicrobial agent. It demonstrated antimicrobial activity against *B. cereus*, *E. coli* and *S. aureus* (Friedman, 2006). concerning the antimicrobial activity against *B. subtilis*, the oil supported the previous results. However, the oil that showed a weak activity against against *S. aureus*. This may be due to its percentage (45.79 %) in the oil which did not reach to the extent necessary to inhibit the growth of those microorganisms.

Table 16. MIC and antibiofilm activities of the essential oil and methanol extract of *R.suaveolens*

	Essential oil						**Methanol extract**					
	Planktonic	% inhibition on biofilms					Planktonic	% inhibition on biofilms				
Microorganism	MIC µL/mL	MIC	MIC/2	MIC/4	MIC /8	MIC/16	MIC mg/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16
Staphylococcus aureus ATCC 25923	20	9.48	*NI*	*NI*	*NI*	*NI*	12.5	20.75	*NI*	*NI*	*NI*	*NI*
Staphylococcus aureus ATCC 6538 P	80	4.96	*NI*	*NI*	*NI*	*NI*	12.5	27.25	7.37	*NI*	*NI*	*NI*
Staphylococcus epidermidis MU 30	20	50.30	32.96	*NI*	*NI*	*NI*	25	49.06	19.72	11.92	*NI*	*NI*
Bacillus subtilis ATCC 6633	40	45.55	13.20	*NI*	*NI*	*NI*	1.56	*NI*	*NI*	*NI*	*NI*	*NI*
Bacillus cereus RSKK 863	10	30.35	7.06	*NI*	*NI*	*NI*	12.5	50.49	13.01	8.43	*NI*	*NI*
Micrococcus luteus NRRL B-4375	40	47.43	39.34	11.65	*NI*	*NI*	25	58.34	42.95	38.59	18.36	12.33
Streptococcus mutans CNCTC 8/77	40	24.04	*NI*	*NI*	*NI*	*NI*	6.25	8.86	*NI*	*NI*	*NI*	*NI*
Candida albicans ATCC 10239	80	18.53	*NI*	*NI*	*NI*	*NI*	3.125	21.29	11.42	*NI*	*NI*	*NI*

NI : no inhibition

4. Results of *Centaurea furfuracea*

4.1. GC/MS of the essential oil of *C. furfuracea*

The table 17 shows the chemical composition of the essential oil of *C. furfuracea* obtained by hydrodistillation method, the yield of essential oil was 0.52 % (volume/dry-weight) and having yellow color. A total of 69 constituents comprising 96.94 % were characterized from the essential oil. The components listed in order of elution on a DB-1 column. Caryophyllene oxide, Z-10-Pentadecen-1-ol and Farnesyl methylester were major compounds representing 12.01 %, 8.11% and 7.79 % respectively.

Caryophyllene oxide (12.01 %), the major component of the aerial part oil of *C. furfuracea* has been also identified as the major fraction (38.5 %) of essential oil of aerial part of *C. pullata* growing in Blida, North Algeria (Djeddi *et al.*, 2011); the chloroform extract of aerial parts of *C. ensiformis* (28.72%), *C. austro-anatolica* (21.32 %) and *C. cariensis* subsp. *niveo-tomentosa* (20.79 %) collected in Mugla, Turkey (Ugur *et al.*, 2009a; Ugur *et al.*, 2009b; Ugur *et al.*, 2010); aerial parts of *C. athoa* oils (17.1 %) (Erel *et al.*, 2013); *C. aucheri* (17.4 %) (Asadipour *et al.*, 2005); *C. raphanina* subsp. *mixta* (10.3 %) (Lazari *et al.*, 1999) and *C. thessala*. subsp. *Drakiensis* (7.8 %) (Lazari *et al.*, 2000). Aslo, presented in many *Centaurea* essential oils by different percentage. Such as: Aerial parts of *C. solstitialis* (25.2 %); *C. depressa* (4.0 %) (Esmaeili *et al.*, 2006); capitula of *C. deflexa* (12.8 %); *C. aladaghensis* (7.5 %); *C. cheirolepidoides* (6.1 %); flower heads of *C. chrysantha* (9.5 %) (Flamini *et al.*, 2006); *C. eryngioides* (4.3 %) (Senatore *et al.*, 2005) and seeds of *C. huber-morathii* (3.3 %) (Baser *et al.*, 2006). Wherease, Farnesyl methylester 8.11 %, Z-10-Pentadecen-1-ol 7.79 % and trans-2-hexadecenoic acid 6.08 % have not been identified in *Centaurea* essential oils previously.

Table 17. The Chemical composition (%) of essential oil of *C. furfuracea*

N°	Compounds	Composition (%)	RI[a]
1	Furan, 2-pentyl-	0.10	984
2	2-Nonen-1-ol	0.17	1088
3	Linalool	0.09	1100
4	Camphor	0.06	1141
5	p-cymen-8-ol	0.06	1186
6	Safranal	0.06	1189
7	(E)-2-Decenal	0.29	1202
8	Perilla aldehyde	0.43	1270
9	Chroman	0.15	1286
10	Carvacrol	0.07	1300
11	2,4-Decadienal, (E,E)-	0.10	1305
12	α-cubebene	0.13	1350
13	Alloaromadendrene	0.06	1372
14	β-Elemene	1.61	1389
15	7-tetradecene	0.09	1394
16	(2Z)-3,7-Dimethyl-2-octenyl-2-methylpropanoate	0.14	1405
17	Cedrene	0.23	1410
18	**β-Caryophyllene**	**5.57**	1420
19	Isopropenyl-2,3,4,5-tetra methyl benzene	0.81	1428
20	γ(tau)-Elemene	2.64	1436
21	di-epi-α-cedrene	0.22	1440
22	α-humulene	0.51	1445
23	Z-β-Farnesene	0.96	1448
24	α-Himachalene	0.14	1450

25	gamma-elemene	0.31	1456
26	α-Guaiene	1.33	1476
27	Epi-bicyclosesquiphellandrene	2.48	1479
28	Eremophilene	0.43	1483
29	Ledene	0.10	1487
30	γ-Gurjunene	0.66	1495
31	**Z-10-pentadecen-1-ol**	**7.79**	1498
32	Isocaryophyllene	0.50	1509
33	Aromadendrene oxide-2	0.12	1514
34	5,6-Decadien-3-yne, 5,7-diet	0.53	1519
35	β-Cadinene	0.33	1530
36	β-Guaiene	0.17	1532
37	Epiglobulol	0.23	1539
38	tau-Gurjunene	1.46	1550
39	aromadendrene oxide	0.62	1569
40	Spathulenol	1.76	1577
41	**Caryophyllene oxide**	**12.01**	1556
42	Z-α-bisabolene epoxide	2.52	1590
43	Longifolenaldehyde	0.31	1613
44	8-cedrene-13-ol	1.68	1624
45	β-Eudesmol	3.68	1649
46	Santalol	0.22	1657
47	5β-7β-10α-Eudesm-11-en-1α-ol	1.32	1663
48	7,10,13-Hexadecatrienoic acid methyl ester	5.51	1670
49	Z-9,17-Octadecadienal	2.10	1674
50	Murolan-3,9(11)-diene-10-peroxy	1.43	1693

51	(2Z, 13E)-Octadecadien-1-ol	0.87	1725
52	6,8,8-trimethyl-2-methylenetricylo(5,2,2,0)undecan-3-ol	1.22	1736
53	Geranyllinallol	1.52	1751
54	Eudesma-4,11-diene-2-ol	1.12	1773
55	γ-costol	0.90	1775
56	**Farnesyl methylester**	**8.11**	1780
57	3Z,15Z-Octadecadien-1-ol acetate	0.18	1805
58	Hexahydro farnesylacetone	0.88	1838
59	1-Eicosanol	3.05	1874
60	Methyl 9,10-epoxystearate	0.10	1955
61	Verticiol	0.14	2014
62	1,2,3,4-tetrahydrophenanthren-9-ol	0.21	2044
63	n-Hexadecanoic acid	0.47	2103
64	9-Hexadecenoic acid	0.42	2125
65	**trans-2-hexadecenoic acid**	**6.08**	2213
66	3,7,11,15-tetramethyl-2-hexadecen-1-ol	1.96	2235
67	2-Octadecoxyethanol	3.94	2475
68	3-ethyl-5(2-ethylbuthyl) Octadecane	0.49	2598
69	1-docosanol	1.00	984
	Total identified:	**96.94**	
	Monoterpenoids:	**1.15**	
	Sesquiterpenes:	**19.83**	
	Sesquiterpenoids:	**52.30**	
	Fatty acids:	**6.97**	
	Other:	**16.69**	

[a]Kovats index on DB-1 fused silica column.

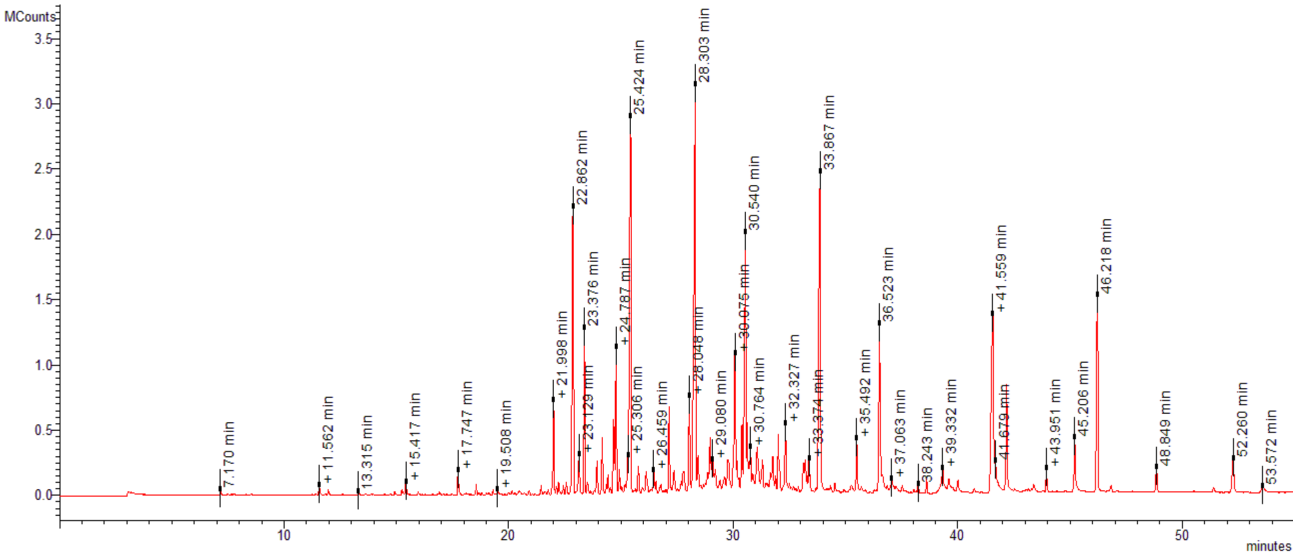


Figure 50. GC/MS chromatogram of *C. furfuracea* essential oil showing the detection of chemical components using DB-1 column

Components of the essential oil of were separated into five classes (figure 51), including monoterpenoids (1.15 %), sesquiterpenes (19.83 %), sesquiterpenoids (52.3 %); fatty acids (6.97 %) and others (16.69 %). The essential oil consisted mainly of oxygenated sesquiterpenes (52.3 %). caryophyllene oxide (12.01 %) and Z-10-Pentadecen-1-ol (7.79 %) were the prevailing oxygenated sesquiterpenes. Different parts of *C. zuvandica*; *C. cariensis* subsp. *niveo-tomentosa*; *C. ensiformis* and *C. athoa* were rich in oxygenated sesquiterpenes (Erel *et al.*, 2013; Salmanpour *et al.*, 2009; Ugur *et al.*, 2009a; Ugur *et al.*, 2010). The essential oil of *C. furfuracea* also resembled to those oils from classification side.

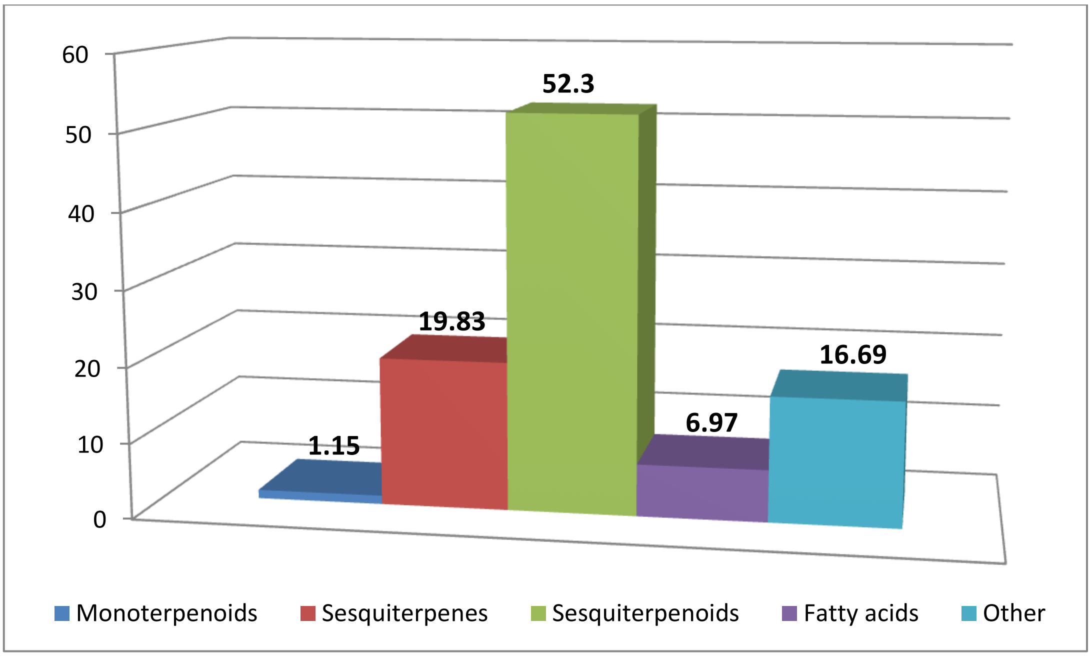


Figure 51. Histogram of *C. furfuracea* essential oil showing the chemical groups

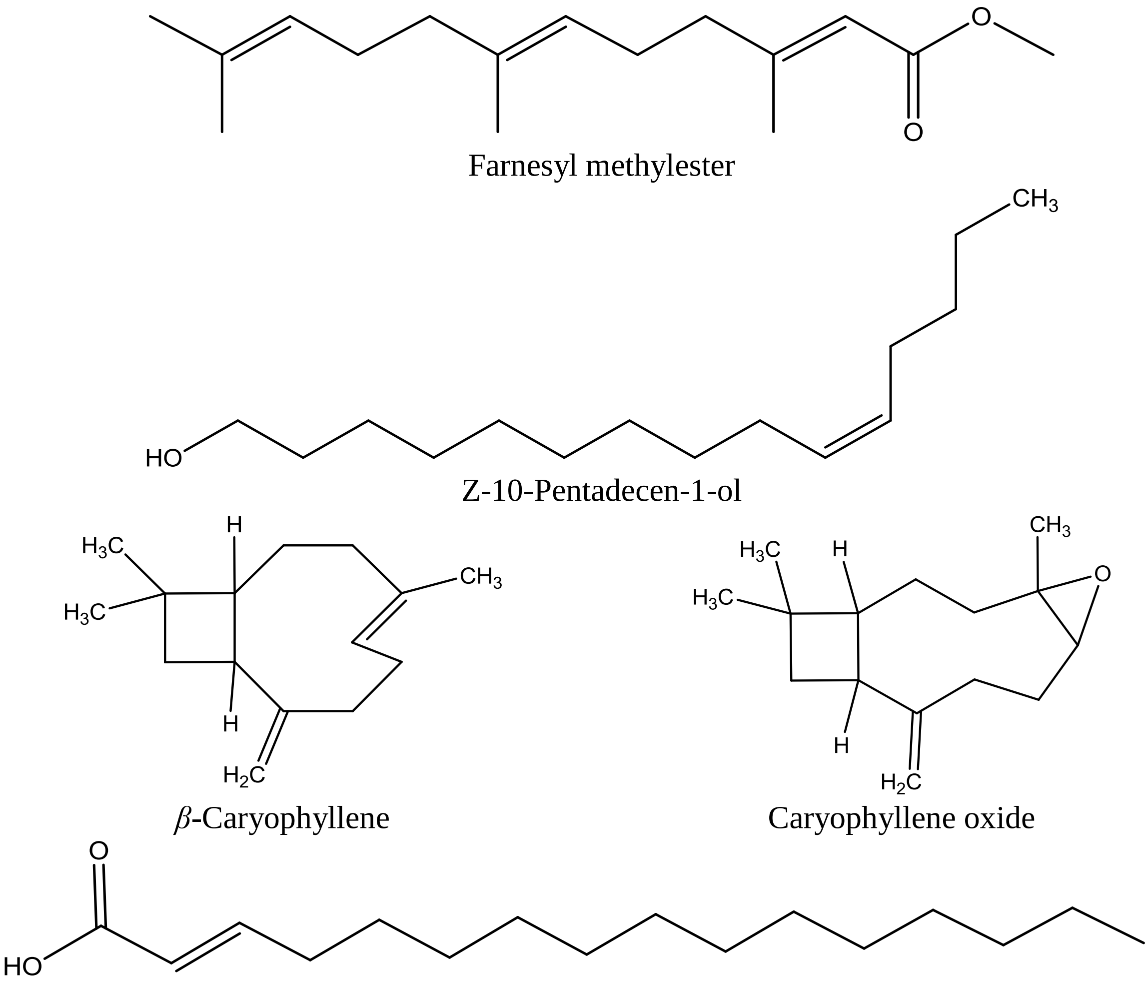


Figure 52. Chemical structures of major compounds of *C. furfuracea* essential oil

4.2. Total phenolic and total flavonoid contents and Antioxidant activity of *C. furfuracea*

There are many methods for assessment of antioxidant potential, and we cannot reliance of single universal method. Thus, we tested three antioxidant assays, that would give a better understanding into the true antioxidant potential of the essential oil and methanol extract. Table 18 summed the results of antioxidant activity of the extracts. In β-carotene-linoleic acid assay, methanol extract of *C. furfuracea* showed the best lipid peroxidation inhibition activity with an IC_{50} of 18.6 ± 4.11 µg/mL followed by its essential oil (91.25 ± 0.14 µg/mL). Some literatures reported that the inhibition capacity of *Centaurea* species such as *C. mucronifera* (35.2%) (Tepe *et al.*, 2006), *C. ensiformis* (85.15% of ethyl acetate, 72.51% of chloroform extract) (Ugur *et al.*, 2009a) and 63.60% of methanol extract in *C. pulchella* (Zengin *et al.*, 2010). In contrast, in DPPH assay, the methanol extract and essential oil showed moderate to low activity with IC_{50} = 190.47 ± 0.99 µg/mL for methanol extract and 1664.95±32 µg/mL for essential oil. The results of cupric reducing antioxidant capacity assay were given as absorbances (Figure 53). Higher absorbance exhibited higher activity. Generally, the extracts showed weak absorbances compared with BHA and α-tocopherol. The values $DPPH^{\bullet}$ scavenging activity of *Centaurea* species which plants growing in Scotland was found as ranging from 0.018 mg/mL and 0.095 mg/mL (Kumarasamy *et al.*, 2007). From the results it can be concluded that *C. furfuracea* has lower free radical scavenging activity than growing in Scotland.

