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"Composition, antimicrobial and antioxidant activity of the essential oils of *Thymus algeriensis, Thymus capitatus, Satureja thymbra* and *Salvia fruticosa* from Libya"

Doctoral dissertation

Belgrade, 2013

УНИВЕРЗИТЕТ У БЕОГРАДУ

БИОЛОШКИ ФАКУЛТЕТ

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“Састав, антимикробна и антиоксидативна активност етарских уља *Thymus algeriensis, Th. capitatus, Salvia fruticosa* и *Satureja thymbra* из Либије)”

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Сажетак

У овом раду анализиран је састав етарских уља неких биљних врста из фамилије Lamiaceae које расту као самоникле у Либији (*Thymus capitatus, Th. algeriensis, Satureja thymbra* и *Salvia fruticosa*) коришћењем GC и GC-MS техника. Уља *Thymus capitatus, Th. algeriensis* и *Salvia fruticosa* карактеришу се присуством оксигенованих монотерепена, који су заступљени са 87.60%, 54.67% и 64.89%. Монотерпенски угљоводоници су главна група једињења код врсте *Satureja thymbra* , чинећи 58.57% од укупног уља.

Главна једињења етарског уља *Thymus capitatus* су карвакрол (68.19%) и тимол (12.29%), док етарско уље *Th. algeriensis* карактерише тимол (38.50%) који је главна компонента. Уље *Satureja thymbra* као водеће компоненте садржи γ-терпинен (39.23%), тимол (25.16%) и *p*-цимен (7.17%). Висок садржај 1.8-цинеола (49.34%) и камфора (7.53%) је присутан у уљу *Salvia fruticosa*.

Такође је испитивано антиоксидативно дејство уља и главних компонената етарских уља коришћењем DPPH теста. Највиши антиоксидативни потенцијал показало је уље *Satureja thymbra* (IC_{50} = 0.0967 mg/ml раствора), затим *Thymus capitatus* (IC_{50} = 0.119 mg/ml раствора), *Th. algeriensis* sa IC_{50} od 0.299 mg/ml, а најнижа активност добијена је са уљем *Salvia fruticosa* (IC_{50} = 15.53 mg/ml раствора). Добијене IC_{50} вредности за тимол и карвакрол су 0.403 и 0.105 mg/ml, и 0.0717 mg/ml раствора за BHA. Коришћењем методе микродилуције испитивана је антимикробна активност на осам бактерија и осам гљива. Као тест организми коришћено је четири врсте Грам-негативних бактерија - *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Proteus mirabilis* (хумани изолат) и четири Грам-позитивне бактерије – *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (клинички изолат), *Micrococcus flavus* (ATCC 10240), и *Staphylococcus aureus*

(ATCC 6538). Најбољу активност међу уљима анализираних биљних врста, показало је уље *Thymus capitatus*, где је минимална инхибиторна активност на тестиране бактерије била 0.001-0.002 mg/ml, док је бактерицидна активност (MBC) постигнута у концентрацији 0.001-0.04 mg/ml. Уље *Th. algeriensis* имало је вредности MIC од 0.001-0.05 mg/ml, док је MBC постигнута са вредностима уља од 0.0025-0.05 mg/ml. Уље *Satureja thymbra* је имало бактериостатско деловање у концентрацији од 0.001-0.1 mg/ml и бактерицидно од 0.002-0.2 mg/ml. Етарско уље *Salvia fruticosa* било је ефикасно у распону од 0.125-1.5 mg/ml за MIC и од 0.5-2.0 mg/ml за MBC. За утврђивање антифунгалне активности етарских уља, тестиране су следеће микромицете: *Aspergillus flavus* (ATCC 9643), *Aspergillus fumigatus* (хумани изолат), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) и квасац *Candida albicans* (хумани изолат). *Thymus capitatus* је и са овог аспекта показао највећу активност, где се минимална инхибиторна концентрација кретала од 0.0002 до 0.001 mg/ml и минимална фунгицидна концентрација од 0.002-0.025 mg/ml, у поређењу са MIC за *Th. algeriensis* од 0.0005 до 0.025 mg/ml и MFC 0.001-0.05 mg/ml. Уље *Satureja thymbra* је показала фунгистатичку активност од 0.001-0.025 mg/ml и фунгицидну од 0.001-0.1 mg/ml. MIC за *Salvia fruticosa* уље кретала се од 0.125 do 1.0 mg/ml, а MFC у распону од 0.125 до 1.5 mg/ml.

Кључне речи: *Thymus capitatus*; *Thymus algeriensis*; *Satureja thymbra*; *Salvia fruticosa*; етарско уље; антимикробна активност; антиоксидативна активност.

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Abstract

The essentiasl oils composition of some essential oils from Lamiaceae family wild growing in Libya (*Thymus capitatus, Thymus algeriensis, Satureja thymbra* and *Salvia furticosa*) have been analyzed using GC and GC-MS. The oils of *Thymus capitatus, Th. algeriensis* and *Salvia fruticosa* were characterised with domination of oxygenated monoterepenes with percentage 87.60%, 54.67% and 64.89 % respectively. Monoterpene hydrocarbons was the major group of the compounds of the oil of *S. thymbra* represented 58.57% of the total oil.

The main compounds of *Th. capitatus* essential oil were carvacrol (68.19%) followed by thymol (12.29%), while *Th. algeriensis* essential oil was characterized by thymol (38.50%) as the major component. The oil of *S. thymbra* was demonstrated by γ-terpinene (39.23%), thymol (25.16%) and *p*-cymene (7.17%) as the major constituents. *S. fruticosa* oil was characterized by contained 1,8-cineole (49.34%) and camphor (7.53%) as the main compounds. The oils were also, screened for antioxidant activity by DPPH assay, and compared with their main compounds. The highest antioxidant activity showed *S. thymbra* oil with IC_{50} = 0.0967 mg/ml of solution, followed by *Th. capitatus* (IC_{50} = 0.119 mg/ml, *Th. algeriensis* with the IC_{50} of 0.299 mg/ml and the lowest activity was found for *S. fruticosa* (IC_{50} = 15.53 mg/ml of solution), comparing with 0.403 and 0.105 mg/ml for thymol and carvacrol, and 0.0717 mg/ml of solution for BHA. Furthermore, antimicrobial activity was tested using microdilution method against eight bacteria and eight fungal species. Gram-negative from bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Proteus mirabilis* (human isolate) and from Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 6538) were used. Among the oils, *Th. capitatus* oil showed the best activity, where the inhibitory activity against tested bacteria was at 0.001-0.002 mg/ml, while bactericidal activity (MBC) was achieved at 0.001- 0.04 mg/ml. *Th. algeriensis* oil showed MIC at 0.001-0.05 mg/ml, while bactericidal activity (MBC) was achieved at 0.0025-0.05 mg/ml, oil of *Satureja thymbra* showed

bacteriostatic activity at 0.001–0.1 mg/ml and bactericidal at 0.002–0.2 mg/ml. The essential oil of *S. fruticosa* was efficient with MIC range from 0.125-1.5 mg/ml and MBC from 0.5-2.0 mg/ml. For antifungal assay the oils were tested against the following fungi: *Aspergillus flavus* (ATCC 9643), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) and yeast *C. albicans* (human isolate). Again *Th. capitatus* was the most powerful with MICs ranged from 0.0002-0.001 mg/ml and MFC 0.002-0.025 mg/ml, compared with activity MICs for *Th. algeriensis,* ranged 0.0005-0.025 mg/ml and MFC 0.001-0.05 mg/ml. *S. thymbra* showed fungistatic effects at 0.001–0.025 mg/ml and fungicidal effects at 0.001–0.1 mg/mL and *S. fruticosa* exhibited fungistatic (MIC) at 0.125-1.0 mg/ml and fungicidal effect (MFC) at 0.125-1.5 mg/ml.

Key words: *Thymus capitatus*; *Thymus algeriensis*; *Satureja thymbra*; *Salvia fruticosa*; essential oil; antimicrobial activity; antioxidant activity.

Scientific field: Biology

Specific scientific field: Morphology, phytochemistry and systematics of plants

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Introduction

Plants from various families have been used from ancient time as food, spices, and for medicines purpose (Balunas *et al.,* 2005). In more recent history, the use of plants in folk medicine has involved the extraction of some active compounds, the begining was the isolation of morphine in early 19th century (Srinivasan et al., 2005; Sylvestre *et al.*, 2005). Furthermore, plants being used by traditional healers have been found to contain properties that inhibit the growth of bacteria, viruses, and other microbes (Ndubani and Hojer, 1999).

Nowadays, there has been increasing interest in naturally occurring compounds which have biological activities and antioxidant nutrients (Vuorelaa *et al*., 2004). The most important source for such these kinds of bioactive phytochemicals are the plant materials (Elless *et al*., 2000).

Scientific researchers have been found that the plants have valuable chemical compounds (Morrison and Boyd, 1987). Such these natural chemical compounds and their synthetic have been used in pharmaceutical, food, and chemical industry; others are applied as food flavors and fragrances, sweeteners, or even pesticides.

Consequently, these days, there is a considerably focus on swapping synthetic food additives which could have side effects with those of plant-based natural ones (Paradiso *et al*., 2008).

Over the last few decades, a substantial body of scientific evidence is available demonstrating wide range of pharmacological and nutraceutical activities of medicinal herbs (Burt, 2004; Celiktas *et al*., 2007). These include antioxidant, anticancer, anti-inflammatory activities.

The infectious diseases generally caused by to microbial contamination of foods are becoming a main problem in the world, especially in the developing countries (Burt, 2004). A serious challenge is facing the consumers of microbes-infected food and threats their health (Hussain *et al.,* 2008). The microbial growth in foods has the ability to generate several toxins that are harmful for the health of humans, also these kinds of toxins leads to decrease the value of food commodities (Celiktas *et al*., 2007).

Plant essential oils, also called volatile or ethereal oils (Guenther, 1948) are "volatile, natural, mixture compounds are formed by aromatic plants as secondary metabolites and have a strong odor" (Bakkali *et al*., 2008; Celiktas *et al*., 2007). Also, essential oils and extracts have been used for thousands of years, especially in food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997) and have been reported as potential sources of novel antimicrobial compounds against bacterial and fungi pathogens (Prabuseenivasan *et al*., 2006).

Due to the new attention which has been paid to natural products such as essential oils, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment. Some of them contain effective alternatives compounds to synthetic compounds of the chemical industry, without displaying the same side effects (Carson and Riley, 2003). Furthermore, the essential oils and herbs-derived extracts are gaining much recognition as a potential source of natural and safer antioxidants and bioactives (Burt *et al.,* 2003; Burt, 2004; Cantore, *et al.,* 2004).

There are more than 2.000 plant species belong to about 60 plant families, have the ability to form essential oils as a secondary metabolisms. However, economically only 100 species are important in the world (Hussain, 2009). Lamiaceae (syn. Labiatae) family consists of more than 252 genera and about 7.000 species (Hedge, 1992), considered as one of the richest families in essential oils. Furthermore, plants belong to this family generally aromatic in all parts, and most of them broadly use as culinary herbs, such as sage, thyme, rosemary, oregano, basil, mint, lavender, marjoram, savory, and perilla (Wink, 2003; Celiktas *et al*., 2007; Hussain *et al*., 2008).

Despite a rich tradition of medicinal plants used for the treatment of various infectious diseases, inflammations, and injuries in many parts of Libya (Al-Kathe and Al-Ramah, 1997), there are only a few reports on their antibacterial or antifungal activities (Hussein *et al*., 1990; Hussain and Tobji, 1997; Hussein *et al*., 1989). Therefore, it is of great interest to carry out a chemical composition, antimicrobial and antioxidant screening of some Lamiaceae species wild growing in Libya, which are not investigated yet in order to validate their pharmacological use and reveal their active constituents.

Chapter one

Review of literature

1.1. The genus *Thymus* L.

The genus *Thymus* is one of the largest genera within the Lamiaceae (= Labiatae) family, and economically most important genera within it.

Thymus species are distributed throughout the arid, temperate and cold regions of the Old World north of the equator, and on the coasts of Greenland (Morales, 1989). The number of species within this genus is assumed to be more than 200 (Morales, 2002). Thyme is a woody, perennial herb native to the Mediterranean region. Its name probably derives from the Latin "thymus" which means "perfumed", or from Greek "thymos" which means "courage" or "strength" (Morales, 2002; Lawrence and Tucker, 2002). The genus has been subdivided into eight sections: *Mastichina, Micantes, Piperella, Pseudothymbra, Thymus, Teucrioides, Hyphodromi* and *Serpyllum* (Jalas *et al.*, 1971).

The stems tend to be narrow or even wiry; leaves are evergreen in most species, and arranged in opposite pairs, oval, entire, and small, 4–20 mm long, and usually aromatic. *Thymus* flowers are in dense terminal heads, with an uneven calyx, with the upper lip three-lobed, yellow, white or purple (Jafri and El-gadi, 1985). Many *Thymus* species are extensively used dry or fresh as culinary herbs.

The essential oils obtained from these species were utilized as flavor ingredients in a wide variety of food, beverage and confectionery products, as well as in perfumery for the scenting of soaps and lotions. Several *Thymus* species are used as medicinal herbs, and they are known to possess antispasmodic, sedative, antiphlogistic, antiviral, antioxidant, antibacterial and antifungal activities (Giordiani *et al*., 2008; Hazzit *et al*., 2009; Adorjan *et al*., 2010; Ali *et al*., 2010; Dandlen *et al.,* 2010; Belaqziz, 2010; Miguel *et al*., 2010; Kunicka-Styczyńska, 2011; Nedorostova, 2011).

From a chemical point of view thymus essential oils are characterized by a considerable amount of monoterpenes, which, generally, account about 90% of total oil. The thymol and carvacrol (phenolic monoterpenes) occur more frequently, always accompanied by the ρ-cymene/γ-terpinene, the four monoterpenes which are considered biogenetically. Linalool, 1,8-cineole and borneol are the other oxygenated monoterpenes, in order of importance singled out in thymus essential oils (Stahl-Biskup, 2002). In particular, concerning the capitatum species or *Th. capitatus*,

carvacrol mostly proves to be the major component (Karousou *et al.,* 2005; Bounatirou *et al.,* 2007). However, as mentioned, different chemotypes have been stated for many *Thymus* species, thus this species also shows different chemical profiles: thymol, carvacrol and thymol/ carvacrol (Karousou *et al*., 2005; Miceli *et al.,* 2006).

In Libya, the genus *Thymus* is represented by only two species - *Thymus algeriensis* Boiss. et Reut., and *Thymus capitatus* Hoffms. et Link. They are known in Libya under the common name "Zaatar" (Jafri and El-gadi, 1985). These species are commonly used fresh or dry as spicy herbs, for medicine purpose to treat respiratory system disorder, and against illnesses of the digestive tube and antiabortion (Pottier-Alapetite, 1981; Le Floch and Boulos, 2008)

1.1.1. *Thymus capitatus*

Thymus capitatus Hoffms. et Link., [Bas. *Satureja capitata* L.; Syn. *Thymbra capitata* (L.) Cav., *Corydothymus capitatus* Rechenb. f.], (Jafri and El-gadi, 1985). This species is typical of garrigues, dry slopes and Mediterranean pine forests and it is considered a good indicator of the dry Mediterranean area (Pignatti, 1982).

Fig 1.1. *Thymus capitatus* Hoffms. et Link.

Thymus capitatus is a low shrub 20-60 cm; stems much branched, compact; old branches stiff, spine scent, whitish; leaves 3-8 x 0.5-1 mm, linear, keeled and boat-shaped, gland-dotted, ciliate at the base, sessile; flowers in ovoid heads 0.6-1.2

cm; bracteoles 5-6 x 1.5 mm, ovate, the margins ciliate; calyx 4-5 mm, bilabiate, 13-veined; upper lip with 3 triangular teeth, the lower 2-fid, with subulate teeth; corolla 0.8-1 cm, bilabiate, purplish-mavue, with red glandular dots outside, exserted; stamens exerted; style branches equal (Bolous, 2002).

This species is used fresh or dry as spicy herbs, also for medicine purpose to treat respiratory system disorder (Le Floch, 2008; Pottier-Alapetite, 1981).

There are a number of reports on the chemical composition of *Th. capitatus* essential oil, and its antibacterial and antioxidant activities from different parts of Mediterranean region. In all studies the species found to be rich in thymol and/or carvacrol, with γ-terpinene and p-cymene as the other major compounds. The oil possessed good antibacterial activity (Katz *et al.,* 1987; Karpouhtsis *et al.*, 1998; Hedhili *et al.,* 2002)**.** Moreover, previous studies of this species collected from other parts of Mediterranean region (Karousou *et al.,* 2005; Goren *et al.,* 2003; Schulz *et al.,* 2005), reported that *Th. capitatus* essential oil contain mainly carvacrol as a principal component with thymol content more than 9%. On the other hand, there are some findings showing that the main constiuent of *Th. capitatus* essential oil was carvacrol with small amount of thymol (Katz *et al.,* 1987; Karpouhtsis *et al.*, 1998; Hedhili *et al.,* 2002; Skoula *et al.*, 2005; Faleiro *et al*, 2005; Hedhili *et al.,* 2005; Miguel *et al.,* 2005; Miceli *et al.,* 2006). Also, in some cases thymol was the main compound of the *Th. capitatus* essential oil, with a trace amount of carvacrol (Karousou *et al.,* 2005).

1.1.2. *Thymus algeriensis*

Thymus algeriensis Boiss. et Reut., is endemic to North Africa (Libya, Algeria and Tunisia). It is a short lived, diploid (2n¼2x¼30) and gynodioecious shrub ((Jafti and El-Gadi, 1985). *Th. algeriensis* populations could be found from the sub-humid to the lower arid bioclimates, at altitudes ranging from 120 to 1100 m. The species grows on low fertile calcareous soils and befalls in scattered and could be found in small populations, screening different levels of demolition, mainly due to overharvesting and overgrazing (Ben El Hadj Ali *et al.,* 2010).

Fig 1.2. ***Thymus algeriensis*** Boiss. et Reut.

The flowers, with ovate bracts and pink purplish or whitish purple corolla, are small (5-7 mm). Flowering takes place between April and June (Ben El Hadj Ali *et al.,* 2010).

It has been reported that, *Th. algeriensis* is used fresh or dried as a culinary herb (Hazzit *et al.*, 2009; Pottier-Alapetite, 1981; Le Floc'h and Boulos, 2008). Furthermore, this herb is also largely used in folk medicine against some diseases such as digestive disorders tube and antiabortion (Le Floc'h, 1983).

The chemical compositions have been previously established for *Th. algeriensis* (Aboutabl and El-dahmy, 1995; Benjilali *et al.*, 1987; Houmani *et al.*, 2002; Hazzit *et al.*, 2009). The studies investigated the chemical composition and biological properties of *Th. algeriensis* essential oil from some regions of North Africa (Giordiani *et al.,* 2008; Hazzit *et al*., 2009; Ben El Hadj Ali *et al.*, 2010). Six chemotypes could be identified according to the main compounds of *Th. algeriensis* essential oil, caryophyllene oxide/1,8-cineole/a-pinene, 1,8-cineole/a-pinene, 1,8-cineole/a-pinene/camphor, borneol/1,8-cineole/a-pinene, linalool, and Carvacrol and

thymol chemotypes. "The spatial chemotype distribution was linked to the geographic distance among populations rather than to bioclimates, indicating that local selective environmental factors act on the chemotype diversity"(Ben El Hadj Ali et al., 2010).

1.2. The genus *Satureja* L.

The genus *Satureja* belongs to the family Lamiaceae, sub-family Nepetoideae, and the tribe Mentheae. The genus embraces over 30 species whose center of distribution is located in the eastern part of the Mediterranean. These species are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky habitats (Slavkovska *et al*., 2001).

Species of this genus are widely distributed in the Mediterranean area, regularly in sunny dry rocky habitats, contain about 200 species, usually aromatic herbs and shrubs (Bezic *et al.,* 2009). The genus *Satureja* is native to the Mediterranean region of Europe, western Asia, North Africa, the Canarian Islands and South America (Satil *et al.*, 2002). They have commercial importance as they are used in pharmaceutical industry and cosmetics (Ball and Getliffe, 1972).

Due to occurrence of secondary metabolites such as flavonoids, steroids, essential oils, and tannins *Satureja* species have been known for their medicinal properties for a long time. These species have been used as traditional folk remedies, to treat various diseases such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases. Also, their antimicrobial activity against a wide range of multidrug resistant pathogens has been confirmed (Bezić *et al.*, 2009; Skocibusic *et al.,* 2006).

The essential oils obtained from the leaves and flowers of *Satureja* spp. have varied industrial applications as flavouring materials, medicine and perfumes (Teklu *et al.,* 1998).

This genus represented by only two species in Libya (Satureja *thymbra* and *Satureja fortii*), which the second one is endemic to Libya. The two species could found only in the Green mountain eastern part of Libya (Jafti and El-Gadi, 1985).

1.2.1 *Satureja thymbra* L.

Satureja thymbra L., known as the most common *Satureja* species as home remedy, due to its antimicrobial, gastro sedative and diuretic properties (Loizzo *et al.,* 2008; Capone *et al.,* 1998). It is much branched, usually grey-puberulent dwarf shrub, 20-35 cm (Ball and Getliffe, 1972).

Fig 1.3. *Stureja thymbra* L.

The essential oils yield of this species ranged between (4.8 and 7.3% v/w). Furthermore, essential oil of *S. thymbra* possesses good activity in inhibiting mycelia growth. It's also, used in folk remedy against antiseptic, stimulation, tonic, gastric sedative and diuretic (Capone *et al.,* 1989). In addition, it has been found to have a good antifungal (Goren *et al.,* 2004), and antibacterial properties (Vagionas *et al.*, 2007). Furthermore, *S. thymbra* considered as a very important from economical point of view (Karpuhtsis *et al.,* 1998).

The chemical composition of *S. thymbra* essential oils have been reported by many authors, in most cases carvacrol or/ and thymol were the major compounds (Sokovic *et al.*, 2002; Goren *et al.*, 2004 ; Skoula *et al.,* 2005 ; Ayvaz *et al.,* 2010). Good antimicrobial activity of *S. thymbra* essential oil was established (Chorinpoulos *et al.,* 2006).

1.3. The genes *Salvia* L.

The genus *Salvia*, belonging to Lamiaceae, is considered one of the largest genera in this family. It comprises about 900 species that grow in the Oriental Mediterranean, South-West Asia, South Africa and America (Standley and Williams, 1973; Kelen *et al.,* 2008; Maksimovic *et al.,* 2007).

High attention has been paid to *Salvia* species due to the wide range of its biological activities (Askun *et al.,* 2010). Many authors have focused on the biological properties of the essential oils obtained from *Salvia* species and their major compounds, such as antibacterial, cytostatic (Janssen *et al.,* 1987; Gonzalez *et al.,* 1989; Darias *et al.,* 1990), antiviral (Tada *et al.,* 1994) and antioxidant activities (Weng and Wang, 2000).

Moreover, they are frequently used in traditional medicine to treat diarrhea, eye diseases, gonorrhea, also they possess antiseptic and antispasmodic activities.

The essential oils of *Salvia* species are used as cosmetics and as flavoring agents in perfumery (Basaif *et al.,* 2004; Longaray Delamaer *et al.,* 2007; Kelen *et al.,* 2008).

Plant products that have antimicrobial activity gained special interest due to the resistance of some microorganisms to antibiotics (Essawi and Srour, 2000). Thus, several authors have focused on the biological properties of the essential oils obtained from *Salvia* species and their major compounds, such as antibacterial, cytostatic (Janssen *et al.,* 1987; Gonzalez *et al.,* 1989; Darias *et al.,* 1990), and antiviral activities (Tada *et al*., 1994).

In Libya, the genus *Salvia* is represented by ten species, out of which three are cultivated (Jafri and El-Gadi, 1985).

1.3.1. *Salvia fruticosa*

Salvia fruticosa Mill. (Syn. *S. triloba* L.), is a native species of the eastern Mediterranean basin (Carmona *et al.,* 2005; Ali-Shtayeh *et al.,* 2000). It is an aromatic, perennial herb and commercially considered one of the most important *Salvia* species for culinary and medicinal purposes (Kosar *et al.,* 2005). This herb (especially the leaves) has a folk standing in the eastern Mediterranean region for the

treatment of various skin, blood, and infectious ailments as well as ailments of the digestive, circulatory, respiratory, and osteomuscular systems (Carmona *et al.,* 2005; Ali-Shtayeh *et al.,* 2000). It is also used as a hypoglycemic herb and against inflammations, hepatitis, and tuberculosis (Pitarokili *et al.,* 2003).

Several authors have been reported the chemical composition of *S. fruticosa* essential oil, in most investigations 1,8-cineole was the main compound (Skoula *et al.,* 2000; Pitarokili *et al.,* 2003; Kosar *et al.,* 2005; Papageoriou *et al.,* 2008; Longaray Delamare *et al.,* 2007).

Fig 1.4. ***Salvia furticosa*** Mill.

Evidence suggests that the essential oil from *S. fruticosa* has antimicrobial properties. Longaray Delamare *et al.*, (2007) reported that the essential oil obtained from *S. fruticosa* possessed good antimicrobial activity against foodborne bacteria. Also, Pitarokili *et al.,* (2003) found that *S. fruticosa* possesed antifungal activity. There are many studies carried out on the chemical composition of the essential oil of *S. fruticosa* (Longaray Delamare *et al.,* 2007; Sivropoulou *et al.,*1997; Al-Kalaldeh *et al.,* 2010), antimicrobial (Janssen *et al.,* 1987; Gonzalez *et al.,* 1989; Darias *et al.,* 1990) and antioxidant activities (Papageorgiou *et al.,* 2008).

1.4. Essential oils

Essential oils, also called volatile or ethereal oils (Guenther, 1948) are "volatile, natural, mixture compounds are formed by aromatic plants as secondary metabolites and have a strong odor" (Bakkali *et al*., 2008; Celiktas *et al*., 2007). Also essential oils have been defined by Geunther, (1948) as "aromatic oily liquids obtained from plant materials (flowers, buds, seeds, roots, leaves, twigs, barks, herbs, wood and fruits)" They could be obtained by expression, fermentation, enfleurage or extraction, however, steam distillation is the most commonly used method for commercial production of essential oil (Van de Braak and Leijten, 1999), and the proportion of different essential oils extracted by steam distillation is 93% and the remaining 7% is extracted by the other methods (Masanago, 2001).

Or it can be define as "they are a mixture of fragrant of liquids obtained by distillation of aromatic plant materials" (Burt, 2004).

The term 'essential oil' is thought to comes from the name invented in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; he called the active constituent of a drug Quinta essential (Guenther, 1948). Among 3.000 essential oils known, of which about 300 are important for commercial scale and basically destined for the flavours and fragrances market (Van de Braak and Leijten, 1999).

It has been known that essential oils have biological properties such as antimicrobial activities (Guenther, 1948) but the relatively recent augmentation of interest in 'green' consumerism has led to a renewal of scientific interest in these substances (Nychas, 1995; Tuley de Silva, 1996).

Essential oils are wellknown from the past time to have antimicrobial properties (Guenther, 1948; Boyle, 1955) and these have been reviewed in the past (Shelef, 1983; Nychas, 1995) as have the antimicrobial properties of spices (Shelef, 1983) but the relatively recent enhancement of interest in 'green' consumerism has led to a renewal of scientific interest in these substances (Nychas, 1995; Tuley de Silva, 1996). Further, it has antibacterial properties (Deans and Ritchie, 1987; Carson *et al.,* 1995b; Mourey and Canillac, 2002), the components of essential oils have been reported to exhibit antiviral (Bishop, 1995), antimycotic (Azzouz and Bullerman, 1982; Jayashree and Subramanyam, 1999; Mari *et al.,* 2003), antitoxigenic (Ultee and Smid, 2001), antiparasitic (Pandey *et al.,* 2000; Pessoa *et al.,* 2002), and insecticidal

(Konstantopoulou *et al.,* 1992; Karpouhtsis *et al.,* 1998) activities. These properties are possibly related to the act of these compounds in plants (Guenther, 1948; Mahmoud and Croteau, 2002).

1.4.1. The history of essential oil investigation

In the East began the history of essential oils, for the process of distillation the technical basis of the essential oil industry was conceived. In the Orient was the first use of Distillation as a method to produce the essential oils, especially in Egypt, India and Persia(Guenther, 1948) before more than 2000 years ago and it has improved by the Arabs in the 9th century (Bauer *et al.,* 2001).

The great Greek historian, Herodotus (484-425 B.C.), as well as the Roman historian of natural history, Pliny and his contemporary, Dioscorides the author of the treatise "De Materia Medica" which dominated therapy for more than 1,500 years mention oil of turpentine and give partial information about methods of producing it. They do not describe any other oil.

Until the early Middle Ages (and even later) the art of distillation was used primarily for the preparation of distilled waters. "Where this process resulted in a precipitation of essential oils, as in the crystallization of rose oil on the surface of distilled rose water, it is likely that the oil was regarded as an undesired by-product rather than as a new and welcome one" (Guenther, 1948).

The first authentic written account of distillation of essential oil is attributed to Villanova (ca. 1235–1311), a Catalan physician (Guenther, 1948). The being of produce the essential oils by pharmacies was by the 13th century; also its pharmacological effects were described in pharmacopoeias (Bauer *et al.,* 2001). However, the use of essential oils did not become general until the second half of the sixteenth century (Guenther, 1948), from the time that they were traded in the City of London (Crosthwaite, 1998). In that century the two Strassburg physicians, Brunschwig and Reiff Published separately and they mentioned only to a number of oils between them; turpentine, juniper wood, rosemary, spike (lavender), clove, mace, nutmeg, anise and cinnamon (Guenther, 1948).

In the 17th century the essential oils preparation was well known and about 15-17 essential oils stocked in pharmacies (Guenther, 1948).

The medical purposes use of the tea tree oil has been documented since the occupation of Australia at the end of the 18th century, though it is seems to have been used by the native Australians before that (Carson and Riley, 1995a).