Total phenolic and flavonoids contents of the extracts were investigated spectrophotometrically. The methanol extract had 4.75 ± 0.009 µg pyrocatechol equivalents as its phenolic content, and demonstrated 3.17 ± 0.001 µg quercetin equivalents as its flavonoid content. The amount of phenolics was very small with the results described in the literature for other *Centaurea* species. For example, the higher content was detected as 348.56 mg GAE g^{-1} for MeOH extract of *C. pulcherrima* var. *pulcherrima* growing in Turkey (Aktumsek *et al.*, 2013b) and lower content found with MeOH of *C. ammocyanus* as 10.9 mg GAE g^{-1} from Jordan (Alali *et al.*, 2007). The same thing with total flavonoid content, the other *Centaurea* species showed high amount than *C. furfuracea*, ranging from 13.12 ± 1.01 mg Rutin Equivalent/g extract to 182.56 ± 2.13 mg RE g^{-1} in MeOH extract of *C. babylonica* and *C. pulcherrima* var. *pulcherrima*, respectively (Aktumsek *et al.*, 2013b). These differences may due to genotype and growing location according to study carried out by Mpofu *et al.* (2006) on wheat. Also, Giorgi *et al.* (2010) reported that higher altitude may constitute a highly effective way to significantly enhance the levels of phenolic acids. Noticeable thing, the hight phenolic and favonoid contnets showed with *C. pulcherrima* var. *pulcherrima* collected from Kardeştep village, Arpacay, Kars in Turkey at 2245 m altitude.whereas, *C. furfuracea* collected from Algerian Sahara at 33 m altitude.

Table 18. Antioxidant activity (%) of the essential oil and methanol extract of *C. furfuracea* by the DPPH and *β*-carotene/linoleic acid assays

	Concentration µg/mL	**Inhibition %**			
		MeOH	**EO**	**BHA**	**α-Tocopherol**
DPPH	12.5	3.98±0.87	*NA*	31.15±0.65	90.7±0.23
	25	5.72±0.97	*NA*	38.56±0.81	91.16±0.17
	50	14.76±0.90	2.59±1.19	43.78±0.21	92.03±0.55
	100	29.46±0.45	3.80±1.59	59.9±0.35	93.77±0,07
	200	51.16±0.30	4.97±0.64	79.83±0.51	95.9±0.05
	400	65.36±0.57	5.98±1.12	90.58±0.24	96.1±0.9
	800	68.73±0.21	8.43±1.22	94.16±0.15	96.7±0.21
	IC_{50}	**190.47±0.99**	**1664.95±32**	**45.4 ± 0.47**	**7.31 ± 0.17**
β-carotene /linoleic acid	12.5	38.9±6.51	18.27±4.01	90.1±0.2	89.15±0.1
	25	51.56±5.48	40.26±2.41	91.56±0.22	90.6±0.3
	50	66.56±1.18	46.23±1.53	92.68±0.3	91.89±0.27
	100	72.09±1.56	50.61±0.36	93.6±0.16	92.1±0.51
	200	75.64±1	52.99±3.04	94.8±0.21	93.32±0.33
	400	75.41±1.98	57.59±2.22	95.8±0.15	94.22±0,28
	800	77.12±1.06	62.31±0.24	97.7±0.16	96.02±0,30
	IC_{50}	**18.6±4.11**	**91.25±0.14**	**1.34 ± 0.04**	**2.10 ± 0.08**

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

NA: not active

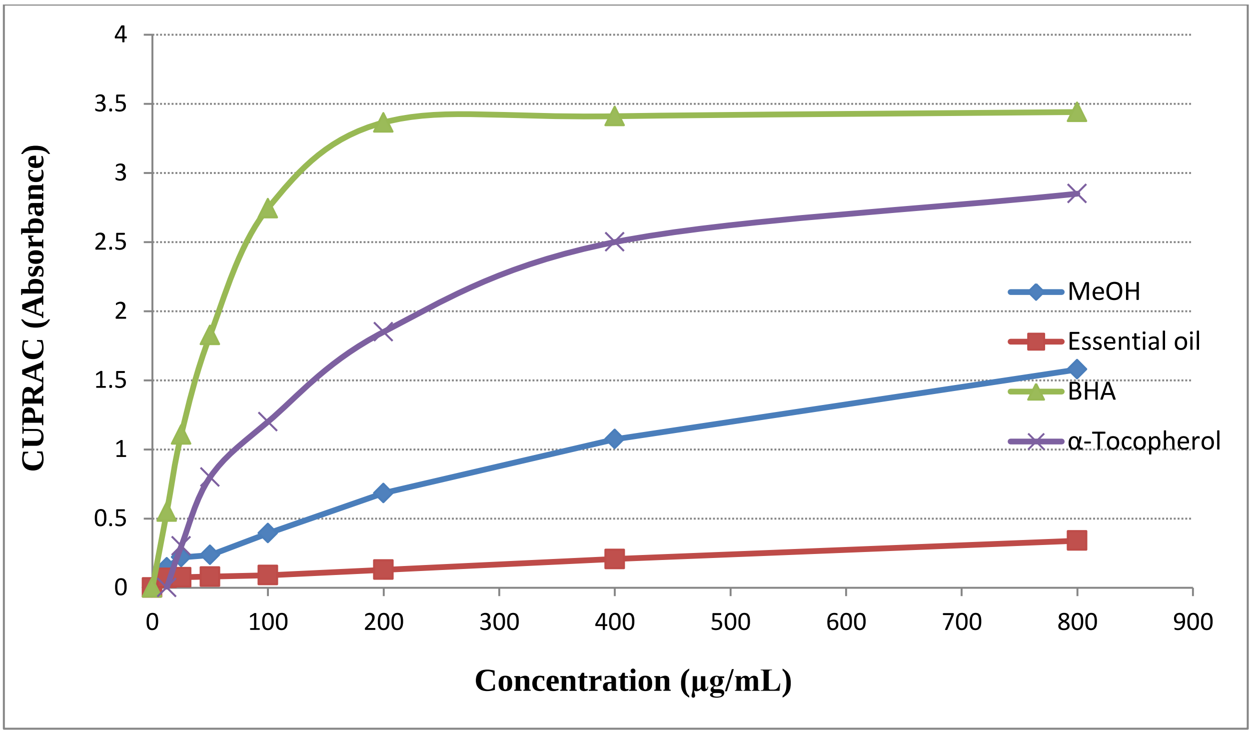


Figure 53. Cupric reducing antioxidant capacity of the essential oil and methanol extract of *C. furfuracea*

Values expressed as absorbance at 450 nm are means ± standard deviation of three parallel measurements. ($p < 0.05$).

4.3. Anticholinesterase activity of *C. furfuracea*

AChE inhibitors are used for the treatment of mild to moderate AD (Birks, 2006). Currently, the three cholinesterase inhibitors licensed Donepezil, Rivastigmine, and Galantamine are widely recommended for clinical use (Kaduszkiewicz *et al.*, 2005). Recently, there is a growing interest from the researchers about finding new AChE inhibitors from the plant resources. Anticholinesterase activity of some *Centaurea* species has been studied by Aktumsek *et al.* (2013a). This study inspected the anticholinesterase activity of *C. furfuracea* for the first time. Table 19 shows the results of AChE and BChE inhibitory activities of the extracts compared with that of Galantamine used as a standard drug. The tests were screened for AChE inhibitory activity using Ellman's colorimetric method in a 96-well plate by microplate reader at 25, 50 and 100 µg/mL concentrations. As shown in table 19 the methanol extract indicated a noticeable inhibition against AChE and BChE at all concentrations, the IC_{50} values were found be 164.4 ± 5.69 and 82.4 ± 1.75 µg/mL, respectively. These values are comparable to the value reported in the literature for other *Centaurea* species such as *C. polypodiifolia* var. *pseudobehen* (24.54% and 45.50% for AChE and BChE at 2 mg/mL)

(Aktumsek *et al.*, 2013a). while the essential oil showed almost no activity against AChE and BChE. Whereas, essential oils of other *Centaurea* species showed moderate activity (Ertas *et al.*, 2014).

Table 19. Acetylcholinesterase and butyrylcholinesterase inhibitory activities (%) of the essential oil and methanol extract of *C. furfuracea*.[a]

		Inhibition %		
	Concentration µg/mL	**MeOH**	**EO**	**Galantamine**
AChE	3.125	9.59±2.79	*NA*	41.75±0.65
	6.25	10.67±0.53	*NA*	52.32±1.20
	12.5	18.62±0.3	*NA*	62.21±0.32
	25	21.22±1.6	*NA*	68.36±1.10
	50	22.92±2.31	*NA*	74.38±0.65
	100	41.27±0.26	*NA*	78.59±0.47
	200	55.9±2.42	*NA*	80.4±0.9
	IC_{50}	**164.4±5.69**	*NA*	**5.01±0.09**
BChE	3.125	*NA*	*NA*	17.44±1.08
	6.25	*NA*	*NA*	21.35±0.66
	12.5	*NA*	*NA*	29.62±1.30
	25	13.56±1.78	*NA*	40.59±2.88
	50	34.52±1.07	*NA*	48.73±0.90
	100	59.14±1.73	*NA*	65.02±0.44
	200	-[b]	-	82.2±1.6
	IC_{50}	**82.4±1.75**	**-**	**53.9±0.56**

NA: not active

[a] IC_{50} values represent the means ± SD. of three parallel measurements ($p < 0.05$).

[b] Could not be determined due to turbidity in the well.

4.4. Minimum inhibitory concentrations and antibiofilm activity of *C. furfuracea*

Biofilms can provide a protective environment for pathogenic bacteria and reduce the effectiveness of inhibitory agents, Which leads to cause diseases in humans and animals (Hall-Stoodley *et al.*, 2004). The antibiofilm activity and MIC of the essential oil and methanol extract of *C. furfuracea* were studied in this work. The results are shown in Table 20. Statistically, the essential oil inhibited the growth of all tested microorganisms between 6.25 and 25 µL/mL concentrations, better than methanol extract. MIC values detrmined for methanol extract between 6.25 to 50 mg/mL. The extract has low activity on the growth of *S. aureus* ATCC 6538 P, *S. epidermidis* MU 30, *B. cereus* RSKK 863 and *M. luteus* NRRL B-4375 which are only inhibited at high concentration (50 mg/mL). The antimicrobial activity of essential oils and extracts of some *Centaurca* species have been investigated before. Ethyl acetate, acetone, chloroform, and ethanol extracts from *C. ptosomipappoides*, *C. odyssei*, *C. ptosomipappa*, *C. amonicola* and *C. kurdica* were investigated by agar-well diffusion assay, and all of the extracts exhibited an antimicrobial effect against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans* and *Candida globrata*. When, the high antimicrobial activity shown in ethyl acetate extract with MIC values between 62.5 and 250 mg/mL (Güven *et al.*, 2005).

In other study, The essential oils of *C. sessilis* and *C. armena* showed antibacterial activity against *Yersinia pseudotuberculosis* ATCC 911, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 6633 with inhibition zones between 5.5 and 10 mm (Yayli *et al.*, 2005). Whereas, essential oils of *C. appendicigera* and *C. helenioides* showed antimicrobial activity against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231 with MIC between 80 and 330 µg/mL (Yaylı *et al.*, 2009).

In other study by Bülent Köse *et al.* (2007). High antimicrobial activity of *Centaurea aladagensis* essential oil have shown against *Staphylococcus epidermidis* ATCC 1228 with MIC = 0.11 mg/mL. However, extraxts and essential oils of *Centaurea* species showed noticible antimicrobial activity against some microorganism with various percentages.

In the presence of 25 µL/mL essential oil (MIC), the mean biofilm formation values were equal to 40.75 % for *B. subtilis* ATCC 6633 and 39.80 % for *C. albicans* ATCC 10239. Essential oil did not exhibit inhibitory effect against *S. aureus* ATCC 6538-P biofilm formation.

The highest antibiofilm activity has been found 87.90 % with MeOH extract against *S. aureus* ATCC 6538 P and 87.53 % against *B. subtilis* ATCC 6633 at 50 mg/mL and 25 mg/mL concentrations (MIC), respectively. *C. furfuracea* essential oil has shown a good antimicrobial activity against test bacteria while extract has shown good antibiofilm activity.

Table 20. MIC and antibiofilm activities of the essential oil and methanol extract of *C. furfuracea*

	Essential oil						**Methanol extract**					
	Planktonic	% inhibition on biofilms					Planktonic	% inhibition on biofilms				
Microorganism	MIC µL/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16	MIC mg/mL	MIC	MIC/2	MIC/4	MIC/8	MIC/16
Staphylococcus aureus ATCC 25923	12.5	11.82	*NI*	*NI*	*NI*	*NI*	6.25	14.51	*NI*	*NI*	*NI*	*NI*
Staphylococcus aureus ATCC 6538 P	6.25	*NI*	*NI*	*NI*	*NI*	*NI*	50	87.90	34.41	18.60	*NI*	*NI*
Staphylococcus epidermidis MU 30	12.5	28.09	21.22	*NI*	*NI*	*NI*	50	80.13	38.35	18.49	*NI*	*NI*
Bacillus subtilis ATCC 6633	25	40.75	16.28	*NI*	*NI*	*NI*	25	87.53	56.74	34.01	10.09	*NI*
Bacillus cereus RSKK 863	6.25	6.94	*NI*	*NI*	*NI*	*NI*	50	83.03	72.34	47.94	15.60	7.47
Micrococcus luteus NRRL B-4375	25	10.40	*NI*	*NI*	*NI*	*NI*	50	79.07	58.85	51.20	22.91	13.47
Streptococcus mutans CNCTC 8/77	6.25	18.08	*NI*	*NI*	*NI*	*NI*	25	31.09	19.57	6.42	*NI*	*NI*
Candida albicans ATCC 10239	25	39.80	9.01	*NI*	*NI*	*NI*	12.5	36.11	14.44	9.51	*NI*	*NI*

NI: no inhibation

Chapter 6

General discusion

VI. GENERAL DISCUSSION

Essential oils and their various components have received much attention as a source of new drugs, because many have been demonstrated to have significant antibacterial, antifungal antimycotic, antihelmintic, and antiseptic properties against a wide range of microorganisms. Essential oils have also been shown to have antispasmodic and antioxidant properties, and to function as effective food preservatives (Burt, 2004; Djilani and Dicko, 2012; Lopes-Lutz *et al.*, 2008). Synthetic antioxidants such as butylhydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are commonly used in food and beverages, have toxic effects on the lungs and increase the incidence of liver tumors. Moreover, many infectious diseases have become difficult to treat because of antibiotic resistance, which is increasing at an alarming rate. In some cases, there are no treatment options. Therefore, to combat increasing antibiotic resistance and to meet the increasing demand for safe antioxidants, it is important to search for, and develop, new and natural antimicrobial and antioxidant agents (Ud-Daula *et al.*, 2016).

Free radicals are known to play a pivotal role in the onset and exacerbation of several pathologies (Demir *et al.*, 2014). By counteracting these free radicals, antioxidants help in preserving good health. Indeed, phytochemicals have received much interest owing to their molecular structure which consists of hydroxyl groups on aromatic rings and this has been associated with their functionality as oxidant scavengers (Tukun *et al.*, 2014). Phytochemicals act by inhibiting oxidative chain reactions at cellular level thereby increasing their therapeutic efficacy (Akhtar *et al.*; Zengin *et al.*, 2015).

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity and phenolic compounds are also known as powerful chain breaking antioxidants (Shahidi *et al.*, 1992). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily is ingested from a diet rich in stems and vegetables (Tanaka *et al.*, 1988). It is well known that polyphenols are widely distributed in plants, they are sometimes present in high concentrations, especially in medicinal plant and many edible plants (Hagerman *et al.*, 1998). Flavonoids and phenolic compounds are the main antioxidative compounds of fruits and vegetables (Huang *et al.*, 1998), which exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron-donating agents, and metal ion chelating properties (Rice-Evans *et al.*, 1996). The biological properties, scavenging and antioxidant activities of flavonoids have been extensively reviewed (Boumerfeg, 2010; Kumar and Pandey, 2013).

In this regard, the phenolic content of the plant extracts was estimated using the Folin-Ciocalteau method. This method is rapid and simple. Flavonoids are the major class of phenolic compounds and are known to exhibit strong antioxidant activities. The concentration of phenolics and flavonoids in the extract was expressed as micrograms of pyrocatechol and micrograms of quercetin equivalents per milligrams of the extract, respectively. Interestingly, in the present study, it was observed that MeOH extract of *M. deserti* showed high phenolic and flavonoid content.

The methanolic extracts had values ranging between 4.75±0.009 and 58.166±0.033 µg pyrocatechol equivalents as its phenolic content (µg pyrocatechol Eq/mg), in the following order:

M. deserti > *R. suaveolens* > *A. stiparum subsp. sabulicola* > *C. furfuracea*

The flavonoids content in the four plants were varied from 3.17±0.001 to 5.94±0.011 µg Q-Eq/mg. In general flavonoids levels have not exhibited a great difference among various plant extracts.

The phenolic content of MeOH extract of *R. suaveolens* was 35.58 ± 0.04 µg pyrocatechol equivalents/mg extract and the flavonoid content was 5.5 ± 0.02 µg quercetin equivalents/mg extract. It can be said that the *R. suaveolens* is poor in flavonoids. Supportively, in a previous study (Bouaziz *et al.*, 2009). 625 ± 75 mg pyrogallol/100 g and trace milligrams of quercetin/100 g were reported for the same plant collected in November of Douz area in Tunisia. The season and area of collection of the plant may lead to the different results.

The antioxidant capacity cannot be fully described using a single method because antioxidant capacity is influenced by many factors. Therefore, various assays are performed for determination of antioxidant capacity (Wong *et al.*, 2006). Accordingly, we have practiced three chemical assays for antioxidant properties of plant extracts.

2,2-Diphenyl-1-picrylhydrazyl radical is one of the few stable organic nitrogen radicals, which has a deep purple color. It does not have to be generated before assay. The DPPH assay is mainly based on an electron transfer reaction, and hydrogen-atom abstraction (Prior *et al.*, 2005). This assay is based on the measurement of the reducing ability of antioxidants toward DPPH.

Table 21: Results of total phenolic and flavonoid contents and antioxidant and anticholinesterease activities of essential oils and methanol extracts of all studied plants

Plants	Total phenolic content µg PE mg^{-1}	Total Flavonoid content µg QE mg^{-1}	DPPH assay IC_{50} (µg/mL)		*β*-carotene /linoleic acid assay IC_{50} (µg/mL)		CUPRAC assay [a] $A_{0.50}$ (µg/mL)		AChE inhibition IC_{50} (µg/mL)		BChE inhibition IC_{50} (µg/mL)	
			MeOH	EO	MeOH	EO	MeOH	EO	MeOH	EO	MeOH	EO
M. deserti	56.25	5.19	12.87	559.78	2.15	21.42	32.28	349.38	277.39	/	93.3	/
A. stiparum subsp. *sabulicola*	13.19	5.91	92.69	917.69	9.96	619.98	131.83	1508.13	490.46	142.07	212.14	/
R. suaveolens	34.40	5.47	36.54	373.86	11.55	17.97	56.04	802.23	169.29	/	40.94	/
C. furfuracea	4.56	3.15	190.47	1664.9	18.6	91.25	162.47	1334.72	164.4	/	82.4	/

[a]: The sample concentration providing 0.50 absorbance ($A_{0.50}$) was calculated from the graph of theabsorbance of cupric reducing antioxidant capacity against the sample concentration .

µg PE mg^{-1}: µg pyrocatechol equivalents /mg of extract

µg QE mg^{-1}: µg quercetin equivalents /mg of extract

DPPH assay of methanolic extracts and essential oils of four plants was investigated using Blois method with slight modification (Blois, 1958). IC_{50} value is the effective concentration at which DPPH radicals were scavenged by 50% and was calculated from the graph plotting inhibition percentage against extract concentration. IC_{50} value is inversely related to antioxidant ability of extracts. Results are given in Tables 6, 10, 14 and 18. As can be seen in the tables, all EOs and extracts exhibited a concentration-dependent DPPH radical scavenging activity. The highest activity showed with MeOH extract of *M. deserti* (IC_{50} = 12.87±0.25 µg/mL). Antioxidant activity of MeOH extracts showed the following order:

M. deserti > *R. suaveolens* > *A. stiparum subsp. sabulicola* > *C. furfuracea*

Results revealed a high correlation (R = 0.9598) between DPPH radical scavenging activity and phenolic content in different extracts ($P \leq 0.05$), indicating that polyphenols might be the main hydrogen donating responsible for the antioxidant activities. While, the oils showed weak activity ranging between 373.86±3.47 µg/mL for EO of *R. suaveolens* and 1664.95±32 µg/mL for EO of *C. furfuracea*. However, the IC_{50} values of MeOH extracts were between 12.87±0.25 µg/mL for MeOH of *M. deserti* and 190.47±0.99 µg/mL for MeOH of *C. furfuracea*.

In *β*-Carotene/linoleic acid bleaching assay, the linoleic acid free radical formed attacks the highly unsaturated *β*-carotene molecules and in the absence of an antioxidant rapidly bleached the orange color of *β*-carotene. from linoleic acid. The linoleic acid free radical attacks the highly unsaturated *β*-carotene models. The presence of different antioxidants can hinder the extent of *β*-carotene-bleaching by neutralizing the linoleate- and other free radicals formed in the system (Shon *et al.*, 2003; Wettasinghe and Shahidi, 1999). The extent of discoloration is monitored spectrophotometrcially at 470 nm (Jayaprakasha *et al.*, 2001; Miller, 1971). The lowest *β*-carotene discoloration rate exhibited the highest antioxidant activity. The inhibition capacities measured in the MeOH extracts were better than that from EOs. The inhibition value reached 94.03% in MeOH extract of *A. stiparum* subsp. *Sabulicola*.

The results of CUPRAC assay are depicted in Figures 37, 39, 41 and 43. The method is based on measures cupric ion reducing ability of polyphenols and was developed by Apak *et al.* (2004). The assay based on reduction of Cu^{2+} to Cu^{1+} by antioxidant compounds in the presence of neocuproine. The test system has distinct advantages such as, simplicity, clarity of end point and mechanism, readily available instrumentation, good intra- and inter-assay reproducibility (Prior *et al.*, 2005). As shown in fingures aforesaid, reducing power of MeOH extract of *M. deserti* showed high activity better than standards at 800 µg/mL concertation with Abs_{450nm} = 3.81. Whereas, the rest of extracts and essential oils were less than standards.

Acetylcholinesterase (AChE) catalyzes the hydrolysis of acetylcholine (ACh) in the brain, which is one of the most important neurotransmitter in the synaptic gap. Alzheimer's diseases (AD) patients have low levels of acetylcholine. From this point, if AChE is inhibited, an increased amount of ACh will accumulate in the synaptic area. This phenomenon is called as "cholinergic hypothesis" and thus improving mental function AD patients (Zengin, 2016). To this end, several AChE inhibitors (galatamine, tacrin and rivastigmine) are synthetically produced, but they exhibited several side effects including gastrointestinal problems (Chopra *et al.*, 2011).

AChE inhibitors are well utilized for the management of mild to moderate AD, and there are several researchers focused on the search of new AChE inhibitors from the herbal resources (Aktumsek *et al.*, 2013a; Giacobini, 2004). This study examined the anticholinesterase activity of four Saharian plants for the first time. The MeOH extracts and EOs were screened for AChE inhibitory activity using Ellman's colorimetric method in a 96-well plate (Ellman *et al.*, 1961). The results of AChE and BChE inhibitory activities of tested *Centaurea*species were expressed as percentage of inhibition. IC_{50} of BChE inhibitory ranged between 40.94 µg/mL and 142.07 µg/mL when for MeOH extract of *R. suaveolens* and *A. stiparum* subsp. *sabulicola*, respectively. While, essential oils did not record any inhibation except *A. stiparum* subsp. *sabulicola* essential oil with IC_{50} = 212.14 µg/mL. Concerning AChE inhibition, MeOH extract of *C. furfuracea* demonstrated higher AChE activity than other extracts (IC_{50} = 164.4 µg/mL). The essential oils were not active against AChE enzyme.

Foodborne illnesses are a major concern for consumers, the food industry, and food safety authorities. In recent years, considerable effort has been made to find natural antimicrobials that can inhibit bacterial and fungal growth in foods in order to improve quality and shelf-life. Similarly, consumers have become concerned about the safety of synthetic preservatives used in food. As a result, there is increasing demand for natural products that can serve as alternative food preservatives (Tajkarimi *et al.*, 2010). This, in turn, has led to a search for antimicrobials derived from a variety of natural sources. Natural antimicrobials can be obtained from different sources including plants, animals, bacteria, algae and fungi. Several studies related to plant antimicrobials have demonstrated the efficacy of plant-derived compounds in food applications, as well as factors influencing this effectiveness (Gyawali and Ibrahim, 2014; Tajkarimi *et al.*, 2010).

A biofilm is a complex matrix of microorganisms in which cells bind together and attach to biotic or abiotic surface (Costerton *et al.*, 1999; Hall-Stoodley *et al.*, 2004). Biofilms usually create a sticky gel composed of polysaccharides, proteins and other organic components on a wet surface, found in different environments including clinical and industrial, food processing environments, and drinking water distribution systems . Bacteria within biofilms are more resistant to antibiotics and chemical agents than planktonic cells in suspension (Ceri *et al.*, 1999; Rounds *et al.*, 2012). Chemical agents penetrating into the biofilm matrix are less effective, because most of the chemicals are active only against unattached microorganisms. In order to penetrate and degrade biofilms, it is necessary to hydrolyze the biofilm matrix. Restricting the growth and development of food borne and nosocomial pathogens such as *Staphylococcus aureus* and *Escherichia coli* is very important, however the eradiation of these organisms is not always successful because of their ability to form biofilms on a various range of surfaces (Bazargani and Rohloff, 2016; Nostro *et al.*, 2007).

In the study of antimicrobial and antibiofilm formation activities against six bacterial strains and a yeast (*Candida albicans*) were performed, the MeOH extract of *A. stiparum* subsp. *sabulicola* showed best minimum inhibitory concentration (MIC) against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 with 1.56 mg/mL. And the same MIC showed with MeOH extract of *R. suaveolens* against *B. subtilis* ATCC 6633. As far as the antibiofilm formation activity, MeOH extract of *C. furfuracea* exibited highest percentage of biofilm formation of *Staphylococcus aureus* ATCC 6538 P (87.90 %) at 50 mg/mL.

CONCLUSION
AND
PERSPECTIVES

VII. Conclusion and perspectives

The aim of this work was to conduct the chemical composition of essential oils of four medicinal plants from Algerian Sahara and investigate the antioxidant, anticholinesterase, antimicrobial and antibiofilm activities of their essential oils and methanol extracts. The resutls of antioxidant activity indicate that extracts contain active compounds. Esspecially, methanol extract of *M. deserti* and *R. suaveolens*. Apparently, there is strong positif relation between polyphenol content and antioxidant activity, which suggests that the active compounds on extracts are phenolic compounds. The investigated essential oils have no almost phenolic compounds. Therefore, they behave to be slightly effective as antioxidants. Anticholinesterase activity screening leads to the conclusion that extracts have moderate to good activity against AChE and BChE. The highest activity was given away with methanol extract of *R. suaveolens* against BChE and was nearly similar with galantamine. Thus, the plant can be used as an anticholinesterase agent, particularly against BChE. Further studies are necessary to evaluate the origin of the activity. The studied oils were almost inactive, exept those of *A. stiparum* subsp. *sabulicola* which inhibit moderate activity against BChE.

In results of antimicrobial activity, methanol extracts and essential oils showed a certain inhibition of growth attributed to all tested microoganisms with different percentages. Statistically, methanol extract of *A. stiparum* subsp. *sabulicola* showed higher antimicrobial activity. Whereas, in antibiofilm activty, methanol extracts showed better activity than essential oils and highest antibiofilm activity have reached to 87.90 % with methanol extract of *C. furfuracea* against *Staphylococcus aureus* ATCC 6538 P at 50 mg/mL. these results indicate that the essential oils and methanol extracts may have a potential for the development of new anti-biofilm, as well as antimicrobial agents, and demonstrate the importance of such medicinal plant in pharmaceutical production.

These results are preliminary and it would be interesting to test the activity of high purified fractions and isolate the responsible molecules which underlie the various detected activities in different extracts by more efficient methods. In any case it is important to highlight that the tests were performed *in vitro*. It is thus mandatory to confirm these findings by *in vivo* studies so as to obtain useful information for eventual therapeutic or dietary interventions.

References

References

Adams, R.P., **(2007**). Identification of essential oil components by gas chromatography/mass spectrometry. Allured publishing corporation.

Adorjan, B., Buchbauer, G., **(2010)**. Biological properties of essential oils: an updated review. Flavour and Fragrance Journal 25, 407-426.

African Plants Database, Home Page. http://www.ville-ge.ch/musinfo/bd/cjb/africa/. Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute, Pretoria.

Akhlaghi, H., **(2008)**. The essential oils from flowers, stems and leaves of *Ferulago angulata* from Iran. Chemistry of Natural Compounds 44, 396-397.

Akhtar, N., Ihsan ul, H., Mirza, B., **(2015)**. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arabian Journal of Chemistry.

Akkal, S., Benayache, F., Benayache, S., Medjroubi, K., Jay, M., Tillequin, F., Seguin, E., **(1999)**. A new flavone glycoside from *Centaurea furfuracea*. Fitoterapia 70, 368-370.

Akkal, S., Benayache, F., Medjroubi, K., Tillequin, F., **(2007)**. Flavonol glycosides from *Centaurea furfuracea*. Antiplasmodial and cytotoxic activities. Chemistry of Natural Compounds 43, 319-320.

Akkal, S., Benayache, F., Medjroubi, K., Tillequin, F., Seguin, E., **(2003)**. Flavonoids from *Centaurea furfuracea* (Asteraceae). Biochemical systematics and ecology 31, 641-643.

Aktumsek, A., Zengin, G., Guler, G.O., Cakmak, Y.S., Duran, A., **(2013a)**. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. Food and Chemical Toxicology 55, 290-296.

Aktumsek, A., Zengin, G., Guler, G.O., Cakmak, Y.S., Duran, A., **(2013b)**. Assessment of the antioxidant potential and fatty acid composition of four *Centaurea* L. taxa from Turkey. Food Chemistry 141, 91-97.

Al-Easa, H.S., **(2004)**. Fatty acids composition of fifty-one plant species growing in Qatar. Journal of Chemistry (Calcutta, India) 14, 195-204

Al-Mazroa, S.A., Al-Wahaibi, L.H., Mousa, A.A., Alkhathlan, H.Z., **(2006)**. Essential oils of *A. factorovskyi, K. linearis, C. arvensis, P. babylonica, P. cyanocarpa, L. nudicaulis* and *R. epapposu*. Journal of Saudi Chemical Society 9, 693-698

Alali, F.Q., Tawaha, K., El-Elimat, T., Syouf, M., El-Fayad, M., Abulaila, K., Nielsen, S.J., Wheaton, W.D., Iii, J.O.F., Oberlies, N.H., **(2007)**. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. Natural Product Research 21, 1121-1131.

Ali Asgar, M., **(2013)**. Anti-Diabetic Potential of Phenolic Compounds: A Review. International Journal of Food Properties 16, 91-103.

Alokan, J., Aletor, V., **(1997)**. Plant tannins-Their role in forage legume quality, Proceedings XVIII International Grassland Congress (IGC), Winnepeg, Manitoba.

Amessis-Ouchemoukh, N., Madani, K., Falé, P.L.V., Serralheiro, M.L., Araújo, M.E.M., **(2014)**. Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Industrial Crops and Products 53, 6-15.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E., **(2004)**. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry 52, 7970-7981.

Asadipour, A., Mehrabani, M., Najafi, M.L., **(2005)**. Volatile oil composition of Centaurea aucheri (DC.) Wagenitz. DARU Journal of Pharmaceutical Sciences 13, 160-164.

Azar, M., Verette, E., Brun, S., **(1987)**. Identification of Some Phenolic Compounds in Bilberry Juice *Vaccinium myrtillus*. Journal of Food Science 52, 1255-1257.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., **(2008)**. Biological effects of essential oils–a review. Food and Chemical Toxicology 46, 446-475.

Bardaweel, S.K., Tawaha, K.A., Hudaib, M.M., **(2014)**. Antioxidant, antimicrobial and antiproliferative activities of *Anthemis palestina* essential oil. BMC complementary and alternative medicine 14, 1.

Barja, G., **(2004)**. Free radicals and aging. Trends in Neurosciences 27, 595-600.

Baser, K.C., Özek, T., Demirci, F., Boydag, I., **(2002)**. The Essential Oil of *Anthemis cretica* L. subsp. *leucanthemoides* (Boiss.) Grierson. Acta Pharmaceutica Turcica 44, 189-194.

Baser, K.H.C., Ozek, G., Ozek, T., Duran, A., **(2006)**. Composition of the essential oil of *Centaurea huber-morathii* Wagenitz isolated from seeds by microdistillation. Flavour and Fragrance Journal 21, 568-570.