De la Croix in 1881. has done the first experimental to test the antibacterial activities of the vapours of essential oil (Boyle, 1955). However, the use of essential oils as flavour and aroma had much interested, and use of essential oils in a medicine purpose became secondary (Guenther, 1948).

1.4.2. Sources of essential oils

There are more than 2.000 plant species belong to about 60 plant families, have the ability to form essential oils as a secondary metabolites. However, economically only 100 species are important in the world (Hussain, 2009).

Essential oils are obtained from numerous aromatic plants usually localized in warm to temperate countries, such as Mediterranean and tropical regions, which are considered to be an important part of the traditional pharmacopoeia.

Essential oils are volatile, liquid, limpid and seldom coloured, they are resolvable and soluble in organic solvents and low density than water. They formed by all plant parts and stored in security cells, for instance cavities, glandular trichomes or epidermic cells (Masotti *et al.,* 2003; Angioni *et al.,* 2006; Bakkali *et al*., 2008).

Gymnosperms and Angiosperms have a quite high ability to accumulate essential oils. Even though, Angiosperms are the most important commercial aromatic plants and essential oils source. And the most of important families among Angiosperms as source of commodities oils and aromatic plants in the world trade are Lamiaceae, Umbelliferae and Compositae (Burt, 2004; Celiktas *et al*., 2007; Hussain *et al*., 2008).

Essential oils are isolate from different parts of plant, such as flowers, buds, seeds, roots, leaves, twigs, barks, herbs, wood and fruits by various methods (Burt, 2004; Hussain *et al*., 2008).

1.4.3. Lamiaceae essential oils

Lamiaceae (syn. Labiatae) family consists of more than 252 genera and 7.000 species (Hedge, 1992). It has been known to have a many of species with medicinal properties, which have been used since ancient times, and many of these species are common in Mediterranean region (Ali *et al*., 2000). In Libya, this family is represented by 22 genera and 65 species (Jafri and El-gadi, 1985).

The plants of Lamiaceae are perennial herbs or undershrubs and rarely shrubs (Jafri and El-gadi, 1985). Plants belong to this family generally aromatic in all parts, and most of them broadly use as culinary herbs, such as sage, thyme, rosemary, oregano, basil, mint, lavender, marjoram, savory, and perilla (Wink, 2003; Celiktas *et al*., 2007; Hussain *et al*., 2008). Some of them are shrubs, and a very few are vines or trees (Jafri and El-gadi, 1985).

The aromatic essential oils could be found in leaves which emerge oppositely with each pair positioned at right angles to the previous one (called *decussate*), and in the cross section of stems is square in shape. The flowers are symmetrical with 5 united sepals and 5 united petals. Such plants are mostly bisexual and verticillastrate (a flower cluster that looks like a whorl of flowers but actually consists of two crowded clusters) (Cantino *et al*., 1992; Heywood *et al*., 2007).

1.4.4. Chemistry of the essential oils

Essential oils are very complex natural mixtures of compounds such as terpenoids, esters, phenols, aldehydes, ketones, and alcohols (Radulescu *et al.*, 2004). Essential oils can comprise about 20–60 components or more at quite different concentrations, and usually characterized by two or three main compounds at high concentrations (20–70%) comprehension with other components present in trace amounts. For example, carvacrol (53.7%), γ-terpinene (17.6%) and thymol (13%) were the main compounds of essential oil of *Satureja thymbra* (Ayvaz, 2010) and the major compound of *Th. algeriensis* essential oil collected from different locations in Morocco was carvacrol (80.40%) (Jaaffari *et al.,* 2007).

The monoterpenes are, generally, formed by the combination of two isoprene unit (C5) **Fig 1.1**. They are the mainly representative molecules constituting 80-90% of the essential oils and allow a vast variety of structures (Burt, 2004).

The oxygenated constituents that contain oxygen determined the taste and the odour of an essential oil, by giving them some solubility in water and considerable solubility in alcohol (Tisserand *et al*., 1995). The main group is composed of terpenes and terpenoids (monoterepenes) and the other of aromatic and aliphatic constituents (Bakkali *et al,* 2008).

1.4.4.1. Terpenes

"Terpenes are made up just by hydrogen and carbon atoms. All terpenes are based on the isoprene unit (C5) an essential building block in plant biochemistry (Tisserand *et al*., 1995). The main terpenes are the monoterpenes which contain two isoprene units (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) also exist. A terpene containing oxygen is called oxygenated monoterpenes (Bakkali *et al.,* 2008).

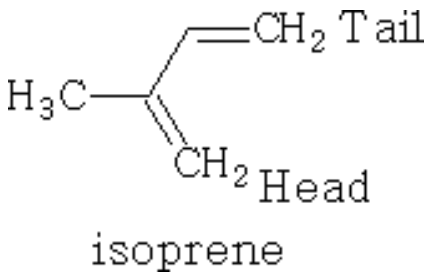


Fig 1.5. The chemical structure of the isoprene unit

1.4.4.1.1. The monoterpenes

The monoterpenes are made up from two isoprene units (C10). They are the most molecules exist in the essential oil, and they are constituting about 80- 90% of the total essential oils, and allow a great variety of structures and they gave many

function groups (Bakkali *et al.,* 2008). Leland, *et al,.* (2006) has cited the function groups as following:

1- Aldehydes – group of compounds have a carbonyl group (C=O group) in which the carbon atom is bonded to at least one hydrogen atom. Such as geranial, neral, citronellal.

2- Alcohols **–** any class of compounds characterized by the presence of a hydroxyl group (-OH group) bonded to saturated carbon atom.

3- Esters – Esters are any class of compounds structurally related to carboxylic acids but in which the hydrogen atom in the carboxyl group (-COOH group) was replaced by a hydrocarbon group, resulting in a –COOR structure where R is the hydrocarbon. linalyl acetate or propionate, citronellyl acetate.

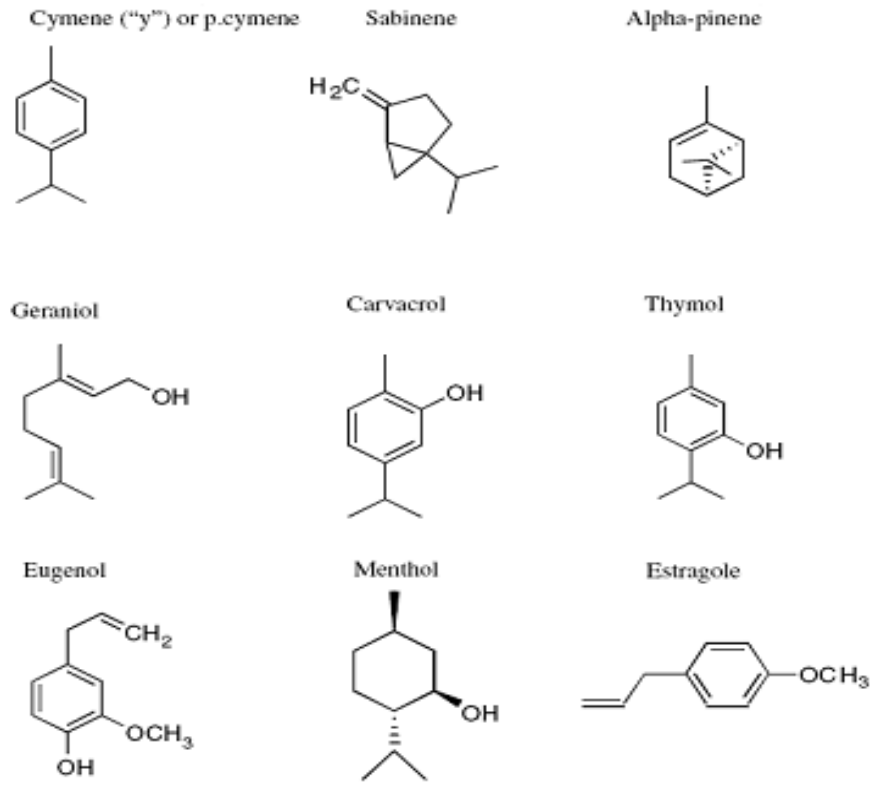
4- Phenols **-** they constitute a large class of compounds in which a hydroxyl group (-OH group) is bound to an aromatic ring for instance carvacrol, thymol... etc.

1.4.4.1.2. Sesquiterpenes

The sesquiterpenes are formed from the of three isoprene units (C15) **Fig 1.2**, which mean that, they have 15 carbon atoms. The chain extension leads to increases the number of cyclisation which allows a great variation of structures. The sesquiterpenes have similar function and structure to those of the monoterpenes.

Examples of sesquiterpenes characteristic of essential oils: hydrocarbons (β-bisabolene), alcohols (farnesol), ketones (nootkatone), aldehydes (sinensals) and esters (cedryl acetate) (Bakkali, et al, 2008). **Fig 1.2** showed chemical structure of some major components of essential oils.

Monoterpenes



Sesquerpitenes

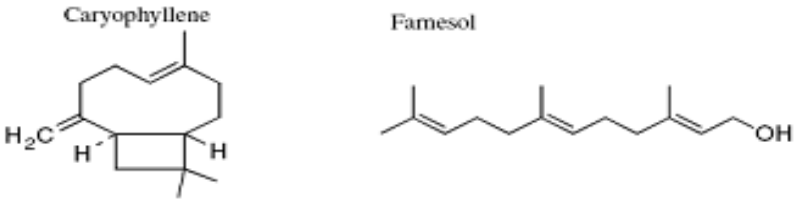


Fig 1.6. Some selected monoterepenes compounds.

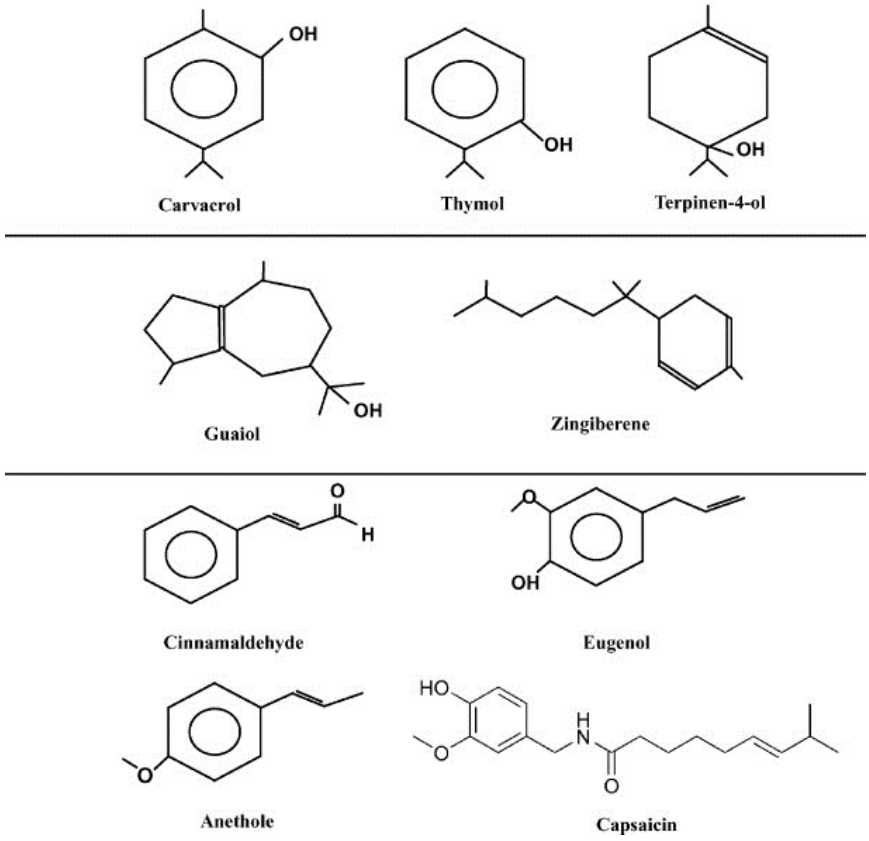


Fig 1.7. Chemical structure of some major components of essential oils.

(Calsamiglia et al., 2007)

1.4.4.2. Aromatic compounds

Aromatic compounds derived from phenylpropane, the aromatic compounds arise less frequently than the terpenes. The biosynthetic pathways concerning terpenes and phenylpropanic derivatives commonly are independent in plants but may coexist sometimes with one main pathway.

Aromatic compounds comprise:

Aldehyde: cinnamaldehyde

Alcohol: cinnamic alcohol

Phenols: chavicol, eugenol

Methoxy derivatives: anethole, elemicine, estragole, methyleugenols

Methylene dioxy compounds: apiole, myristicine, safrole.

The principal plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon, and some botanical families (Apiaceae, Lamiaceae, Myrtaceae, Rutaceae).

Nitrogenous or sulphured components such as glucosinolates or isothiocyanate derivatives (garlic and mustard oils) are also characteristic as secondary metabolites of diverse plants or of torrefied, grilled or roasted products.

1.4.4.3. Aliphatic compounds

Essential oils may contain various aliphatic compounds, generally of low molecular weight, which are extracted during steam distillation: hydrocarbons (linear or remified, saturated or not, rarely specific), acids (C3 to C10), alcohols, aldehydes, acyclic esters or lactones. Nitrogen or sulfur containing compounds are characteristic of roasted or grilled products and are exceptional among products (Norsita, 2003).

1.4.5. Factors affecting essential oil accumulation

The composition and yield of the essential oil obtained from plant materials always controlled by many factors. The separation of these factors from each other sometimes is difficult, since many are interdependent and influence one another (Terblanche, 2000). These variations in chemical composition could include seasonal variation, geographical origin, genetic variation, vegetation stages, part of plant utilized and postharvest drying and storage (Marotti *et al.,* 1994; Hussain *et al.,* 2008; Anwar *et al.,* 2009).

Also Variation in chemical composition of essential oils, in particular, and extracts of medicinal plants may be observed due to the origin the environmental conditions, and the developmental stage of collected plant materials (Burt, 2004).

1.4.5.1. Seasonal variations

In literature there are numerous reports regarding the variation in the chemical composition of essential oils obtained from many plants collected during different seasons (Kofidis *et al*., 2004; Celiktas *et al*., 2007; Hussain *et al*., 2008).

Thymus vulgaris from Jordan were harvested during different growth stages including vegetation, beginning of blooming, full blooming and fruit maturation. Results indicated that oil yields of thyme were affected by growth stage (Abu-Darwish *et al.,* 2012). Moreover, a study carried out to evaluate the effect of harvesting time on essential oil composition of *Satureja thymbra* collected from the same location at different vegetation stages. The findings showed that the major compounds in the vegetation phase were thymol (27.88%), followed by γ-terpinene (17.02%) and carvacrol (11.88%), while in full flowering phase carvacrol (29.18%), thymol (17.22%) and γ-terpinene (12.45%) were the main compounds, and in the fruiting phase thymol (20.73%) was again the main compound, followed by γ-terpinene 14.91% and carvacrol 12.8% (Chorianopoulos *et al.,* 2006). The findings of a study carried out by (Hussain, 2009) showed that, the variation in the chemical composition of *Mentha spicata* essential oils collected from Pakistan with regard to different seasons of the year. Previous investigations have demonstrated that harvesting seasons can vary the chemical composition of the essential oils of *Mentha spicata* and *Mentha pulegium* (Muller-Riebau *et al*., 1997; Kofidis *et al*., 2004). In Addition, Pala-Paul *et al*. (2001) reported month-to-month essential oil composition variations of *Santolina rosmarinifolia,* which could be due to precipitation and temperature. Findings obtained by Badi *et al.*, (2004) for *Thymus vulgaris* (thyme), also indicated that the harvesting time is critical to both oil composition and yield.

The variation in the chemical profile at different growth stages including pre-flowering, flowering and post-flowering of the essential oil isolated from *Artemisia annua* has been reported by Mohammadreza *et al.,* (2008).

1.4.5.2. Geographical variation

Plants of a single species growing in different habitat types have different oil composition (Karousou *et al.,* 2005). Also Uribe-Hernandez *et al.* (1992) reported

that, the yield and composition of essential oil varied significantly, due to the locations where the plants grew.

In the literature there are many reports mentioned to the variation in the yield and chemical profile of the essential oil, with respect to geographical regions (Souto-Bachiller *et al.,* 1997; Celiktas *et al.,* 2007; Van vuuren *et al*., 2007). Viljoen *et al*. (2006) and Hussain, (2009) reported that, chemical composition variations of essential oils of *Mentha spicata, Mentha longifolia, Ocimum sanctum, Ocimum gratissimum* and *Ocimum basilicum* samples collected from different geographical regions in Pakistan were observed.

In *Mentha longifolia*, the variation in most of the essential oil components with respect to geographical regions was significant ($p < 0.05$) (Hussin, 2009). Moreover, variations in the chemical profile of essential oils from *Mentha longifolia* and *Tagetes minuta* populations, collected from different geographical locations has been reported by Chalchat *et al*., (1995). Such these differences could be due to the variation of the soil textures and possible adaption response of different populations, resulting in different chemical profile, without morphological differences being observed in the plants (Hussain *et al*., 2008). In addition, Lamiaceae common in Crete, have shown that the variation of their quantitative oil composition follows the geographic direction from the western to the eastern part of the island (Kokkini *et al.,* 1997; Karousou *et al.,* 1998).

Altitude could be another environmental factor effecting chemical composition and oil yield of the essential oils (Vokou *et al*., 1993). The yield and chemical composition of essential oils from *Thymus algeriensis* oils showed a large variability and showed different chemical profiles, from two samples collected from the same location at two different levels 800 m and 1500 m (Hazzit, 2009). Also Houmani *et al*., (2002) his findings showed that, two *Thymus algeriensis* samples collected from the same location, of which one was thymol-rich (62.7%) and the other linalool-rich (78.8%). The *Origanum vulgare* ssp. *hirtum* essential oils from twenty three localities, scattered all over Greece were varied significantly (Vokou *et al*., 1993). Climatic factors such as heat and drought were also related to the essential oil profiles obtained (Uribe-Hermandez *et al*., 1992; Milos *et al*., 2001).

1.4.5.3. Genetic variation

The genetic make-up of the plant has been suggested to have a greater influence on the chemical profile of the oil produced, rather than the soil-type in which it is growing (Graven *et al*., 1990; Terblanche, 2000; Milos *et al*., 2001). Also, it has been shown that, essential oil content and composition is related to genetic (Shafie *et al.*, 2009).

Generally genotype is defined as, "the genetic make-up of an organism as characterized by its physical appearance or phenotype". Whereas chemotype is topically defined as "a group of organisms that, produce the same chemical profile for a particular class of secondary metabolites". The difference in chemical profiles were observed from oils produced from specimens collected from the same population and location, demonstrating the presence of different chemotypes, within this species (Catalan and De Lampasona, 2002; Fouche *et al.,* 2002; Juliani *et al.,* 2002; Wink, 2003; Ahmad *et al.,* 2006).

The significant variation of the chemical composition of the essential oil of Mousami (*Citrus sinensis*), Eureka lemon (*C. limon*) and Grapefruit (*C. paradisi*) could be attributed to the variation in their genetic make-up. A study of growing 24 wild and 19 cultivated caraway populations under the same conditions showed significant variances between the chemical composition and yields of the cultivated and wild types (Galambosi and Peura, 1996).

1.4.5.4. Other factors effecting the chemical composition of the essential oils

There are some other factors which could affect the growing plants. Such these factors could lead to make variation in essential oils profile. For instance the part of plant used to extract the essential oil (Chalchat *et al*., 1995; Santos-Gomes and Fernandes-Ferreira, 2001). The harvesting methods used may also change the oil composition and yield (Bonnardeaux, 1992). Furthermore, Galambosi and Peura, (1996) reported that, time of snowing effect the oil composition. Also plant density has been reported as a factor influence the oil composition (Graven *et al.*, 1990; Clark and Menary, 1979).

The chemical composition variation of essential oil obtained from stems, leaves and flowers of *Salvia officinalis* L. collected from the northern Portugal has

been reported by Santos-Gomes and Fernandes-Ferreira, (2001). Besides, their findings showed significant variations in the major components of the essential oils from shoots sampled over the year at two different locations in Northern Portugal. Also, the day light could influence the quantity of specific chemical compounds of peppermint oil (Clark and Menary, 1979).

1.4.6. Biological effects of essential oils

Essential oils have gained great importance due to their properties as antibacterial, antifungal, antitumor and insecticidal (Burt, 2004).

1.4.6.1. Antimicrobial activity

There are two kinds of antimicrobial agents have been reported by Microbiologists are used to cure infections by microorganisms. The first one is antibiotics and mycotics, which are produced by certain groups of microorganisms, and second is chemotherapeutic agent, which is synthesis chemically (Davidson and Harrison, 2002). The selective toxicity is considered to be the most property for the antimicrobial agent. This means that, the biochemical action of the agent in the bacteria is different somehow from the act of this agent in the animal cells. This advantage of differentiation could be considered in chemotherapy (Burt, 2004).
However, it seems that, there is no standard test has been developed to assume the antimicrobial activity of possible preservatives against food-born microorganisms, although the need for such has been indicated (Davidson and Parish, 1989).

The tests of antimicrobial activity of essential oils can be characterized as diffusion, dilution or bio-autographic methods (Rios *et al.,* 1988). Measurement of minimum inhibitory concentration (MIC) are the most used method for assessing the antimicrobial activity of essential oils (Juliano *et al*., 2000; Lambert *et al*., 2001; Burt, 2004; Holley and Patel, 2005; Bakkali *et al*., 2008).

The old NCCLS method for antibacterial sensitivity testing has been developed for measuring essential oil activity (Hammer *et al.,* 1999; NCCLS, 2000). Researchers adjust experimental methods to better represent possible future applications in their field.

Nevertheless, since the outcome of the fact that some factors for instance the method of isolation of essential oil, volume of inoculum, growth phase, culture medium used, pH of the media and incubation time and temperature have the ability to effect a test (Rios *et al.,* 1988), comparison of published findings is difficult (Janssen *et al.,* 1987; Friedman *et al.,* 2002).

In the literature the disk diffusion method is used for screening of essential oils for antibacterial activity. The principle of this method, in which a paper disk saturated with known concentration of essential oil, is placed on the agar plate. This is mainly use as first check for the activity of the oils to check if there is a necessity for more studies.

There are many factors must be considerable between studies such as the amount of essential oil placed on the paper discs, and the thickness of the agar layer. Usually, this method is suitable for screening between essential oils, but direct comparison of published results is not possible (Senatore *et al*., 2000; Elgayyar *et al*., 2001).

When huge numbers of essential oils or large number of organisms isolates are to be tested, the agar well diffusion assay is mostly used, in which well are formed by cutting wells in agar, and the essential oils are put in that wells (Deans *et al*., 1993; Dorman and Deans, 2000). Both methods, disc diffusion (Sentore *et al*., 2001; Elgayyar *et al*., 2001) and well diffusion (Wan *et al*., 1998; Dorman and Deans, 2000; Ruberto *et al*., 2000) are reported to test antimicrobial activity of the essential oils.

The most important method used to evaluate the antimicrobial activity of essential oils is the measurement of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which gives us the precise, exact and reproducible data.

In literature there are many definitions for MIC and MBC, for instance Carson *et al*., (1995b) has defined MIC as "lowest concentration resulting in maintenance or reduction of inoculum viability" and MBC as "Ccncentration where 99.9% or more of the initial inoculum is killed", furthermore, MIC is "lowest concentration inhibiting visible growth of test organism" (Hammer *et al.,* 1999; Delaquis *et al.,* 2002). Also MBC has been defined as "lowest concentration at which no growth is observed after subculturing into fresh broth" (Onawunmi, 1989).

The antimicrobial strength can be determined by dilution of essential oils in broth or agar (Ruberto *et al*., 2000; Pintore *et al*., 2002). There are number of protocols used in broth dilution studies to determine the MIC and MBC. The measurement of optical density and the enumeration of colonies by viable count is the most used methods (Sivropoulou *et al*., 1997; Chaibi *et al*., 1997; Lambert *et al*., 2001; Skandamis *et al*., 2001; Ultee and Smid, 2001). A new micro-dilution method for determining the MIC of oils based composites uses the Resazurin (redox indicator) (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide) as a visual indicator of the MIC (Sarker *et al*., 2007). The results compare positively with those gotten by optical density and viable count measurement, and the method is more sensitive than the agar dilution method (Mann and Markham, 1998).

Resazurin, an oxidation-reduction indicator, is used for the assessment of cell growth. It is a violet/blue non-fluorescent and non-toxic dye that becomes purple-pink and fluorescent by oxidoreductases enzyme when reduced to Resorufin within viable cells **Fig 1.3.** Resorufin can also decrease to hydroresorufin, a non-fluorescent and uncolored (Sarker *et al*., 2007). A resazurin reduction assay has been mainly used to assess bacterial/yeast pollution in milk (McNicholl *et al.,* 2006).

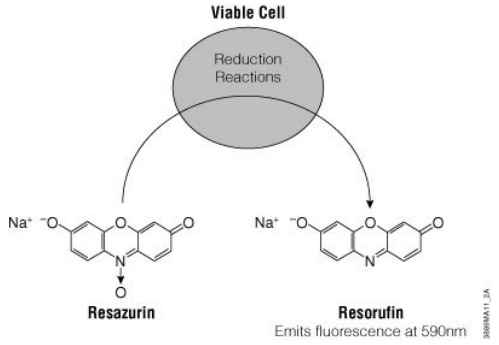


Fig 1.8. Reduction of resazurin to resorufin by oxidoreductases from viable cells.

There is a new method has been found appropriate for testing combinations of antibacterial substances by calculating the MIC from optical density measurements

(Lambert *et al*., 2001). The percentage of essential oil resulting in a 50% reduction in the viable count was determined in one study by plots of concentration against percentage kill (Friedman *et al*., 2002). The variety of methods of recording the antibacterial activity of essential oils limits comparison between studies and could lead to duplication of work.

One property of test methods that, varies largely is whether or not a solvent /emulsifier is used to dissolve the essential oils/extracts or to stabilize it in water based culture media. For this purpose a quite a few materials have been used, such as ethanol/ methanol (Pol and Smid, 1999; Marino *et al*., 2001; Packiyasothy and Kyle, 2002), n- hexane (Senatore *et al*., 2000), or dimethyl sulfoxide (Firouzi *et al*., 1998), agar (Delaquis *et al*., 2002; Gill *et al*., 2002; Burt and Reinders, 2003), and Tween-20/Tween-80 (Mann and Markham, 1998; Cosentino *et al*., 1999; Hammer *et al*., 1999; Mourey and Canillac, 2002; Bassole *et al*., 2003; Wilkinson *et al*., 2003). Nevertheless, some researchers reported, it is unnecessary to use an additive (Tassou *et al*., 2000; Elgayyar *et al*., 2001; Cimanga *et al*., 2002; Mejlholm and Dalgaard, 2002).

Remmal *et al*., (1993) has compared the effect of the most common used solvents, ethanol and Tween-80 with that of agar, the influence of the stabilization of clove and oregano oils. The use of 0.2% agar was found to produce as homogenous dispersion as a true solution in absolute ethanol (Remmal *et al*., 1993). The minimum inhibitory concentrations (MIC) of clove and oregano essential oils were considerably lower in the presence of agar than in the presence of ethanol or Tween-80. It was concluded that solvents and detergents could reduce the antibacterial effect of essential oils (Remmal *et al*., 1993; Juven *et al*., 1994). Tween-80 also has been revealed to protect *Listeria monocytogenes* from the antibacterial activity of essential oil components during freeze–thaw cycles (Cressy *et al*., 2003). A more disadvantage of using Tween-80 to dissolve essential oils, is the fact that the turbidity of the resulting dispersion can hamper visual observations, and optical density measurements (Carson *et al*., 1995b). This is confirmed by the fact that, Tween-80 has been recommended as a neutralizer of phenolic disinfectants (Cremieux *et al.,* 1981) and this has been confirmed in a study on the action of thyme oil against *Salmonella typhimurium* (Juven *et al.,* 1994; Sokovic et al., 2006).

1.4.6.1.1. Mode of antimicrobial action

However, the antimicrobial properties of essential oils and their components have been reported before (Shelef, 1983; Nychas, 1995). The mode of antimicrobial action has not been studied in great detail (Lambert *et al.,* 2001).

Since there is a large number of different groups of chemical compounds existent in essential oils, it is most likely that their antibacterial activity is not due to one specific mechanism, its seems there are many targets in the cell (Skandamis *et al.,* 2001; Carson and Riley, 2003). The targets or mechanisms in the bacterial cell could be sites of action for essential oil constituents are shown in **Fig 1.4.**

Not all of these mechanisms have different targets; some of them are affected as a consequence of another one being targeted.

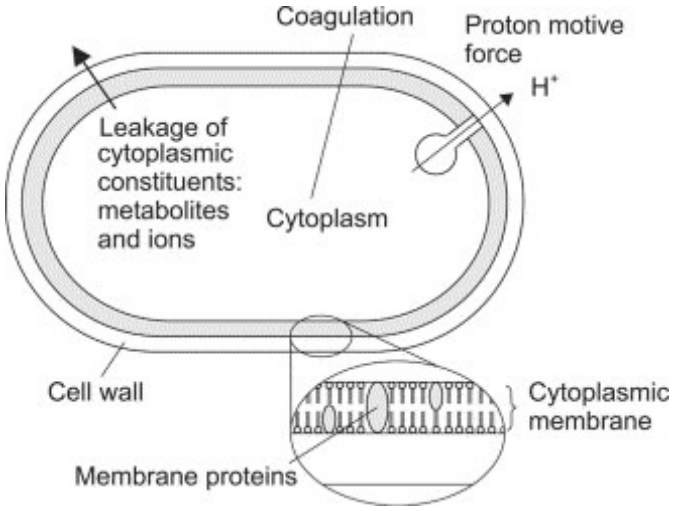


Fig 1.9. Locations and mechanisms in the bacterial cell thought to be sites of action for EO components: degradation of the cell wall; damage to cytoplasmic membrane ; damage to membrane proteins; leakage of cell contents; coagulation of cytoplasm and depletion of the proton motive force (Burt, 2004).

One of the important action of essential oils and their compounds is their hydrophobicity, which leads them to partition in the lipids of the cell membrane of the

bacteria, disturbing the structures and making them leakier (Knobloch *et al.,* 1986; Sikkema *et al.,* 1994).

The ions and other cell contents can then be escaped out the cell (Oosterhaven *et al.,* 1995; Cox *et al.,* 2000; Lambert *et al.,* 2001; Skandamis *et al.,* 2001; Carson *et al.,* 2003; Ultee *et al.,* 2002). The loss of certain amount from the cell could be tolerated without loss of the ability to survivor, but extensive loss of cell contents or critical molecules and ions will lead to death (Denyer and Hugo, 1991).

Commonly, the essential oils possessing the highest antibacterial properties against foodborne pathogens contains a high amount of phenolic compounds, for instance carvacrol, eugenol (2-methoxy-4-(2-propenyl)phenol) and thymol (Farag *et al.,* 1989; Cosentino *et al.,* 1999; Dorman and Deans, 2000; Juliano *et al.,* 2000; Lambert *et al.,* 2001). It seems reasonable that, their mechanism of action would therefore be similar to other phenolics, this is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents (Denyer and Hugo, 1991; Sikkema *et al.,* 1995; Davidson, 1997).

The chemical structure of the individual essential oil compound affects their particular mode of action, and antibacterial activity (Dorman and Deans, 2000). The importance of the existence of the hydroxyl group in phenolic compounds such as carvacrol and thymol has been confirmed (Knobloch *et al.,* 1986; Dorman and Deans, 2000; Ultee *et al.,* 2002).

The relative position of the hydroxyl group on the phenolic ring does not appear strongly to affect the degree of antibacterial activity, the action of thymol against *B. cereus*, *S. aureus* and *P. aeruginosa* appears to be comparable to that of carvacrol (Ultee *et al.,* 2002). However, in one study Gram-positive and Gram-negative species have been affected differently by carvacrol and thymol (Dorman and Deans, 2000). The importance of the phenolic ring itself (destabilized electrons) is demonstrated by the lack of activity of menthol compared to carvacrol (Ultee *et al.,* 2002).

In one study the addition of an acetate moiety to the molecule appeared to increase the antibacterial activity, geranyl acetate was more active against a range of Gram-positive and negative species than geraniol (Dorman and Deans, 2000). As far

as non-phenolic components of essential oils are concerned, the type of alkyl group has been found to effect activity (alkenyl > alkyl). For example, limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) is more active than *p*-cymene (Dorman and Deans, 2000). Also, the cell proteins which considered as a part of cytoplasmic membrane appear to effect by the components of essential oils (Knobloch *et al.,* 1989). For instance ATP-ases enzymes are known to be found in the cytoplasmic membrane, and to be bordered by lipid molecules. Two possible mechanisms have been proposed whereby cyclic hydrocarbons could act on these. The molecules lipophilic hydrocarbon could accumulate in the lipid bilayer and change the lipid-protein interaction alternativelly, direct interaction of the lipophilic compounds with hydrophobic parts of the protein is possible (Juven *et al.,* 1994; Sikkema *et al.,* 1995).

Some essential oils have been found to encourage the growth of pseudo mycelia (a series of cells antibacterial properties of essential oils adhering end-to-end as a result of incomplete separation of newly formed cells) in certain yeasts. This could indicate that essential oils work on the enzymes involved in the energy regulation, or synthesis of structural constituents (Conner and Beuchat, 1984). The oil of Cinnamon and its constituents have been reported to prevent amino acid decarboxylases in *Enterobacter aerogenes*. The mechanism of action was supposed to be the binding of proteins (Wendakoon and Sakaguchi, 1995), indications that essential oils components could act on proteins were also reported in studies using milk containing different protein levels (Pol *et al.,* 2001).

1.4.6.1.2. Essential oils as natural antimicrobial agents

The natural substances which have antimicrobial activities such as essential oils are attractive to the food industry for the following reasons:

- High expense of toxicological testing of the new compounds before will be approved for use as food antimicrobials.
- There exists a important need for expanded antimicrobial activity both in terms of spectrum of activity and of broad food applications.
- High interest in producing “green” labels, i.e., once without any chemical additives.

- There are many health benefits comes to the consumption by using of some naturally occurring antimicrobials.

Lately, essential oils and extracts of some plants have been reported to have antimicrobial properties, as well as imparting flavour to foods (Burt, 2004). Some essential oils have shown promising as potential food safety compounds when added to processed and raw foods were some of the most effective natural antimicrobials are extracted from spices, herbs and essential oils and isolates of the different plant families (Juliano *et al*., 2000; Lambert *et al*., 2001; Burt, 2004; Holley and Patel, 2005; Bakkali *et al*., 2008). Extracts and essential oils from dietary herbal species belonging to the family Lamiaceae, including thyme, have been used as sources of medicine and food preservatives for over 4000 years (Burt, 2004; Rota *et al*., 2008).

Recently, some of the bioactive coupled to these medicinal and preservative functions have been determined to be phenolic metabolites (Dorman and Deans, 2000; Lambert *et al*., 2001; Ultee *et al*., 2002; Burt, 2004). Some phenolic metabolites from *Lamiaceae,* like thymol (from thyme and oregano), rosmarinic acid (from rosemary,) have antiseptic and anti-inflammatory properties in addition to their antimicrobial potentials (Lambert *et al*., 2001; Ultee *et al*., 2000).

1.4.6.2. Antioxidant activity

1.4.6.2.1. What are the antioxidants?

Antioxidants are a substances or compounds in food, that they play important roles as a protection factor. Also they have the ability to reduce the risk of chronic diseases, including heart disease and cancer. Plants considered the most important source of antioxidants compounds, which have the ability to trap free radicals that are already exist in the biological system. These free radicals have the ability to oxidize the proteins, lipids and nucleic acids. Furthermore, they can initial deteriorating diseases (Hussin, 2009)

Antioxidants activity has been defined as "materials that could stop or delay the oxidative deterioration when they added to food". Also, from biological view, antioxidant have been defined as "any substance that, when present at low concentration compared to those of an oxidisable substance (e.g., lipids, proteins and

DNA), significantly delay or prevent oxidation of that substrate, and act as a free radical scavenger" (Benzic and Strain, 1996).

1.4.6.2.2. Measurement of antioxidant activity

Natural antioxidant compounds exhibit their antioxidant activity by different mechanisms like: (1) Chain breaking by donation of hydrogen atoms or electrons, which change free radicals in to more constant species. (2) Chelating metal ions which are involved in the generation of reactive oxygen species. (3) Decomposing lipid peroxides into stable final products, and (4) preventing the deleterious action of pro oxidant enzymes (Cai *et al.*, 2006; Siquet *et al.,* 2006).

Due to complexity of the composition of plants and plant based foods, that the process of separation and study of each compound separately is tedious and time-consuming. Researchers are looking for creative methods, which can speed up determination of antioxidant activity in the food and other biological systems. However, such kind of methods is up to now in the development phases (Natella *et al.*, 1999; Wright *et al.*, 2001).

1.4.6.2.3. *In vitro assay* of essential oil antioxidant activity

In the assessment of antioxidant activity, the use of different methods is necessary (Frankel *at el*., 1994; Koleva *et al.,* 2002; Hussain *et al*., 2008; Hussain, 2009). The common method use to evaluate the antioxidant activity of plant essential oils and extracts are:

- 2,2-di(4-*tert*-octaphenyl)-1-picrylhydrazyl (DPPH) radical scavenging assay
- Inhibition of linoleic acid peroxidation
- Bleaching of β-carotene in linoleic acid system assays.

Some of these methods are proposed to be considered for standardization, or as reference methods when assessing antioxidant capacity. Huang and Prior, (2005) reported that, a standardizer method of evaluating antioxidant activity of plant products should achieve the following requirements; "measuring chemistry that actually occurs in potential applications, employing a biologically relevant source of

radicals, simplicity and reproducibility, defined endpoint and known chemical mechanism and moderate/less instrumentation".

Schlesier *et al*., (2002) proposed that it is very important in measuring antioxidant activity to use at least two methods depending on the expected antioxidant potential, and/or on the origin of the substance. Kulisic *et al.,* (2004) suggested the DPPH radical scavenging activity assay and β-carotene bleaching as the best methods for standardizing valuation of antioxidant capacity of essential oils.

1.4.6.2.4. Antioxidant potential of essential oils

In recent decades, the concern about the safety of synthetic antioxidant as a food preservation has more attention due their side effects. These artificial antioxidants are known to have carcinogenic effects on health and food system (Siddhuraju, 2006; Paradiso *et al*., 2008; Descalzo and Sancho 2008). In additional, chio *et al.,* (2007) and Fan, *et al*., (2007) reported that, synthetic antioxidants could lead to liver swelling and effect on liver system and activities cerebrovascular diseases.

For these reasons there is a strong need for effective and safer antioxidants, to stop the oxidation. Also to avoid these harm effects which are accrue by using synthetic antioxidants. For the best results, the natural sources could be the right choice. Many studies reported that, essential oils and extracts from natural sources considered to have a strong antioxidant activity (Descalzo and Sancho 2008; Paradiso *et al.,* 2008). Furthermore, essential oils and extracts from plant material reported to have different ability as antioxidant activity, especially those that have a high level of phenolic continents (Descalzo and Sancho 2008; Tabata *et al.,* 2008). Hussian *et al.*, (2008) reported that, some essential oils obtained from plant material were more effective or similar to some synthetic antioxidants. Also the activity of plant essential oil could be due to presence of hydroxyl group in their chemical structures (Shahidi *et al*, 2000).

The potential of antioxidants to prevent oxidation in the body has therefore attracted much attention. Vitamins, such as vitamin C, b-carotene, and vitamin E, which are essential micronutrients and cannot be synthesized by the human body, are well-known antioxidants. It has been suggested that some plant extracts or products,

such as green tea (Sawai *et al.,* 2005), rooibos tea (Joubert *et al*, 2005) and red wine (Kanner et al., 1994), and components containing phenolics have the activity to control oxidation in the body (Aliaga and Lissi, 2004; Katsube *et al.,* 2006; Kim and Lee, 2004), and decrease the risk of developing illnesses, such as cardiovascular disease and atherogenesis.

1.4.7. Methods of essential oils isolation

The isolation of essential oils from plant material can be categorized in to: Enfleurage, steam distillation, solvent extraction, and supercritical fluid extraction.

1.4.7. 1. Solvent extraction

This method usually used to extract the essential oils from very sensitive plant materials, such as flowers. The procedure of this method is by soaking the plant material in non-polar solvent, such as hexane at room temperature, or they can be extracted by Soxhlet extractor. After removing the solvent at reduced pressure the sticky remainder is named concrete. The concrete comprises together volatile and non-volatile/non-polar compounds upon dissolving in ethanol, the non-polar and non-volatile materials are precipitated, which can be separated by filtration. The filtrate is concentrated under reduced pressure is named absolute, and it contains the essential oil. This method has less application in comparison to steam distillation (Guenther, 1948).

1.4.7.2. Supercritical fluid extraction method (SCFE).

In 1879 Hanny and Hogarth established the solubilizing properties of supercritical fluids. Lately, this method of extraction has been applied for many classes of natural products, counting essential oils. The advantage of carbon dioxide (CO2) supercritical fluid extraction for the isolation of the essential oils over steam and hydro-distillation, and solvent extraction are the mild conditions and the cost is low, also low toxicity, and the problems of disposal of the waste organic are absent (Jarvis, 1997).

In the Supercritical Fluid Extraction apparatus (SCFE) the plant material is extracted by fluid CO2 at different densities, controlled by pressure and temperature of the CO2 (Vilegas**,** 1994).

1.4.7. 3. Hydro and steam distillation

The most common methods used to isolate the essential oils from botanical material are hydro or steam distillation (Whis, 1996; Masango, 2004).

When the two immiscible liquids mixed together, every exert vapour pressure as if each liquid were a pure (Houghton and Raman, 1998). The boiling mixture has the sum of vapours pressers exerted by each of the individual compound of the mixture. The mixture start boiling , when the total vapour pressure reaches the atmospheric pressure, that mean, that the mixture will reach the boiling point at lower temperature than the boiling point of the separated compound, by reducing boiling points. Thus the steam distillation could separate the volatile compounds from non-volatile (Baker *et al.,* 2000). Furthermore, the compounds oxidation protection occurred, because of the displacement of atmospheric oxygen by the steam, which add plus point to this method (Krell, 1982).

However, in commercial scale steam distillation considered to be the much common method to produce essential oils, and about 93% of the oils isolated by this method, it's not common method in the research laboratories (Masango, 2004). This is most likely due to unavailability of steam suitable distillation vessels and generators. Most studies which focus on the essential oil of plants have made use of hydro-distillation in Clevenger-type apparatus (Kulisic *et al*., 2004; Sokovic and Griensven, 2006; Hussain *et al*., 2008).

In hydro-distillation, the plant material immersed in water, and then heated to the boiling point by using external source of heat. In the both steam and hydro-distillation procedures, the vapour passed through the condenser, and then the oil could be separate from the aqueous phase (Hougthon and Raman, 1998). To prevent the loss of more volatile oil, care must be taken by making sure that the condenser is sufficient for condensation.

In the literature, there are several reports on the significance of hydro and steam distillation processes. Water and plant material are combined together in the still in hydro-distillation method , and all things heated to boil, hot water draw out the oils, just as steam dose, and it is taken to condenser and cooled to hydrosol and essential oil. However, this method produces a finer, more complete product, as hot water is collar than steam distillation and shocks the plant material less (Ackerman, 2001).

Steam distillation has been suggested as more effective than hydro-distillation in removing essential oils from plant material, and give higher yield than hydro-distillation (Charles and Simon, 1990), although the same composites were detected. They also approved the hydro-distillation, a simpler and more rapid method for oil isolation. In contrast, Sefidkon *et al.,* (2007) reported that, the highest oil yield was obtained by hydro-distillation and the lowest was by the steam distillation, when he isolated the essential oil from the aerial parts of *Staureja rechigeri* by both methods. Furthermore, Khanavi *et al.,* (2004) reported that, the essential oils yield obtained from *Stachys persica* and *S. byizantine* by using hydro-distillation method was higher than the yield that got by stem distillation method. In contrast, no difference between the oil yields, when the oil isolated from *Melaleuca alternifolia* (tea tree) by hydro and steam distillation (Whish, 1996), only slight difference were appeared between the essential oils composition obtained from *Lippa scaberrima,* isolated by hydro-distillation and microwave distillation. Also the yields obtained by hydro-distillation method were generally higher.

Several studies compared the chemical composition, which obtained by different procedures, their finding showed that, hydro and steam distillation the oils contained higher percentage of terpene hydrocarbons. On the other hand, super critical extracted oil contained a higher percentage of oxygened compounds (Reverchon, 1997; Donelian *et al*., 2009). Also variation in the chemical composition of *Carum copticum* essential oil isolated by supercritical fluid extraction and hydro-distillation methods have been reported by Khajeh *et al*. (2004). In addition, the essential oils from leaves of *Ocimum gratissimum*, *O. micranthum* and *O. selloi* produced by microwave oven distillation, supercritical and steam distillation, showed

different composition, however, they reported the same main compounds but different in relative amount (Silva *et al*., 2004) .

The duration of the distillation process is another an important factor that effect on the composition and yield of essential oils (Koedam, 1982; Masango, 2004).

Because of a small increase in the oil yields obtained towards the end of the cycle, the avoided of long distillation should be considered, which is counteracted by the huge loss of polar compounds to the increased aqueous fraction (Masango, 2004). The use of shorter distillation cycle in commercial scale could reduction in production costs.

Koedam, (1982) has shown in hydro distillation experiments that a longer the length of distillation process, the acidity of the distillation water also influenced the composition of the oil obtained.

1.4. Aims of this study

Significant differences were reported in essential oils composition in samples from different regions, and different species. The medicinal plants from Libyan flora are still insufficiently investigated.

To the best of our knowledge, there are no previous reports on chemical composition and biological activity of essential oils of the species under investigation (*Thymus capitatus, Thymus algeriensis, Satureja thymbra* and *Salvia furticosa*) originating from this area, except of the study of the chemical composition and antimicrobial activities of *Thymus algeriensis* essential oil (Aboutabl and El-Dahmy, 1985).

The aims of this study were:

-Quantification and qualification analysis of the essential oils of some wild-growing *species* from Libya using GC and GC-MS technics.

-Comparison it with other reported results of samples of these species collected from various areas of their natural distribution, which were published so far.

-Testing the antioxidant, antifungal and antibacterial activities of the analyzed essential oils as a potentially new source of biologically active natural products.

Chapter two

Material and Methods

2.1. Materials

2.1.1. Chemical and standard compounds

2, 2,-diphenyl-1-picrylhydrazyl, homologous series of C9-C24 *n*-alkanes and various reference chemicals: (3,5-Di-*tert*-butyl-4-hydroxytoluene (BHT), 2,2-dyphenyl-1-picrylhydrazyl (DPPH), *p*-cymene, γ-terpinene, thymol, carcacrol, Tween 80 (Polyoxyethylene (20) sorbitan monooleate ($C_{64}H_{124}O_{26}$), antibiotic Streptomycin, Anhydrous sodium sulphate (Na_2SO_4) were obtained from Sigma Chemical Co. (St Louis, MO,USA).

All culture media (Malt-agar (MA) and Mueller-Hinton) were purchased from Oxoid Ltd., (Hampshire, UK).

Dimethyl sulfoxide (DMSO) was obtained from Merck (Darmstadt, Germany), Bifonazol from (Srbolek, Belgrade, Serbia) and all organic solvents were purchased from (Zorka pharma, Serbia).

2.1.2. Instruments

The instruments used for different analyses during the study alongwith their company identification are listed in **Table 2.1.**

Table 2.1 Instruments used in experimental work.

Name of instrument	Manufacturing company
PH-5890II apparatus	Hewlett-packard, USA
HP.G 1800C II GCD system	Hewlett-packard, USA
Spectrophotometer Jenway 6305 UV/Vis	Sineks Laboratory, UK
96-well microplates	Spectra Cacak, Serbia
Binocular Microscope	Laica type, Leika Mikroscopic and Systeme GmbH Wetlzar, Germany
Microplate hanger 4.0 (Bio Rad Lab)	Hercults, Calfornia
Electric Balance type: AV264CM	Ohaus corporation, USA
Orbitrary shaker	Tehtinca-zelezniki (Super lab), Serbia
Sterilization autoclaves AES-75	Raypa Company, Spain.

2.1.3. Plant collection

The wild growing samples of investigated plants were collected during the flowering stage in May 2010 for *Thymus capitatus* and in April 2010 for *Thymus algeriensis* from Zentan (Libya), which located on the top of Western mountain (Aljabel Algarbi) at altitude about 700 m above sea level (Jafti and El-Gadi 1985). *Staureja thymbra* and *Salvia frutcosa* collected from Biadda city near Wadi-Akofa in (Green Mountain) Eastern Libya in March 2010.

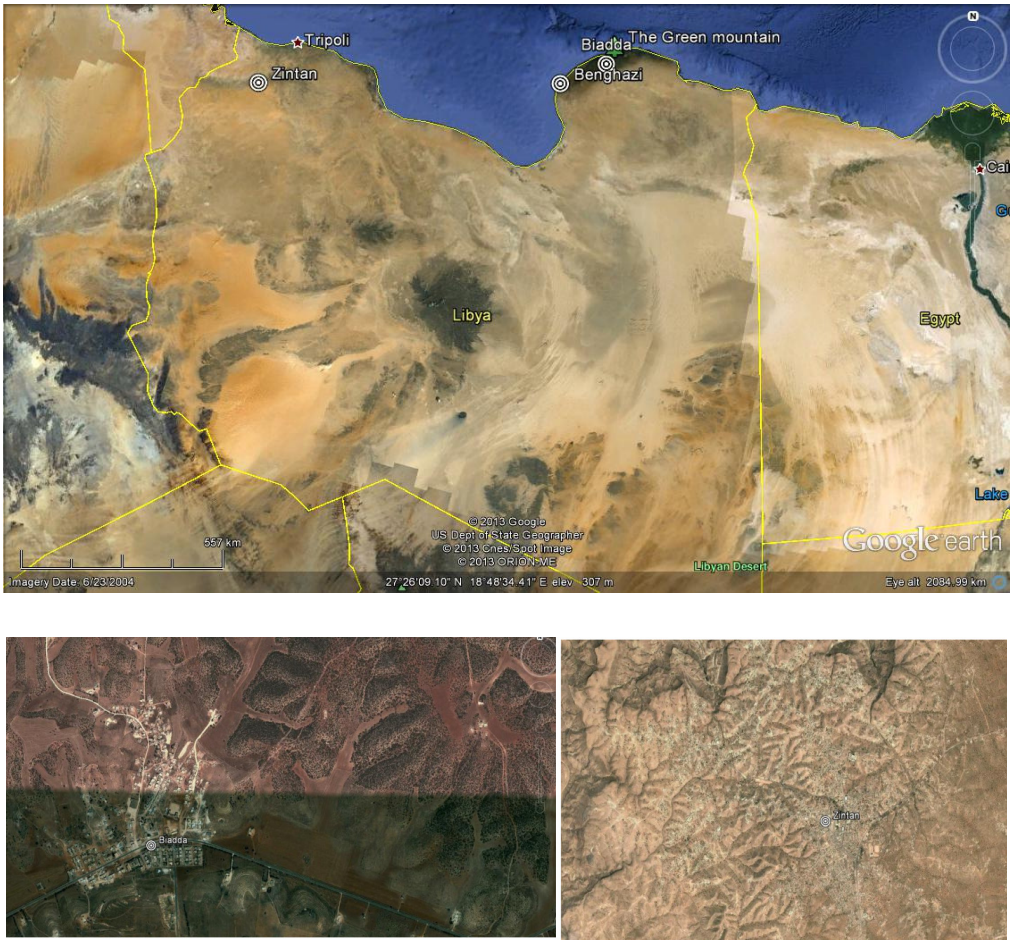


Fig 2.1. Map of Libya with the two localities of plants collection.

The plants were identified by Dr. A. Felaly, Faculty of Science, Al-Gabel Al-Garbi University Libya, and later confirmed by (P. D. Marin). Voucher specimens where deposited in Herbarium of Institute of Botany and Botanical Garden

"Jevremovac" (BEOU), (vouchers No. 16614 - *Thymus algeriensis*; 16615 - *Thymus capitatus;* 16618- *Satureja thymbra*; 16702 – *Salvua furticosa*).

The samples were dried in shadow at room temperature for 10 days.

Table 2.2. Plant material collections employed in the present study.

Species	**Parts used**	**collection date**	**Location**	**Vouchers No**
Thymus capitaus	Arial parts	May 2010	Zintan - Libya	16615 BEOU
Thymus algeriensis	Arial parts	April 2010	Zintan - Libya	16614 BEOU
Satureja thymbra	Arial parts	March 2010	Biadda City near Wadi-Akofa in Green mountain, Eastern Libya	16618 BEOU
Salvia furticosa	Arial parts	March 2010	Biadda near Wadi-Akofa in Green mountain, Eastern Libya	16702 BEOU

2.1.4. Microorganisms utilized to access the antimicrobial activity of essential oil

2.1.4.1. Gram-negative bacteria:

1- *Escherichia coli* (ATCC 35210).
2- *Pseudomons aeruginosa* (ATCC 27853).
3- *Salmonella typhimurium* (ATCC 13311)

4- *Proteus mirabilis* (human isolate).

2.1.4.2. Gram-positive bacteria:

1- *Bacillus cereus* (clinical isolate).
2- *Micrococcus flavus* (ATCC 10240).
3- *Staphylococcus aureus* (ATCC 6538).
4- *Listeria monocytogenes* (NCTC 7973).

2.1.4.3. Fungal Species:

1- *Aspergillus flavus* (ATCC 9643)
2- *Aspergillus fumigatus* (human isolate)
3- *Aspergillus niger* (ATCC 6275).
4- *Aspergillus ochraceus* (ATCC 12066).
5- *Penicillium funiculosum* (ATCC 36839).
6- *Penicillium ochrochloron* (ATCC 9112).
7- *Trichoderma viride* (IAM 5061).
8- *Candida albicans* (human isolate).

The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

2.2. Methods (Experimental Protocol)

2.2.1. Essentail oils isolation

As we have mentioned, there are many methods used to isolate essential oils from plant materials. However, the most common methods used to isolate the essential oils from botanical material are hydro or steam distillation (Whis, 1996; Masango, 2004). Most studies which focus on the essential oil of plants have made use of hydro-distillation in Clevenger-type apparatus (Kulisic *et al*., 2004; Sokovic and Griensven, 2006; Hussain *et al*., 2008).

In our study we extracted the essential oil by using hydrodistillation method. Amount of (100g) of air-dried aerial parts of plant samples deprived from wooden parts, were submitted to hydrodistillation, using Clevenger-type apparatus for 3 h, according to the standard procedure reported earlier in the (Europaean Pharmacopea, 2004).

The obtained essential oil was dried over anhydrase sodium sulphate, and stored in a sealed dark vials, then kept at 4ºC prior to further analysis. The oils yield (v/w) on a dry weight basis were reported.

2.2.2. Gas chromatography (GC) and gas chromatography - mass spectrometry (GC/MS):

Capillary gas chromatograph (GC) has been for many years as the method of choice to analysis the essential oils (Tabacchi and Garnero, 1987). Constituents existing in the essential oil can be identified by comparison of their relative retention indices and their mass spectra (MS) (Sheille *et al.,* 2002). Some authors have also assessed different techniques for essential oil analysis, like the more comprehensive two-dimensional gas chromatography (GC×GC) (Dimandja *et al.,* 2000; Sheille *et al.,* 2002). However, GC-MS analysis is still the most widely used method for routine analysis of essential oils, and care must be taken to optimize the chromatographic conditions in order to obtain the most accurate results.

Here qualitative and quantitative analyses of the oils were performed using GC and GC-MS. The essential oils were analyzed by using GC HP-5890 II apparatus, equipped with a flam ionization injector HP-5 capillary column (25 m x 0.32 mm, film thickness 0.52 µm), and fitted to FID. Helium (H_2) was used as a carrier gas with flow rate (1 ml/min). A sample of 1.0 µL was injected, using slit mode (split ratio, 1:30). Injector and detector temperatures were set at 250 and 300 °C, respectively. Column oven temperature was programmed from 40 °C to 240 °C at the rate of 4°C/min. The same analytical conditions were employed for GC-MS analysis, where HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-400. Identification of the

individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with those held in Wiley275 library of mass spectra. Confirmation was performed using AMDIS software and literature (Adams, 2007). For the purpose of quantitative analysis area percent obtained by FID were used as a base. Quantification was performed by normalizing the area under GC peak areas.

2.2.3. Antioxidant activity (Spectrophotometric DPPH *assay*)

Kulisic *et al.,* (2004) suggested the DPPH radical scavenging activity assay as one of the best methods for standardizing valuation of antioxidant capacity of essential oils.

Radical scavenging using DPPH radical considered to be one of the main mechanisms by which antioxidant act in food system. The reduction ability of DPPH radicals' formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable dimagnetic molecule (Soares *et al*., 1997).

The antioxidant activity of the *Lamiaceae* essential oils was assessed by measuring their ability to scavenging 2, 2 ′-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The assay was carried out spectrophotometrically as described by Blois (1958). A methanolic solution (0.04 mg/ml) of DPPH was prepared, and then 1800 µl of this solution was added to 200 µl of different essential oils in methanol at different concentrations. Synthetic antioxidant BAH was used as a positive control.

The absorbance was measured at 517 nm in a Jenway 6305 UV/Vis spectrophotometer (Sineks Laboratory, UK), after 30 min in dark at room temperature for all samples. Methanol was used to zero the spectrophotometer. All determinations were taken in triplicate and special care was taken to minimize the loss of free radical

activity of the DPPH. The percentage inhibition of the DPPH radical by samples was calculated according to following equation:

% inhibition = [$(A_0 - A_1)/A_0$] × 100

Where A_0 is the absorbance of control sample (without essential oils), and A_1 is the absorbance of the samples with essential oils at different concentrations. Oils concentrations (mg/ml) providing 50% inhibition (IC_{50}) was calculated from graph plotting scavenging activity against oil concentration.

2.2.4. Antimicrobial activity

2.2.4.1. Antibacterial activity

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Proteus mirabilis* (human isolate) and the following Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The antibacterial assay was carried out by microdilution method, in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria (Daouk *et al.,* 1995; Hanel and Raether, 1988; Espinel-Ingroff, 2001). The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 x 10^5 CFU/ml. From 1000 µl of the overnight culture (10^9) 100 µl is taken and added in pure clean medium 900 µl. This procedure was repeated 4 times to obtain final concentration of 10^5 cells/ml. The inocula were prepared daily and stored at +4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

2.2.4.2. Microdilution Test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 x 10^5 CFU/ml. The microplates were filed with 180 µl of Tryptic Soy Broth medium in first row and all the rest rows were filled with 100 µl of TSB medium. Essential oils or compounds tested were added in 10 µl in first row, and 10 µl of appropriate bacterial inoculum, 1.0 x 10^4 cfu per well, was added. The final volume in first raw was 200 µl and 100 µl was removed to another raw until the end of the plate as serial dilution order, to obtain double lower concentration in every raw. The microplates were incubated for 24 h at 37°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The following day, 30 µl of 0.2 mg/ml solution of INT (*p*-iodonitrotetrazolium violet) was added, and the plates were returned to the incubator for at least one-half hour to ensure adequate color reaction. Inhibition of growth was indicated by a clear solution or a definite decrease in color reaction. Streptomycin was used as a positive control (1 mg/ml DMSO). Two replicates were done for each compound.

2.2.4.3 Antifungal Activity

For the antifungal bioassay eight fungi were used: *Aspergillus flavus* (ATCC 9643), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) and *C. albicans* (human isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The micromycetes were maintained on malt agar and the cultures stored at 4°C and sub-cultured once a month (Booth, 1971). In order to investigate the antifungal activity of the extracts, a modified microdilution technique was used (Daouk *et al*., 1995; Hanel and Raether, 1988; Espinel-Ingroff, 2001). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 µl per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum. Minimal inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in 5% DMSO (1 mg/ml) and added in broth Malt medium with inoculum. The microplates were incubated for 72 h at 28°C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 µl into microtiter plates containing 100 µl

of broth per well and further incubation 72 h at 28℃. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, commercial fungicide, bifonazole, was used as positive controls (1-3000 μg/ml).