Baxter, H., Harborne, J.B., Moss, G.P., **(1998**). Phytochemical dictionary: A handbook of bioactive compounds from plants. CRC Press.

Bazargani, M.M., Rohloff, J., **(2016)**. Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control 61, 156-164.

Benaissa, O., **(2011)**. Etude des métabolismes terpénique et flavonique d’espèces de la famille des composées, genres *Chrysanthemum* et *Rhantherium*. Activité Biologique., DEPARTEMENT DE CHIMIE. Universite Mentouri, Constantine-Algeria, p. 271.

Benhammou, N., Bekkara, F.A., Coustard, J.-M., **(2009)**. Antioxidant activity of methanolic and water extracts from *Marrubium deserti* (de Noë) AND *Thymelaea microphylla* from Algerian Sahara. Advances in Food Sciences 31, 194.

Benhouhou, S., **(2005**). A Guide to Medicinal Plants in North Africa. IUCN.

Berger, R.G., **(2007**). Flavours and fragrances: chemistry, bioprocessing and sustainability. Springer Science & Business Media.

Bialecki, A., Smadja, J., **(2014**). Identification of Volatile Compounds from Flowers and Aromatic Plants: How and Why?, in: Gupta Bhowon, M., Jhaumeer-Laulloo, S., Li

Kam Wah, H., Ramasami, P. (Eds.), Chemistry: The Key to our Sustainable Future. Springer Netherlands, pp. 15-39.

Birks, J.S., **(2006)**. Cholinesterase inhibitors for Alzheimer's disease. The Cochrane Library.

Blois, M.S., **(1958)**. Antioxidant Determinations by the Use of a Stable Free Radical. Nature 181, 1199-1200.

Bouaziz, M., Dhouib, A., Loukil, S., Boukhris, M., Sayadi, S., **(2009)**. Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. African Journal of Biotechnology 8.

Bouheroum, M., Benayache, S., Benayache, F., Zaiter, L., Barrera, J., Francisco, L., **(2007)**. Terpenoids and triynepoxide from the aerial part of *Rhantherium adpressum*. Chemistry of Natural compounds 43, 110-111.

Boumerfeg, S., **(2010)**. Antioxidative properities of *Tamus communis* L., *Carthamus caeruleus* L. AND *Ajuga iva* L. EXTRACTS, Biology Departement. University ferhat Abbas, Setif- Algeria, p. 164.

Bravo, L., **(1998)**. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews 56, 317-333.

Breitmaier, E., **(2006**). Terpenes: flavors, fragrances, pharmaca, pheromones. John Wiley & Sons.

Bruni, R., Medici, A., Andreotti, E., Fantin, C., Muzzoli, M., Dehesa, M., Romagnoli, C., Sacchetti, G., **(2004)**. Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) flower calices. Food Chemistry 85, 415-421.

Buckle, J., **(2003**). Chapter 3 - Basic Plant Taxonomy, Chemistry, Extraction, Biosynthesis, and Analysis, in: Buckle, J. (Ed.), Clinical Aromatherapy (Second Edition). Churchill Livingstone, Saint Louis, pp. 38-75.

Buckle, J., **(2015**). Chapter 3 - Basic Plant Taxonomy, Basic Essential Oil Chemistry, Extraction, Biosynthesis, and Analysis, Clinical Aromatherapy (Third Edition). Churchill Livingstone, St. Louis, pp. 37-72.

Bülent Köse, Y., İşcan, G., Demirci, B., Başer, K.H.C., Çelik, S., **(2007)**. Antimicrobial activity of the essential oil of *Centaurea aladagensis*. Fitoterapia 78, 253-254.

Burt, S., **(2004)**. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology 94, 223-253.

Ceri, H., Olson, M., Stremick, C., Read, R., Morck, D., Buret, A., **(1999)**. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. Journal of clinical microbiology 37, 1771-1776.

ChemSpider, Home Page. http://www.chemspider.com/. Royal Society of Chemistry.

Chopra, K., Misra, S., Kuhad, A., **(2011)**. Current perspectives on pharmacotherapy of Alzheimer's disease. Expert Opinion on Pharmacotherapy 12, 335-350.

Chung, K.-T., Wong, T.Y., Wei, C.-I., Huang, Y.-W., Lin, Y., **(1998)**. Tannins and Human Health: A Review. Critical Reviews in Food Science and Nutrition 38, 421-464.

Ciccarelli, D., Noccioli, C., Pistelli, L., **(2013)**. Chemical Composition of Essential Oils and Aromatic Waters from Different Italian *Anthemis maritima* Populations. Chemistry & Biodiversity 10, 1667-1682.

Clarke, S., **(2008**). Chapter 4 - Processing, extraction and purity, in: Clarke, S. (Ed.), Essential Chemistry for Aromatherapy (Second Edition). Churchill Livingstone, Edinburgh, pp. 79-93.

Clinical Laboratory Standards Institute, **(2006)**. Quality control minimal inhibitory concentration (MIC) limits for broth dilution and MIC interpretative breakpoints (M27-S2). Wayne, Pennsylvania, USA

Costerton, J.W., Stewart, P.S., Greenberg, E.P., **(1999)**. Bacterial Biofilms: A Common Cause of Persistent Infections. Science 284, 1318-1322.

Crozier, A., Jaganath, I.B., Clifford, M.N., **(2009)**. Dietary phenolics: chemistry, bioavailability and effects on health. Natural product reports 26, 1001-1043.

Csupor, D., Blazsó, G., Balogh, Á., Hohmann, J., **(2010)**. The traditional Hungarian medicinal plant *Centaurea sadleriana* Janka accelerates wound healing in rats. Journal of Ethnopharmacology 127, 193-195.

Dai, J., Mumper, R.J., **(2010)**. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15, 7313-7352.

Demir, N., Yildiz, O., Alpaslan, M., Hayaloglu, A.A., **(2014)**. Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. LWT - Food Science and Technology 57, 126-133.

Dendougui, H., Seghir, S., Belloum, Z., Benayache, F., Leon, F., Brouard, I., Bermejo, J., Benayache, S., **(2011)**. A new labdane diterpene and other constituents from *Marrubium deserti* Noe ex coss. Records of Natural Products 5, 300-304.

Djeddi, S., Argyropoulou, C., Skaltsa, H., **(2008)**. Secondary metabolites from *Centaurea grisebachii* ssp. *grisebachii*. Biochemical systematics and ecology 36, 336-339.

Djeddi, S., Sokovic, M., Skaltsa, H., **(2011)**. Analysis of the Essential Oils of Some *Centaurea* Species (Asteraceae) Growing Wild in Algeria and Greece and Investigation of their Antimicrobial Activities. Journal of Essential Oil Bearing Plants 14, 658-666.

Djeridane, A., Yousfi, M., Brunel, J.M., Stocker, P., **(2010)**. Isolation and characterization of a new steroid derivative as a powerful antioxidant from *Cleome arabica* in screening the in vitro antioxidant capacity of 18 Algerian medicinal plants. Food and Chemical Toxicology 48, 2599-2606.

Djilani, A., Dicko, A., **(2012**). The therapeutic benefits of essential oils. INTECH Open Access Publisher.

Dohi, S., Terasaki, M., Makino, M., **(2009)**. Acetylcholinesterase Inhibitory Activity and Chemical Composition of Commercial Essential Oils. Journal of Agricultural and Food Chemistry 57, 4313-4318.

Dorman, H., Deans, S., **(2000)**. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88, 308-316.

Edziri, H., Mastouri, M., Aouni, M., Verschaeve, L., **(2012)**. Polyphenols content, antioxidant and antiviral activities of leaf extracts of *Marrubium deserti* growing in Tunisia. South African Journal of Botany 80, 104-109.

El Bardai, S., Morel, N., Wibo, M., Fabre, N., Llabres, G., Lyoussi, B., Quetin-Leclercq, J., **(2003)**. The Vasorelaxant Activity of Marrubenol and Marrubiin from *Marrubium vulgare*. Planta Med 69, 75-77.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., **(1961)**. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology 7, 88-95.

Erel, S.B., Demirci, B., Demir, S., Karaalp, C., Hüsnü Can Baser, K., **(2013)**. Composition of the essential oils of *Centaurea aphrodisea, C. polyclada, C. athoa, C. hyalolepis* and *C. iberica*. Journal of Essential Oil Research 25, 79-84.

Ertas, A., Gören, A.C., Boga, M., Demirci, S., Kolak, U., **(2014)**. Chemical Composition of The Essential Oils of Three *Centaurea* Species Growing Wild in Anatolia and Their Anticholinesterase Activities. Journal of Essential Oil Bearing Plants 17, 922-926.

Esmaeili, A., Rustaiyan, A., Akbari, M.T., Moazami, N., Masoudi, S., Amiri, H., **(2006)**. Composition of the Essential Oils of *Xanthium strumarium* L. and *Cetaurea solstitialis* L. from Iran. Journal of Essential Oil Research 18, 427-429.

Fakhfakh, J.A.e., Damak, M., **(2007)**. Sesquineolignans from the flowers of *Centaurea furfuracea* Coss. et Dur. (Asteraceae). Natural Product Research 21, 1037-1041.

Fakhfakh, J.A.E., Martin, M.T., Damak, M., **(2005)**. Flavonol triglycosides from the leaves of *Hammada scoparia* (POMEL) ILJIN. Journal de la Société Chimique de Tunisie 7, 11-18.

Fitzpatrick, D.F., Bing, B., Rohdewald, P., **(1998)**. Endothelium-Dependent Vascular Effects of Pycnogenol. Journal of Cardiovascular Pharmacology 32, 509-515.

Fiuza, S.M., Gomes, C., Teixeira, L.J., Girão da Cruz, M.T., Cordeiro, M.N.D.S., Milhazes, N., Borges, F., Marques, M.P.M., **(2004)**. Phenolic acid derivatives with potential anticancer properties—a structure–activity relationship study. Part 1:

Methyl, propyl and octyl esters of caffeic and gallic acids. Bioorganic & Medicinal Chemistry 12, 3581-3589.

Flamini, G., Tebano, M., Cioni, P.L., Bagci, Y., Dural, H., Ertugrul, K., Uysal, T., Savran, A., **(2006)**. A multivariate statistical approach to *Centaurea* classification using essential oil composition data of some species from Turkey. Plant Systematics and Evolution 261, 217-228.

Friedman, M., **(2006)**. Antibiotic activities of plant compounds against non-resistant and antibiotic-resistant foodborne human pathogens, ACS symposium series. Oxford University Press, pp. 167-183.

Gali-Muhtasib, H., Hilan, C., Khater, C., **(2000)**. Traditional uses of *Salvia libanotica* (East Mediterranean sage) and the effects of its essential oils. Journal of Ethnopharmacology 71, 513-520.

Gao, H., Huang, Y.-N., Xu, P.-Y., Kawabata, J., **(2007)**. Inhibitory effect on α-glucosidase by the fruits of *Terminalia chebula* Retz. Food Chemistry 105, 628-634.

Geiger, J.L., **(2005)**. The essential oil of ginger, *Zingiber officinale*, and anaesthesia. International Journal of Aromatherapy 15, 7-14.

Giacobini, E., **(2004)**. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacological Research 50, 433-440.

Giada, M.d.L.R., **(2013)**. Food phenolic compounds: main classes, sources and their antioxidant power. Oxidative Stress and Chronic Degenerative Diseases—A Role for Antioxidants, InTech, Rijeka, Croatia, 87-112.

Giorgi, A., Madeo, M., Speranza, G., Cocucci, M., **(2010)**. Influence of environmental factors on composition of phenolic antioxidants of Achillea collina Becker ex Rchb. Natural Product Research 24, 1546-1559.

Glišić, S.B., Mišić, D.R., Stamenić, M.D., Zizovic, I.T., Ašanin, R.M., Skala, D.U., **(2007)**. Supercritical carbon dioxide extraction of carrot fruit essential oil: Chemical composition and antimicrobial activity. Food Chemistry 105, 346-352.

Gonzalez, A.G., Darias, V., Alonso, G., Boada, J.N., Feria, M., **(1978)**. Cytostatic Activity of Sesquiterpene Lactones from Compositae of the Canary Islands. Planta Med 33, 356-359.

Green, R.C., **(2007)**. Physicochemical properties and phenolic composition of selected Saskatchewan fruits: buffaloberry, chokecherry and sea buckthorn. University of Saskatchewan Saskatoon.

Gürbüz, İ., Yesilada, E., **(2007)**. Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis* by using various in vivo and biochemical techniques. Journal of Ethnopharmacology 112, 284-291.

Güven, K., Çelik, S., Uysal, İ., **(2005)**. Antimicrobial Activity of *Centaurea*. Species. Pharmaceutical Biology 43, 67-71.

Gyawali, R., Ibrahim, S.A., **(2014)**. Natural products as antimicrobial agents. Food Control 46, 412-429.

Hagerman, A.E., Butler, L.G., **(1978)**. Protein precipitation method for the quantitative determination of tannins. Journal of Agricultural and Food Chemistry 26, 809-812.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L., **(1998)**. High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. Journal of Agricultural and Food Chemistry 46, 1887-1892.

Halis, y., **(2007**). Plant Encyclopedia in area Oued Souf: desert plants common in the big East race (in Arabic). El Walid, El Oued- Algeria.

Hall-Stoodley, L., Costerton, J.W., Stoodley, P., **(2004)**. Bacterial biofilms: from the Natural environment to infectious diseases. Nat Rev Micro 2, 95-108.

Halliwell, B., **(1999)**. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and the effects of nutrition. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 443, 37-52.

Hamia, C., Gourine, N., Boussoussa, H., Saidi, M., Gaydou, E.M., Yousfi, M., **(2013)**. Chemical composition and antioxidant activity of the essential oil and fatty acids of the flowers of *Rhanterium adpressum*. Natural product communications 8, 1171-1174.

Hammiche, V., Maiza, K., **(2006)**. Traditional medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. Journal of Ethnopharmacology 105, 358-367.

Hanbali, E.F., Mellouki, F., Akssira, M., H, B., M. A, B., **(2007)**. Composition and Antimicrobial Activity of Essential Oil of *Anthemis tenuisecta* Ball. Journal of Essential Oil Bearing Plants 10, 499-503.

Handa, S.S., **(2008**). 1 An Overview of Extraction Techniques for Medicinal and Aromatic Plants, Extraction Technologies for Medicinal and Aromatic Plants. International Centre for Science and High Technology, pp. 21-54.

Harborne, J.B., **(1989**). Methods in plant biochemistry. Volume 1. Plant phenolics. Academic Press Ltd.

Hatam, N.A.R., Porzel, A., Seifert, K., **(1995)**. Polyodonine, a prefuranic labdane diterpene from *Marrubium polydon*. Phytochemistry 40, 1575-1576.

Hennebelle, T., Sahpaz, S., Skaltsounis, A., Bailleul, F., **(2007)**. Phenolic compounds and diterpenoids from *Marrubium peregrinum*. Biochemical systematics and ecology 35, 624-626.

Hertog, M.G., Sweetnam, P.M., Fehily, A.M., Elwood, P.C., Kromhout, D., **(1997)**. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. The American Journal of Clinical Nutrition 65, 1489-1494.

Hibasami, H., Achiwa, Y., Fujikawa, T., Komiya, T., **(1995)**. Induction of programmed cell death (apoptosis) in human lymphoid leukemia cells by catechin compounds. Anticancer research 16, 1943-1946.

Higley, C., Higley, A., **(1998**). Reference guide for essential oils. Abundant Health.

Howes, M.-J.R., Simmonds, M.S.J., Kite, G.C., **(2004)**. Evaluation of the quality of sandalwood essential oils by gas chromatography–mass spectrometry. Journal of Chromatography A 1028, 307-312.

Hu, C., Kitts, D., **(2001)**. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems in vitro. Mol Cell Biochem 218, 147-155.

Huang, W.-Y., Cai, Y.-Z., Zhang, Y., **(2009)**. Natural Phenolic Compounds From Medicinal Herbs and Dietary Plants: Potential Use for Cancer Prevention. Nutrition and Cancer 62, 1-20.

Huang, Y., Tan, A., Shen, Y., Lu, J., **(1998)**. Scavenging effect of total flavonoids of *Lycium barbarum* L on active oxygen radicals and inhibitory effects on heat output from L1210 cells. Wei Sheng Yen Chiu 27, 109-111, 115.

Hunter, M., **(2009**). Essential oils: art, agriculture, science, industry and entrepreneurship: a focus on the Asia-Pacific region. Nova Science Publishers.

Hüsnü, K., Başer, C., Demirci, F., **(2007**). Chemistry of Essential Oils, in: Berger, R. (Ed.), Flavours and Fragrances. Springer Berlin Heidelberg, pp. 43-86.

Hussain, A., **(2009)**. Characterization and biological activities of essential oils of some species of Lamiaceae (Ph. D. Thesis) Department of Chemistry and Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan.

Javidnia, K., Miri, R., Kamalinejad, M., Sarkarzadeh, H., Jamalian, A., **(2004)**. Chemical composition of the essential oils of *Anthemis altissima* L. grown in Iran. Flavour and Fragrance Journal 19, 213-216.

Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., **(2001)**. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chemistry 73, 285-290.

Jham, G.N., Dhingra, O.D., Jardim, C.M., Valente, V.M.M., **(2005)**. Identification of the major fungitoxic component of cinnamon bark oil. Fitopatologia Brasileira 30, 404-408.

Kaduszkiewicz, H., Zimmermann, T., Beck-Bornholdt, H.-P., van den Bussche, H., **(2005)**. Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. BMJ 331, 321-327.

Kala, A., Gherraf, N., Belkacemi, D., Ladjel, S., Zellagui, A., Samir, H., Chihi, S., Brahim, L., **(2009)**. Composition of the essential oil of *Rhanterium adpressum* Coss. and Durieu. from Algeria. Archives of Applied Science Research 1, 115-118.

Kalemba, D., Kunicka, A., **(2003)**. Antibacterial and Antifungal Properties of Essential Oils. Current Medicinal Chemistry 10, 813-829.

Kar, A., **(2003**). Pharmacognosy and pharmacobiotechnology. New Age International.

Karamenderes, C., Bedir, E., Pawar, R., Baykan, S., Khan, I.A., **(2007)**. Elemanolide sesquiterpenes and eudesmane sesquiterpene glycosides from *Centaurea hierapolitana*. Phytochemistry 68, 609-615.

Karioti, A., Heilmann, J., Skaltsa, H., **(2005)**. Labdane diterpenes from *Marrubium velutinum* and *Marrubium cylleneum*. Phytochemistry 66, 1060-1066.

Kaul, T.N., Middleton, E., Ogra, P.L., **(1985)**. Antiviral effect of flavonoids on human viruses. Journal of Medical Virology 15, 71-79.

Keszei, A., Brubaker, C.L., Foley, W.J., **(2008)**. A molecular perspective on terpene variation in Australian Myrtaceae. Australian Journal of Botany 56, 197-213.

Khokhar, Z.-u., Munir, S., Ahmad, S.A., Ehsan-Ul-Haq, A.A., Ali, S.S., Qadir, M., **(2011)**. Isoamyl salicylate: Synthesis and use in beauty soap as a fragrance. Science International 23.

Klein, J.A., Ackerman, S.L., **(2003)**. Oxidative stress, cell cycle, and neurodegeneration. The Journal of clinical investigation 111, 785-793.

Kobayashi, Y., Suzuki, M., Satsu, H., Arai, S., Hara, Y., Suzuki, K., Miyamoto, Y., Shimizu, M., **(2000)**. Green Tea Polyphenols Inhibit the Sodium-Dependent Glucose Transporter of Intestinal Epithelial Cells by a Competitive Mechanism. Journal of Agricultural and Food Chemistry 48, 5618-5623.

Koca, U., Süntar, I.P., Keles, H., Yesilada, E., Akkol, E.K., **(2009)**. In vivo anti-inflammatory and wound healing activities of *Centaurea iberica* Trev. ex Spreng. Journal of Ethnopharmacology 126, 551-556.

Koroch, A.R., Juliani, H.R., Zygadlo, J.A., **(2007**). Bioactivity of essential oils and their components, Flavours and fragrances. Springer, pp. 87-115.

Kubeczka, K.-H., **(2010**). History and sources of essential oil research, Handbook of essential oils: science, technology, and applications. CRC Press/Taylor & Francis, Boca Raton, pp. 3-38.

Kumar, S., Pandey, A.K., **(2013)**. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal 2013, 16.

Kumarasamy, Y., Byres, M., Cox, P.J., Jaspars, M., Nahar, L., Sarker, S.D., **(2007)**. Screening seeds of some Scottish plants for free radical scavenging activity. Phytotherapy Research 21, 615-621.

Laouer, H., Yabrir, B., Djeridane, A., Yousfi, M., Beldovini, N., Lamamra, M., **(2009)**. Composition, antioxidant and antimicrobial activities of the essential oil of *Marrubium deserti*. Natural Product Communications 4, 1133-1138.

Lazari, D.M., Skaltsa, H.D., Constantinidis, T., **(1999)**. Volatile constituents of *Centaurea raphanina* Sm. subsp. *mixta* (DC.) Runemark and *C. spruneri* Boiss. & Heldr.(Asteraceae), growing wild in Greece. Flavour and Fragrance Journal 14, 415-418.

Lazari, D.M., Skaltsa, H.D., Constantinidis, T., **(2000)**. Volatile constituents of *Centaurea pelia* DC., *C. thessala* Hausskn. subsp. *drakiensis* (Freyn & Sint.) Georg. and C. zuccariniana DC. from Greece. Flavour and Fragrance Journal 15, 7-11.

Li, Y., Fabiano-Tixier, A.-S., Chemat, F., **(2014**). Essential Oils: From Conventional to Green Extraction, Essential Oils as Reagents in Green Chemistry. Springer, pp. 9-20.

Li, Y., Peng, L., Zhang, T., **(2006**). Progress of Studies on the Natural Cembranoids from the Soft Coral Species of *Sarcophyton* Genus, Medicinal Chemistry of Bioactive Natural Products. John Wiley & Sons, Inc., pp. 257-300.

Lis-Balchin, M., Deans, S.G., **(1997)**. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. Journal of Applied Microbiology 82, 759-762.

Lopes-Lutz, D., Alviano, D.S., Alviano, C.S., Kolodziejczyk, P.P., **(2008)**. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. Phytochemistry 69, 1732-1738.

Makang'a, O.B., **(2012)**. composition and repellency of essential oils of *Tagetes minuta* from different zones in kenya against brown ear tick (*Rhipicephalus appendiculatus*). Kenyatta University, Kenya.

Masango, P., **(2005)**. Cleaner production of essential oils by steam distillation. Journal of Cleaner Production 13, 833-839.

Merritt, J.H., Kadouri, D.E., O'Toole, G.A., **(2005**). Growing and Analyzing Static Biofilms, Current protocols in microbiology. John Wiley & Sons, Inc.

Miana, G.A., Al-Hazimi, H.M.G., **(1983)**. Chemical investigations of Saudi Arabian plants. I. Scopoletin from *Rhanterium epapposum* olive. Journal of the Chemical Society of Pakistan, 223-225.

Miller, H., **(1971)**. A simplified method for the evaluation of antioxidants. Journal of the American Oil Chemists Society 48, 91-91.

Mimica-Dukic, N., Popovic, M., Jakovljevic, V., Szabo, A., Gašic, O., **(1999)**. Pharmacological Studies of *Mentha longifolia* Phenolic Extracts. II. Hepatoprotective Activity. Pharmaceutical Biology 37, 221-224.

Mojab, F., Nickavar, B., **(2010)**. Composition of the Essential Oil of the Root of *Heracleum persicum* from Iran. Iranian Journal of Pharmaceutical Research, 245-247.

Mpofu, A., Sapirstein, H.D., Beta, T., **(2006)**. Genotype and Environmental Variation in Phenolic Content, Phenolic Acid Composition, and Antioxidant Activity of Hard Spring Wheat. Journal of Agricultural and Food Chemistry 54, 1265-1270.

Musa Özcan, M., Chalchat, J.C., **(2007)**. Chemical composition of carrot seeds (*Daucus carota* L.) cultivated in Turkey: characterization of the seed oil and essential oil. Grasas y aceites 58, 359-365.

Nascimento, M., Santana, A., Maranhão, C., Oliveira, L., Bieber, L., **(2013**). Phenolic Extractives and Natural Resistance of Wood, in: Chamy, R.F., Rosenkranz (Ed.), Biodegradation - Life of Science. InTech, p. 378.

Noguchi, Y., Fukuda, K., Matsushima, A., Haishi, D., Hiroto, M., Kodera, Y., Nishimura, H., Inada, Y., **(1999)**. Inhibition of Df-Protease Associated with Allergic Diseases by Polyphenol. Journal of Agricultural and Food Chemistry 47, 2969-2972.

Nostro, A., Roccaro, A.S., Bisignano, G., Marino, A., Cannatelli, M.A., Pizzimenti, F.C., Cioni, P.L., Procopio, F., Blanco, A.R., **(2007)**. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. Journal of Medical Microbiology 56, 519-523.

Oberprieler, C., **(1998)**. The systematics of *Anthemis* L.(Compositae, Anthemideae) in W and C North Africa. Bocconea 9, 1-328.

Olthof, M.R., Hollman, P.C., Katan, M.B., **(2001)**. Chlorogenic acid and caffeic acid are absorbed in humans. The Journal of nutrition 131, 66-71.

Orhan, I.E., Belhattab, R., Şenol, F.S., Gülpinar, A.R., Hoşbaş, S., Kartal, M., **(2010)**. Profiling of cholinesterase inhibitory and antioxidant activities of *Artemisia absinthium, A. herba-alba, A. fragrans, Marrubium vulgare, M. astranicum, Origanum vulgare* subsp. *glandulossum* and essential oil analysis of two *Artemisia* species. Industrial Crops and Products 32, 566-571.

Oueslati, M.H., Ben Jannet, H., Abreu, P.M., Mighri, Z., **(2004)**. Alcools tetrahydropyraniques polyacetyleniques antibacteriens de la plante *Rantherium suaveolens* poussant dans le sud tunisien. Journal de la Société Algérienne de Chimie 14, 245-258

Oueslati, M.H., Ben Jannet, H., Mighri, Z., Matthew, S., Abreu, P.M., **(2007)**. A new C9 nor -isoprenoid glucoside from *Rantherium suaveolens*. Natural Product Research 21, 884-888.

Oueslati, M.H., Mighri, Z., Ben Jannet, H., Abreu, P.M., **(2005)**. New ceramides from *Rantherium suaveolens*. Lipids 40, 1075-1079.

Ould El Hadj, M.D., Hadj-Mahammed, M., Zabeirou, H., **(2003)**. Place des plantes spontanées dans la médicine traditionnelle de la région d'Ouargla (Sahara septentrional Est). Courrier du Savoir 3, 47-51.

Ozenda, P., **(1977)**. Flora of the Sahara. Flore du Sahara.

Ozenda, P., **(2004**). Flore et végétation du Sahara, 3 ed. CNRS.

Öztürk, M., Kolak, U., Topçu, G., Öksüz, S., Choudhary, M.I., **(2011)**. Antioxidant and anticholinesterase active constituents from *Micromeria cilicica* by radical-scavenging activity-guided fractionation. Food Chemistry 126, 31-38.

Pandey, K.B., Rizvi, S.I., **(2009)**. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity 2, 270-278.

Paré, P.W., Tumlinson, J.H., **(1999)**. Plant Volatiles as a Defense against Insect Herbivores. Plant Physiology 121, 325-332.

Park, Y.C., Rimbach, G., Saliou, C., Valacchi, G., Packer, L., **(2000)**. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF-α secretion, and NF-κB-dependent gene expression in RAW 264.7 macrophages. FEBS Letters 465, 93-97.

Park, Y.K., Koo, M.H., Ikegaki, M., Contado, J., **(1997)**. Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arq. Biol. Tecnol 40, 97-106.

Pavlović, M., Lakušić, D., Kovačević, N., Tzakou, O., Couladis, M., **(2010)**. Comparative Analysis of Essential Oils of Six *Anthemis* Taxa from Serbia and Montenegro. Chemistry & Biodiversity 7, 1231-1244.

Pengelly, A., **(2004**). The Constituents of Medicinal Plants: An Introduction to the Chemistry and Therapeutics of Herbal Medicine, 2nd ed. CABI.

Perry, N.S.L., Bollen, C., Perry, E.K., Ballard, C., **(2003)**. *Salvia* for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacology Biochemistry and Behavior 75, 651-659.

Prior, R.L., Wu, X., Schaich, K., **(2005)**. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. Journal of Agricultural and Food Chemistry 53, 4290-4302.

Quézel, P., Santa, S., **(1963**). Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales.

Radulović, N.S., Blagojević, P.D., Zlatković, B.K., Palić, R.M., **(2009)**. Chemotaxonomically Important Volatiles of the Genus *Anthemis* L. – a Detailed GC and GC/MS Analyses of *Anthemis Segetalis* Ten. from Montenegro. Journal of the Chinese Chemical Society 56, 642-652.

Rao, C.V., Desai, D., Simi, B., Kulkarni, N., Amin, S., Reddy, B.S., **(1993)**. Inhibitory Effect of Caffeic Acid Esters on Azoxymethane-induced Biochemical Changes and Aberrant Crypt Foci Formation in Rat Colon. Cancer Research 53, 4182-4188.

Reed, J.D., Horvath, P.J., Allen, M.S., Van Soest, P.J., **(1985)**. Gravimetric determination of soluble phenolics including tannins from leaves by precipitation with trivalent ytterbium. Journal of the Science of Food and Agriculture 36, 255-261.

Rice-Evans, C.A., Miller, N.J., Paganga, G., **(1996)**. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20, 933-956.

Rimm, E.B., Katan, M.B., Ascherio, A., Stampfer, M.J., Willett, W.C., **(1996)**. Relation between Intake of Flavonoids and Risk for Coronary Heart Disease in Male Health Professionals. Annals of Internal Medicine 125, 384-389.

Rounds, L., Havens, C.M., Feinstein, Y., Friedman, M., Ravishankar, S., **(2012)**. Plant Extracts, Spices, and Essential Oils Inactivate *Escherichia coli* O157:H7 and

Reduce Formation of Potentially Carcinogenic Heterocyclic Amines in Cooked Beef Patties. Journal of Agricultural and Food Chemistry 60, 3792-3799.

Ruberto, G., Baratta, M.T., **(2000)**. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chemistry 69, 167-174.

Saad, H.E.A., El-Sharkawy, S.H., Halim, A.F., **(1995)**. Composition of the essential oils of the leaves and stems of *Torilis arvensis*. Pharmaceutica Acta Helvetiae 70, 85-87.

Saija, A., Tomaino, A., Cascio, R.L., Trombetta, D., Proteggente, A., De Pasquale, A., Uccella, N., Bonina, F., **(1999)**. Ferulic and caffeic acids as potential protective agents against photooxidative skin damage. Journal of the Science of Food and Agriculture 79, 476-480.

Sajjadi, S.E., Ghassemi, N., Shokoohinia, Y., Moradi, H., **(2013)**. Essential Oil Composition of Flowers of *Anthemis odontostephana* Boiss. var. *odontostephana*. Journal of Essential Oil Bearing Plants 16, 247-251.

Sajjadi, S.E., Mehregan, I., **(2006)**. Volatile constituents of flowers and leaves of *Anthemis hyalina*. Chemistry of Natural Compounds 42, 531-533.

Salmanpour, S., Khalilzadeh, M.A., Sadeghifar, H., **(2009)**. Chemical Composition of the Essential Oils From Leaves, Flowers, Stem and Root of *Centaurea zuvandica* Sosn. Journal of Essential Oil Research 21, 357-359.

Santos-Buelga, C., Scalbert, A., **(2000)**. Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture 80, 1094-1117.

Sarikurkcu, C., Tepe, B., Daferera, D., Polissiou, M., Harmandar, M., **(2008)**. Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum* subsp. *globosum* (lamiaceae) by three different chemical assays. Bioresource Technology 99, 4239-4246.

Saroglou, V., Dorizas, N., Kypriotakis, Z., Skaltsa, H.D., **(2006)**. Analysis of the essential oil composition of eight *Anthemis* species from Greece. Journal of Chromatography A 1104, 313-322.

Scandalios, J.G., **(2002)**. The rise of ROS. Trends in Biochemical Sciences 27, 483-486.

Senatore, F., Arnold, N.A., Bruno, M., **(2005)**. Volatile components of *Centaurea eryngioides* Lam. and *Centaurea iberica* Trev. var. hermonis Boiss. Lam., two *Asteraceae* growing wild in Lebanon. Natural Product Research 19, 749-754.

Shahidi, F., Chi-Tang, H., **(2005**). Phenolics in food and natural health products: an overview, Phenolic compounds in foods and natural health products, pp. 1-8.

Shahidi, F., Janitha, P.K., Wanasundara, P.D., **(1992)**. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 32, 67-103.

Shahidi, F., Naczk, M., **(2003**). Phenolics in food and nutraceuticals. CRC press.

Shan, B., Cai, Y.-Z., Brooks, J.D., Corke, H., **(2007)**. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology 117, 112-119.

Shoeb, M., Celik, S., Jaspars, M., Kumarasamy, Y., MacManus, S.M., Nahar, L., Thoo-Lin, P.K., Sarker, S.D., **(2005)**. Isolation, structure elucidation and bioactivity of schischkiniin, a unique indole alkaloid from the seeds of *Centaurea schischkinii*. Tetrahedron 61, 9001-9006.

Shon, M.-Y., Kim, T.-H., Sung, N.-J., **(2003)**. Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of Hymenochaetaceae) extracts. Food Chemistry 82, 593-597.

Simon, J.E., Quinn, J., Murray, R.G., **(1990)**. Basil: a source of essential oils. Advances in new crops, 484-489.

Slinkard, K., Singleton, V.L., **(1977)**. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture 28, 49-55.

Smid, E.J., Gorris, L.G., **(1999**). Natural antimicrobials for food preservation, in: Rahman, M.S. (Ed.), Handbook of Food Preservation. CRC press, pp. 285-308.

Strack, D., **(1997**). 10 - Phenolic Metabolism, in: Harborne, P.M.D.B. (Ed.), Plant Biochemistry. Academic Press, London, pp. 387-416.

Tajkarimi, M.M., Ibrahim, S.A., Cliver, D.O., **(2010)**. Antimicrobial herb and spice compounds in food. Food Control 21, 1199-1218.

Tanaka, M., Kuei, C.W., Nagashima, Y., Taguchi, T., **(1988)**. Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi 54, 1409-1414.

Tepe, B., Sokmen, M., Akpulat, H.A., Yumrutas, O., Sokmen, A., **(2006)**. Screening of antioxidative properties of the methanolic extracts of *Pelargonium endlicherianum* Fenzl., *Verbascum wiedemannianum* Fisch. & Mey., *Sideritis libanotica* Labill. subsp. *linearis* (Bentham) Borm., *Centaurea mucronifera* DC. and *Hieracium cappadocicum* Freyn from Turkish flora. Food Chemistry 98, 9-13.

Thormar, H., **(2010**). Lipids and essential oils as antimicrobial agents. John Wiley & Sons.