Chapter three

Results

3.1. *Thymus capitatus*

3.1.1. Chemical composition of essential oil

The chemical composition of the essential oil of *Th. capitatus* analyzed by GC and GC-MS is reported in **Table 3.1.** Twenty nine compounds representing 99.71% of the total oil were identified. The oil yield obtained was 4.97 % (w/v), and the essential oil was rich in monoterpenes (98.06%). The oxygenated monoterpenes class being the most representative (87.60%), followed by monoterpene hydrocarbons 10.46% and sesquiterpenes reached (1.06%) of the total oils.

Th. capitatus essential oil analysis showed that carvacrol (68.07%) was main compounds, followed by thymol, *p*-cymene, γ-terpene, *p*-cymene-7-ol, borneol, linalool and β-caryophyllene (12.27%, 3.24%, 3.09%, 2.83%, 1.58%, 1.21% and 1.01% respectively). Other compounds were represented in a trace amount less than 1% of the total oil.

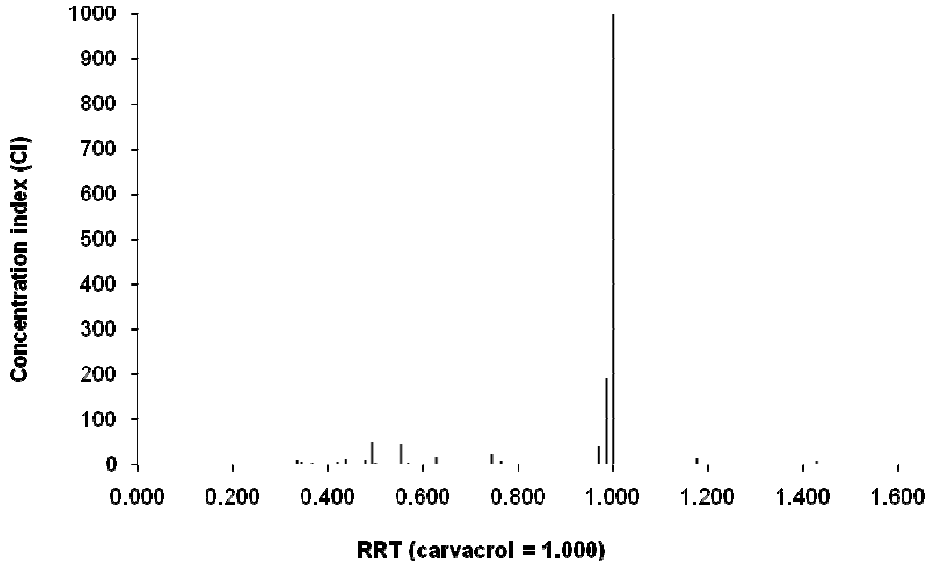


Fig 3.1. Normalised chromatogram of *Th. capitatus* essential oil.

Table 3.1. Chemical composition of *Th. capitatus* essential oil.

Constituents	KIE	KIL	*Th. capitatus*		
α-thujene	917.4	924	0.67	±	0.0052
α-pinene	923.1	932	0.49	±	0.0039
Camphene	937.2	946	0.33	±	0.0034
Sabinene	962.0	969	0.12	±	0.0022
β-pinene	965.5	974	0.33	±	0.0045
β-myrcene	983.4	988	0.91	±	0.0096
α-phellandrene	995.5	1002	0.12	±	0.0017
δ^3-carene	1001.1	1008	0.05	±	0.0005
α-terpinene	1007.7	1014	0.75	±	0.0071
p-cymene	1016.2	1020	3.24	±	0.0262
β-phellandrene	1019.5	1025	0.25	±	0.0022
1,8-cineole	1022.0	1026	0.08	±	0.0009
trans-β-ocimene	1040.6	1044	0.03	±	0.0006
γ-terpinene	1049.9	1054	3.09	±	0.0244
cis-sabinene hydrate	1059.4	1065	0.29	±	0.0037
α-terpinolene	1078.9	1086	0.1	±	0.0006
Linalool	1093.1	1095	1.21	±	0.0038
Camphor	1134.4	1141	0.17	±	0.0036
Borneol	1157.6	1165	1.58	±	0.0211
terpinene-4-ol	1168.5	1174	0.65	±	0.0119
cis-dihydro carvone	1189.5	1191	0.09	±	0.0027
trans-dihydrocarvone	1195.0	1200	0.12	±	0.0023
p-cymene -7-ol	1283.2	1287	2.83	±	0.0128
thymol	1292.7	1289	12.27	±	1.1357
carvacrol	1298.7	1298	68.07	±	0.9775
thymol acetate	1346.2	1349	0.08	±	0.0004
β-caryophyllene	1407.0	1417	1.01	±	0.0037
α-humulene	1441.5	1452	0.05	±	0.0016
caryophyllene oxide	1569.9	1582	0.51	±	0.0068
Total					**99.71 %**
Yield (v/w)%					**4.97**
Number of constituents					29
Monoterpene hydrocarbons					10.46%
Oxyentaed monoterpenes					87.60%
Sesquiterpene hydrocarbons					1.06%
Oxygenated hydrocarbons					0.51%
Other					0.08%

[a]KIE=Kovats (retention) index experimentally determined (AMDIS);

[b] KIL=Kovats (retention) index - literature data;

3.1.2. Antimicrobial activity

3.1.2.1 Antibacterial activity

Results of antibacterial activity of essential oils of *Thymus capitatus* are presented in **Table 3.2.** The oil showed inhibitory activity at 0.001-0.002 mg/ml and bactericidal at 0.001-0.04 mg/ml. Thymol exhibited antibacterial activity with MIC at 0.01-0.1 mg/ml and MBC at 0.05-0.15 mg/ml, while carvacrol showed MIC = 0.0025–0.05 mg/ml and MBC= 0.005–0.1 mg/ml. It can be seen that, the oil tested showed higher antibacterial activity than thymol and carvacrol. Streptomycin showed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml.

Table 3.2. Antibacterial activity of *Th. capitatus* and its main compounds tested by microdilution method (MIC and MBC in mg/ml).

Bacteria	*Th. capitatus*		thymol		carvacrol		streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram (+) bacteria								
Bacillus cereus	0.001	0.002	0.025	0.05	0.0125	0.025	0.0005	0.0005
Micrococcus flavus	0.001	0.001	0.025	0.05	0.0025	0.005	0.0005	0.001
Staphylococcus aureus	0.002	0.002	0.025	0.05	0.025	0.05	0.001	0.001
Listeria monocytogenes	0.001	0.001	0.1	0.1	0.05	0.05	0.001	0.002
Gram (-) bacteria								
Escherichia coli	0.001	0.003	0.1	0.15	0.05	0.05	0.0005	0.001
Pseudomonas aeruginosa	0.002	0.04	0.1	0.15	0.05	0.1	0.001	0.002
Proteus mirabilis	0.002	0.03	0.01	0.15	0.05	0.1	0.001	0.002
Salmonella typhimurium	0.002	0.025	0.05	0.1	0.05	0.05	0.001	0.001

3.1.2.2 Antifungal activity

Results of antifungal activity of oil tested are presented in Table 3.3. As in the case of the antibacterial activity, the oil showed strong antifungal potential. The exhibited inhibitory effect at 0.0002-0.001 mg/ml and fungicidal effect at 0.002-0.025mg/ml. Thymol showed MIC at 0.01-0.05 mg/ml and MFC at 0.01-0.05 mg/ml, while carvacrol exhibited inhibitory activity at 0.005-0.025 mg/ml and fungicidal activity at 0.005-0.05 mg/ml. Bifonazole exhibited much lower antifungal activity than oils tested. MIC was at 0.15-0.20 mg/ml and MFC 0.20-0.25 mg/ml.

Table 3.3. Antifungal activity of *Th. capitatus* essential oil and its main compounds tested tested by microdilution method (MIC and MFC in mg/ml).

Fungi	*Th. capitatus*		thymol		carvacrol		Bifonazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium funiculosum	0.001	0.002	0.0125	0.025	0.0125	0.0125	0.20	0.25
Penicillium ochrochloron	0.001	0.002	0.025	0.025	0.0025	0.005	0.15	0.20
Aspergillys fumigatus	0.001	0.003	0.025	0.05	0.025	0.025	0.15	0.20
Aspergillus niger	0.001	0.003	0.01	0.02	0.025	0.025	0.15	0.20
Aspergillus flavus	0.001	0.002	0.01	0.01	0.005	0.01	0.15	0.20
Aspergillus ochraceus	0.001	0.002	0.01	0.015	0.005	0.01	0.15	0.20
Candida albicans	0.001	0.025	0.05	0.05	0.025	0.05	0.15	0.20
Trichoderma viride	0.0002	0.0025	0.01	0.01	0.005	0.01	0.20	0.25

3.1.3. Antioxidant activity

The findings of DPPH scavenging assay of the *Th. capitatus* essential oil, it's principal components and BHA are shown in **Fig 3.2**. and **Table 3.4**. Our results showed that the, oil tested have a strong antioxidant activity. *Th. capitatus* essential oil was more active with IC_{50} = 0.1192 mg/ml of solution compared with thymol (IC_{50}= 0.403 mg/ml of solution) and quite similar to carvacrol (IC_{50} = 0.105 mg/ml of solution). *Th. capitatus* showed slightly lower antioxidant activity than BHA which gained IC_{50} = 0.0717 mg/ml of solution.

Table 3. 4. The IC_{50} value (mg/ml) of *Thymus capitatus* essential oil, thymol, carvacrol and BHA on DPPH assay.

Sample	IC_{50} % mg/ml
Th. capitatus	0.1192
Thymol.	0.403
Carvacrol.	0.105
BHA	0.0717

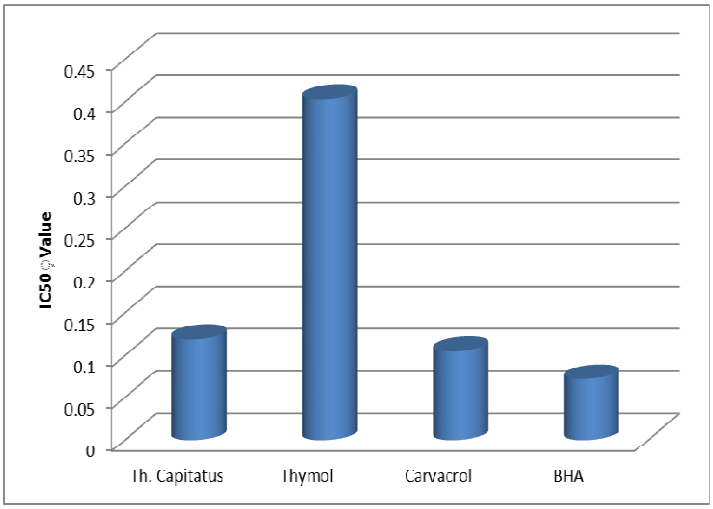


Fig 3.2. Comparison between the IC_{50} value (mg/ml) of *Thymus capitatus* essential oil, thymol, carvacrol and BHA on DPPH assay.

3.2. *Thymus algeriensis*

3.2.1 Chemical composition of essential oil

According to GC-MS analysis of *Th. algeriensis* forty-five compounds were identified representing 99.67% of the total oil. And the oil yield was 2.5 % (v/w). The results of GC-MS analysis and oil yield are listed in **Table 3.5**.

The essential oil of *Th. algeriensis* was characterized by domination of monoterpenes, representing 80.80% of the total oil. The class of oxygenated monoterpenes is being the most representative 54.67% in essential oil, followed by monoterpene hydrocarbons with percentage 26.13% of the total oil. Sesquiterpenes in the oil reached 3.08%.

The essential oil composition of *Th. algeriensis* was characterized by high percentage of thymol (38.50%) as the main compound, followed by *p*-cymene (8.91%), γ-terpinene (7.19%) bornyl acetate (7.03%), borneol (6.03%), carvacrol (4.69%), thymol methyl ether (3.81%), thymol acetate (2.75%), linalool (2.42%), myrcene (1.34%), β-caryophyllene (1.32%) and β-bisabolene (1.03%).

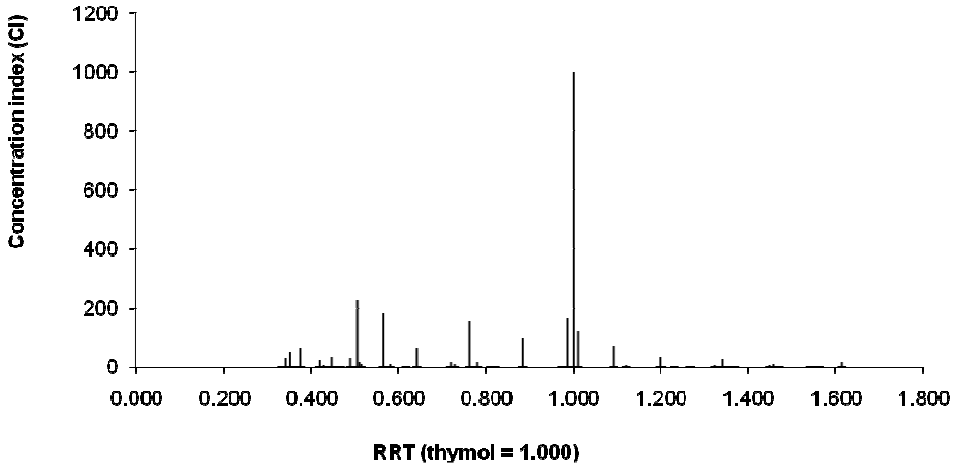


Fig 3.3. Normalised chromatogram of *Thymus algeriensis* essential oil.

Table 3.5. Chemical composition of *Thymus algeriensis* essential oil.

Constituents	KIE	KIL	*Th. algeriensis*
tricyclene	911.8	921	0.10 ± 0.00066
α-thujene	917.4	924	1.13 ± 0.00832
α-pinene	923.1	932	1.98 ± 0.01407
camphene	937.2	946	2.40 ± 0.01498
sabinene	962.0	969	0.84 ± 0.02444
β-pinene	965.5	974	0.23 ± 0.01556
1-octen-3-ol	976.2	974	0.11 ± 0.00794
myrcene	983.1	988	1.34 ± 0.00754
α-phellandrene	995.5	1002	0.15 ± 0.00683
δ^3-carene	1001.1	1008	0.07 ± 0.00607
α-terpinene	1007.7	1014	1.12 ± 0.00284
p-cymene	1016.2	1020	**8.91** ± 0.04083
limonene	1019.4	1024	0.60 ± 0.00296
1,8-cineole	1022.0	1026	0.43 ± 0.00381
trans-β-ocimene	1040.6	1044	0.05 ± 0.00072
γ-terpinene	1049.9	1054	**7.19** ± 0.03081
cis-sabinene hydrate	1059.4	1065	0.45 ± 0.00317
α-terpinolene	1078.9	1086	0.18 ± 0.00977
Linalool	1093.1	1095	2.42 ± 0.02042
camphor	1134.4	1141	0.69 ± 0.02099
cis-chrysanthenol	1138.8	1147	0.36 ± 0.01034
borneol	1157.6	1165	**6.03** ± 0.03969
terpinene-4-ol	1168.5	1174	0.68 ± 0.017
α-terpineol	1184.2	1186	0.13 ± 0.00119
cis-dihydro carvone	1189.5	1191	0.11 ± 0.00663
thymol methyl ether	1226.4	1232	3.81 ± 0.01071
isobornyl acetate	1276.4	1283	0.14 ± 0.00472
bornyl acetate	1280.7	1287	**7.03** ± 0.8612
Thymol	1292.7	1289	**38.50** ± 0.95129
carvacrol	1298.7	1298	**4.69** ± 0.03265
thymol acetate	1346.2	1349	2.75 ± 0.00704
carvacrol acetate	1364.5	1370	0.25 ± 0.00141
β-caryophyllene	1407.0	1417	1.32 ± 0.00342
aromadendrene	1426.3	1439	0.08 ± 0.00228
alloaromadendrene	1448.3	1458	0.13 ± 0.00033
bicyclogermacrene	1484.4	1500	0.23 ± 0.00102
β-bisabolene	1497.4	1505	1.03 ± 0.00272
γ-cadinene	1502.0	1513	0.09 ± 0.00035
δ-cadinene	1511.5	1522	0.20 ± 0.00102
spathulenol	1565.7	1577	0.28 ± 0.0528
caryophyllene oxide	1569.9	1582	0.35 ± 0.00124
α-guaiol	1579.3	1587	0.11 ± 0.0031
epi-α-cadinol (τ-cadinol)	1629.6	1638	0.14 ± 0.00169
α-eudesmol	1637.9	1652	0.20 ± 0.00064
eudesm-3-en-6-ol	1677.8	1679	0.61 ± 0.01034
Total			**99.67%**
Yield (v/w)%			**2.5**
Number of constituents			**45**
Monoterpene hydrocarbons			**26.13%**
Oxyentaed monoterpenes			**54.67%**
Sesquiterpene hydrocarbons			**3.08%**
Oxygenated hydrocarbons			**1.07%**
Other			**14.72%**

[a]KIE=Kovats (retention) index experimentally determined (AMDIS);

[b] KIL=Kovats (retention) index - literature data;

3.2.2. Animicrobial activity

3.2.2.1 Antibacterial activity

Results of antibacterial activity of essential oil tested are presented in **Table 3.6.** Oil of *Th. algeriensis* showed very strong antibacterial activity against all species. The oil was effective at 0.001-0.05 mg/ml and bactericidal activity was achieved at 0.0025-0.05 mg/ml. Thymol exhibited antibacterial activity with MIC at 0.025-0.1 mg/ml and MBC at 0.05-0.1 mg/ml, while cavacrol exhibited high antibacterial activity with MIC at 0.025-0.05 mg/ml and MBC at 0.025-0.1 mg/ml. Streptomycin showed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml.

Table 3.6. Antibacterial activity of *Th. algeriensis* essential oil and its maincompounds tested by microdilution method (MIC and MBC in mg/ml).

Bacteria	*Th. algeriensis*		thymol		carvacrol		streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram (+) bacteria								
Bacillus cereus	0.001	0.0025	0.025	0.05	0.0125	0.025	0.0005	0.0005
Micrococcus flavus	0.001	0.0025	0.025	0.05	0.0025	0.005	0.0005	0.001
Staphylococcus aureus	0.002	0.003	0.025	0.05	0.025	0.05	0.001	0.001
Listeria monocytogenes	0.001	0.05	0.1	0.1	0.05	0.05	0.001	0.002
Gram (-) bacteria								
Escherichia coli	0.002	0.004	0.1	0.15	0.05	0.05	0.0005	0.001
Pseudomonas aeruginosa	0.003	0.05	0.1	0.15	0.05	0.1	0.001	0.002
Proteus mirabilis	0.003	0.05	0.01	0.15	0.05	0.1	0.001	0.002
Salmonella typhimurium	0.05	0.05	0.05	0.1	0.05	0.05	0.001	0.001

3.2.2.2 Antifungal activity

Results of antifungal activity of oil tested are presented in **Table 7**. As in the case of the antibacterial activity, the oil showed strong antifungal potential. *Th. algeriensis* oil showed antifungal potential with MIC 0.0005-0.025mg/ml and MFC 0.001-0.05 mg/ml. *Th. algeriensis* oil again showed better potential than. Thymol which gained MIC at 0.0125-0.05 mg/ml and MFC at 0.025-0.05 mg/ml, while carvacrol exhibited inhibitory activity at 0.0125-00.025 mg/ml and fungicidal activity at 0.0125-0.05 mg/ml. Bifonazole exhibited much lower antifungal activity than oil tested. MIC was at 0.15-0.20 mg/ml and MFC 0.20-0.25 mg/ml.

Table 3.7. Antifungal activity of *Th. algeriensis* essential oils and its main compounds tested by microdilution method (MIC and MFC in mg/ml).

Fungi	*Th. algeriensis*		thymol		carvacrol		Bifonazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium. funiculosum.	0.001	0.002	0.0125	0.025	0.0125	0.0125	0.20	0.25
Penicillium ochrochloron	0.001	0.0025	0.025	0.025	0.0025	0.005	0.15	0.20
Aspergillus fumigatus	0.002	0.003	0.025	0.05	0.025	0.025	0.15	0.20
Aspergillus niger	0.001	0.003	0.01	0.02	0.025	0.025	0.15	0.20
Aspergillus flavus	0.002	0.004	0.01	0.01	0.005	0.01	0.15	0.20
Aspergillus ochraceus	0.001	0.0025	0.01	0.015	0.005	0.01	0.15	0.20
Candida albicans	0.025	0.05	0.05	0.05	0.025	0.05	0.15	0.20
Trichoderma viride	0.0005	0.001	0.01	0.01	0.005	0.01	0.20	0.25

3.2.3. Antioxidant activity

The results of DPPH scavenging assay of the *Thymus algeriensis*, its principal components and BHA are shown in **Table 3.8.** and **Fig 3.4**. Our findings showed that

the *Th. algeriensis* essential oil possessed a strong antioxidant activity with IC_{50} = 0.299 mg/ml of solution, and it was better than thymol 0.403 mg/ml of solution and less than carvacrol (IC_{50} = 0.105 mg/ml of solution) and BHA (IC_{50} = 0.0717 mg/ml of solution).

Table 3.8. The IC_{50} value (mg/ml) of *Thymus algeriensis* essential oil, thymol, carvacrol and BHA on DPPH assay.

Sample	IC_{50} % mg/ml
Thymus algeriensis	0.2991
Thymol.	0.403
Carvacrol.	0.105
BHA	0.0717

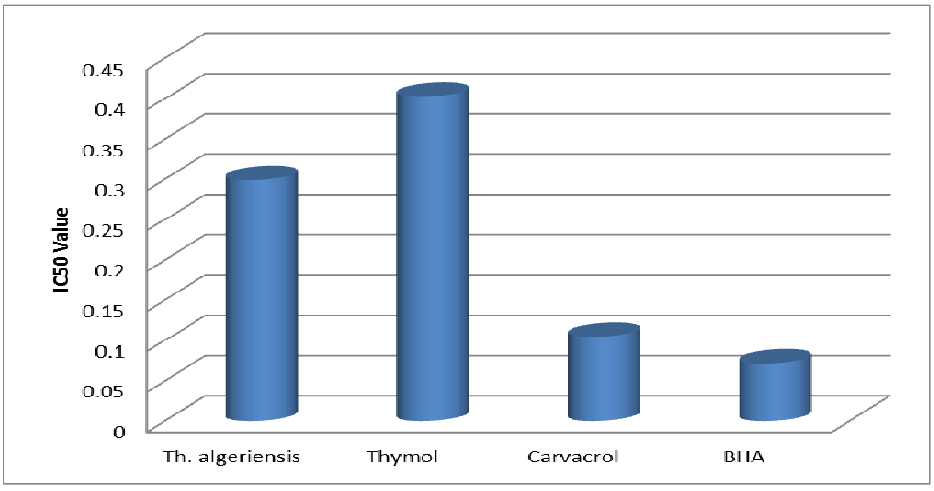


Fig 3.4. Comparison between the IC_{50} value (mg/ml) of *Thymus algeriensis* essential oil, thymol, carvacrol and BHA on DPPH assay.

3.3. *Satureja thymbra*

3.3.1. Chemical composition of essential oil

The essential oil components identified by using GC and GC/MS analysis are given in **Table 3.9**. Thirty three compounds were identified representing 99.81% of the total oil. The oil yield reached 4.9% (v/w).

The class of monoterpene hydrocarbons was the major class, flowed by oxygenated monoterpene represented 58.57% and 31.21% respectively. The main compounds were γ-terpinene (39.23%), thymol (25.16%), *ρ*-cymene (7.17%), α-terpinene 3.26%), carvacoral (4.18%) and β- caryophyllene (2.76%).

The presence of other monoterpene hydrocarbons, such as myrcene, *α*-terpinene, was also assayed in small quantitie. *β*-caryophyllene was detected as major component of the oil tested .

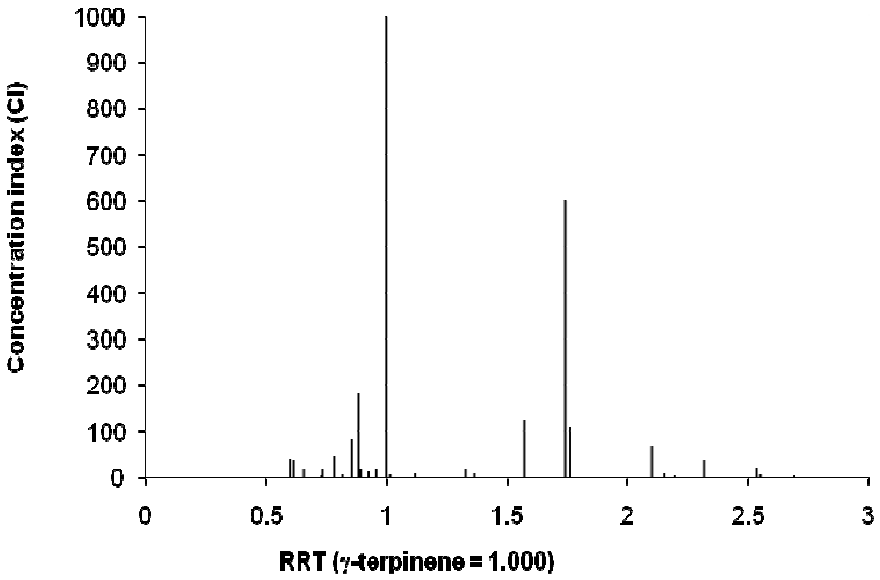


Fig 3.5. Normalised chromatogram of *Satureja thymbra* essential oil.

Table 3.9. Chemical composition of *S. thymbra* essential oil.

Constituents	[a] **KIE**	[b] **KIL**	***Satureja thymbra***
α-thujene	917.7	924	1.55±0.02
α-pinene	923.4	932	1.48±0.03
camphene	937.4	946	0.69±0.01
sabinene	963.6	969	0.14±0.00
β-pinene	965.7	974	0.70±0.01
myrcene	983.2	988	1.76±0.01
α-phellandrene	995.4	1002	0.28±0.00
δ^3-carene	1001.2	1008	0.10±0.01
α-terpinene	**1007.8**	**1014**	**3.26±0.02**
p-cymene	**1016.1**	**1020**	**7.17±0.05**
sylvestrene	1019.5	1025	0.78±0.01
cis-β-ocimene	1030.6	1032	0.52±0.00
trans-β-ocimene	1040.6	1044	0.81±0.01
γ-terpinene	**1051.5**	**1054**	**39.23±0.27**
cis-sabinene hydrate	1060.6	1065	0.23±0.01
terpinolene	1079.1	1086	0.11±0.00
linalool	1051.5	1095.6	0.34±0.00
borneol	1157.2	1165	0.82±0.00
terpinen-4-ol	1169.4	1174	0.36±0.01
α-terpineol	1185.3	1186	0.05±0.01
neo-dihydrocarveol	n/a	1193	0.06±0.04
carvacrol methyl ether	**1235.6**	**1241**	**3.33±1.88**
thymol	**1291.0**	**1289**	**25.16±1.95**
carvacrol	**1297.9**	**1298**	**4.18±0.23**
thymyl acetate	1346.9	1349	0.21±0.21
β-caryophyllene	**1407.1**	**1417**	**2.76±0.03**
aromadendrene	1426.5	1439	0.36±0.01
α-humulene	1441.3	1452	0.15±0.00
alloaromadendrene	1448.4	1458	0.22±0.22
cis-β-guaiene	1484.6	1492	1.56±0.02
spathulenol	1565.9	1577	0.95±0.03
caryophyllene oxide	1570.0	1582	0.32±0.01
cis-α-bergamotene	[c] n/a		0.17±0.16
Total			**99.81%**
Yield (v/w) %			**4.9**
Number of constituents			**33**
Monoterpene hydrocarbons			**58.57%**
Oxygenated monoterpenes			**31.21%**
Sesquiterpene hydrocarbons			**5.23%**
Oxygenated hydrocarbons			**1.27%**
Other			**3.53%**

[a] KIE=Kovats (retention) index experimentally determined (AMDIS)

[b] KIL=Kovats (retention) index-literature data; [c] n/a-not available.

3.3.2. Animicrobial activity

3.3.2.1 Antibacterial activity

Results of antibacterial activity of essential oil tested are presented in **Table 3.10**. The oil was active against all the bacteria tested. *S. thymbra* essential oil showed inhibitory effect at 0.001-0.1 mg/ml and bactericidal at 0.002-0.2 mg/ml. Thymol exhibited antibacterial activity with minimum inhibitory concentration (MIC) at 0.01-0.1 mg/ml and minimum bactericidal concentration (MBC) at 0.05-0.15 mg/ml, while carvacrol showed stronger inhibitory activity at 0.0025-0.05 mg/ml and fungicidal activity at 0.005-0.1 mg/ml. Inhibitory and bactericidal concentrations for γ-terpinene were slightly higher than for previous compounds (MIC at 0.05-0.2 mg/ml, and MBC at 0.07-0.3 mg/ml). Streptomycin expressed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml.

Table 3.10. Antibacterial activity of *S. thymbra* essential oil and its main compounds tested by microdilution method (MIC and MBC in mg/ml).