Tisserand, R., Young, R., **(2014**). 2 - Essential oil composition, in: Young, R.T. (Ed.), Essential Oil Safety (Second Edition). Churchill Livingstone, St. Louis, pp. 5-22.

Tukun, A.B., Shaheen, N., Banu, C.P., Mohiduzzaman, M., Islam, S., Begum, M., **(2014)**. Antioxidant capacity and total phenolic contents in hydrophilic extracts of selected Bangladeshi medicinal plants. Asian Pacific Journal of Tropical Medicine 7, Supplement 1, S568-S573.

Ud-Daula, A.F.M.S., Demirci, F., Abu Salim, K., Demirci, B., Lim, L.B.L., Baser, K.H.C., Ahmad, N., **(2016)**. Chemical composition, antioxidant and antimicrobial activities of essential oils from leaves, aerial stems, basal stems, and rhizomes of *Etlingera fimbriobracteata* (K.Schum.) R.M.Sm. Industrial Crops and Products 84, 189-198.

Ugur, A., Duru, M.E., Ceylan, O., Sarac, N., Varol, O., Kivrak, I., **(2009a)**. Chemical composition, antimicrobial and antioxidant activities of *Centaurea ensiformis* Hub.-Mor. (Asteraceae), a species endemic to Mugla (Turkey). Natural Product Research 23, 149-167.

Ugur, A., Sarac, N., Ceylan, O., Duru, M., **(2009b)**. Chemical composition of endemic *Centaurea austro-anatolica* and studies of its antimicrobial activity against multi-resistant bacteria. Acta pharmaceutica 59, 463-472.

Ugur, A., Sarac, N., Ceylan, O., Emin Duru, M., **(2010)**. Antimicrobial activity and chemical composition of endemic *Centaurea cariensis* subsp. *niveo-tomentosa*. Natural Product Research 24, 861-872.

Vakili, E., Amjad, L., Karbasizade, V., **(2013)**. Antibacterial properties of *Anthemis gayana* leaves essential oil. International Journal of Agriculture and Crop Sciences 5, 1464.

Vermerris, W., Nicholson, R., **(2006**). Families of phenolic compounds and means of classification, Phenolic Compound Biochemistry. Springer, pp. 1-34.

Vermerris, W., Nicholson, R., **(2007**). Phenolic compound biochemistry. Springer Science & Business Media.

Visioli, F., Bellosta, S., Galli, C., **(1998)**. Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. Life Sciences 62, 541-546.

Vladimir-Knežević, S., Blažeković, B., Kindl, M., Vladić, J., Lower-Nedza, A.D., Brantner, A.H., **(2014)**. Acetylcholinesterase inhibitory, antioxidant and phytochemical properties of selected medicinal plants of the *Lamiaceae* family. Molecules 19, 767-782.

Waris, G., Ahsan, H., **(2006)**. Reactive oxygen species: role in the development of cancer and various chronic conditions. Journal of Carcinogenesis 5, 14.

Waterhouse, A.L., **(2001**). Determination of Total Phenolics, Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc.

Wettasinghe, M., Shahidi, F., **(1999)**. Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage *(Borago officinalis* L.) seeds. Food Chemistry 67, 399-414.

Whish, J.P.M., **(1996)**. A Flexible Distillation System for the Isolation of Essential Oils. Journal of Essential Oil Research 8, 405-410.

Wiklund, A., **(1986)**. The genus *Rhanterium* (Asteraceae: Inuleae). Botanical journal of the Linnean Society 93, 231-246.

Wilkes, J., **(1827**). Encyclopaedia Londinensis, or, Universal dictionary of arts, sciences, and literature.

Wong, S.P., Leong, L.P., William Koh, J.H., **(2006)**. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry 99, 775-783.

Xu, M., Sun, X., Tong, W., **(1994)**. Medical research and development of Seabuckthorn. Hippophae 7, 32-40.

Yaghmai, M.S., Kolbadipour, S., **(1987)**. Volatile components of *Rhanterium epapposum* oliv. Flavour and Fragrance Journal 2, 29-32.

Yayli, N., Yaşar, A., Güleç, C., Usta, A., Kolaylı, S., Coşkunçelebi, K., Karaoğlu, Ş., **(2005)**. Composition and antimicrobial activity of essential oils from *Centaurea sessilis* and *Centaurea armena*. Phytochemistry 66, 1741-1745.

Yaylı, N., Yaşar, A., Yaylı, N., Albay, C., Aşamaz, Y., Coşkunçelebi, K., Karaoğlu, Ş., **(2009)**. Chemical composition and antimicrobial activity of essential oils from *Centaurea appendicigera* and *Centaurea helenioides*. Pharmaceutical Biology 47, 7-12.

Yordi, E.G., Pérez, E.M., Villares, E.U., Matos, M.J., **(2012**). Antioxidant and pro-oxidant effects of polyphenolic compounds and structure-activity relationship evidence, in: Bouayed, J. (Ed.), Nutrition, Well-Being and Health. INTECH Open Access Publisher, pp. 23-48.

Zaabat, N., Darbour, N., Bayet, C., Michalet, S., Doléans-Jordhem, A., Chelr-Ghedlra, L., Akkal, S., Dijoux-Franca, M.G., **(2010)**. Étude préliminaire de *Marrubium deserti* de Noé, une Lamiaceae endémique algérienne. Phytothérapie 8, 353-358.

Zaabat, N., Hay, A.-E., Michalet, S., Darbour, N., Bayet, C., Skandrani, I., Chekir-Ghedira, L., Akkal, S., Dijoux-Franca, M.-G., **(2011)**. Antioxidant and antigenotoxic properties of compounds isolated from *Marrubium deserti* de Noé. Food and Chemical Toxicology 49, 3328-3335.

Zengin, G., **(2016)**. A study on in vitro enzyme inhibitory properties of *Asphodeline anatolica*: New sources of natural inhibitors for public health problems. Industrial Crops and Products 83, 39-43.

Zengin, G., Aktumsek, A., Guler, G.O., Cakmak, Y.S., Kan, Y., **(2012)**. Composition of essential oil and antioxidant capacity of *Centaurea drabifolia* Sm. subsp. *detonsa* (Bornm.) Wagenitz, endemic to Turkey. Natural Product Research 26, 1-10.

Zengin, G., Cakmak, Y.S., Guler, G.O., Aktumsek, A., **(2010)**. In vitro antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from Central Anatolia region of Turkey. Food and Chemical Toxicology 48, 2638-2641.

Zengin, G., Guler, G.O., Aktumsek, A., Ceylan, R., Picot, C.M.N., Mahomoodally, M.F., **(2015)**. Enzyme Inhibitory Properties, Antioxidant Activities, and Phytochemical Profile of Three Medicinal Plants from Turkey. Advances in Pharmacological Sciences 2015, 8.

Zucker, W.V., **(1983)**. Tannins: Does Structure Determine Function? An Ecological Perspective. The American Naturalist 121, 335-365.

Taylor & Francis
Taylor & Francis Group

Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Chemical constituents of essential oil of endemic Rhanterium suaveolens Desf. growing in Algerian Sahara with antibiofilm, antioxidant and anticholinesterase activities

Ahmed Elkhalifa Chemsa, Ebru Erol, Mehmet Öztürk, Amar Zellagui, Ceylan Özgür, Noureddine Gherraf & Mehmet Emin Duru

To cite this article: Ahmed Elkhalifa Chemsa, Ebru Erol, Mehmet Öztürk, Amar Zellagui, Ceylan Özgür, Noureddine Gherraf & Mehmet Emin Duru (2015): Chemical constituents of essential oil of endemic Rhanterium suaveolens Desf. growing in Algerian Sahara with antibiofilm, antioxidant and anticholinesterase activities, Natural Product Research, DOI: 10.1080/14786419.2015.1110705

To link to this article: http://dx.doi.org/10.1080/14786419.2015.1110705

NATURAL PRODUCT RESEARCH, 2015
http://dx.doi.org/10.1080/14786419.2015.1110705

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

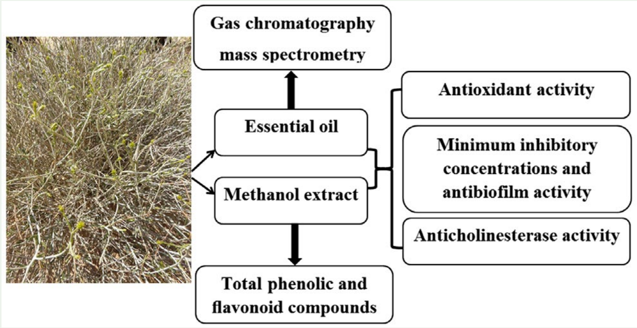
Chemical constituents of essential oil of endemic *Rhanterium suaveolens* Desf. growing in Algerian Sahara with antibiofilm, antioxidant and anticholinesterase activities

Ahmed Elkhalifa Chemsa[a,b], Ebru Erol[c], Mehmet Öztürk[c], Amar Zellagui[b], Ceylan Özgür[d], Noureddine Gherraf[b] and Mehmet Emin Duru[c]

[a]Faculty of Life and Natural Sciences, Department of Biology, El Oued University, El-Oued, Algeria; [b]Laboratory of Biomolecules and Plant Breeding, Faculty of Exact Science and Life Science and Nature, Life Science and Nature Department, University of Larbi Ben Mhidi, Oum El Bouaghi, Algeria; [c]Faculty of Science, Department of Chemistry, Mugla Sitki Kocman University, Mugla, Turkey; [d]Department of Plant and Animal Breeding, Ula Ali Kocman Vocational School, Mugla Sitki Kocman University, Mugla, Turkey

ABSTRACT

Twenty compounds were detected in the essential oil of *Rhanterium suaveolens* representing 98.01% of the total oil content. Perillaldehyde (45.79%), caryophyllene oxide (24.82%) and *β*-cadinol (5.61%) were identified as the main constituents. In β-carotene–linoleic acid assay, both the oil and the methanol extract exhibited good lipid peroxidation inhibition activity, with IC_{50} values of 17.97 ± 5.40 and 11.55 ± 3.39 μg/mL, respectively. In DPPH and CUPRAC assays, however, the methanol extract exhibited a good antioxidant activity. The highest antibiofilm activity has been found 50.30% against *Staphylococcus epidermidis* (MU 30) at 20 μg/mL for essential oil and 58.34% against *Micrococcus luteus* (NRRL B-4375) at 25 mg/mL concentration for methanol extract. The *in vitro* anticholinesterase activity of methanol extract showed a moderate acetylcholinesterase inhibitory (IC_{50} = 168.76 ± 0.62 μg/mL) and good butyrylcholinesterase inhibitory (IC_{50} = 54.79 ± 1.89 μg/mL) activities. The essential oil was inactive against both enzymes.



ARTICLE HISTORY
Received 31 July 2015
Accepted 3 September 2015

KEYWORDS
Rhanterium suaveolens; essential oil; antibiofilm; anticholinesterase; antioxidant activity

CONTACT Ahmed Elkhalifa Chemsa ✉ khalifa-chemsa@univ-eloued.dz

Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2015.1110705

1. Introduction

The genus *Rhanterium* is distributed over western North Africa, the Arabian Peninsula, Iraq and Iran. Three species; namely, *R. adpressum* Coss. & Durieu, *R. epapposum* Oliver and *R. suaveolens* Desf. of this genus have been reported in the literature. *R. intermedium* Coss. & Durieu ex Pomel is another species, but it is a hybrid between *R. suaveolens* and *R. adpressum*. *R. suaveolens* Desf., a member of the Asteraceae (Compositae), is locally known as 'Arfadja'. Quézel and Santa reported that it is an endemic desert plant growing in North Africa (Quézel & Santa 1963; Wiklund 1986). Some of the *Rhanterium* species are used in folk medicine as an antidiuretic (Hamia et al. 2013).

There are some studies on the *Rhanterium* species in the literature. In one study, β-eudesmol, 16β-hydroxylupeolyl-3-hexadecanoate, stigmasterol and (+)-3-[3′-(nona-1″-en-3″,5″,7″-trynyl) oxiran-2′-yl] propan-2-ol were isolated from *R. adpressum* (Bouheroum et al. 2007). In another study, scopoletin was isolated from *R. epapposum* (Miana & Al-Hazimi 1983). The composition of *R. adpressum* and *R. epapposum* was also studied (Yaghmai & Kolbadipour 1987; Al-Easa 2004; Al-Mazroa et al. 2006; Kala et al. 2009). Ranthenone glucoside, 9-hydroxylinaloyl glucoside, scopolin, fraxetin, scopoletin and sitosterol-3β-O-[6′-palmitoyl-β-*D*-glucopyranoside] (Oueslati et al. 2007), antimicrobial polyacetyleneic alcohols (Oueslati et al. 2004) and ceramides (Oueslati et al. 2005) were isolated from the *R. suaveolens*.

The essential oil composition of the *R. suaveolens* and biological activities have not been reported earlier, except the antiradical activity using ABTS and DPPH assays (Bouaziz et al. 2009).

This paper is the first study of the chemical composition of essential oil of *R. suaveolens*. Antibiofilm and anticholinesterase activity of essential oil and methanol extract of *R. suaveolens* against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), the chief enzymes of Alzheimer's disease, were also reported for the first time. Hence, the main objectives of this work were: determine the chemical composition of hydrodistillated oil of the aerial parts of *R. suaveolens* using gas chromatography and gas chromatography/mass spectrometry (GC/MS). The antibiofilm effect of sub-MICs of essential oil and methanol extract was assessed by the microplate biofilm assay. The antioxidant capacity of the essential oil and methanol extract was evaluated using three complementary assays; namely, β-carotene-linoleic acid, DPPH$^{\cdot}$ scavenging and CUPRAC assays.

2. Results and discussion

2.1. Essential oil

The EO having yellow colour was obtained from aerial parts using hydrodistillation method and the yield was 0.14% (volume/dry-weight). A total of 20 compounds were identified representing 98.01% of the total oil content. The major compounds were perillaldehyde (45.79%), caryophyllene oxide (24.82%), β-cadinol (5.61%), β-caryophyllene (5.17%) and 8-cedren-13-ol (4.98%). β-pinene (3.21%) and α-irone (1.62%) were also determined as a constituent of the EO (Table S1). Monoterpenoids accounted for 48.25% of the total oil content while sesquiterpenoids amounted to 37.97% of the total oil. The sesquiterpenes and monoterpenes were detected in the ratio of 7.40% and 4.39%, respectively. The EOs of *R. adpressum* and *R. epoppposum* were rich in monoterpenoids (Yaghmai & Kolbadipour 1987; Hamia et al. 2013). The EO of *R. suaveolens* also resembled to those oils from classification side.

2.2. Anticholinesterase activity

The anticholinesterase activity of the EO and MeOH extract of *R. suaveolens*, against AChE and BChE enzymes was given in Table S2. Galantamine was the standard drug used for comparison. The MeOH extract exhibited moderate to good inhibitory activity against AChE and BChE enzymes. The IC_{50} values were 168.76 ± 0.62 and 54.79 ± 1.89 μg/mL, respectively. On the contrary, the EO was inactive against both AChE and BChE.

2.3. Total phenolic and total flavonoid contents and antioxidant activity

The total phenolic and total flavonoid contents were performed spectrophotometrically using pyrocatechol and quercetin as standard compounds, respectively. The phenolic content of MeOH extract of *R. suaveolens* was 35.58 ± 0.04 μg pyrocatechol equivalents/g extract and the flavonoid content was 5.5 ± 0.02 μg quercetin equivalents/g extract. It can be said that the *R. suaveolens* is poor in flavonoids. Supportively, in a previous study (Bouaziz et al. 2009), 625 ± 75 mg pyrogallol/100 g and trace milligrams of quercetin/100 g were reported for the same plant collected in November in Douz area in Tunisia. The season and area of collection of the plant may lead to the different results.

Table S3 shows the $DPPH^{\cdot}$ scavenging activity and lipid peroxidation inhibitory activity by β-carotene/linoleic acid assay of the EO and the MeOH extract of *R. suaveolens*. Antioxidant activity of the extract and oil increased dose dependently in both assays. In DPPH assay, the MeOH extract demonstrated good $DPPH^{\cdot}$ scavenging activity ($IC_{50} = 0.017 \pm 0.004$ mg/mL), while the EO exhibited weak activity. In the previous study, the DPPH assay was performed only for MeOH extract and the IC_{50} value was calculated as 1.09 ± 0.19 μg/mL (Bouaziz et al. 2009). These differences may be due to the collection time and locality of the plant.

In lipid peroxidation inhibition assay, the MeOH extract exhibited higher inhibition (IC_{50} = 11.55 ± 3.39 μg/mL) against lipid peroxidation, while the EO exhibited (IC_{50} = 17.97 ± 5.40 μg/mL). The EO composition was given in Table S1. As seen, there are no phenolic compounds to scavenge the DPPH radicals. However, compounds as well as the conjugated mono and sesquiterpenoids are responsible for the lipid peroxidation activity. These compounds can scavenge the singlet oxygen and therefore protect the β-carotene colour against bleaching, indirectly.

Figure S1 shows the results of the CUPRAC (cupric reducing antioxidant capacity) of both the MeOH extract and the EO of *R. suaveolens*. α-Tocopherol and BHT were used as positive controls. As shown in (Figure S1), the activity of the MeOH extract of the *R. suaveolens* was compatible with α-tocopherol at all concentrations. As expected the EO indicated a less reducing activity.