Bacteria	*S. thymbra*		γ-terpenene		thymol		carvacrol		streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram (+) bacteria										
Bacillus cereus	0.05	0.1	0.05	0.07	0.025	0.05	0.0125	0.025	0.0005	0.0005
Micrococcus flavus	0.001	0.002	0.05	0.07	0.025	0.05	0.0025	0.005	0.0005	0.001
Staphylococcus aureus	0.05	0.1	0.05	0.1	0.025	0.05	0.025	0.05	0.001	0.001
Listeria monocytogenes	0.1	0.2	0.1	0.2	0.1	0.1	0.05	0.05	0.001	0.002
Gram (-) bacteria										
Escherichia coli	0.05	0.1	0.15	0.2	0.1	0.15	0.05	0.05	0.0005	0.001
Pseudomonas aeruginosa	0.05	0.1	0.15	0.3	0.1	0.15	0.05	0.1	0.001	0.002
Proteusmira bilis	0.05	0.1	0.2	0.3	0.01	0.15	0.05	0.1	0.001	0.002
Salmonella typhimurium	0.05	0.1	0.1	0.2	0.05	0.1	0.05	0.05	0.001	0.001

3.3.2.2 Antifungal activity

Results of antifungal activity of compounds tested are presented in **Table 3.11.** As in the case of the antibacterial activity, the oils showed strong antifungal potential. *Satureja thymbra* oil possessed strong activity with minimum inhibitory concentration (MIC) 0.001-0.0 25mg/ml and fungicidal effect at 0.001-0.1mg/ml. MIC and MFC for thymol are at 0.01-0.05 mg/ml, while carvacrol showed MIC at 0.0025-0.025 mg/ml and MFC at 0.005-0.05 mg/ml. MIC and minimum fungicidal concentration (MFC) for γ-terpinene were at 0.015-0.05 mg/ml and 0.02-0.1 mg/ml. Bifonazole showed lower antifungal activity than oils tested. MIC was at 0.15-0.20 mg/ml and MFC 0.20-0.25 mg/ml.

Table 3.11. Antifungal activity of *S. thymbra* essential oil and its main compounds tested by microdilution method (MIC and MFC in mg/ml)

Fungi	*S. thymbra*		γ-terpenene		thymol		carvacrol		Bifonazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium funiculosum	0.025	0.05	0.025	0.07	0.0125	0.025	0.0125	0.0125	0.20	0.25
Penicillium ochrochloron	0.001	0.001	0.025	0.07	0.025	0.025	0.0025	0.005	0.15	0.20
Aspergillus fumigatus	0.025	0.05	0.05	0.07	0.025	0.05	0.025	0.025	0.15	0.20
Aspergillus niger	0.025	0.05	0.02	0.03	0.01	0.02	0.025	0.025	0.15	0.20
Aspergillus flavus	0.025	0.05	0.02	0.03	0.01	0.01	0.005	0.01	0.15	0.20
Aspergillus ochraceus	0.025	0.05	0.015	0.02	0.01	0.015	0.005	0.01	0.15	0.20
Candida albicans	0.025	0.1	0.05	0.1	0.05	0.05	0.025	0.05	0.15	0.20
Trichoderma viride	0.025	0.05	0.05	0.07	0.01	0.01	0.005	0.01	0.20	0.25

3.3.3 Antioxidant activity

Antioxidant activity was analyzed using DPPH free radical scavenging method. The results of DPPH scavenging assay of *Satureja thymbra* essential oil, its principal components and synthetic antioxidant butylatedhydroxyanisole (BHA) are shown in **Fig 3.6.** and **Table 3.12.** Present results showed that, *S. thymbra* essential oil possessed strong antioxidant activity as seen from the concentrations at which 50 % radical scavenging occurred (IC_{50} = 0.0967 mg/ml of solution), being better than γ-terpinene and thymol and quite similar to BHA.

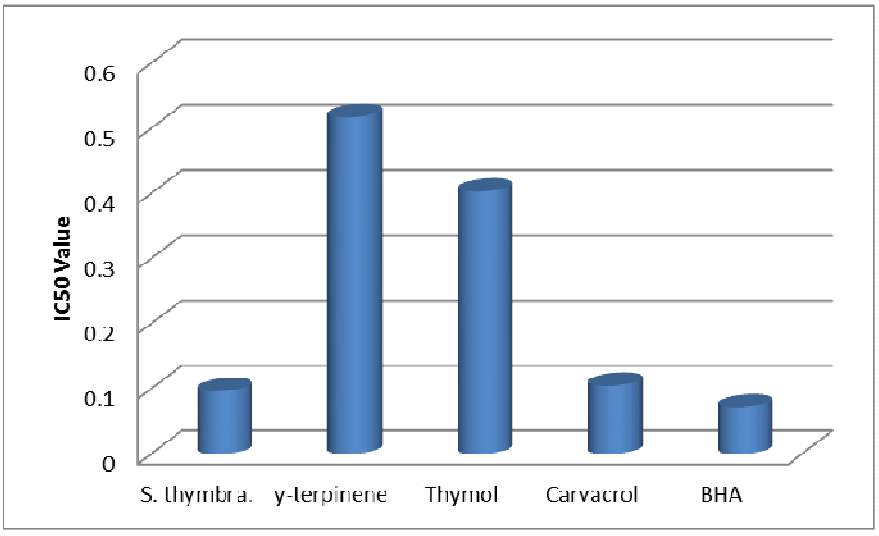


Fig 3.6. Comparison between the IC_{50} values (mg/ml) of *S. thymbra* essential oil, thymol, carvacrol, γ-terpinene and BHA on DPPH assay.

Table 3.12. The IC_{50} value (mg/ml) of *S. thymbra* essential oil, thymol, carvacrol and BHA on DPPH assay.

Samples	IC_{50} % mg/ml
S .thymbra. essential oil	0.0967
γ-terpinene.	0.517
Thymol	0.403
Carvacrol	0.105
BHA	0.0717

3.4 *Salvia fruticosa*

3.4.1 Chemical composition of essential oil

Forty-five compounds were identified in *S. fruticosa* essential oil, accounting 99.76% of the whole essential oil. The yield of essential oil was 1.56% (v/w). The composition of essential oil and oil yield are listed in **Table 3.13.** It can be seen that oxygenated monoterpene was the main class represented by 64.89% of the total of oil. The high percentage of this class is due to the high percentage of 1,8-cineole (49.34%), camphor (7.53%) and α-terpineol (3.25%). Other major compounds belongs to monoterpene hydrocarbons such as β-pinene 7.38%, myrcene (7.38%), α-pinene (5.15%) and camphene (2.72%). β-caryophyllene (4.13%) belongs to sesquiterpene hydrocarbons. Other compounds are detected in a trace amount, less than 1%.

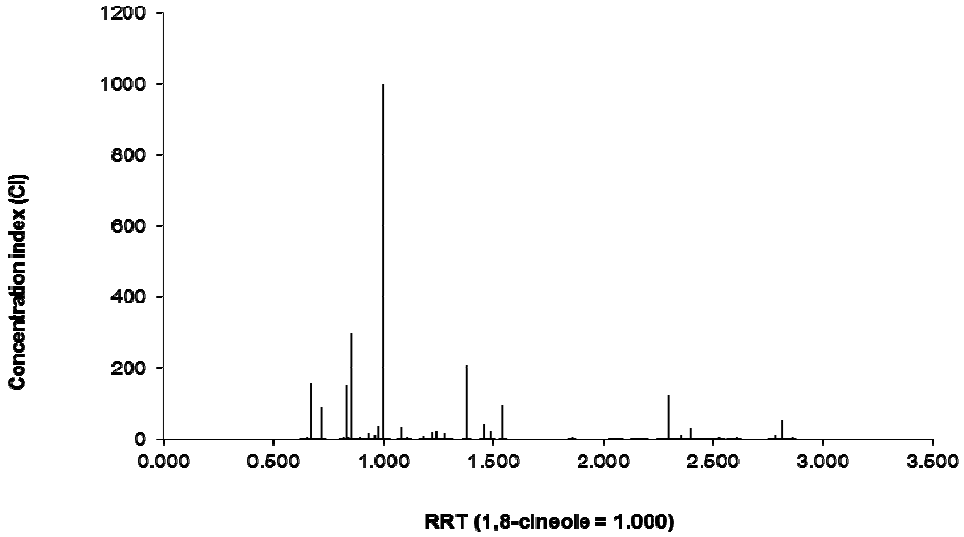


Fig 3.7. Normalised chromatogram of *Salvia furticosa* essential oil.

Table 3.13. Chemical composition of essential oil of *Salvia fruticosa.*

Constituents	[a] **KIE**	[b] **KIL**	***S. furticosa***		
tricyclene	911.7	921	0.08	±	0.0181
α-thujene	917.6	924	0.25	±	0.0005
α-pinene	**922.9**	**932**	**5.15**	**±**	**0.0376**
camphene	936.9	946	2.72	±	0.0200
sabinene	965.2	969	0.35	±	0.0014
β-pinene	**970.8**	**974**	**7.38**	**±**	**0.0529**
1-octen-3-ol	979.9	974	0.11	±	0.0014
myrcene	**982.9**	**988**	**7.38**	**±**	**0.0530**
α-phellandrene	995.1	1002	0.12	±	0.0036
α-terpinene	1007.6	1014	0.41	±	0.0041
p-cymene	1017.6	1020	0.40	±	0.0033
limonene	1019.1	1024	0.85	±	0.0031
1,8-cineole	**1023.4**	**1026**	**49.34**	**±**	**0.6247**
cis-β-ocimene	1031.1	1032	0.04	±	0.0022
γ-terpinene	1049.5	1054	0.77	±	0.0067
cis-sabinene hydrate	1059.8	1058	0.16	±	0.0021
α-terpinolene	1079.0	1086	0.28	±	0.0024
trans-sabinene hydrate	1090.5	1098	0.09	±	0.0059
linalool	1093.6	1095	0.58	±	0.0562
β-thujone (cis-thujone)	1096.1	1101	0.66	±	0.0058
α-thujone (trans-thujone)	1107.2	1112	0.48	±	0.0176
cis-*p*-menth-2-en-1-ol	1114.0	1118	0.05	±	0.0013
camphor	**1133.9**	**1141**	**7.53**	**±**	**0.0543**

borneol	**1156.3**	**1165**	**1.71**	**±**	**0.0221**
terpinen-4-ol	1168.0	1174	0.63	±	0.0223
α-terpineol	**1182.1**	**1186**	**3.25**	**±**	**0.0239**
bornyl acetate	1276.0	1287	0.19	±	0.0009
α-copaene	1364.1	1374	0.03	±	0.0015
α-burbonene	1373.1	1387	0.04	±	0.0022
α-gurjunene	1397.0	1409	0.03	±	0.1521
β-caryophyllene	**1406.8**	**1417**	**4.13**	**±**	**0.0192**
β-gurjunene	1415.7	1431	0.07	±	0.0109
selina-4(15),5-diene	1421.7	1433	0.05	±	0.0056
aromadendrene	1426.2	1439	0.43	±	0.0098
selina-5,11-diene	1430.7	1444	0.05	±	0.0071
α-humulene	1441.0	1452	0.97	±	0.0219
alloaromadendrene	1448.2	1458	0.10	±	0.0188
γ-muurolene	1465.1	1478	0.05	±	0.0084
viridiflorene	1483.1	1496	0.21	±	0.0211
α-selinene	1487.1	1498	0.05	±	0.0113
γ-cadinene	1502.2	1513	0.05	±	0.0174
trans-calamenene	1510.8	1521	0.15	±	0.0174
caryophyllene oxide	1569.1	1582	0.49	±	0.0120
viridiflorol	**1578.2**	**1592**	**1.73**	**±**	**0.0346**
humulene epoxide II	1594.7	1608	0.15	±	0.0171
Total			99.76 %		
Yield (v/w) %			1.56 %		
Number of constituents			45		
Monoterpene hydrocarbons			25.89 %		
Oxygenated monoterpene			64.89 %		
Sesquiterpene hydrocarbons			6.90 %		
Oxygenated hydrocarbons			1.88 %		
other			0.19 %		

[a] KIE=Kovats (retention) index experimentally determined (AMDIS);

[b] KIL=Kovats (retention) index - literature data;

3.4.2 Antimicrobial activity

3.4.2.1 Antibacterial activity

Findings of antibacterial activity of essential oil and its main compounds tested are presented in **Table 3.14**. The oil tested showed antibacterial activity against all the species tested. Essential oil from *Salvia fruticosa* possessed antibacterial activity, and showed minimal inhibitory concentration (MIC) effect at 0.125-1.5 mg/ml and minimal bactericidal concentration (MBC) at 0.5-2.0 mg/ml. The monoterpenic hydrocarbon β-pinene showed antibacterial activity with MIC of 0.05-0.10 mg/ml and MBC of 0.05-0.13 mg/ml. Camphor exhibited inhibitory activity at 0.05-0.07 mg/ml

and bactericidal effect was at 0.06-0.10 mg/ml. The best activity among components and essential oil tested, was achieved for 1,8-cineole (bacteriostatic activity at 0.04-0.07 mg/ml and bactericidal at 0.05-0.09 mg/ml). Streptomycin showed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml.

Table 3.14. Antibacterial activity of *Salvia fruticosa* essential oil and its main compounds tested by microdilution method (MIC and MBC in mg/ml).

Bacteria	*S. fruticosa*		1,8-cineole		Camphor		β-pinene		streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram (+) bacteria										
Bacillus cereus	0.5	1	0.04	0.05	0.05	0.06	0.05	0.06	0.0005	0.0005
Micrococcus flavus	0.25	0.5	0.04	0.05	0.05	0.06	0.05	0.05	0.0005	0.001
Staphylococcus aureus	0.25	0.5	0.05	0.06	0.06	0.07	0.06	0.08	0.001	0.001
Listeria monocytogenes	0.5	1	0.05	0.06	0.07	0.07	0.09	0.10	0.001	0.002
Gram (-) bacteria										
Escherichia coli	1.5	2.0	0.06	0.08	0.7	0.08	0.08	0.10	0.0005	0.001
Pseudomonas aeruginosa	0.125	2.0	0.07	0.09	0.07	0.10	0.10	0.13	0.001	0.002
Proteus mirabilis	0.25	0.5	0.06	0.08	0.07	0.09	0.09	0.10	0.001	0.002
Salmonella typhimurium	0.5	1	0.05	0.06	0.06	0.07	0.08	0.09	0.001	0.001

3.4.2.2 Antifungal activity

Results of antifungal activity of the essential oil of *Salvia fruticosa* and the main compounds tested are presented in **Table 3.15**. As in the case of the antibacterial activity, the oil showed antifungal potential. *S. fruticosa* oil possessed good activity with MIC 0.125-1.0 mg/ml and showed minimal fungicidal concentration (MFC) at 0.125-1.5 mg/ml; β-pinene showed MIC at 0.05-0.07 mg/ml and fungicidal at 0.05-0.11 mg/ml, while camphor showed MIC at 0.04-0.06 mg/ml and MFC at 0.04-0.10 mg/ml. The best activity is obtained for 1, 8-cineole (MIC at 0.03-0.06 mg/ml and

MFC at 0.04-0.09 mg/ml). Antifungal potential could be presented as follows: essential oil < β-pinene < camphor < 1,8-cineole. Bifonazole showed lowest antifungal activity MIC was at 0.15-0.20 mg/ml and MFC 0.20-0.25 mg/ml.

Table 3.15. Antifungal activity of *Salvia fruticosa* essential oil and its main compounds tested tested by microdilution method (MIC and MFC in mg/ml).

Fungi	*S. fruticosa*		1,8 cineole		Camphor		β-pinene		Bifonazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium funiculosum	0.5	0.5	0.03	0.04	0.04	0.05	0.06	0.06	0.20	0.25
Penicillium ochrochloron	0.15	0.25	0.04	0.04	0.04	0.05	0.05	0.05	0.15	0.20
Aspergillus fumigatus	0.15	0.3	0.04	0.05	0.04	0.06	0.05	0.07	0.15	0.20
Aspergillus niger	1.0	1.5	0.06	0.07	0.06	0.08	0.07	0.09	0.15	0.20
Aspergillus flavus	0.125	0.125	0.05	0.09	0.05	0.10	0.06	0.11	0.15	0.20
Aspergillus Ochraceus	0.125	0.25	0.05	0.07	0.05	0.08	0.06	0.09	0.15	0.20
Candida Albicans	0.25	0.5	0.05	0.06	0.05	0.07	0.06	0.08	0.15	0.20
Trichoderma viride	0.25	0.5	0.03	0.05	0.04	0.04	0.07	0.09	0.20	0.25

3.4.3 Antioxidant activity

Antioxidant activity was analyzed using DPPH free radical scavenging method. The results of DPPH scavenging assay of *Salvia. fruticosa* essential oil and synthetic antioxidant butylated hydroxyanisole (BHA) are shown in **Fig 3.8** and **Table 3.16**. Present results showed that antioxidant activity of *S. fruticosa* essential oil, as seen from the concentrations at which 50% radical scavenging occurred (IC_{50} = 15.53 mg/ml of solution), which mean that it has lower antioxidant activity compared to BHA, thymol, crvacrol and γ-terpenene.

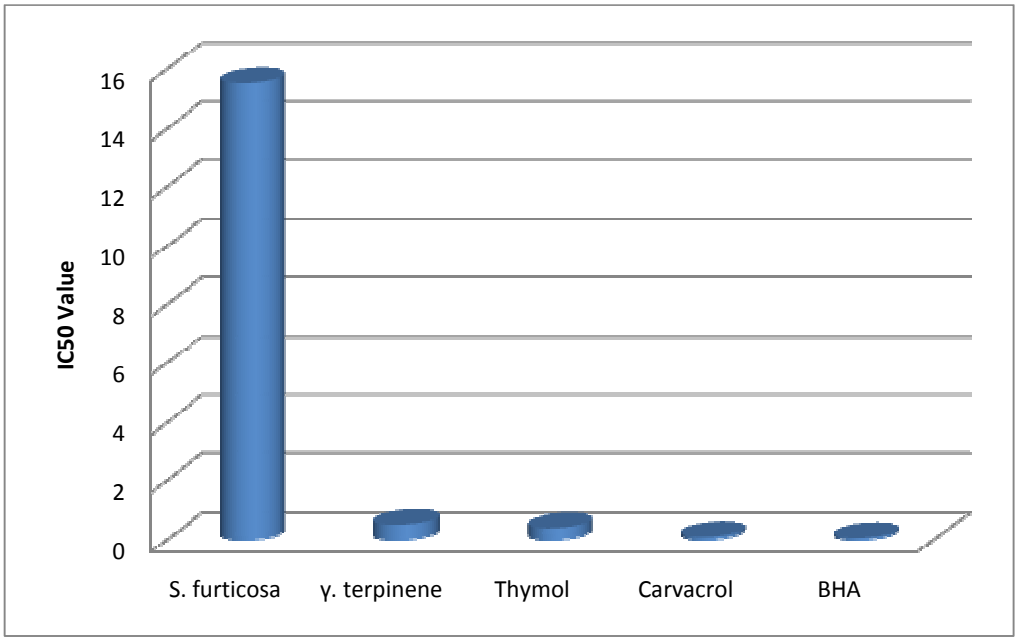


Fig 3.8. Comparison between the IC_{50} values (mg/ml) of *Salvia fruticosa* essential oil, thymol, carvacrol, γ-terpinene and BHA on DPPH assay.

Table 3.16. The IC_{50} value (mg/ml) of *Salvia fruticosa* essential oil, thymol, carvacrol, γ-terpinene and BHA on DPPH assay.

Samples	**IC_{50} % mg/ml**
S. fruticosa. Es	15.53
γ-terpinene.	0.517
Thymol	0.403
Carvacrol	0.105
BHA	0.0717

Chapter four

Discussion

4.1. Essential oils yield

The essential oils yield obtained from the species investigated by hydro-distillation method using Clavenger- approuates, showed that, the highest yield obtained was 4.97% (v/w) from *Th. capitatus*, *S. thymbra* 4.97 % (v/w), *Th. algeriensis* 2.5% v/w and the lowest was from *S. fruticosa* 1.56% (v/w). However, all the species were rich in essential oil.

4.2. Chemical composition

According to GC and GC/MS analysis of the investigated species (29, 45, 33 and 45) compounds could be identified representing 99.71%, 99.67%, 99.81% and 99.76% of the total oils of (*Thymus capitatus, Th. algeriensis, Satureja thymbra* and *Salvia fruticosa* respectively) The results of the essential oils composition and the oils yield of the investigated species are summarized in **Tables. 3.1, 3.5, 3.9** and **3.13.**

4.2.1 *Thymus capitatus*

Results of the the chemical composition of the essential oil of *Th. capitatus* reported in **Table 3.1**, showed that the oil was dominated by the monoterpenes 98.06%. Among monoterpenes oxygenated monoterpenes class being the most representative (87.60%) flowed by monoterpene hydrocarbons 10.46% and sesquiterpenes reached (1.06%) of the total oil.

Th. capitatus essential oil analysis showed that, carvacrol (68.07 %) was main compounds, followed by thymol, p-cymene, γ-terpene, p-cymene-7-ol, borneol, linalool and β-caryophyllene (12.27 %, 3.24%, 3.09%, 2.83%, 1.58%, 1.21% and 1.01% respectively). Other compounds were represented in a trace amount less than 1% of the total oil. This indicated that, *Th. capiataus* essential oil growing wild in Zintan is carvacrol/thymol chemo-type. Our results are similar with previous studies on essential oil composition of *Th. capitatus* collected from other parts of Mediterranean region (El-Ajjouri *et al.* 2008; Goren *et al.,* 2003; Miceli *et al.,* 2006; Karousou *et al.,* 2005; Schulz *et al.,* 2005). These authors reported that *Th. capitatus*

essential oil contain mainly carvacrol as a major compound with thymol percent more than 9%. In the other hand, there are some findings showed that the main content of *Th. capitatus* essential oil was carvacrol with small amount of thymol (Miguel *et al.,* 2005; Hedhili *et al.,* 2005; Skoula and Grayer, 2004; Hedhili *et al.,* 2002; Faleiro *et al.,* 2005; Machado *et al.,* 2010; Miceli *et al.,* 2006; Karpouhtsis *et al.,* 1998). Also, Karousou *et al.* (2005), Katz *et al.* (1987) and Skoula and Grayer (2004) cited that, thymol was the main compound of the *Th. capitatus* essential oil, with a trace amount of carvacrol.

4.2.2. *Thymus algeriensis*

The finding obtained from the essential oil from *Thymus algeriensis* summarized in **Table 3.5.**, showed that, the group of monoterpenes reached 80.80% of the total oil, among them the class oxygenated monoterpene was the most abundant with percentage 54.67%, folowed by monoterepene hydrocarbons 26.13%. Sesquiterpene hydrocarbons don't exceed 3.08% of the total oil.

Th. algerinsis, essential oil characterized by thymol as the main compound with percentage (38.50%) followed by p-cymene (8.91%), γ-terpinene (7.19%) bornly acetate (7.03%), borneol (6.03%), carvacrol (4.69%), thymol methyl ether (3.81%), thymol acetate (2.75), linalool (2.42%), β-caryophyllene (1.32%) and β-bisabolene (1.03%) which mean that, essential oil of wild growing in Libyan *Th. algeriensis* collected from Zintan is thymol chemo-type. Our results are similar with data wich have been reported by Hazzit *et al.* (2009), They finding out that, *Th. algeriensis* essential oil from Algeria was thymol chemotype. In contrast, Aboutabl and EL-Dahmy (1995) reported that, the main compound of Libyan *Th. algeriensis* essential oil collected from Grian 100 Km from Zentan were carvacrol followed by thymol. However, they did not detect bornyl actate, which was (7.03%) in our study. Also Jaafarri *et al.,* (2007) reported that, the major compounds of the *Th. algeriensis* essential oil collected from Algeria were Carvacrol (80.40%). In other study done by Amarit *et al.* (2010) showed that the main compounds of *Th. algerinsis* essential oil from Morocco were camphor (27.7%) and α-pinene (20.5%).

4.2.3. *Satureja thymbra*

The essential oil of *S. thymbra* components identified by using GC and GC/MS analyses are given in **Table 3.9**. Thirty three compounds could be identified representing 99.81% of the total oil. The class of monoterpene hydrocarbons was the major class, flowed by Oxygenated monoterpene represented (58.57% and 31.21 % respectively).

The major compounds of the essential oil were, γ- terpinene (39.23%), thymol (25.16%), *p*-cymene (7.17%), carvacoral (4.18%), carvacrol methyle ether (3.33%), α-terpinene (3.26%), and β-caryophyllene (2.76%) the content of the compounds such as cis-β-guaiene, α-pinene, α-thjene, myrecene do not exceed 2% for each one. *β*-caryophyllene was detected as major component of the oil tested. The isomeric phenolic monoterpinenes (carvacrol and thymol) and their biosynthetic monoterpenes (*p*-cymene and γ- terpinene) representing about 75.63%, which are in accordance with most previous studies.

The composition of essential oil isolated from *S. thymbra* is given in **Table 3.9.** The essential oil was characterized by γ-terpinene (39.26%), thymol (25.16%), *p*-cymene (7.17%) and carvacrol (4.18%) as the major constituents. Our findings are in contrast with some previous observations of *S. thymbra* essential oil (Loizzo *et al.,* 2008; Ayvaz *et al.,* 2010; Karousou *et al.,* 2005; Sokovic *et al.,* 2002; Skoula and Grayer, 2005; Schulz *et al.,* 2005; Chorianopoulos *et al.,* 2006; Michaelakis *et al.,* 2007; Karpuhtsis *et al.,* 1998; Ravid *et al.,* 1983). In most references mentioned, carvacrol and/or thymol were the major compounds. Also, the essential oil composition of *S. thymbra* from Turkey contains mainly carvacrol and γ-terpinene (Schulz *et al.,* 2005). *S. thymbra* essential oil from Lebanon was characterized by similar amount of *p*-cymene (10.76%), α-pinene (10.15%), thymol (9.92%) and sabinene (8.64%) (Loizzo *et al.,* 2008). According to essential oil composition of this species collected from Crete from two locations, the authors found different chemotypes. In the sample from Aktotiri the major compounds were carvacrol, thymol, *p*-cymene and γ-terpinene (44.6%, 0.3%, 11.9% and 25.5% respectively), while in the other sample the main compounds were thymol (35.5%), γ-terpinene (27.6%), *p*-cymene (10.4%) and carvacrol (3.2%) (Skoula *et al.,* 2005). Also, the

essential oil of the same species growing wild in Turkey, the oil dominated by carvacrol (53.7%) followed by γ-terpinene (17.6%), thymol (13%) and *p*-cymene (10.1%) (Ayvaz *et al.,* 2010). Furthermore, the variation in essential oils composition reported by studying 13 essential oils of *S. thymbra* belonging to different locations in Crete, the finding showed a high variation in essential oils composition, which carvacrol varied significantly (5.2-65%), thymol (0.1- 65.6%), γ-terpinene (20 - 4.4%) and cymene (5.5 - 15%) (Karousou *et al.,* 2005). Moreover, a study carried out to evaluate the effect of harvesting time in essential oil composition of *S. thymbra* collected from the same location at different vegetation stages, the finding showed that the major compounds in vegetation phase were thymol (27.88%), followed by γ-terpinene (17.02%) and carvacrol (11.88%), while in full flowering phase carvacrol (29.18%), thymol (17.22%) and γ-terpinene (12.45%) were the main compounds, in fruiting phase thymol again 20.73% was the main compound, γ-terpinene 14.91% and carvacrol 12.8% (Chorianopoulos *et al.,* 2006).

4.2.4. *Salvia fruticosa*

Forty-five compounds were identified in *S. fruticosa* essential oil, accounting 99.76% of the whole essential oil. The yield of essential oil was 1.56% (v/w). The composition of essential oil and oil yield are listed in **Table 3.13.** It can be seen that oxygenated monoterpene was the main class represented by 64.89% of the total of oil. The high percentage of this class is due to the high percentage of 1,8-cineole (49.34%), camphor (7.53%) and α-terpineol (3.25%). Other major compounds belongs to monoterpene hydrocarbons such as β-pinene 7.38%, myrcene (7.38%), α-pinene (5.15%) and camphene (2.72%). β-caryophyllene (4.13%) belongs to sesquiterpene hydrocarbons. Other compounds are detected in a trace amount, less than 1%.

In our sample of *S. furticosa* oil, 1,8-cineole (49.34%) was the most abundant compound followed by camphor (7.53%), β-pinene 7.38%, myrcene (7.38%), and α-pinene (5.15%). These results are in full agreement with Kosar *et al.* (2005) who

reported that 1,8-cineole and camphor are dominated compounds of *S. furticosa* essential oil from Turkey. Pitarokili *et al.,* (2003) studied the essential oil composition of *S. furticosa* from Greece in 15 different locations noticeable variation in the amounts of the five main components 1,8-cineole, β-thujone, α-thujone, camphor, and β-caryophyllene. Also Al-kalaldeh *et al.,* (2010) reported that *S. furticosa* collected from Amman-Jordon dominated by 1,8-cineole as a major compound and Skoula *et al.* (2000) reported that 1,8-cineole the major constitute. However, Sivropoulou *et al.,* (1997) reported 1,8-cineole α- and β-thujone as main constitutes. We reported a small amount of β-thujone dose not exceed 0.25%. On the other hand, Longaray Delamare, *et al.* (2007) reported that α-thujone (20.1%) was the major compound in essential oil of *S. fruticosa* cultivated in south Brazil and Pierozan *et al.* (2009) found α-thujone as the main compound which is in contrast with our results.

According to literature data and our results, it is obvious that the essential oil composition of *S. furticosa* differ according the different geographic location. The variation in the essential oil compositions could be due to the several environmental conditions (climatic, seasonal, geographical) and genetic differences (Perry *et al*., 1999). Moreover, it has been reported that the oil quantitative composition is related to the plant habitat (Karousou *et al.,* 2005).

In most of the investigated on essential oil composition of *S. furticosa* 1,8-cineole was the main compound (Skoula *et al.,* 2000; Pitarokili *et al.,* 2003; Kosar *et al.,* 2005; Papageoriou *et al.,* 2008; Longaray Delamare *et al.,* 2007).

It's obvious, that all species under investigation are dominated by monoterpenes which represented (98.06%, 80.80%, 89.73% and 90.88%) for *Th. capiataus, Th. algeriensis, S. thymbra* and *S. fruticosa* respectively. Among monoterpenes the class oxygenated monoterpene was the most abundant with percentage 87.60% for *Th. capitatus*, 54.67% for *Th. algerinesis* and 64.89% for *S. fruticosa*. In the case of *S. thymbra* monoterpene hydrocarbons was major class represented 58.57% of the total oil.

4.3. Antioxidant activity

4.3.1. *Thymus capitatus*

The antioxidant activity of essential oil of *Th. capitatus* and its main compounds measured by using DPPH scavenging assay given in **Fig 3.2**. and **Table 3.4**., showed that the oil gained high activity with IC_{50} = 0.119 mg/ml of solution. The antioxidant activity of this species was better than thymol 0.403 mg/ml of solution, and quite similar to carvacrol (IC_{50} = 0.105 mg/ml of solution), however, it was slightly lower antioxidant activity than BHA (IC_{50} = 0.0717 mg/ml of solution).

The strong activity of this species could be due to high percentage of carvacrol (68.07%) and thymol (12.27%) **Table 3.1** which have been reported to have antioxidant activity (Hazzit *et al.,* 2009). However, Zouari *et al.* (2011) reported that essential oil of Tunisian *Thymus* possess high antioxidant activity even in the case of absent of thymol and caravcrol. Which mean that minor compounds could be responsible for the activity.

4.3.2. *Thymus algeriensis*

The results of DPPH scavenging assay of the *Thymus algeriensis*, its principal components and BHA are shown in **Fig 3.4** and **Table 3.8**. Our findings showed that the *Th. algeriensis* essential oil was able to reduce DPPH radicals into the DPPH-H form, and this activity was dose-dependent. The oil possessed a strong antioxidant activity with IC_{50} = 0.299 mg/ml of solution, and it was better than thymol 0.403 mg/ml and less than carvacrol (IC_{50} = 0.105 mg/ml of solution). That can be attributed to high percentage of oxygenated monoterepenes especially thymol and carvacrol (Hazzit *et al.,* 2009), which represent together 43.19% of the total oil. The oil also showed lower antioxidant activity than BHA. The DPPH scavenging activity of Algerian thyme species oils was claimed to be attributed to the attendance of phenolic compounds such as thymol or carvacrol (Hazzit *et al.,* 2009). The key role of phenolic compounds as scavengers of free radicals is also reported in earlier papers. On the contrary, it was reported that essential oil of Tunisan *Th. algeriensis* displayed

relatively high DPPH radical-scavenging activity, despite of absence of thymol and carvacrol (Zouari *et al.,* 2011). These results imply that non-phenolic contents could be also responsible for this activity. Nevertheless, it is hard to attribute the antioxidant activity to one or few active compounds of total essential oil since both slight and major constitute should make a significant contribution to the activity of oil (Wang *et al.,* 2008). Moreover, radical-scavenging activity is one of various mechanisms to contribute overall activity, thereby creating a synergistic effect (Sokmen *et al.,* 2004).

4.3.3. *Satureja thymbra*

Results of *S. thymbra* essential oil reported in **Fig 3.6. and Table 3.12.** The oil possessed strong antioxidant activity, as seen from the concentrations at which 50% radical scavenging occurred (IC_{50} = 0.0967 mg/ml of solution), being better than γ-terpinene and thymol and quite similar to BHA. It has been shown that the IC_{50} of *Origanum vulgare* essential oil, which was dominated by γ-terpinene and thymol (34.4% and 31.8%, respectively) was better than BHA (Galego *et al.,* 2008). Moreover, *O. vulgare* essential oil, containing 49% of γ-terpinene and 15% of thymol, showed an IC_{50} value quite similar to our results. Also, carvacrol, present in relative high amounts in *Thymbra capitata* oil can be partly responsible for high antioxidant activity, whereas in *O. vulgare* oil the activity may be attributed to two components, γ-terpinene and thymol (Albano *et al.,* 2012). According to earlier reports, some structural features, such as the presence of strongly activated methylene groups in the molecule, are probably the reason for antioxidant activity of monoterpene hydrocarbons (Ruberto *et al.,* 2000).

4.3.4. *Salvia furticosa*

Antioxidant activity was analyzed using the DPPH free radical scavenging method. The results of DPPH scavenging assay of *S. fruticosa* essential oil and synthetic antioxidant butylated hydroxyanisole (BHA) are shown in **Fig 3.8** and **Table 3.16**. The antioxidant activity of the *S. fruticosa* essential oil was lower than

the BHA (IC50 = 15.53 mg/ ml of solution). This could be attributed to the absence of phenolic compounds such as thymol and carvacrol. Papagergiou, *et al.,* (2008) reported that 1,8-cineole was not active. Also, Burits, *et al.* (2001) studied the antioxidant activity of 1,8-cineole and camphor and found that they did not exhibit strong antioxidant activity.

The activity of the essential oil of *S. fruticosa* could not be attributed to the main compounds nor to the other minor components, such as β-caryophyllene 4.13%, which has been reported to have antioxidant activity with IC_{50} = 18.6 g/L (Papagergiou *et al.,* 2008).

In conclusion, the results of DPPH scavenging assay of the species, their principal components and BHA are shown in **Table 4.1**. Our findings showed that, all species possessed a strong antioxidant activity except *S. fruticosa*, which showed very low antioxidant activity IC_{50} = 15.53 mg/ml of solution compared with other species and BHA. The high antioxidant activity of *Thymus* species and *S. thymbra* could be due to the high percentsge of phenolic compounds thymol and carvacrol (Hazzit *et al.,* 2009) and for γ-terpinene and thymol in the case of *S. thymbra* (Albano, 2012). The low antioxidant activity of this *S. furticosa* could be to due the nonappearance of phenolic compounds and the activity could be attribute to the minore constites such as β-caryophyllene, which has been reported to has antioxidant activity (Papagergiou *et al.,* 2008).

S. thymbra was the most active with IC_{50} = 0.0967 mg/ml of solution compared with *Th. algeriensis,* thymol and γ-terpinene (IC_{50} 0.2991, 0.403 mg/ml and 0.517 mg/ml of solution respectively) and quite similar to *Th. capitatus* and carvacrol IC50 = (0.119 mg/ml and 0.105 mg/ml respectively). BHA was more active than all essential oils with IC_{50} = 0.0707 mg/mL.

For *Thymus* species the high activity of these species could be related to high percentage of phenolic compounds. The DPPH scavenging activity of Algerian thyme species oils was claimed to be attributed to the attendance of phenolic compounds such as thymol or carvacrol and their synthesis (γ-terpinene and *p*-cymen) (Hazzit *et al.,* 2009), which represented in high percentage in the essential oils of these species

Table 4.1. The antioxidant activity of the oils tested and their main compounds comparison with BHA

Essential oil + compounds tested	IC_{50} mg/ml
Th. capitatus	0.1192
Th. algeriensis	0.2991
S. thymbra	0.0967
S. fruticosa	15.53
Thymol	0.403
Carvacrol	0.105
γ- terpinene	0.517
BHA	0.0717

(86.63% in *Th. capitatus*, 59.28% in *Th. algeriensis* oil and 75.70% for *S. thymbra* essential oil. The key role of phenolic compounds as scavengers of free radicals is also reported in earlier papers. On the contrary it was reported that Tunisan *Th. algeriensis* essential oil displayed relatively high DPPH radical activity, despite of absence of thymol and carvacrol, (Zouari *et al.,* 2011). These results imply that, non-phenolic contents could be also responsible for this activity. According to earlier reports, some structural features, such as the presence of strongly activated methylene groups in the molecule, are probably the reason for antioxidant activity of monoterpene hydrocarbons (Ruberto *et al.,* 2000).

Nevertheless, it is hard to attribute the antioxidant activity to one or few active compounds of total essential oil since both slight and major constitute should make a significant contribution to the activity of oil (Wang *et al.,* 2008). Moreover, radical-scavenging activity is one of various mechanisms to contribute overall activity, thereby creating a synergistic effect (Sokmen *et al.,* 2004).

4.4. Antimicrobial activity

4.4.1. *Thymus algeriensis*

4.4.1.1. Antibacterial activity

Results of antibacterial activity of essential oil of *Th. algeriensis* are presented in **Table 3.6**. Tested oil showed very strong antibacterial activity against all strains. It was effective at 0.001-0.05 mg/ml and bactericidal activity was achieved at 0.0025-0.05 mg/ml. It could be related to oxygenated monoterpenes and monoterpene hydrocarbons components (Cox *et al.,* 2000), which constitute together about 80.80% in the oil **Table 4.2.**

Table 4.2. The persntage of monoterpene hydrocarbons and oxygenated monoterpenes in the essential oils of different species

Species	Monoterpene hydrocarbons	Oxygenated monoterpenes	Total
Thymus capitatus	10.46	87.6	98.6
Thymus algeriensis	26.13	54.67	80.8
Satureja thymbra	58.57	31.21	89.78
Salvia. furticosa	25.89	64.89	90.78

Thymol exhibited high antibacterial activity with MIC at 0.01-0.1 mg/ml and MBC at 0.05-0.15 mg/ml. The oil tested showed higher antibacterial activity than thymol and carvacrol, which gained MIC at 0.0025-0.05 mg/ml and MBC at 0.005-0.1 mg/ml. It is obvious that essential oil possessed better antibacterial activity than compounds thymol and carvacrol which could be explained by synergistic activity of other compounds presented. Streptomycin showed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml. *Th. algeriensis* essential oil showed slightly lower antibacterial activity than streptomycin and better than thymol and carvacrol.

4.4.1.2. Antifungal activity

Results of antifungal activity of oil tested are presented in **Table 3.7**. As in the case of the antibacterial activity, the oil showed strong antifungal potential, with MIC 0.0005-0.025 mg/ml and MFC 0.001-0.05 mg/ml. *Th. algeriensis* oil again showed better potential than thymol which gained MIC at 0.01-0.05 mg/ml and MFC at 0.01-0.05 mg/ml, while carvacrol exhibited inhibitory activity at 0.0025-0.025 mg/ml and fungicidal activity at 0.005-0.05 mg/ml. Bifonazole exhibited much lower antifungal activity than oil tested. MIC was at 0.15-0.20 mg/ml and MFC at 0.20-0.25 mg/ml. Fungi were more sensitive than bacteria **Tables. 3.6** and **3.7.**

Comparing the previous data with the chemical composition of the oil, it becomes evident that there is a relationship between the high activity of the *Thymus* type oils and the presence of phenolic components, such as thymol and carvacrol. The high antimicrobial activity of this essential oil could be explained by the high percentage of phenolic components. It seems possible that phenolic components may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α- and β-glucanases of the fungus (Adams *et al,* 1996). Consequently, the high content of phenol components may account for the high antifungal activity of oils (Adam *et al,* 1998).

From our results it can be seen that essential oil of investigated species as well as individual phenolic monoterpenes carvacrol and thymol have very high antifungal activities, even higher than the commercial fungicide bifonazole.

According to obtained results it is obvious that the oil showed very good either antibacterial or antifungal activity. These results could be related to the high percentage of phenolic compounds, such as carvacrol and thymol (Giordiani *et al.,* 2008; Bouchra *et al.,* 2003; Daferera *et al.,* 2003; Sokovic *et al.,* 2002; Daouk *et al.,* 1996). Furthermore, it has been reported that the antifungal activity of *Th. pallescens* from Oued Rhiou or El-Asnam regions in Algeria attributed by their high content in thymol and carvacrol (Hazzit *et al.,* 2009). In fact, it is difficult to attribute the antimicrobial activity of *Th. algeriensis* essential oil, characterized by a complex mixture, to a single or particular constituent. Some studies have concluded that whole

essential oils have a greater antibacterial activity than the major components mixed (Gill *et al.,* 2002).

4.4.2. *Thymus capitatus*

4.4.2.1. Antibacterial activity

The findings of the antibacterial activity of *Th. capiataus* essential oil are presented in **Table 3.3**. It can be seen that, the showed the highest activity among the oils investigated with MIC ranged between 0.001-0.002 mg/ml and MBC = 0.001-0.04 mg/ml. It can be seen that tested oil showed higher antibacterial activity than thymol and carvacrol. Which mean that minor components, as well as a possible interaction between the substances could also affect the antimicrobial activity. In fact, other constituents, such as γ-terpinene, have been considered to display relatively good activity due to their possible synergistic or antagonistic effects (Vardar-Unlu *et al.,* 2003). As in the case of *Th. algeriensis* the high activity could be due the oxygenated monoterpenes and monoterpene hydrocarbons components (Cox *et al.,* 2000), which constitute together about 98.06% of the total oil **Table 4.2.** especially the two phenolic compounds thymol and carvacrol, which represent together (80.34%) of the total oil **Table 4.2**.

However, Streptomycin showed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml. and showed slightly higher antibacterial activity than *Th. capiataus* essential oil, *Th. capitatus* oil which showed similar bactericidal potential against *M. flavus* and *L. monocytogenes* **Tables. 3.6** and **3.2**.

Our results are in agreement with (Faleiro *et al.,* 2005; Bouzouita *et al.,* 2003). Furthermore, Figueiredo *et al*. (2008) reported that, thyme essential oils or some of their constituents are indeed effective against a large variety of fungi, particularly the oils with high amounts of thymol and/or carvacrol, such as those from *Th. zygis* subsp. *zygis* (thymol type), *Th. pulegioides* (thymol/carvacrol type) and *Thymbra*

Table 4.3. The sum of percentage of the main phenolic compounds (carvacrol and thymol) in the essential oils of plants tested.

Samples	Total of thymol and carvacrol %
Thymus capitatus	80.34
Thymus algeriensis	43.19
Satureja thymbra	29.34
Salvia furticosa	0

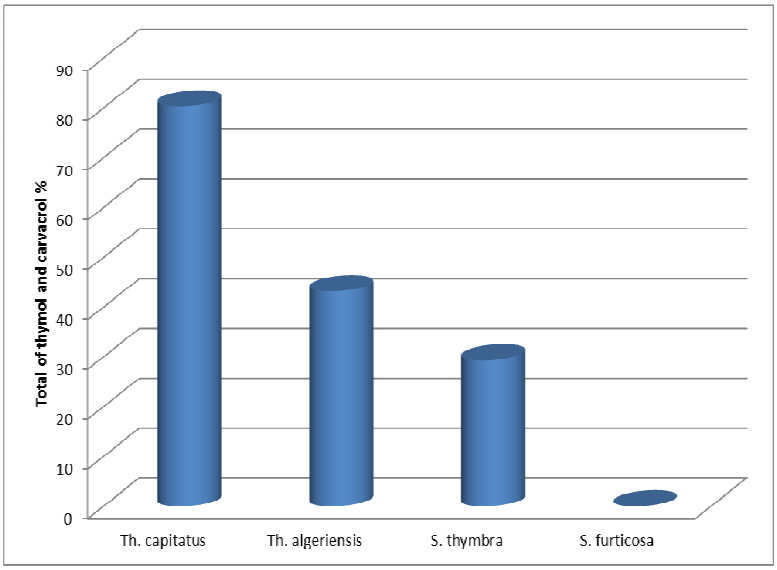


Fig 4.1. The sum of percentage of the main phenolic compounds (carvacrol and thymol) in the essential oils of plants tested.

capitata (carvacrol type) For these oils MIC values ranged from 0.08 to 0.64µL/mL, which are higher than our results (Figueiredo *et al.,* 2008). Nevertheless, it is reasonable to associate the activity of these oils with the presence of carvacrol and thymol.

The most of the species of *Thymus* genus have been studied for their antimicrobial activities against some bacteria and fungi, depending on thymol and its isomer carvacrol (Mohammed *et al.,* 2009; Talei *et al.,* 2007; Safaei-Ghomi *et al.,* 2009; Toroglu, 2007) and other species depending on thymol as major compounds (Hazzit *et al.,* 2009; Cavara *et al*., 2009; Mojab *et al*., 2008; Reddy *et al.,* 1998). Also it can be seen that our results showed that the oil was active more than its main compounds, these results are in contrast with Figueiredo *et al.* (2008) who suggested that carvacrol and thymol was more active than the essential oil of *Th. capitaus* (carvacrol chemotype) from Portugal.

4.4.2.2. Antifungal activity

Results of antifungal activity of oils tested are presented in **Table 3.3.** As in the case of the antibacterial activity the oils showed strong antifungal potential. Oil of *Th. capitatus* exhibited inhibitory effect at 0.0002-0.001 mg/ml and fungicidal effect at 0.002-0.025 mg/ml. *Th. capitatus* oil again showed better potential than its main compounds Thymol and Carvacrol (MIC and MFC = 0.01-0.05 mg/ml for thymol and MIC= 0.0025-0.025 mg/ml and fungicidal activity at 0.005-0.05 mg/ml). Bifonazole exhibited much lower antifungal activity than oils tested. MIC was at 0.15-0.20 mg/ml and MFC 2.0-2.5 mg/ml.

Essential oil of *Thymus* species showed even 10-100 times better antifungal activity **Table 3.6**. It seems possible that phenolic components which present in high concentration may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α- and β-glucanases of the fungus (Adams *et al.,* 1996). Therefore, the high content of phenol components may account for the high antifungal activity of oils (Adam *et al.,* 1998). From our results it can be seen that essential oils of investigated *Thymus* species as well as individual phenolic monoterpenes carvacrol and thymol have very high antifungal activities, even higher than the commercial fungicide bifonazole.

However, Cutler *et al*.,(1996) and Arras and Usai, (2001) have been reported that *Thymus* and *Origanum* like *Thymus capitatus*, *Thymus zygis* and *Origanum syriacum* displayed excellent antifungal activities. Our results were better than them.

In addition, *Th. capitatus* characterized by carvacrol (70.92%) showed a strong antifungal activity against all tested fungi (El Ajjouri *et al.,* 2008), which agrees with our results.

In the comprehension between our results with the chemical composition and previous studies, it becomes clear that, the main compounds of the oil are the main factor determining the activity of the oil, it can be seen that the high antimicrobial activity of the essential oil could be explained by the high percentage of phenol components. These results are in agree with the results published by Faleiro *et al.* (2005) who suggested that the essential oils of *Th. capitatus* was the most active against all strains, independently of their Gram staining and their morphology. The antimicrobial activity of thyme is possibly due to a phenolic constituent carvacrol 81.4%. Also, several authors have pointed to the antimicrobial activity of carvacrol (Curtis *et al.,* 1996; Kim *et al.,* 1995). Cosentino *et al.,* (1999) have compared the antimicrobial activity of *Th. capitatus* with 48.9% content of phenols with, *Th. herba-barona* content of phenols 67.5 % and he found that *Th. herba-barona* has high antimicrobial activity than *Th. capitaus* .Also, the relatedness between antimicrobial activity and phenolic compounds present in some plant essential oils was already observed when using other microorganisms (Baratta *et al*., 1998), in particular *Bacillus cereus* (Delgado *et al.,* 2004) and *Helicobacter pylori* (Chun *et al*., 2005). Furthermore, Arras and Usai (2001) demonstrated that the antifungal activity of *Th. capitatus* essential oil was due to carvacrol.

It's obvious from our findings that the oil of *Th. capiataus* obtained showed very strong antibacterial or antifungal activity. This activity could be attributing to presence of phonlic compounds, which have been reported to have excellent antimicrobial activity ((Jalsenjak *et al*., 1987; Sivropoulou *et al*., 1997; Tepe *et al*., 2005).

Overall, the oil and single compounds were more active aganist yeasts and Gram positive bacteria than Gram negatives, which have been reported in similar studies (Burt, 2004; Cosentino *et al.,* 1999; Smith-Palmer *et al.,* 1998; Farag *et al.,* 1989).

4.4.3. *Satureja thymbra*

4.4.3.1. Antibacterial activity

Antibacterial activity results of the tested essential oil are presented in **Table 3.10.** The oil was active against all the bacteria tested. *S. thymbra* essential oil showed bacteriostatic effects at 0.001–0.1 mg/ml and bactericidal ones at 0.002–0.2 mg/ml. Thymol exhibited high antibacterial activity with a minimum inhibitory concentration (MIC) at 0.01–0.1 mg/ml and minimum bactericidal concentration (MBC) at 0.05–0.15 mg/ml, while carvacrol showed stronger bacteriostatic activity at 0.0025–0.05 mg/ml and bactericidal activity at 0.005–0.1 mg/ml. Inhibitory and bactericidal concentrations for γ-terpinene were slightly higher than for previous compounds (MIC at 0.05–0.2 mg/ml, and MBC at 0.07–0.3 mg/ml). Streptomycin expressed inhibitory effects at 0.0005–0.001 mg/ml and bactericidal activity at 0.0005–0.002 mg/ml. It can be seen that the essential oil showed the highest bactericidal activity (MBC = 0.002 mg/ml) against *Micrococcus flavus* and the lowest (MBC = 0.2 mg/ml) against *Listeria monocytogenes*. However, *S. thymbra* essential oil showed lower antibacterial activity than streptomycin, and was quite similar to thymol (MIC = 0.01–0.1 mg/ml and MBC= 0.05–0.15 mg/ml) and slightly lower or similar to carvacrol (MIC = 0.0025–0.05 mg/ml and MBC = 0.005–0.1 mg/ml), and it was stronger or similar to γ-terpinene (MIC at 0.05–0.2 mg/ml, and MBC at 0.07–0.3 mg/ml).

4.4.3.2. Antifungal activity

Results of antifungal activity of compounds tested are presented in **Table 3.** As in the case of the antibacterial activity, the oils showed strong antifungal potential. *S. thymbra* oil possessed strong activity with (MIC) 0.001–0.025 mg/ml and showed fungicidal effects at 0.001–0.1 mg/ml. MIC and the MFC for thymol are 0.01–0.05 mg/ml, while carvacrol showed MIC at 0.0025–0.025 mg/ml and MFC at 0.005–0.05 mg/ml. MIC and (MFC) for γ-terpinene were at 0.015–0.05 mg/mL and 0.02–0.1 mg/ml. Bifonazole showed lower antifungal activity than the tested oils. MIC was at 0.15–0.20 mg/ml and MFC 0.20–0.25 mg/ml. *Penicillium ochrochloron* was the most

sensitive fungus, with MBC at 0.001 mg/ml, while *Candida albicans* was the most resistant species when treated with this oil. Fungi were in general more sensitive than bacterial species **Tables. 3.10** and **3.11**. In general, *S. thymbra* essential oil showed antifungal activity similar or lower than thymol (MIC and MFC 0.01–0.05 mg/ml) and lower than carvacrol (MIC at 0.0025–0.025 and MFC at 0.005–0.05 mg/ml), and it was slightly better or similar to γ-terpinene (0.015–0.05 mg/ml and 0.02–0.1 mg/ml).

Previous investigations showed that the essential oil of *S. thymbra* was found to be active against the bacteria *E. coli, P. aeruginosa, S. typhimurium, S. sonnei* and *S. aureus* and the yeast *C. albicans* (Gören *et al.,* 2004). *S. thymbra* essential oil from Greece possessed very good antifungal properties with low MIC (0.1–1.0 μl/ml) and MFC (0.2–2.0 μl/ml) values (Sokovic *et al.,* 2002). The antifungal activity results of *S. thymbra* oil against *M. perniciosa,* a contaminator of *Agaricus bisporus,* obtained by the micro atmosphere method, showed MIC of 0.001–0.05 μl/ml and MFC of 0.1–0.25 μl/ml (Glamočlija *et al.,* 2006). More recently, it has been shown that the oil of *S. thymbra* exhibited minimum inhibitory concentration (MIC) in the range of 0.6–5.0 μg/ml, and minimum bactericidal concentration (MBC) in the range of 2.5–10.0 μg/ml, while fungi were more sensitive with MIC and MFC at 1.25–2.5 μg/ml, and fungicidal activity in range of 2.5–5.0 μg/ml (Marković, *et al,* 2011).

It should be noted that the antimicrobial activity of *S. thymbra* against the pathogenic bacteria and fungi was lower (or equal) as compared to the essential oils compounds tested here thymol and carvacrol. Presumably, this activity is not derived only from the presence of these phenols, but part of the activity resulted from the effect of minor active constituents.

4.4.4. *Salvia furticosa*

4.4.4.1. Antibacterial activity

Findings of antibacterial activity of essential oil and its main compounds tested are presented in **Table 3.14.** The oil tested showed antibacterial activity against all the species tested. Essential oil from *S. fruticosa* possessed antibacterial activity,

and showed minimal inhibitory concentration MICt at 0.125-1.5 mg/ml and minimal bactericidal concentration (MBC) at 0.5-2.0 mg/ml. The monoterpenic hydrocarbon β-pinene showed antibacterial activity with MIC of 0.05-0.10 mg/ml and MBC of 0.05-0.13 mg/ml. Camphor exhibited inhibitory activity at 0.05-0.07 mg/ml and bactericidal effect was at range 0.06-0.10 mg/ml. The best activity among components and essential oil tested, it was achieved for 1,8-cineole (bacteriostatic activity at 0.0-0.07 mg/ml and bactericidal at 0.05-0.09 mg/ml). Streptomycin showed inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml. The oil tested showed lower antibacterial activity than all tested compounds and streptomycin. It can be seen that the most sensitive bacteria to essential oil was *Pseudomonas aeruginosa* (MBC = 0.25 mg/ml) and lowest bactericidal activity (MBC=2.0 mg/ml) against *Escherichia coli.* It is obvious that, among the components tested, hydrocarbon monoterpenes show the lowest antibacterial activity, while oxygenated compounds possess a higher potential.

4.4.4.2. Antifungal activity

Results of antifungal activity of essential oil of *S. fruticosa* and the main compounds tested are presented in **Table 3.15.** As in the case of the antibacterial activity, the oil showed antifungal potential. *S. fruticosa* oil possessed good activity with MIC 0.125-1.0 mg/ml and showed minimal fungicidal concentration (MFC) at 0.125-1.5 mg/ml; β-pinene ishowed MIC at 0.05-0.07 mg/ml and fungicidal at 0.05-0.11 mg/ml, while camphor showed MIC at 0.04-0.06 mg/ml and MFC at 0.04-0.10 mg/ml. The best activity is obtained for 1,8-cineole (MIC at 0.03-0.06 mg/ml and MFC at 0.04-0.09 mg/ml). Antifungal potential could be presented as follows: essential oil < β-pinene < camphor < 1,8-cineole. Bifonazole showed lowest antifungal activity MIC was at 0.15-0.20 mg/ml and MFC 0.20-0.25 mg/ml and it can be seen that antifungal capacity is the same as in the case of antibacterial activity. The most sensitive fungus to the essential oil was *Aspergillus flavus* with MFC = 0.125 mg/ml and the most resistant species was *A. niger*. However, *S. fruticosa* essential oil

has antifungal activity lower than its main compounds. It showed better activity than the Bifonazole.

The effectiveness of the essential oil of *S. fruticosa* (MIC and MBC), against susceptible bacteria was higher than that previously reported for this species (Sivropoulou *et al.,* 1997; Longaray Delamare *et al.,* 2007), and for *Salvia, S. pratensis, S. glutinosa,* and *S. aethiopis* (Velickovic *et al.,* 2002), *S. tomentosa* (Tepe *et al*., 2005). 1,8-cineole and camphor, three monoterpenes with well documented antibacterial and antifungal potential (Jalsenjak *et al.,* 1987; Sivropoulou *et al.,* 1997; Sur, *et al.,* 1991). The stronger activity of the essential oil of *S. fruticosa (=S. triloba*) against almost all the susceptible bacteria may be due to the presence of a high concentration of β-caryophyllene, since the antimicrobial properties of caryophyllene and caryophyllene oxide (Azaz, *et al.,* 2002). Other than the major compounds, α-pinene and borneol, as well as other minor constituents of the essential oil of *S. officinalis* and *S. triloba* have antimicrobial activity (Dorman and Deans, 2000). Also the essential oil of *Artemisia afra* Jacq., which has a qualitative composition similar to that of *S. fructicosa* (α- and *β*-thujone, 52%; 1,8-cineole, 13%; and camphor, 15%), showed moderate antimicrobial and antifungal activities (Graven *et al.,* 1992) comparable in magnitude to these reported here for *S. fructicosa*. In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity.

Comparing the results of all species investigated, it can be seen that the most active oils against all bacteria strains and fungi was the oil obtained from *Th. capitaus* , which was even better than main compounds and very close to carvacrol. However, other species also showed high activity against all bacteria strains and fungi except *S. furticosa* which showed moderate antimicrobial activity compared to other species.

The high activity antimicrobial activity of these essential oils could be explained by the high percentage due the oxygenated monoterpenes and monoterpene hydrocarbons components (Cox *et al.,* 2000), which constitute together about 98.06% in *Th. capitatus,* 80.8% *in Th. algeriensis,* 89.78% in *S. thymbra and* 90.87% *in S.*

furticosa **Table 4.2** and **Fig 4.1**, especially the two phenolic compounds thymol and carvacrol. From the results it can be suggested that the difference of antimicrobial activity among the species could be due to the difference of presence of these compound in the essential oils, it can be seen that the best activity was obtained from *Th. capitaus* oil which has the highest amount of these compounds followed by *Th. algeriensis* then *S. thymbra* and finely *S. furticosa*. Also even in the oil which has higher percentage of oxygenated monoterpenes and monoterpene hydrocarbons components, the percentage (thymol and crvacrol) determined the activity of the oil and that could be noted when you compare *S. thymbra*, *Th. algeriensis* and *S. furticosa*, it can be seen that *S. furticosa* essential oil has the highest percentage of oxygenated monoterpenes and monoterpene hydrocarbons components 90.87%, but it has less activity. This could be because of the absence of phenolic compounds, while, *Th. algeriensis* which has lowest percentage of these classes, but higher percentage of phenolic compounds, has the highest activity among them. This results are in agreement with some authors, who reported that, oils of *Myrtus communis* and *Laurus nobilis* oils which are completely devoid of phenols and characterized by very high concentrations of 1,8-cineole (61.0% and 42.3%, respectively), which suggests that oils with high 1,8-cineole levels are less active, when compared with *Th. capitaus* which is rich in carvacrol 81% (Biondi *et al*., 1993; Carson *et al*., 1995a; Akgül, *et al.,* 1998).