2.4. Determination of MIC's and antibiofilm activity

The MIC and antibiofilm activity results of the EO and methanol extract against six bacteria species and *Candida albicans* are given in Table S4. The oil inhibited the growth of all micro-organisms between 10 and 80 μg/mL concentrations. EO at the MIC's inhibited biofilm formations of all microorganisms tested in various percentages. The oil exhibited the highest antimicrobial activity against *S. epidermidis* MU 30 at 20 μg/mL (MIC/1) and at 10 μg/mL (MIC/2) concentrations with 50.3% and 32.96%, respectively.

According to the results, *B. subtilis* was found to be the most susceptible strain against MeOH extract of *R. suaveolens*. The extract has low activity on the growth of *M. luteus* NRRL B-4375 and *S. epidermidis* MU 30 which were only inhibited at high concentration (25 mg/mL). In the presence of 25 mg/mL extract (MIC), the mean biofilm formation values were equal to 58.34% for *M. luteus* NRRL B-4375 and 49.06% for *S. epidermidis* MU 30.

In the current investigation, the EO exhibited antimicrobial activity, particularly against *S. epidermidis* MU 30, *M. luteus* NRRL B-4375 and *B.* subtilis ATCC 6633. Perillaldehyde, the major compound of the oil is an antimicrobial agent. It demonstrated antimicrobial activity against *B. cereus*, *E. coli* and *S. aureus* (Friedman et al. 2006). Concerning the antimicrobial activity against *B. subtilis*, the oil supported the previous results. However, the oil that showed a weak activity against against *S. aureus*. This may be due to its percentage (45.79%) in the oil which did not reach to the extent necessary to inhibit the growth of those micro-organisms.

3. Conclusion

The anticholinesterase activity against both enzymes was performed for the first time in this study. According to the results the EO indicated no activity. However, the methanol extract exhibited good activity against BChE. Thus, the plant can be used as an anticholinesterase agent, particularly against BChE. However, further studies are necessary to evaluate the origin of the activity. Also, the methanolic extract and the oil proved to be effective antioxidants and antimicrobials in different *in vitro* assays and can be suggested as a natural additive in food and pharmaceutical industries. In the case of antioxidant activities, results obtained from β-carotene/linoleic acid bleaching test were found to be stronger than those obtained from DPPH and CUPRAC systems.

Supplementary material

Experimental details relating to this paper are available online, alongside Tables S1–S4 and Figure S1.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Ahmed Elkhalifa Chemsa http://orcid.org/0000-0002-2220-0877

Acknowledgements

This study is a part of Mr. Chemsa's PhD thesis. The authors wish to thank the Ministry of Higher Education and Scientific Research in Algeria for the financial support during the training period in Turkey. Also the authors are grateful to Dr. Youcef Halice for identifying the plant sample. GC and GC-MS spectra were performed at the Department of Chemistry, Faculty of Sciences, Muğla Sıtkı Koçman University.

References

Al-Easa HS. 2004. Fatty acids composition of fifty-one plant species growing in Qatar. J Chem (Calcutta, India). 14:195–204.

Al-Mazroa SA, Al-Wahaibi LH, Mousa AA, Alkhathlan HZ. 2006. Essential oils of *A. factorovskyi, K. linearis, C. arvensis, P. babylonica, P. cyanocarpa, L. Nudicaulis* and *R. epapposu*. J Saudi Chem Soc. 9:693–698.

Bouaziz M, Dhouib A, Loukil S, Boukhris M, Sayadi S. 2009. Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. Afr J Biotechnol. 8:7017–7027.

Bouheroum M, Benayache S, Benayache F, Zaiter L, Barrera JM, Francisco L. 2007. Terpenoids and triynepoxide from the aerial part of *Rhantherium adpressum*. Chem Nat Compd. 43:110–111.

Friedman M, Buick R, Elliott CT. 2006. Antimicrobial activities of plant compounds against antibiotic-resistant *Micrococcus luteus*. Int J Antimicrob Agents. 28:156–158.

Hamia C, Gourine N, Boussoussa H, Saidi M, Gaydou EM, Yousfi M. 2013. Chemical composition and antioxidant activity of the essential oil and fatty acids of the flowers of *Rhanterium adpressum*. Nat prod commun. 8:1171–1174.

Kala A, Gherraf N, Belkacemi D, Ladjel S, Zellagui A, Samir H, Chihi S, Brahim L. 2009. Composition of the essential oil of *Rhanterium adpressum* Coss. and Durieu. from Algeria. Arch Appl Sci Res. 1:115–118.

Miana GA, Al-Hazimi HMG. 1983. Chemical investigations of Saudi Arabian plants. I. scopoletin from *Rhanterium epapposum* olive. J Chem Soc Pakistan. 5:223–225.

Oueslati MH, Ben Jannet H, Abreu PM, Mighri Z. 2004. Alcools tetrahydropyraniques polyacetyleniques antibacteriens de la plante *Rantherium suaveolens* poussant dans le sud tunisien. Journal de la Société Algérienne de Chimie [Journal of the Algerian Society of Chemistry]. 14:245–258.

Oueslati MH, Ben Jannet H, Mighri Z, Matthew S, Abreu PM. 2007. A new C 9 nor -isoprenoid glucoside from *Rantherium suaveolens*. Nat Prod Res. 2007 Aug 01. 21:884–888.

Oueslati MH, Mighri Z, Jannet H, Abreu PM. 2005. New ceramides from *Rantherium suaveolens*. Lipids. 40:1075–1079.

Quézel P, Santa S. 1963. Nouvelle Flore de l’Algérie et des Régions Désertiques Méridionales. Vol. 2. Paris, France: Éditions du Centre National de la Recherche Scientifique; p. 948.

Wiklund A. 1986. The genus *Rhanterium* (Asteraceae: Inuleae). Bot J Linn Soc. 93:231–246.

Yaghmai MS, Kolbadipour S. 1987. Volatile components of *Rhanterium epapposum* oliv. Flavour Fragrance J. 2:29–32.

J. Mater. Environ. Sci. 7 (3) (2016) 993-1000 *Chemsa et al.*
ISSN : 2028-2508
CODEN: JMESCN

Antibiofilm formation, antioxidant and anticholinesterase activities of essential oil and methanol extract of *Marrubium deserti* de Noé

Ahmed Elkhalifa Chemsa[1,2,3]*, Amar Zellagui[2], Mehmet Öztürk[3], Ebru Erol[3], Ozgür Ceylan[4], Mehmet Emin Duru[3], Noureddine Gherraf[2]
[1]*Department of Biology, Faculty of Life and Natural Sciences, El Oued University, Algeria*
[2]*Laboratory of Biomolecules and Plant Breeding, Faculty of Exact Science and Life Science and Nature, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria*
[3]*Department of Chemistry, Faculty of Science, Mugla Sitki Kocman University, Mugla, Turkey*
[4]*Department of Plant and Animal Breeding, Ula Ali Kocman Vocational School, Mugla Sitki Kocman University, Mugla, Turkey*

Received 21 Aug 2015, Revised 06 Dec 2015, Accepted 18 Dec 2015
**Corresponding Author: E-mail chemsakhalifa@gmail.com, khalifa-chemsa@univ-eloued.dz, Tel: +213-3222-3009 Fax: +213-3222-3003*

Abstract

The essential oil obtained from the aerial parts of *Marrubium deserti* de Noé. (Lamiaceae), growing in the North fringe of the Algerian Sahara, was analyzed by GC-MS. Thirty-eight compounds were identified, representing 99.70% of the total oils. The GC-MS analysis revealed the presence of tetracosane, germacrene D, *Δ*-cadinene, *α*-cadinol and *t*-cadinol as the main constituents, representing 31.11%, 7.91%, 6.52%, 6.26% and 5.81%, respectively. Minimum inhibitory concentrations (MICs) of essential oil and methanol extract were calculated by microtitre broth dilution method, and antibiofilm effects by microplate biofilm assay. The highest antibiofilm activity was found to be 69.31% against *Micrococcus luteus* NRRL B-4375 at 25 mg/mL for methanol extract and 36.62% against *Candida albicans* ATCC 10239 at 25 µL/mL concentration for essential oil. The antioxidant activity was determined using three complementary tests namely: β-carotene-linoleic acid, DPPH free radical scavenging, and CUPRAC assays. In β-carotene-linoleic acid assay, both the oil and the extract exhibited good lipid peroxidation inhibition activity, demonstrating 76.81 ± 0.59 and 86.33 ± 0.27% at 200 µg/mL concentration, respectively. In DPPH and CUPRAC assays, however, the methanol extract exhibited high antioxidant activity; however, the essential oil showed weak activity. The *in vitro* anticholinesterase activity, was carried out against acetylcholinesterase and butyrylcholinesterase enzymes spectrophotometrically using Elman method. Methanol extract showed weak acetylcholinesterase and butyrylcholinesterase inhibitory activities, while the essential oil was inactive against both enzymes.

Keywords: Marrubium deserti, antibiofilm, anticholinesterase, antioxidant, essential oil.

1. Introduction

The genus *Marrubium* includes six species and one hybrid in Algeria: *Marrubium vulgare* L., *M. spinum* L., *M. peregrinum* L., *M. alysson* L., *M. alyssoides* Pomel, *M. willkommu* Magn. (*M. supinum* X *vulgare*) and *M. deserti* de Noé. [1], the latter is the subject of this study.

M. deserti is a small perennial shrub (20-30 cm high), very branched, with woolly leaves and stems. The flowers are pale violet or pink. The calyx is bright green and evergreen around the fruit, which is a tetra-achene typical of the Labiatae. The leaves are velvety and opposite, and are generally terminated by three large teeth of variable form [1, 2]. The species is endemic of central and north Algerian Sahara. It grows on desert pastures and flowers in spring (March-April) [1-3]. The plant grows in an arid and semi-arid climate, with an annual rainfall of 100 mm It is usually found in non-saline wadis on gravelly-sandy soils [3, 4]. The plant is used by the

local people in traditional medicine in Algeria. The leaves, stems and flowers are used against intestinal disorders, respiratory diseases, fever, cough, dysmenorrhea, scorpion stings and Allergies [5, 6].
Recently, the flavonoids, phenyl ethanoid and diterpenoids have been isolated from *Marrubium* species which some of them are considered as therapeutic (anti-tumor) and pharmacological [7-11]. In a recent study 6-dehydroxy-19-acetyl-marrubenol, 19-acetyl-marrubenol, 6-acetyl-marrubenol and 16-epoxy-9-hydroxy-labda-13(16), 14-diene, *β*-sitosterol, stigmasterol, *β*-sitosterol 3-*O*-glucoside, and phytol were isolated from *M. deserti* [12]. In other study, marrulibacetal A, desertine, 15-epi-cyllenin A, marrubiin, marrulactone, marrulibacetal, β-stigmasterol, apigenin, apigenin-7-*O*-*β*-neohesperidoside, apigenin-7-*O*-glucoside, terniflorin and apigenin-7-*O*-glucuronide, acteoside and forsythoside B were isolated and elucidated [13]. The chemical composition of essential oil, however, gave six major compounds: germacrene D (45.7%), *β*-bourbonene (4.0%), α-terpinolene (3.9%), *Δ*-cadinene (3.8%), 1-octen-3-ol (3.7%) and α-copaene (3.5%) [14]. The antioxidant effect, antiviral, antibacterial and antigenotoxic activities of aqueous extracts of *M. deserti* were also reported [13, 15-18]. However, to the best of our knowledge, there has been no scientific report on antibiofilm formation and anticholinesterase activities of the extracts of *M. deserti* yet. Therefore, this paper presents the first study on the antibiofilm formation and anticholinesterase activity of essential oil and methanol extract of *M. deserti* together with the chemical composition of hydrodistillated oil of the aerial parts of *M. deserti* growing in El-Oued - Algeria.

2. Experimental methods

2.1. Plant material

The aerial parts (leaves, flowers and steams) of *M. deserti* were collected during the flowering period in April 2012 from Meguibra, El-Oued, Algeria (34°14'0"N, 6°1'0"E) at -11 m altitude and taxonomic identification of plant was confirmed by Dr. Youcef Halis. A voucher sample was deposited in the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria (*M. deserti* voucher number ZA 143).

2.2. Extraction of the essential oil

The essential oil of dried aerial parts (300g) of *M. deserti* was obtained via hydrodistillation by using a Clevenger type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and stored under nitrogen until required.

2.3. Gas chromatography analysis

GC analysis of the oil were performed using a Shimadzu GC-17 AAF, V3, 230V LV Series (Kyoto, Japan) gas chromatography, equipped with a FID and a DB-1 fused silica column [30m x 0.25 mm (i.d.), film thickness 0.25 μm]; the oven temperature was held at 60°C for 5 min, then programmed to 240°C at 4°C/min and held isothermal for 10 min; injector and detector temperatures were 250°C and 270°C respectively; carrier gas was He at a flow rate of 1.3 mL/min; Sample size, 1.0 μL; split ratio, 50:1. The percentage composition of the essential oil was determined with a Class-GC 10 computer program.

2.4. Gas chromatography–mass spectrometry (GC–MS)

The analysis of the essential oil was performed using a Varian Saturn 2100 (Old York Rd., Ringoes, NJ, USA), ion trap machine, equipped with a DB-1 MS fused silica non-polar capillary column [30 m x 0.25 mm (i.d.), film thickness 0.25 μm]. Carrier gas was helium at a flow rate of 1.4 mL/min. The oven temperature was held at 60°C for 5 min, then increased up to 240°C with 4°C/min increments and held at this temperature for 10 min. Injector and transfer line temperatures were set at 250 and 180°C, respectively. Ion trap temperature was 200°C. The injection volume was 0.2 μL and split ratio was 1:30. EI-MS measurements were taken at 70 eVionisation energy. Mass range was from *m/z* 28 to 650 amu. Scan time was 0.5 s with 0.1 s inter scan delays. Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [19] and, whenever possible, by co-injection with authentic compounds.GC and GC-MS spectra were performed at the Department of Chemistry, Faculty of Sciences, Muğla Sıtkı Koçman University.

2.5. Determination of minimum inhibitory concentrations and antibiofilm activity
2.5.1 Microorganisms and condition for cultivation

In the present study, the microorganisms used in the experiments were : Gram positive bacteria (*Staphylococcus aureus* (ATCC 25923, ATCC 6538-P), *Staphylococcus epidermidis* MU 30, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* RSKK 863, *Streptococcus mutans* CNCTC 8/77 and *Micrococcus luteus* NRRL B-4375) and yeast (*Candida albicans* ATCC 10239) were used as test microorganisms. The above-mentioned bacteria except *C. albicans* were grown in nutrient broth (NB, Difco); *C. albicans* was grown in sabouraud dextrose broth (SDB, Difco). *C.albicans* ATCC 10239 was incubated at 30 ± 0.1 °C for 24-48 h. Other bacterial strains were incubated at 37 ± 0.1 °C for 24 h.

2.5.2 Minimal inhibitory concentration (MIC) assay

MICs were determined by a microtitre broth dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [20]. The MIC was defined as the lowest essential oil/extract concentration that yielded no visible growth. The test medium was MHB (Mueller Hinton Broth) and the density of bacteria was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (100 µL) were inoculated in to the wells of 96-well microplatesin the presence of essential oil with different final concentrations (6.25, 12.5, 25, 50, 80, 160 µL/mL) and in the presence of methanol extract with different final concentrations (1.56, 3.12, 6.25, 12.5, 25, 50 mg/mL). The inoculated microplates were incubated at 37ºC for 24 h before being read.

2.5.3 Effect of essential oil and methanol extract on bacterial biofilm formation

The effect of *M. deserti* essential oil and extract at concentrations including 1, 1/2, 1/4, 1/8 and 1/16 MIC on biofilm-forming ability of test microorganisms were tested with a microplate biofilm assay [21]. Briefly, 1% of overnight cultures of isolates were added into 200 µL of fresh Tryptose-Soy Broth (TSB) supplemented with 0.25% glucose and cultivated in the presence and absence of *M. deserti* essential oil/extract without agitation for 48 h at 37 ºC. The wells containing TSB+cells served as control. After incubation, the wells were washed with water to remove planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 min at room temperature. Wells were washed once again to remove the crystal violet solution. 200 µL of 33% glacial acetic acid poured in wells. After shaking and pipetting of wells, 125 µL of the solution from each well transferred to a sterile tube and volume reached to 1 mL with distilled water. Finally optical density (OD) of each well measured at wavelength of 550 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). Percentage of inhibition of the tested extracts was calculated using the formula:

$$Biofilm\ inhibition\ (\%) = \frac{OD_{550Control} - OD_{550Sample}}{OD_{550Control}} \times 100$$

2.6. Anticholinesterase activity

The inhibition activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were measured by spectrophotometric method developed by Elman et al., in 1961, with slight modification[22, 23].

2.7. Antioxidant activity
2.7.1. Free radical-scavenging activity (DPPH assay)

The free radical scavenging activity was performed using slightly modified DPPH assay [23, 24].

2.7.2. β-Carotene-linoleic acid assay

The slightly modified β-Carotene-linoleic acid test system was used to assay lipid-peroxidation inhibitory activity [23, 25].

2.7.3. Cupric reducing antioxidant capacity (CUPRAC)

The slightly modified Apak’s method was used to determine the cupric reducing antioxidant capacity [23, 26].

2.8. Determination of total phenolic compounds

The content of phenolic compounds was determined using Folin–Ciocalteu reagent, and expressed as microgramme of pyrocatechol equivalents [27]. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard pyrocatechol graph:

$$Absorbance = 0.006\,\mu g\ pyrocatechol + 0.035 \quad (\mathrm{r}^2 = 0.978)$$

2.9. Determination of total flavonoid concentration

Total flavonoid content was determined according to the aluminum method. The results were expressed as quercetin equivalents [28]. The concentration of flavonoid compounds was calculated according to following equation that was obtained from the standard quercetin graph.

$$Absorbance = 0.051\,\mu g\ quercetin + 0.001 \quad (\mathrm{r}^2 = 0.999)$$

2.10. Statistical analysis

The antioxidant and the anticholinesterase activity assays were in triplicate analyses. The data were recorded as means ± standard error meaning. Student's t-test were used to determine the significant differences between means; $p < 0.05$ were regarded as significant.