However, the organisms tested were sensitive to all essential oils, the most sensitive bacteria to all the oils was *Micrococcus flavus* with MBC ranged between 0.001mg/ml in *Th. capiataus* to 0.5 mg/ml in the case of *S. fruticosa.*

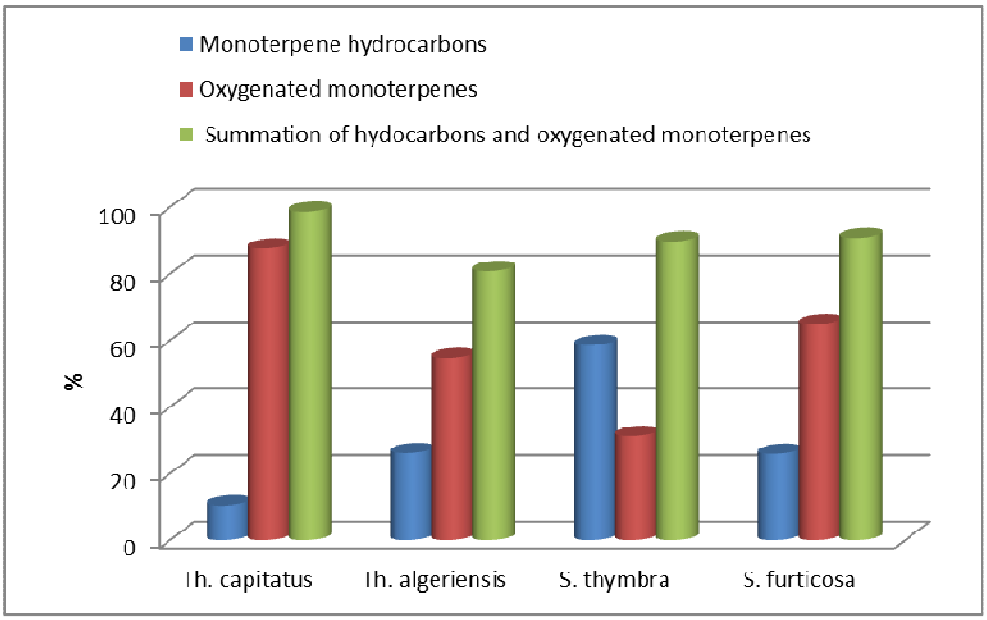


Fig 4.2. The percentage of total hydrocarbons and oxygenated monoterepens % in the total oils in diferent species.

In general, Gram-positive bacteria were more sensitive than Gram-negative species, these results are in accordance with earlier published data (Longaray Dealamare *et al.,* 2007; Marino *et al.,* 2001). This resistance of Gram-negative bacteria could be attributed to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds (Nikaido and Vaara, 1985). In contrast, the absence of this wall in Gram-positive bacteria allow to the essential oil and hydrophobic constituents to be in direct contact with phospholipid bilayer of the cell membrane, where they bring about their effect, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Cowan *et al.,* 1999; Wendakoon and Sakaguchi, 1995). Overall, fungi were more sensitive than bacterial strains for all oils tested.

4.5. Conclusions

1. The selected plants species investigated in this thesis (*Thymus capitatus, Thymus algeriensis, Satureja thymbra* and *Salvia fruticosa*) are popular culinary herbs, and their essential oils have been used extensively for many years in food products, perfumery, and pharmaceutical purposes for dental and oral products. Taken together, our results suggest that the essential oils of species investigated in this thesis are rich in essential oil. Essential oil of *Th. capitatus* and *Satureja thymbra* were reported to have the highest oil yield 4.97 % (v/w).
2. Regarding the chemical composition the oxygenated monoterpene class was the most abundant in most cases, with percentage 87.60% for *Thymus capitatus*, 54.67% for *Th. algerinesis* and 64.89% for *Salvia fruticosa*. In the case of *Satureja thymbra* monoterpene hydrocarbons were the major class represented by 58.57% of the total oil.
3. Phenol compounds such as thymol and carvacrol were the main compounds of the essential oils, which could be responsible for the highest activity of these oils, except in the case of *Salvia fruticosa* essential oil, which possessed 1,8-cineole (49.34%) as the major compound.
4. Our study is the first report of *in vitro* antioxidant activity of the essential oils of some Libyan samples of the investigated species (*Thymus capitatus, Th. algeriensis, Satureja thymbra* and *Salvia fruticosa*). The oils obtained from these species showed strong free radical scavenging activity with exception of *S. fruticosa* oil, which showed moderate activity. All samples could be considered to be a natural source of strong antioxidant substances for use as a natural additive in food and pharmaceutical industries.

5. Screening the antimicrobial activity of the oils obtained. The essential oils showed very strong activity against all microorganisms tested. All oils were active against tested microorganisms. *Th. capitatus* was the most active oil against bacterial and fungi.
6. The results oils and components obtained in this work showed higher antibacterial activity against Gram (+) than towards Gram (−) bacteria. Gram negative bacteria are in general, more resistant than Gram positive ones. Overall, the oils and single compounds were more active against fungi and Gram positive bacteria than Gram negative bacterial species.
7. As is evident from the presented data, this study provides useful information about the composition, antioxidant and antimicrobial activities of essential oils from selected wild-growing Lamiaceae species from Libya.
8. The utilization of indigenously grown Lamiaceae species as potential source of natural antioxidants and antimicrobial agents, as well as food preservating and flavoring agents ought to be encouraged. However, future studies under the *in vivo* conditions are recommended to further elaborate the antimicrobial and antioxidant actions of Lamiaceae essential oils for various potential therapeutic applications.

References

Aboutabl, E.A., and El-Dahmy, S. (1985) Chemical composition and antimicrobial activity of essential oil of *Thymus algeriensis* Boiss., *Bulletin of Faculty of Pharmacy, Cairo University* 33, 87-90.

Abu-Darwish, M. S., Alu'datt, M. H., Al-Tawaha, A. R., Ereifej, K., Almajwal, A., Odat, N., and Al Khateeb, W. (2012) Seasonal variation in essential oil yield and composition from Thymus vulgaris L. during different growth stages in the south of Jordan, *Natural. Product Research* 26, 1310-1317.

Ackerman, D. (2001) In the begning was smell, In: Hydrosols: The Next Aromatherapy Edited by Suzanne Catty. Published by Inner Traditions / Bear & Company, P 27.

Adam, K., Sivropoulu, A., Kokkini, S., Lanaras, T., and Arsenakis, M. (1998) Antifungal activities of Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia and Salvia fruticosa essential oils against human pathogenic fungi, *Journal of Agriculture and. Food Chemistry* 46, 1739-1745.

Adams R.P. (2007) Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, (4^{ed}) Allured Publ. Corp., Carol Stream, IL.

Adams, S., Kunz, B., and Weidenbörner, M. (1996) Mycelial deformations of Cladosporium herbarum due to the application of eugenol and carvacrol, *Essential Oil Research* 8, 535-540.

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

Ahmad, M. M., Salim-ur-Rehman, Z., Iqbal, F., Anjum, M., and Sultan, J. I. (2006) Genetic variability to essential oil composition in four citrus fruit species, *Pakistan Journal of Botany* 319-324.

Akgül, A., and Kivanç, M. (1988) Inhibitory effects of selected Turkish spices and oregano components on some foodborne fungi, *International Journal of Food Microbiology* 6, 263- 268.

Albano, S.M., Lima, A.S., Miguel, M.G., Pedro, L.G., Barroso, J.G., and Figueiredo, A.C. (2012) Antioxidant, anti-5-lipoxygenase and antiacetyl cholinesterase activities of essential oils and decoction waters of some aromatic plants, *Records of Natural Products* 6, 35–48.

Ali, I. B. E., Zaouali, A.B., Bejaoui, A., and Mohamed, B. (2010) Variation of the chemical composition of Essential oils in Tunisia Population of *Thyums algeriensis* Boiss. et Reut. (Lamiacese) and Implication for conservation, *Chemistry and Biodiveristy,* 7, 1276-1289.

Ali, M. S., Saleem, M., Ali, Z., and Ahmad, V. U. (2000) Chemistry of Zataria multifora (Lamiaceae), *Journal of Phytochemistry* 55, 933-936.

Aliaga, C., and Lissi, A. E. (2004) Comparison of the free radical scavenger activities of quercetin and rutin – An experimental and theoretical study, *Canadian Journal of Chemistry* 82, 1668–1673.

Ali-Shtayeh, M. S., Yaniv, Z., and Mahajna, J. (2000) Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants, *Journal of Ethnopharmacol* 73, 221–232.

Al-Kalaldeh, J. Z., Abu-Dahab, R., and Afifi, F. U. (2010) Volatile oil composition and antiproliferative activity of *Laurus nobilis, Origanum syriacum, Origanum*

vulgare, and *Salvia triloba* against human breast adenocarcinoma cells, *Nutrition Research* 30, 271-278.

Al-Kathe, A. A., and Al-Ramah, S. M. (1997) Usages of Some Plants in Libyan Folk-Medicine, Part 1, (2ed). Beirut, Apophthegm House Press, pp. 59, 64, 72, 75, 80, 105, 121.

Angioni, A., Barra, A., Coroneo, V., Dessi, S., and Cabras, P. (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers, *Journal of Agriculture and Food Chemistry* 54, 4364–4370.

Anwar, F., Hussain, A. I., Sherazi, S. T. H., and Bhanger, I. M. (2009) Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (Foeniculum vulgare Mill.) fruit at different stages of maturity, *Journal of Herbs, Spices and Medicinals Plants* 15, 1-16.

Arras, G. and Usai, M. (2001) Fungitoxic activity of 12 essential oils against four post-harvest citrus pathogens: Chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions, *Journal of Food Protection* 64, 1025-1029.

Aşkun, T., Başer, K. H. C., Tümen, G., and Kürkçüoğlu, M. (2010) Characterization of essential oils of some *Salvia* species and their antimycobacterial activities, *Turkish Journal of Biology* 34, 89-95.

Ayvaz, A., Sagdic, O., Karaborklu, S., and Ozturk, I. (2010) Insecticidal activity of the essential oils from different plants against three stored-product insects, *Journal of Insects Science* 10, 1–13.

Azaz, D., Demirci, F., Satil, F., Kurkc¸uoglu, M., Baser, K. H. C. (2002) Antimicrobial activity of some *Satureja* essential oils, *Zeitschrift fur Naturforschung* 57c, 817–821.

Azzouz, M. A. and Bullerman, L. B. (1982) Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents, *Journal of Food Protection* 45,1298-1301.

Badi, H. N., Yazdani, D., ALI, S. M., and Nazari, F. (2004) Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L., *Industrial Crops and Products* 19, 231-236.

Baker, G. R., Lowe, R. F., Southwell, I. A. (2000) Comparison of oil recovered from tea tree by ethanol extraction and steam distillation *Journal of Agricultural Food Chemistry* 48, 4041-4043.

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

Ball, P.W., and Getliffe, F.M. (1972) Satureja L: In Flora Europaea; Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A., (ed.) Cambridge University Press: London, UK, Vol 3, P. 163–165.

Balunas, M., and Kinghorn, A. (2005) Drug discovery from medicinal plants. Life Science Journal, 78, 431-41.

Baratta, M. T., Dorman, H. J. D., Deans, S. G., Figueiredo, A. C., Barroso, J. G., and Ruberto, G.(1998) Antimicrobial and antioxidant properties of some commercial essential oils, *Flavour and Fragrance Journal* 13, 235-244.

Basaif, A.S. (2004). Chemical constituents of *Salvia aegyptiaca*, *Journal of King Abdulaziz University: Science* 16, 33-39.

Bassole, I. H. N., Ouattara, A. S., Nebie, R., Ouattara, C. A. T., Kabore, Z. I., and Traore, S. A. (2003) Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso, *Journal of Phytochemistry* 62, 209-212.

Bauer, K., Garbe, D., and Surburg, H. (2001) Common Fragrance and Flavor Materials: Preparation, Properties and Uses. Wiley-VCH, Weinheim, p. 293.

Belaqziz R., Harrak R., and Romane, A. (2010) Antimicrobial and Insecticidal Activities of the Endemic *Thymus broussonetti* Boiss. and *Thymus maroccanus* Ball., *Records of Natural Products* 4, 230-237.

Benjilali B., Hammouni M., and Richard H. (1987) Chemical polymorphism of Moroccan thyme essential oils: compounds characterization, *Sciences des Aliments* **7**, 77-91.

Benzie I. E. F. and Strain J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, *Analytical Biochemistry* 239, 70-76.

Bezić, N., Šamanić, I., Dunkić, V., Besendorfer, V., and Puizina, J. (2009) Essential Oil Composition and Internal Transcribed Spacer (ITS) Sequence Variability of Four South-Croatian Satureja Species (Lamiaceae), *Molecules* 14, 925-938.

Bishop, C. D. (1995) Antiviral activity of the essential oil of *Melaleuca alternifolia* (Maiden and Betche) Cheel (tea tree) against tobacco mosaic virus, *Journal of Essential Oil Research* 7, 641-644.

Blois M.S., (1985) Antioxidant determinations by the use of a stable free radical, *Nature* 181, 1199–1200.

Bolous, L. (2002) Flora of Egypt Vol. (3) printed al hadara publishing Cai ro, Egypt. P. 13, 34.

Bonnardeaux, J. (1992) The effect of different harvesting methods on the yield and quality of basil oil in the Ord River irrigation area, *Journal of Essential Oil Research* 4, 65-69.

Booth C. (1971) Fungal Culture Media. In: Norris, J. R., Ribbons D. W. (ed.), Methods in Microbiology, IV, 49 - 94. Academic Press, London and New York.

Bouchra C., Achouri M., Idrissi Hassani L.M., and Hmamouchi M.(2003) Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against Botrytis cinerea Pers: Fr., *Journal of Ethanoharmacol* 89, 165-169.

Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G., and Pedro, L.G. (2007) Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link., *Food Chemistry* 105, 146–155.

Bouzouita, N., Kachouri, F., Hamdi, M., and Chaabouni, M. M. (2003) Antimicrobial activity of essential oils from Tunisian aromatic plants, *Flavour and Fragrance Journal* 18, 380–383.

Boyle, W. (1955) Spices and essential oils as preservatives, *The American Perfumer and Essential Oil Review* 66, 25-28.

Burits, M., Asres, K., Bucar, F. (2001). The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperus procera, *Phytotherapy Research* 15, 103-108.

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

Burt, S. A., and Reinders, R. D. (2003) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7, *Letters in Applied Microbiology* 36, 162-167.

Cai, Y.Z., Sun, M., Xing, J., Luo, Q., and Corke, H. (2006) Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants, *Life Science* 78, 2872-2888.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., and Ferret, W.(2007) A.Invited Review: Essential Oils as Modifiers of Rumen Microbial Fermentation, *Journal of Dairy Science* 90, 2580–2595**.**

Cantino, P. D., Harley, R. M., and Wagstaff, S. J. (1992) General of Labiatae: Status and classification: in Advances in Labiate scince, R. M. Harley and T. Reynolds (ed) Royal botanic Cardens, Kew, England. P. 511-522.

Cantore, P. L., Iacobellis, N. S., Marco, A. D., Capasso, F., and Senatore, F. (2004) Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller Var. *vulgare* (Miller) essential oils. *Journal of Agriculture and Food Chemistry,* 52, 7862-7866.

Capone, W., Mascia, C., Spanedda, L., and Chiappini, M.(1989) Chemical composition and antibacterial activity of the essential oil from sardinian *Satureja thymbra*, *Fitoterapia* 60, 90–92.

Carmona, M. D., Llorach, R., Obon, C., and Rivera, D. (2005) "Zahraa", a Unani multicomponent herbal tea widely consumed in Syria: components of drug mixtures and alleged medicinal properties, *Journal of Ethnopharmacol* 102, 344–350.

Carson, C. F. and Riley, T. V. (2003) Non-antibiotic therapies for infectious diseases, *Communicable Disease Intelligence* 27, 143–146.

Carson, C. F., and Riley, T. V. (1995a) Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*, *Journal of Applied Bacteriology* 78,264-269.

Carson, C. F., Cookson, B. D., Farrelly, H. D., and Riley, T. V. (1995b) Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia*, *Journal of Antimicrobial Chemotherapy* 35, 421-424.

Catalan, C. A. N., and DE Lampasona, M. E. P. (2002) The chemistry of the genus Lippia (Verbenaceae). In: Kintzios, S.E. (ed.) Oregano: The genera Origanum and Lippia. 1st ed. London.

Cavara S., Maksimovic, M., Vidic, D. (2009). The essential oil of *Thymus aureopunctatus* (Beck) K. Malý, *Natural Product Communications* 4, 415-420.

Celiktas, O. Y., Hames Kocabas, E.E., Bedir, E., Vardar Sukan F., Ozek T. C., and Baser, K.H.C. (2007) Antimicrobial activities of methanol extracts and

essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations, *Food Chemistry* 100, 553–559.

Chaibi, A., Ababouch, L. H., Belasri, K., Boucetta, S., and Busta, F. F. (1997) Inhibition of germination and vegetative growth of *Bacillus cereus* T and *Clostridium botulinum* 62A spores by essential oils, *Food Microbiology* 14, 161-174.

Chalchat, J. C., Garry, R. P., and Muhayimana, A. (1995) Essential oil of Tagetes minuta from Rwanda and France: chemical composition according to harvesting location, growth stage and part of plant extracted, *Journal of Essential Oil Research* 7, 375-386.

Charles, D. J. and Simon, J. E. (1990) Comparison of extraction methods for the rapid determination of essential oil content and composition, *Journal of the American Society for Horticultural Science* 115, 458-462.

Choi, Y., Jeong, H. S., and Lee, J. (2007) Antioxidant activity of methanolic extracts from some grains consumed in Korea, *Food Chemistry* 103, 130-138.

Chorianopoulos, N., Evergetis, E., Mallouchos, A., Kalpoutzakis, E., Nychas, G.-J., and Haroutounian, S. A. (2006) Characterization of the essential oil volatiles of *Satureja thymbra* and *Satureja parnassica*: Influence of harvesting time and antimicrobial activity, *Journal of Agriculture and Food Chemistry* 54, 3139–3145.

Chun, S.S., Vattem, D. A., Lin, Y.T., and Shety, K. (2005) Phenolic antioxidants from clonal oregano (*Origanum Vulgare*) with antimicrobial activity against *Helicobacter pylori*, *Process Biochem Journal* 40, 809-816.

Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Totté, J., Pieters, L. and Vlietinck, A. J. (2002) Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo, *Journal of Ethnopharmacology* 79, 213-220.

Clark, R. J. and menary, R. C. (1979) The importance of harvest date and plant density on the yield and quality of Tasmanian peppermint oil, *Journal of the American Society for Horticultural Science* 104, 702-706.

Conner, D. E. and Beuchat, L. R. (1984) Effects of essential oils from plants on growth of food spoilage yeasts, *Journal of Food Science* 49, 429-434.

Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E., and Palmas, F. (1999) In vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils, *Letters in Applied Microbiology* 29, 130-135.

Cowan, M. M. (1999). Plant products as antimicrobial agents*, Journal of Clinical Microbiology Reviews* 12, 564–582.

Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R., and Wyllie, S. G. (2000) The mode of antimicrobial action of essential oil of *Melaleuca alternifola* (tea tree oil),m *Journal of Applied Microbiology* 88, 170-175.

Cremieux, A., Guirard-Dauriac, H., and Dumenil, G. (1981) Neutralisation des ntiseptiques et désinfectants, *Journal de Pharmacie de Belgique* 36, 223-226.

Cressy, H. K., Jerrett, A. R., Osborne, C. M., and Bremer, P. J. (2003) A novel method for the reduction of numbers of Listeria monocytogenes cells by

freezing in combination with an essential oil in bacteriological media, *Journal of Food Protection* 66, 390-395.

Crosthwaite, D., (1998) UK trade within the flavour and fragrance industry. International Federation of Essential Oils and Aroma Trades—21st International Conference on Essential Oils and Aroma's. IFEAT, London, P. 6 –12.

Curtis, C.F., Myamba, J., and Wilkes, T.J. (1996) Comparison of different insecticides and fabrics for anti-mosquito bed nets and curtains, *Medical and Veterinary Entomology 10*, *1-11*.

Cutler, H.G., Hill, R.A., Ward, B.G., Rohitha, B.H., and Stewart, A. (1996) Antimicrobial, insecticidal and medicinal properties of natural products. Flavours and fragrances In: Takeoka, G.R., Teranishi, R., Williams, P.J., Kobayashi, A. (ed.) Biotechnologies for Improved Foods and Flavours. American Chemical Society, P. 51–66.

Daferera D.J., Ziagos B.N., and Polissiou M.G. (2003) The effectiveness of plantessential oils on the growth of Botrytis cinerea, *Fusarium sp*. and *Clavibacter michiganensis* subsp. michiganensis, *Journal of Crop Protection* 22, 39-44.

Dandlen A., Lima A.S., Mendes M.D., Miguel M.G., Leonor Faleiro M., and Sousa M.J. (2010) Antioxidant activity of six Portuguese thyme species essential oils, *Flavour and Fragrance Journal* 25, 150–155.

Daouk, K. D., Dagher, M. S., Sattout, J. E. (1996) Antifungal activity of the essential oil of *Origanum syriacum* L., *Journal of. Food Protection* 58, 1147-1149.

Darias, V., Bravo, L., Rabanal, R., Sanchez-Mateo, C. C., and Martin-Herrera, D. A. (1990) Cytostatic and antibacterial activity of some compounds isolated from several Lamiaceae species from the Canary Islands, *Planta Medica* 56, 70-72.

Davidson, P. M. (1997) Chemical preservatives and natural antimicrobial compounds: In M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington. P. 520-556

Davidson, P. M., and Parish M. E. (1989) Methods for testing the efficacy of food antimicrobials, *Food Technology* 43, 148–155.

Davidson, P. M., Harrison. M. A. (2002) Resistance and adaptation to food antimicrobials, sanitizers, and other process controls, *Food Technology* 56, 69–78.

Deans, S. G. and Ritchie, G. (1987) Antibacterial properties of plant essential oils, *International Journal of Food Microbiology* 5,165-180.

Deans, S. G., Simpson, E., Noble, R. C., MacPherson, A., and Penzes, L. (1993) Natural antioxidants from *Thymus vulgaris* (thyme) volatile oil: the beneficial effects upon mammalian lipid metabolism, *Acta Horticulturae* 332, 177-182.

Delaquis, P. J., Stanich, K., Girard, B., and Mazza, G. (2002) Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils, *International Journal of Food Microbiology* 74, 101-109.

Delgado, B., Palop, A., Ferna´ndez, P. S., and Periago, P. M. (2004) Combined effect of thymol and cymene to control the growth of *Bacilllus cereus* vegetative cells, *European Food Research and Technology* 218, 188-193.

Denyer, S. P., and Hugo, W. B. (1991) Biocide-induced damage to the bacterial cytoplasmic membrane, p. 171-188. In S. P. Denyer and W. B. Hugo (ed.), Mechanisms of action of chemical biocides. The Society for Applied Bacteriology, Technical Series No 27. Oxford Blackwell Scientific Publication, Oxford.

Descalzo, A. M., and Sancho, A. M. (2008) A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentine, *Meat Science* 79, 423-436.

Dimandja, J. M., Stanfill S., Grainger, J., and Patterson, D. J. (2000) Application of comprehensive two-dimensional gas chro-matography (GC/GC) to the qualitative ana-lysis of essential oils, *Journal of High Resolution Chromatography* 23, 208-214.

Donelian, A., Carlson, L. H. C., Lopes, T. J., and Machado, R. A. F. (2009) Comparison of extraction of patchouli (Pogostemon cablin) essential oil with supercritical CO2 and by steam distillation, *The Journal of Supercritical Fluids* 48, 15-20.

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

Elgayyar, M., Draughon, F. A., Golden, D. A., and Mount, J. R. (2001) Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms, *Journal of Food Protection 64*, 1019-1024.

Elless, M. P., Blaylock, M. J., Huang, J. W., and Gussman, C. D. (2000) Plants as a natural source of concentrated mineral nutritional supplements. *Food Chemistry*, 71, 181-188.

Espinel-Ingroff, A.(2001) Comparisons of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi, *Journal of Clinical Microbiolology* 39, 1360-1367.

Essawi, T., and Srour, M. (2000) Screening of some Palestinian medicinal plants for antibacterial activity, *Journal of Ethnopharmacol* 3, 343-349.

European Pharmacopoeia (2004) European Directorate for the Quality of Medicines, (5 ed), Council of Europe —, Strasbourg, Cedex, France.

Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., and Teixeira, A. (2005) Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L., *Journal of Agriculture and Food Chemistry* 253, 8162-8168.

Fan, J., Ding, X., and Gu, W. (2007) Radical-scavenging proanthocyanidins from sea buckthorn seed, *Food Chemistry* 102: 168-177.

Farag, R. S., Daw, Z.Y., Hewedi, F. M., and El-Baroty, G .S .A. (1989) Antimicrobial activity of some Egyptian spice essential oils, *Journal of Food Protection* 52, 665–667.

Figueiredo, C., Barroso, J. G., Pedro, L. G., Salgueiro, L., Miguel M. G., and Faleiro, M. L. (2008) Portuguese *Thymbra* and *Thymus* Species Volatiles: Chemical Composition and Biological Activities, *Current Pharmaceutical Design* 14, 3120-3140.

Firouzi, R., Azadbakht, M., and Nabinedjad, A. (1998) Anti-listerial activity of essential oils of some plants, *Journal of Applied Animal Research* 14, 75-80.

Fouche, G., Horak, M. R., Wadiwala, E., and Maharaj, V. J. (2002) Investigation of the commercial potential of *Lippia javanica*. Poster presented at the 36th Convention of the South African Chemical Institute, 1-5 July, Port Elizabeth.

Frankel, E. N., Huang, S. W., Kanner, J., and German, J. B. (1994) Interfacial henomena in the evaluation of antioxidants: bulk oils versus emulsions, *Journal of Agriculture and Food Chemistry* 42, 1054–1059.

Friedman, M., Henika, P. R., and Mandrell,. R. E. (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytog*enes and *Salmonella enterica*, *Journal of Food Protection* 65, 1545-1560.

Galambosi, B. and Peura, P. (1996) Agrobotanical features and oil content of wild and cultivated forms of caraway, *Journal of Essential Oil Research* 8, 389-397.

Galego, L., Almeida, V., Gonçalves, V., Costa, M., Monteiro, I., Matos, F., and Miguel, G. (2008) Antioxidant activity of the essential oils of *Thymbra capitata, Origanum vulgare, Thymus mastichina* and *Calamintha baetica*, *Acta Horticulturae* 765, 325–334.

Gill, A. O., Delaquis, P., Russo, P., and Holley, R. A. (2002) Evaluation of antilisterialaction of cilantro oil on vacuum packed ham, *International Journal of Food Microbiology* 73, 83-92.

Giordiani, R., Hadef, Y., Kaloustina, J. (2008) Compositions and antifungal activities of essential oils of some Algerian aromatic plants. *Fitoterapia* 79, 199-203.

Glamočlija, J., Soković, M., Vukojević, J., Milenković, I., and Van Griensven, .J.L.D. (2006) Chemical composition and antifungal activities of essential oils of *Satureja thymbra* L. and *Salvia pomifera* ssp. calycina (Sm.) Hayek, *Journal of Essential Oil Research* 18, 115–117.

Gonzalez, A. G., Abad, T., Jimenez, I. A., Ravelo, A. G., Luis, J. G., Aguiar, Z., Andres, L. S., Plasencia, M., Herrera, J. R., and Moujir, L.(1989) A first study of antibacterial activity of diterpenes isolated from some *Salvia* species (Lamiaceae), *Biochemical Systematics and Ecology* 17, 293-295.

Gören, A.C., Topçu, G., Bilsel, G., Bilsel, M., Wilkinson, J.M., and Cavanagh, H.M.A. (2004) Analysis of essential oil of *Satureja thymbra* by hydrodistillation, thermal desorber, and headspace GC/MS techniques and its antimicrobial activity, *Natural Product Research* 18, 189–195.

Graven, E. H., Webber, L., Venter, M. and Gardner, J. B. (1990) The development of *Artemisia afra* (Jacq.) as a new essential oil crop, *Journal of Essential Oil Research* 2, 215-220.

Guenther, E. (1948) The essential oils, vol. (1) D. Van Nostrand Company Inc., New York.

Hammer, K. A., Carson, C. F. and Riley, T. V. (1999) Antimicrobial activity of essential oils and other plant extracts, *Journal of Applied Microbiology* 86,985-990.

Hanel H., and Raether W. (1988) A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example, *Mycoses* 31, 148-154.

Hazzit, M., Baaliouamer, A., Verissimo, A.R., Faleiro, M.L., and Miguel, M.G. (2009) Chemical composition and biological activities of Algerian Thymus oils, *Food Chemistry* 116, 714-721.

Hedge, C. (1992) A global survey of the biogeography of the Labiatae. In Harley RM & Reynolds T (ed.) Advances in Labiatae Science. Royal Botanic Gardens, Kew. P. 7–17.

Hedhilia, L., Romdhane M., Planche, H., and Abderrabba, M. (2005) Towards gas chromatography–mass spectrometry coupling protocols for both identifying and quantification essential oils of *Thymus capitatus* Hoff et Link, *Journal of Chromatography* 1064, 129–134

Hedhilia, L., Romdhane, M., Abderrabba, A., Planche, H., Cherif, I. (2002) Variability in essential oil composition of Tunisian *Thymus capitatus* (L.) Hoffmanns. et Link., *Flavour and Fragrance Journal* 17, 26–28.

Heywood, V. H., Brummitt, R. K., Culham, A., and Seberg, O. (2007) Flowering Plant Families of the World. Royal Botanic Gardens, Kew.

Holley, R. A. and Patel, D. (2005) Improvement in shilf-life and safety of perishable foods by plant essential oils and smoke antimicrobials, *Food Microbiology* 22, 273- 292.

Houghton, P. J., and Raman, A. (1998) Laboratory handbook for the fractionation of natural extracts. 1st ed. London: Chapman and Hall.