3. Results and discussion

3.1. Chemical composition

The essential oil having yellow color was obtained from aerial parts using hydrodistillation (0.15%, v/w) method. Totally 38 components were identified representing 99.70% of the oil (Table 1). β-caryophyllene is omnipresent in the essential oils of most *Marrubium* species, followed by germacrene D and bicyclogermacrene [14]. Whereas, the major compounds of essential oil of *M. deserti* were tetracosane (31.11%,), germacrene D (7.91%), Δ-cadinene (6.51%), α-cadinol (6.26%), and t-cadinol (5.81%). bicyclogermacrene (2.84%) and β-caryophyllene (1.32%) were also determined as a constituent of the essential oil (**Table 1**). The monoterpenoids represented 9.26% while sesquiterpenoids 16.45% of the total oil. The monoterpenes and sesquiterpenes were in the ratio of 4.32% and 33.85%, respectively.

3.2. Minimum inhibitory concentrations and antibiofilm activity

The antimicrobial activity of the essential oil and methanol extract against 6 bacteria and *Candida albicans* ATCC 10239 was given in **Table 2**. The essential oil inhibited the growth of all microorganisms between 25 and 80 µL/mL concentrations. The MIC values of methanol extract were in the range of 3.25-25 mg/mL. According to the results, *B. subtilis* ATCC 6633 was found as the most susceptible strain against methanol extract. The extract has low activity on the growth of *M. luteus* NRRL B-4375, *S. mutans* CNCTC 8/77 and *S. epidermidis* MU 30 that were only inhibited at high concentration (25 mg/mL). Essential oil at the MIC's inhibited biofilm formations of all microorganisms tested in various percentages. The essential oil exhibited the highest antibiofilm activity against *C. albicans* ATCC 10239 at 25 µg/mL (MIC) concentration with 36.62%.

In the presence of 25 mg/mL extract (MIC), the mean biofilm formation values were equal to 69.31% for *M. luteus* NRRL B-4375, 29.27% for *S. mutans* CNCTC 8/77 and 56.28% for *S. epidermidis* MU 30. The antibiofilm activity of essential oil on tested strains was lower than that of methanol extract.

3.3. Anticholinesterase activity

The anticholinesterase activity of the essential oil and methanol extract against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes was given in **Table 3**. Galantamine was used to compare the activity as a standard drug. The methanol extract exhibited weak inhibitory activity against AChE and BChE enzymes. The IC_{50} values were calculated as 277.4 ± 13.6, and 93.3 ± 0.7µg/mL, respectively. On the other hand, the essential oil was inactive against both AChE and BChE.

3.4. Total phenolic and total flavonoid contents and Antioxidant activity

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity. The phenolic compounds are also known as powerful chain breaking antioxidants [29]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily is ingested from a diet rich in stems and vegetables [30]. The concentration of phenolics and flavonoids in the extract were expressed as micrograms of pyrocatechol and micrograms of quercetin equivalents per milligrams of the extract, respectively. The methanol extract had 58.17 ± 0.03 µg pyrocatechol equivalents and 5.2 ± 0.03 µg quercetin equivalents.

Table 1: Chemical composition (%) of the essential oil of *M. deserti*

N°	**RI**[a]	**Compounds**	**Composition (%)**[b]
1	890	cyclofenchene	1.52 ± 0.001
2	932	*α*-thujene	0.52 ± 0.0001
3	936	*α* -pinene	2.15 ± 0.0012
4	978	*β*-pinene	0.82 ± 0.0001
5	1025	*D*-limonene	1.05 ± 0.0011
6	1123	camphor	1.94 ± 0.0013
7	1131	citronellal	0.88 ± 0.0001
8	1149	*n*-nonanol	1.06 ± 0.0014
9	1164	terpinen-4-ol	0.62 ± 0.0001
10	1172	myrtenal	0.72 ± 0.0001
11	1176	*α*-terpineol	0.66 ± 0.0001
12	1178	myrtenol	0.28 ± 0.00001
13	1241	*p*-cumic aldehyde (Cuminal)	0.27 ± 0.00001
14	1243	carvone	1.54 ± 0.0014
15	1271	perillaldehyde	1.70 ± 0.002
16	1290	thymol	1.15 ± 0.0013
17	1351	*α*-cubebene	0.74 ± 0.0001
18	1388	*β*-bourbonene	0.56 ± 0.0001
19	1390	*β*-elemene	3.84 ± 0.002
20	1408	*β*-caryophyllene	1.32 ± 0.0009
21	1460	alloaromadendrene	2.54 ± 0.0014
22	1477	*γ*-gurjunene	0.84 ± 0.0003
23	1481	germacrene D	**7.91 ± 0.0023**
24	1486	eremophilene	1.44 ± 0.0003
25	1494	bicyclogermacrene	2.84 ± 0.003
26	1500	*α*-muurolene	0.69 ± 0.0001
27	1513	*γ*-cadinene	1.95 ± 0.0003
28	1523	*Δ*-cadinene	**6.51 ± 0.003**
29	1545	*α*-calacorene	0.49 ± 0.0001
30	1553	*E*-nerolidol	0.86 ± 0.0001
31	1572	spathulenol	0.96 ± 0.0001
32	1578	caryophyllene oxide	0.90 ± 0.0001
33	1633	*t*-cadinol	**5.81 ± 0.003**
34	1635	*α*-muurolol	1.07 ± 0.0001
35	1643	*α*-cadinol	**6.26 ± 0.003**
36	1648	*allo*-himachalol	2.43 ± 0.0003
37	1653	7-*epi*-*α*-Eudesmol	1.57 ± 0.0001
38	2400	tetracosane	**31.11 ± 0.001**
39	2406	unidentified	0.11 ± 0.0001
40	2418	unidentified	0.13 ± 0.0001
41	2422	unidentified	0.05 ± 0.0000
		Total identified:	99.70
		Monoterpenes:	4,32
		Monoterpenoids:	9,26
		Sesquiterpenes:	33.85
		Sesquiterpenoids:	16,45
		Others:	35.84

[a] Kovats index on DB-1 fused silica column.

[b] The percentage concentrations of the compounds are the means of three parallel measurements ± S.E.M. by GC ($p<0.05$).

Table 2: MIC and antibiofilm activity results of the essential oil and methanol extract of *M. deserti*

	Essential oil						**Methanol extract**					
	Planktonic	% inhibition on biofilm formation					Planktonic	% inhibition on biofilm formation				
Microorganisms	MIC (µL/mL)	MIC/1	MIC/2	MIC/4	MIC/8	MIC/16	MIC (mg/mL)	MIC/1	MIC/2	MIC/4	MIC/8	MIC/16
Staphylococcus aureus ATCC 25923	50	19.21	-	-	-	-	6.25	28.90	11.65	-	-	-
Staphylococcus aureus ATCC 6538P	80	4.96	-	-	-	-	12.5	27.25	7.37	-	-	-
Staphylococcus epidermidis MU 30	25	29.81	20.33	-	-	-	25	56.28	28.51	10.78	5.31	-
*Bacillus subtilis*ATCC 6633	50	27.98	11.25	-	-	-	3.25	7.57	-	-	-	-
Bacillus cereus RSKK 863	25	26.66	18.25	4.62	-	-	12.5	49.66	25.68	10.85	-	-
Micrococcusluteus NRRL B-4375	25	23.75	10.70	-	-	-	25	69.31	45.46	27.45	3.32	-
Streptococcus mutans CNCTC 8/77	25	20.36	-	-	-	-	25	29.27	10.88	-	-	-
Candida albicans ATCC 10239	25	36.62	17.69	-	-	-	12.5	34.32	18.47	5.51	-	-

- : no inhibition

Table 3: Acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil and methanol extract of *M. deserti*[a]

Samples	**AChE assay**		**BChE assay**	
	Inhibition (%) (200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (200 µg/mL)	IC_{50} (µg/mL)
Essential oil	*NA*	*NA*	*NA*	*NA*
MeOH Extract	35.4 ± 1.9	277.4 ± 13.6	53.2 ± 0.1	93.3 ± 0.7
Galantamine[b]	81.4 ± 1.0	5.011 ± 0.09	75.5 ± 1.1	53.9 ± 0. 6

NA: not active

[a] IC_{50} values represent the means ± S.E.M. of three parallel measurements ($p< 0.05$).

[b] Standard drug.

In lipid peroxidation inhibition assay, the essential oil exhibited high inhibition (IC_{50} = 35.9 ± 9.3 µg/mL) against lipid peroxidation, while in DPPH assay, the essential oil demonstrated weak DPPH free readical scavenging activity. As seen it the essential oil composition, there are no phenolic compounds (only thymol) to scavenge the DPPH radicals (**Table 4**). However, the compounds particularly the bicyclic compounds as well as the conjugated mono and sesquiterpenoids are responsible for the lipid peroxidation activity. These compounds can scavenge the singlet oxygen. Therefore, they protect the β-carotene color against bleaching, indirectly. The methanol extract showed highest antioxidant activity both in DPPH assay and in Lipid peroxidation inhibition assay. These results are supported the previously studies, where the polar extracts showed higher antioxidant activity than unpolar extracts [17].

Table 4: Antioxidant activity (%) of the essential oil and methanol extract of *M. deserti* by the DPPH and β-carotene/linoleic acid assays[a]

	DPPH Assay				β-carotene/linoleic acid assay			
	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
Essential oil	-	1.34±0.54	3.05±0.58	5.47±0.52	62.29±4.00	72.96±1.37	75.09±0.08	76.81±0.59
MeOH Extract	72.02±0.15	76.77±0.17	77.74±0.26	78.35±0.10	77.62±0.90	81.90±0.45	85.97±0.11	86.33±0.27
BHT[b]	40.43±0.05	53.18±0.51	73.91±0.11	95.1±8,44	93.65±0.06	95.08±0.02	97.83±0,08	99.36±0,09
α-tocopherol	91.16±0.17	92.03±0.55	93.77±0,07	97.25±0,06	92.89±0.27	93.32±0.33	94.22±0,28	96.02±0,30

[a] Values expressed are means ± SEM of three parallel measurements ($p < 0.05$).
[b] BHT: Butylatedhydroxytoluene.

The CUPRAC assay utilized copper(II)-neocuproine (CU(II)-Nc) reagent as the chromogenic oxidizing agent. It is based on the measurement of absorbance at 450 nm by the formation of stable complex between neocuproine and copper (I). As shown in (**Figure 1**). The methanol extract and α-tocopherol had similar activities. Only at 800 µg/mL concentration, the methanol extract (3.81 ± 0.00) exhibited higher reducing power activity than α-tocopherol (2.9 ± 0.00) and close activity to that of BHA (3.8 ± 0.00)) at 800 µg/mL. However, the activity of essential oil was lesser than the positive controls.

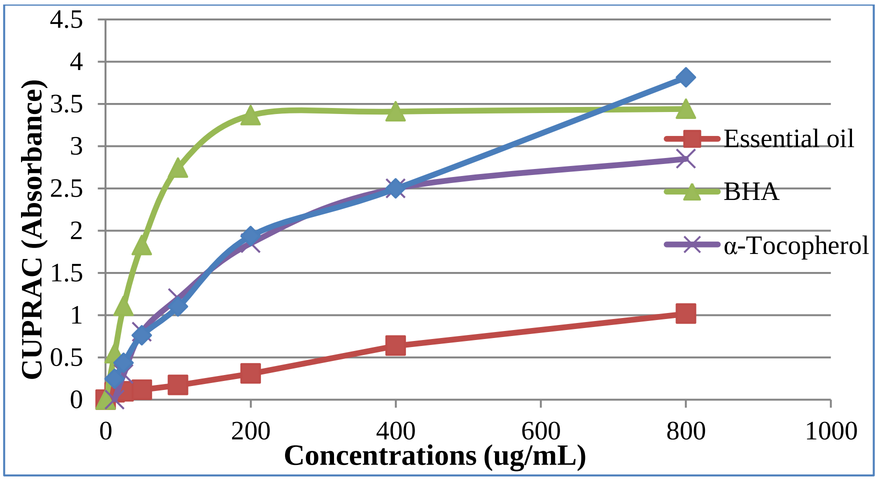


Figure 1: Cupric reducing antioxidant capacity of the methanol extract and essential oil of *M. deserti* Values are means ± S.E.M., $n = 3$, $p < 0.05$, significantly different by Student's *t*-test.

Conclusion

This study deals with the chemical composition of essential oil of *M. deserti* and its biological activities with its methanol extract. Some link could be observed between antioxidant, antibiofilm formation and anticholinesterase activities of methanol extract and essential oil, maybe due the total phenolic and flavonoids contents. The methanolic extract and the oil were found to be effective antioxidants and antimicrobials in different *in vitro* assays and can be suggested as a natural additive in food and pharmaceutical industries. Results obtained from methanol extract were found to be stronger than those obtained from essential oil. However, further studies are necessary to evaluate the origin of the activity.

Acknowledgments- The authors wish to thank the Ministry of Higher Education and Scientific Research in Algeria for the financial support during the training period in Turkey. Also the authors are grateful to Dr. Youcef Halis for identifying the plant sample.

References

1. Quézel P., Santa S., Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales. Éditions du Centre National de la Recherche Scientifique: Paris, France Vol. 2. (1963) 802.
2. Benhouhou S., A Guide to Medicinal Plants in North Africa. IUCN: (2005) 179.
3. Ozenda P., Flore et végétation du Sahara. 3 ed.; CNRS: (2004).
4. Benaradj A., Boucherit H., Bouazza M., *J. Mater. Environ. Sci.* 6 (2015) 3667.
5. Ould El Hadj M. D., Hadj-Mahammed M., Zabeirou H., *Courrier du Savoir* 3 (2003) 47.
6. Hammiche V., Maiza K., *J. Ethnopharmacol.* 105 (2006) 358.
7. Hatam N. A. R., Porzel A., Seifert K., *Phytochemistry* 40 (1995) 1575.
8. El Bardai S., Morel N., Wibo M., Fabre N., Llabres G., Lyoussi B., Quetin-Leclercq J., *Planta Med.* 69 (2003) 75.
9. Karioti A., Heilmann J., Skaltsa H., *Phytochemistry* 66 (2005) 1060.
10. Hennebelle T., Sahpaz S., Skaltsounis A., Bailleul F., *Biochem. Syst. Ecol.* 35 (2007) 624.
11. Sarikurkcu C., Tepe B., Daferera D., Polissiou M., Harmandar M., *Bioresour. Technol.* 99 (2008) 4239.
12. Dendougui H., Seghir S., Belloum Z., Benayache F., Leon F., Brouard I., Bermejo J., Benayache S., *Rec. Nat. Prod.* 5 (2011) 300.
13. Zaabat N., Hay A.-E., Michalet S., Darbour N., Bayet C., Skandrani I., Chekir-Ghedira L., Akkal S., Dijoux-Franca M.-G., *Food Chem. Toxicol.* 49 (2011) 3328.
14. Laouer H., Yabrir B., Djeridane A., Yousfi M., Beldovini N., Lamamra M., *Nat Prod Commun* 4 (2009) 1133.
15. Benhammou N., Bekkara F. A., Coustard J.-M., *Adv. Food Sci.* 31 (2009) 194.
16. Djeridane A., Yousfi M., Brunel J. M., Stocker P., *Food Chem. Toxicol.* 48 (2010) 2599.
17. Edziri H., Mastouri M., Aouni M., Verschaeve L., *S. Afr. J. Bot.* 80 (2012) 104.
18. Zaabat N., Darbour N., Bayet C., Michalet S., Doléans-Jordhem A., Chelr-Ghedlra L., Akkal S., Dijoux-Franca M. G., *Phytothérapie* 8 (2010) 353.
19. Adams R. P., Identification of essential oil components by gas chromatography/mass spectrometry. 4 ed.; Allured publishing corporation: (2007).
20. Clinical Laboratory Standards Institute, Quality control minimal inhibitory concentration (MIC) limits for broth dilution and MIC interpretative breakpoints (M27-S2). In Wayne, Pennsylvania, USA: 2006.
21. Merritt J. H., Kadouri D. E., O'Toole G. A., Growing and Analyzing Static Biofilms. In Current protocols in microbiology, John Wiley & Sons, Inc.: (2005).
22. Ellman G. L., Courtney K. D., Andres V., Featherstone R. M., *Biochem. Pharmacol.* 7 (1961) 88.
23. Öztürk M., Kolak U., Topçu G., Öksüz S., Choudhary M. I., *Food Chem.* 126 (2011) 31.
24. Blois M. S., *Nature* 181 (1958) 1199.
25. Miller H., *J. Am. Oil Chem. Soc.* 48 (1971) 91.
26. Apak R., Güçlü K., Özyürek M., Karademir S. E., *J. Agric. Food Chem.* 52 (2004) 7970.
27. Slinkard K., Singleton V. L., *Am. J. Enol. Vitic.* 28 (1977) 49.
28. Park Y. K., Koo M. H., Ikegaki M., Contado J., *Arq. Biol. Tecnol* 40 (1997) 97.
29. Shahidi F., Janitha P., Wanasundara P., *Crit. Rev. Food Sci. Nutr.* 32 (1992) 67.
30. Tanaka M., Chiu W. K., Nagashima Y., Taguchi T., *Nippon Suisan Gakk* 54 (1988) 1409.

(2016) ; http://www.jmaterenvironsci.com