Houmani, Z., Azzoudj, S., Naxakis, G., and Skoula, M. (2002). The essential oil composition of Algerian Zaâtar: *Origanum* ssp. And *Thymus* ssp, *Journal of Herbs, Spices and Medicinal Plants* 9, 275–280.

Huang, D., Ou, D., and Prior, D. (2005) The chemistry behind antioxidant assays, *Journal of Agriculture and Food Chemistry* 53, 1841-1856.

Hussain, A . (2009) Characterzation and Biological activities of essential oils of some species of Lamiaceae. PhD. Thesis, Faculty of science, University of Agriculture, Faisalabad Pakistan.

Hussain, A. I., Anwar, F., Sherazi, S. T. H., and Przybylski, R. (2008) Chemical composition. Antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations, *Food Chemistry* 108, 986-995.

Hussain, H., and Tobji, R. S. (1997) Antibacterial screening of some Libyan medicinal plants. *Fitoterapi*a 68, 467–470.

Hussein, A. S. M. (1989) Antimicrobial screening of Libyan medicinal plants, *Planta Medica* 55, 650–651.

Hussein, A. S. M. (1989) Antimicrobial screening of Libyan medicinal plants. Planta Medica 55, 650–651.

Jafri, S.M.H., El-gadi A.(1985) Flora of Libya. (ed) Al Faateh University. Faculty of Science. Department of Botany, Tripoli, Libya, vol. 118.

Jalas, J. (1971) Notes on *Thymus* L. (Labiatae) in Europe I. Supraspecific classification and nomenclature, *Botanical* Journal *of the Linnean Society* 64, 199-215.

Jalsenjak, V., Peljnajk, S., and Kustrak, D. (1987) Microcapsules of sage oil, essential oils content and antimicrobial activity, *Pharmazie* 42, 419-420.

Janssen, A. M., Scheffer, J. J. C., and Baerheim Svendsen, A. (1987) Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods, *Planta Medica* 53, 396-398.

Jarvis, A. P., and Morgan, E. D. (1997) Isolation of plant products by supercritical-fluid extraction. *Phytochemical Analysis* 8, 217-222.

Jayashree, T., andSubramanyam, C. (1999) Antiaflatoxigenic activity of eugenol is due to inhibition of lipid peroxidation*, Letters in Applied Microbiology* 28, 179-183.

Joubert, E., Winterton, P., Britz, T.J., and Gelderblom, W.C.A. (2005) Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (Aspalathus linearis), *Journal of. Agriculture and Food Chemistry* 53, 10260–10267.

Juliani, H. R., Karoch, A. R., Juliani, H. R., Trippi, V. S., and Zygadlo, J. A. (2002) Intraspecific variation in the leaf oils of *Lippia junelliana* (mold.) tronc, *Biochemical Systematics and Ecology* 30, 163-170.

Juliano, C., Mattana, A., and Usai, M. (2000) Composition and in vitro antimicrobial activity of the essential oil of *Thymus herba-barona* Loisel growing wild in Sardinia, *Journal of Essential Oil Research* 12, 516–522.

Juven, B. J., Kanner, J., Schved, F., and Weisslowicz, H. (1994) Factors that interact with the antibacterial action of thyme essential oil and its active constituentsm *Journal of Applied Bacteriology* 76, 626-631.

Kanner, J., Granit, R., German, B., and Kinsella, J. E. (1994) Natural antioxidants in grapes and wines, *Journal of Agricultural and Food Chemistry* 42, 64 –69.

Karousou R., Koureas, D.N., and Kokkini S. (2005) Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of Crete, *Phytochemistry* 66, 2668–2673.

Karousou, R., Vokou, D., Kokkini, S. (1998) Variation of *Salvia fruticosa* essential oils on the island of Crete, *Botanica Acta* 111, 250–254.

Karpouhtsis, I., Pardali, E., Feggou, E., Kokkini, S., Scouras, Z.G., and MavraganiI-Tsipidou, P.(1998) Insecticidal and genotoxic activities of oregano essential oils, *Journal of Agriculture and Food Chemistry* 46, 1111–1115.

Katz, D.A., Sneh, B., and Fridman, J. (1987) The allelopathic of Coridothymus capitatus L.(Labiatae). Prelimanary studies on the roles of the shrub in the inhibition of annuals germination and/or to promot allelopathiclly active actiomcetes, *Plant and soil* 98, 53-66.

Kelen, M., and Tepe, B. (2008) Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora, *Bioresource Biores, Technology Journal* 99, 4096–4104.

Khajeh, M., Yamini, Y., Sefidkon, F., and Bahramifar, N. (2004) Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods, *Food chemistry* 86, 587-591.

Khanavi, M., Hadjiakhoondi, A., Amin, G., Amanzadeh, Y., Rustaiyan, A., and Shafiee, A. (2004) Comparison of the volatile composition of *Stachys persica* Gmel. And *Stachys byzantina* C. Koch oils obtained by

hydrodistillation and steam distillation, *Zeitschrift für Naturforschung Z. Naturforsch* 59, 463-467.

Khatabi, K. (1993) The National Report about Biodiversity in Libya. Presented at the Conference on Biodiversity in North Africa Countries, Tunis. (Arabic Document).

Kim, D. O., and Lee, Y. C. (2004) Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship, *Critical Reviews in Food Science and Nutrition* 44, 253–273.

Kim, J. M., Marshall, M. R., Cornell, J. A., Preston, J. F., and Wei, C. I. (1995) Antibacterial activity of carvacrol, citral, and geraniol against *Salmonella*against Salmonella *typhimurium* in culture medium and on fish cubes, *Journal of Food Science* 60, 1364-1368, 1374.

Knobloch, K., Weigand, H., Weis, N., and Schwarm, H.M., Vigenschow, H. (1986) Action of terpenoids on energy metabolism, p. 429-445. In E. J. Brunke (ed.), Progress in Essential Oil Research: 16th International Symposium on Essential Oils. De Gruyter, Berlin.

Koedam, A. (1982) The influence of some distillation conditions on essential oil composition. In: Margaris, N., A. Koedam. and D. Vokou. (eds.). Aromatic plants: basic and applied aspects. The Hague: Martinus Nijhoff Publishers.

Kofidis, G., Bosabalidis, A., and Kokkini, S. (2006) Seasonal variations of essential oils in a linalool-rich chemotype of *Mentha spicata* grown wild in Greece, *Journal of Essential Oil Research* 16: 469-472.

Kokkini, S., Karousou, R., and Lanaras, T. (1997) Essential oils with 1,2- epoxy-p-enthane derivatives from *Mentha spicata* plants growing across the island of Crete, *Botanica Acta* 110, 184–189.

Koleva, I. I., Van Beek, T. A., Linssen, J. P. H., De Groot A., and Evstatieva, L. N.. (2002) Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, *Phytochemical Analysis* 13, 8–17.

Konstantopoulou, I., Vassilopoulou, L., Mavragani-Tsipidou, P., and Scouras, Z. G. (1992) Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on Drosophila auraria, *Experientia* 48, 616-619.

Kosar, M., Tunalier, Z., Ozek, T., Kürcüglu, M., and Can Baser, K. H. (2005) A simple method to obtain essential oils from *Salvia triloba* L. and *Laurus nobilis* L. by using microwave-assisted hydrodistillation, *Zeitschrift für Naturforschung c Z. Naturforsch C* 60, 501-504.

Krell, E. (1982) Techniques and instrumentation in Analytical Chemistry, Vol(2): Handbook of laboratory distillation. 2nd ed. Amsterdam: Elsevier Scientific Publishing Company.

Kulisic, T., Radonic, A., katalinic, V., and Milos, M. (2004) Use of different methods for the testing activity of oregano essential oil, *Food Chemistry* 85, 633-640.

Kunicka-Styczyńska A.(2011) Activity of essential oils against food-spoiling yeast, A review. *Flavour and Fragrance Journal* 26, 326–328.

Lambert, R. J. W., Skandamis, P. N., Coote, P., and Nychas, G. J. E., (2001) A study of the minimum inhibitory concentration and mode of action of oregano

essential oil, thymol and carvacrol, *Journal of Applied Microbiology* 91, 453–462.

Lawrence, B.M., and Tucker, A.O. (2002) The genus *Thymus* as a source of commercial products. Thyme. The genus Thymus. In: Stahl-Bishup, E., Sáez, F. (ed.), Medicinal and Aromatic Plants – Industrial Profiles, vol. (17). Taylor & Francis, London, P. 52–262.

Le Floch, E., and Boulos, L. (2008) Flora of Tunisia synonymous Reviewed Catalog, Montepellier.

Leland, J. C., Ara K., Peter B. K., Sara L. W., James A. D., and Harry L. B. (2006) *Natural products from plants*. Taylor and Francis Group. Florida, USA.

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

Loizzo, M.R., Saab, A.M., Tundis, R., Statti, G.A., Menichini, F., Lampronti, I., Gambari, R., Cinatl, J., and Doerr, H.W. (2008) Phytochemical analysis and in vitro antiviral activities of the essential oils of seven Lebanon species, *Chemistry and Biodiversity* 5, 461–470.

Longaray Delamare, A. P., Moschen-Pistorello, I. T., Artico, L., Atti-Serafini, L., and Echeverrigaray, S. (2007) Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil, *Food Chemistry* 100, 603-608.

Machado M., A. M. Dinis, A. M., L. Salgueiro, L., C. Cavaleiro, C., J. B. A. Custódio, J. B. A., and J. M. D. C. Sousa, J. M. D. C. (2010) Anti-Giardia activity of phenolic-rich essential oils: effects of *Thymbra capitata*,

Origanum virens, Thymus zygis subsp. sylvestris, and Lippia graveolens on trophozoites growth, viability, adherence, and ultrastructure, *Parasitology Research* 106, 1205-1215. .

Mahmoud, S. S., and Croteau, R. B. (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants, *Trends in Plant Science* 7, 366-373.

Maksimović, M., Vidic, D., Miloš, M., Edita Šolić, M., Abadžić, S., and Siljak-Yakovlev, S. (2007) Effect of the environmental conditions on essential oil profile in two Dinaric *Salvia* species: *S. brachyodon* Vandas and *S. officinalis* L., *Biochemical Systematics and Ecologyl* 35, 473-478.

Mann, C. M., and Markham, J. L. (1998) A new method for determining the minimum inhibitory concentration of essential oils, *Journal of Applied Microbiology* 84, 538-544.

Mari, M., Bertolini, P., and Pratella, G. C. (2003) Non-conventional methods for the control of post-harvest pear diseases, *Journal of Applied Microbiology* 94, 761-766.

Marino, M., Bersani, C., and Comi, G. (2001) Impedance measurements to study the antimicrobial activity of essential oils from Lamiacea and Compositae, *International Journal of Food Microbiology* 67, 187– 195.

Marković, T., Chatzopoulou, P., Šiljegović, J., Nikolić, M., Glamočlija, J., Ćirić, A., and Soković, M. (2011) Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components, *Archives of Biological Scince Belgrade* 63, 457–464.

Marotti, M., Piccaglia, R., and Giovanelli, E.(1994) Effects of variety and ontogenic stage on the essential oil composition and biological activity of fennel (*Foeniculum vulgare* Mill.), *Journal of Essential Oil Research* 6, 57-62.

Masango, P. (2001) Towards understanding steam distillation of essential oils by differential quantification of principal components using capillary gas chromatography. PhD thesis, University of Surrey.

Masango, P.(2004) Cleaner production of essential oil by steam distillation, *Journal of Cleaner Production* 13, 833-839.

Masotti, V., Juteau, F., Bessiere, J. M., and Viano, J. (2003) Seasonal and phonological variations of the essential oil from the narrow endemic species *Artemisia ccxli molinieri* and its biological activities, *Journal of Agriculture and Food Chemistry* 51, 7115-7121.

McNicholl B.P., McGrath J.W., and Quinn J.P. (2006). Development and application of a resazurin-based biomass activity test for activated sludge plant management*, Water Research* 41, 127–133.

Mejlholm, O. and Dalgaard, P. (2002) Antimicrobial effect of essential oils on the seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media and fish products, *Letters in Applied Microbiology* 34, 27-31.

Miceli, A., Negro, C., and Tommasi, L. (2006) Essential oil variability in *Thymbra capitata* (L.) Cav. growing wild in Southren Apulia (Italy), *Biochemical Systematics and Ecology* 34, 528-535.

Michaelakis, A., Theotokatofs, S.A., Koliopoulos, G., and Chorianopoulos, N.G. (2007) Essential oils of *Satureja* species: Insecticidal effect on Culex pipiens larvae (Diptera: Culicidae), *Molecules* 12, 2567–2578.

Miguel M. G. (2010) Antioxidant activity of medicinal and aromatic plants, *A* review. *Flavour and Fragrance Journal* 25, 291–312

Miguel M.G., Simoes M.F., Figueiredo A.C., Barroso J.G., and Carvalho, L. M. (2005) Evaluation of the antioxidant activity of *Thymus capitatus, Thymus astichina* and *Thymus camphoratus* essential oil, *Journal of Food Lipids* 12, 181-197.

Milos, M., Radonic, A., Bezic, N., and Dunkic, V. (2001) Localities and seasonal variations in the chemical composition of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten, *Flavour and Fragrance Journal* 16, 157-160.

Mohammadreza, V. R. (2008) Variation in the essential oil composition of *Artemisia annua* L. of different growth stages cultivated in Iran, *African Journal of Plant Science* 2, 016-018.

Mohammed, M. J., and Al-Bayati, F. A. (2009) Isolation and identification of antibacterial compounds from *Thymus kotschyanus* aerial parts and Dianthus caryophyllus flower buds, *Journal of Phytomedicine* 16, 632-637.

Mojab, F., Poursaeed, M., Mehrgan, H., and Pakdaman, S. (2008) Antibacterial activity of *Thymus daenensis* methanolic extract, *Pakistan Journal of Pharmaceutical Sciences* 21,210-223.

Morales R., (2002) The history, botany and taxonomy of the genus Thymus. In: Stahl-Biskup E., Sáez F. (ed.), Thyme: The Genus Thymus. Taylor and Francis, London and New York.

Morales, R. (1989) The genus *Thymus* L. in the western Mediterranean region (Lamiaceae), Biocosme Mésogéen,

Morrison, R. T., and Boyd R. N. (1987) Phenols, In: *Organic Chemistry*, (5ed), Allyn and Bacon, Boston, Ch. 28.

Mourey, A. and Canillacm N. (2002) Anti-Listeria monocytogenes activity of essential oils components of conifers, *Food Control* 13, 289-292.

Muller-Riebau, F., Berger, B., Yegen, O., and Cakir, C. (1997) Seasonal variation in the chemical composition of essential oils of selected aromatic plants growing wild in Turkey, *Journal of Agriculture and Food Chemistry* 45, 4821-4825.

Natella, F., Nardini, I., Di Felice, M., and Scaccini, C. (1999) Benzoic and cinnamic acid derivatives as antioxidants: structure-activity relation, *Journal of Agriculture and Food Chemistry* 47, 1453-1459.

NCCLS. (2000) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—Fifth edition. NCCLS document M7-A5. [ISBN 1-56238-394-9] NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. USA.

Ndubani, P., and Hojer, B. (1999). Traditional healers and the treatment of sexually transmitted illness in rural Zambia, *Journal of Ethnopharmacology*, **67**, 15–25.

Nedorostova L., Kloucek P., Urbanova K., Kokoska L., Smid J., Urban J., Irena, Valterova, I., and Stolcova, M. (2011) Antibacterial effect of essential oil vapours against different strains of *Staphylococcus aureus*, including MRSA, *Flavour and Fragrance Journal* 26, 403–407.

Nikaido, H., and Vaara, M. (1985) Molecular basis of bacterial outer membrane permeability, *Microbiological Reviews* 49, 1–32.

Norsita, T. (2003) The essential oils of *Plumeria* species as new source of perfumery materials. Thesis of Master of Science, University of Malaya.

Nychas, G. J. E. (1995) Natural antimicrobials from plants, P. 58-89. In G. W. Gould (ed.), New methods of food preservation, 1st ed. Blackie Academic & Professional, London.

Onawunmi, G. O. (1989) Evaluation of the antimicrobial activity of citral, *Letters in Applied Microbiology* 9, 105-108.

Oosterhaven, K., Poolman, B., and Smid, E. J. (1995) S-carvone as a natural potato sproutnhibiting, fungistatic and bacteristatic compound, *Industrial Crops and Products* 4, 23-31.

Packiyasothy, E. V., and Kyle, S. (2002) Antimicrobial properties of some herb essential oils, *Food Australia* 54, 384– 387.

Pala-Paul, J., Perez-Alonso, M. J., Velasco-Negueruela, A., Pala-Paul, R., Sanz, J., and Conejero, F. (2001) Seasonal variation in chemical constituents of *Santolina rosmarinifolia* L. ssp. *Rosmarinifolia*, *Biochemical Systematics and Ecology* 29, 663-672.

Pandey, R., Kalra, A., Tandon, S., Mehrotra, N., Singh, H. N., and Kumar, S., (2000) Essential oil compounds as potent source of nematicidal compounds, *Journal of Phytopathology* 148, 501-502.

Papageorgiou, V., Gardeli, C., Mallouchos, A., Papaioannou, M., and Komaitis, M. (2008) Variation of the chemical profile and antioxidant behavior of *Rosmarinus officinalis* L. and *Salvia fruticosa* Miller grown in Greece, *Journal of Agriculture and Food Chemistry* 56, 7254-7264.

Paradiso, V. M., Summo, C., Trani, A., and Caponio, F. (2008) An effort to improve the shelf life of breakfast cereals using natural mixed tocopherols, *Journal of Cereal Science* 47, 322-330.

Paradiso, V. M., Summo, C., Trani, A., and Caponio, F. (2008) An effort to improve the shelf life of breakfast cereals using natural mixed tocopherols. *Journal of Cereal Science*, 47, 322-330.

Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A. J., McGrimpsey, J. A., and Smallfield, B. M. (1999) Essential oil from Dalmation sage (*Salvia officinalis* L.), variations among individuals, plant parts, seasons and sites, *Journal of Agriculture and Food Chemistry* 47, 2048-2054.

Pessoa, L. M., Morais, S. M., Bevilaqua, C. M. L., and Luciano, J. H. S. (2002) Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*, *Veterinary Parasitology* 109, 59-63.

Pierozan, M. K., Pauletti, G. F., Cristina, A., Lerin, L. A., Luccio, M. D. I., Mossi, A. J., Atti-serafini, L., Cansian, L., and Vladimir, J. (2009) Chemical characterization and antimicrobial activity of essential oils of *Salvia* L. species, *Analysis* 29, 764-770.

Pignatti, S. (1982) Flora d'Italia. vol. (1-3) Edagricole, Bologna.

Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R., and Casanova, J. (2002) Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica, *Flavour and Fragrance Journal* 17, 15-19.

Pitarokili, D., Tzakou, O., Loukis, A., and Harvala, C. (2003) Volatile metabolites from *Salvia fruticosa* as antifungal agents in soilborne pathogens, *Journal of Agriculture and Food Chemistry* 51, 3294-3301.

Pol, I. E., Mastwijk, H. C., Slump, R. A., Popa, M. E., and Smid, E. J. (2001) Influence of food matrix on inactivation of *Bacillus cereus* by combinations of nisin, pulsed electric field treatment and carvacrol, *Journal of Food Protection* 64, 1012-1018.

Pottier-Alapetite, G. (1981) Flora of Tunisia, (ed.) Angiosperms Dicotyledons gamopetalous. Tunisia.

Prabuseenivasan, S., Jayakumar, M., and Ignacimuthu, S. (2006) *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary* and *Alternative Medicine,* 6: 39-45.

Radulescu, V., Chiliment, S., and Orea, E. (2004) Capillary gas chromatography-mass spectophotometry of volatile and semi volatile compounds of *Salvia officinalis*, *Journal of chromatography* 1027, 121-126.

Ravid, U. and Putievsky, E. (1983) Constituentsof the essentail oils from *Majorana syriaca, Coridothymus capitatus* and *Satureja thymbra*, *Planta Medica* 49, 248–249.

Reddy M.V.B., Angers, P., Gosselin, A., and Arul, J. (1998) Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits, *Journal of Phytochemistry* 47, 1515–1520.

Remmal, A., Bouchikhi, T., Rhayour, K., and Ettayebi, M. (1993) Improved method for the determination of antimicrobial activity of essential oils in agar medium, *Journal of Essential Oil Research* 5, 179-184.

Reverchon, E. (1997) Supercritical fluid extraction and fractionation of essential oils and related products, *Journal of Supercritical Fluids* 10, 1-37.

Rios, J. L., Recio, M. C., and Villar, A. (1988) Screening methods for natural antimicrobial products with antimicrobial activity: a review of the literature, *Journal of Ethnopharmacology* 23, 127–149.

Rota, M. C., Herrera, A., Martinez, R. M., Sotomayor J. A., and Jordan, M. J. (2008) Antimicrobial activity and chemical composition of *Thymus vulgaris, Thymus zygis* and *Thymus hyemalis* essential oils, *Food Control* 19, 681-687.

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

Ruberto, G., Baratta, M. T., Deans, S. G., Dorman, H. J. D. (2000) Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Medica* 66, 687-693.

Safaei-Ghomi J., Ebrahimabadi, A.H., Djafari-Bidgoli, Z., and Batooli, H. (2009) GC/MS analysis and in vitro antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* Jalas and its main constituent carvacrol, *Food Chemistry* 115, 1524–1528.

Santos-Gomes, P. C., and Fernandes-Ferreira, M. (2001) Organ and season dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites, *Journal of Agriculture and Food Chemistry* 49, 2908-29016.

Sarker, S. D., Nahar L., and Kumarasamy, Y. (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals, *Methods* 42, 321-324.

Satil, F., Tümen, G., Akçelik, A., and Baser, K. H. C. (2002) Comparative morphological, anatomical, ecological and chemical studies on endemic *Satureja parnassica* subsp. sipylea from Turkey, *Acta Botanica. Croat*ica 6, 207–220.

Satou, T., Matsuura, M., Murakami, S., Hayashi, S., and Koike, K. (2009) Composition and seasonal variation of the essential oil from *Abies sachalinensis* from Hokkaido, Japan, *Natural Production Communications* 4, 845-858.

Sawai, ,Y., Moon, H., Sakata, H., and Watanabe, N. (2005) Effects of structure on radical-scavenging abilities and antioxidative activities of tea polyphenols: NMR analytical approach using 1,1-diphenyl-2-picrylhydrazyl radicals, *Journal of Agricultural and Ffood Cchemistry* 53, 3598-3604.

Schlesier, K., Harwat, M., Bohm, V., and Bitsch, R. (2002) Assessment of antioxidant activity by using different in vitro methods, *Free Radical Research* 36, 177– 187.

Schulz, H., Ozkan, G., Baranska, M., Kruger, H., and Ozcan, M. (2005) Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy, *Vibrational Spectroscopy* 39, 249–256.

Sefidkon, F., Abbasi, K., Jamzad, Z., and Ahmad, S. (2007) The effect of distillation methods and stage of plant growth on the essential oil content and

composition of *Setureja rechingeri* Jamzad, *Food Chemistry* 100, 1054-1058.

Senatore, F., Napolitano, F. and Ozcan, M. (2000) Composition and antibacterial activity of the essential oil from *Crithmum maritimum* L. (Apiaceae) growing wild inTurkey, *Flavour and Fragrance Journal* 15, 186– 189.

Shafie, M. S. B., Hasan, Z., and Shah, R. M. (2009) Study of genetic variability of Wormwood capillary (*Artemisia capillaris*) using inter simple sequence repeat (ISSR) in Pahang region, Malaysia, *Plant Scince and Omics Journal* 2, 127–134.

Shahidi, F. (2000) Antioxidant in food and food antioxidants, *Nahrung* 44, 158–163.

Sheille, R., Mondello, L., Marriott, P., and Dugo G. (2002) Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography, *Journal of Chromatography* 970, 225–234.

Shelef, L. A. (1983) Antimicrobial effects of spices, *Journal of Food Safety* 6, 29-44.

Siddhuraju, P. (2006) The antioxidant activity and free radical scavenging capacity of phenolics of raw and dry heated moth bean *(Vigna aconitifoli*a) (Jacq.) Marechal seed extracts, *Food Chemistry* 99, 149–57.

Sikkema, J., De Bont, J. A. M., and Poolman, B. (1995) Mechanisms of membrane toxicity of hydrocarbons, *Microbiological Reviews* 59, 201-222.

Sikkema, J., De Bont, J. A. M., and Poolman. B. (1994) Interactions of cyclic hydrocarbons with biological membranes, *Journal of Biological Chemistry* 269, 8022-8028.

Silva, M. G. V., Matos, F. J. A., Lopes, P. R. O., silva F. O., and Holanda, M. T. (2004) Composition of essential oils from three *Ocimum* species obtained by steam and microwave distillation and supercritical CO2 extraction, *Arkivoc* 6, 66-71.

Siquet, C., Paiva-Martins, F., Lima, J. L. F. C., Reis, S., and Borges, F. (2006) Antioxidant profile of dihydroxy- and trihydroxyphenolic acids – A structure-activity relationship study, *Free Radiccal Research* 40, 433-442.

Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., and Arsenakis, M. (1997) Antimicrobial and cytotoxic activities of *Origanum* essential oils, *Journal of Agricultural and Food Chemistry* 44, 1202-1205.

Skandamis, P., Koutsoumanis, K., Fasseas, K., and Nychas, G. J. E. (2001) Inhibition of oregano essential oil and EDTA on *Escherichia coli* O157:H7, *Italian Journal of Food Science* 13, 65-75.

Skocibusic, M., Bezic, N., and Dunkic V. (2006) Phytochemical composition and antimicrobial activity of the essential oils from *Satureja subspicata* Vis. Growing in Croatia, *Food Chemistry* 96, 20-28.

Skoula, M., Abbes, J., and Johnson, C. (2000) Genetic variation of volatiles and rosmarinic acid in populations of *Salvia fruticosa* mill growing in Crete, *Biochemical Systematics and Ecology* 28, 551-561.

Skoula, M., and Grayer, R. J. (2005) Volatile oils of *Coridothymus capitatus, Satureja thymbra, Satureja spinosa* and *Thymbra calostachya* (Lamiaceae) from Crete, *Flavour and Fragrance Journal* 20, 573–576.

Slavkovska, V., Jancic, R., Bojovic, S., Milosavljevic, S., and Djokovic, D.(2001) Variability of essential oils of *Satureja montana* L. and *Satureja kitaibelii*

Wierzb. ex Heuff. From the central part of the Balkan peninsula, *Journal of Photochemistry* 57, 71-76.

Smith-Palmer, A., Stewart, J., and Fyfe, L. (1998) Antimicrobial properties of plant essential oils and essences against five importantfood-borne pathogens, *Letters in ApplApplied Microbiology* 26, 118–122.

Soares, J. R., Dinis, T. C. P., Cunha, A. P, and Almeida, L. M. (1997) Antioxidant activity of some extracts of *Thymus zygis*, *Free Radical Research* 26, 469–478.

Sokovic, M. and Van Griensven L. J. L. D. (2006) Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*, *European Journal of Plant Pathology* 116, 211-224.

Sokovic, M., Tzakou, O., Pitarakoli, D., and Couladis, M. (2002) Antifungal activities of selected aromatic plants growing wild in Greece, *Food / Nahrung* 46, 317–320.

Souto-Bachiller, F. A., De-Jesus-Echevarria, M., Cardenas-Gonzalez, O. E., Acuna-Rodriguez, M. F., Melendez, P. A., and Romero-Ramsey, L. (1997) Terpenoid composition of *Lippia dulcis*, *Journal of Phytochemistry* 44, 1077-1086.

Srinivasan, K. (2005) Spices as influencers of body metabolism: an overview of three decades of research. *Food Research International,* 38, 77-86.

Stahl-Biskup, E. (2002) Essential oil chemistry of the genus Thymus – a global view. Thyme. The genus Thymus. In: Stahl-Bishup, E., Sáez, F. (eds.), Medicinal

and Aromatic Plants – Industrial Profiles, vol. (17). Taylor & Francis, London, P. 75–124.

Standley, P. and Williams, L. (1973) Flora of Guatemala, *Fieldiana Botany* 24, 237-317.

Sur, S. V., Tuljupa, F. M., and Sur, L. I. (1991) Gas chromatographic determination of monoterpenes in essential oil medicinal plants, *Journal of Chromatography* 542, 451–458.

Sylvestre, M., Legault, J., Dufour, D., Pichette, A., and Lonatin, A. (2005) Chemical composition and anticancer activity of leaf essential oil of Myrica gale L. *Phytomedicine*, 12:299-304.

Tabacchi, R. and Garnero, J. (1987) in Sandra, P. and Bicchi, C. (ed), Capillary Gas Chromatography in Essential oil analysis. Huthing, Heidelberg. P. 1-11.

Tada, M., Okuno, K., Chiba, K., Ohnishi, E., and Yoshii, T. (1994) Antiviral diterpenes from *Salvia officinalis*, *Journal of Phytochemistry* 35, 539-541.

Talei, G. R., and Meshkatalsadat, M. H. (2007) Antibacterial activity and chemical constitutions of essential oils of *Thymus persicus* and *Thymus eriocalyx* from west of Iran, *Pakistan Journal of Biological* Science 10, 3923-3926.

Tassou, C., Koutsoumanis, K., and Nychas, G. J. E. (2000) Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil, *Food Research International* 33, 273-280.

Taylor and Francis (2002) Fouche, G., M.R. Horak, E. Wadiwala and V. J. Maharaj Investigation of the commercial potential of Lippia javanica. Poster presented at the 36th Convention of the South African Chemical Institute, 1-5 July, Port Elizabeth.

Teklu, S., Alemayehu, G., and Abegaz, B. M. (1998) Chemical Composition of the Essential Oil of *Satureja punctate*, *Journal of Essential Oil Research* 10, 339.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., and Polissiou, M. (2005) Antimicrobial and antioxidative activities of the essential oils and various extracts of *Salvia tomentosa* Miller (Lamiaceae), *Food Chemistry* 90, 333-340.

Terblanche, F. C. (2000) The characterization, utilization and manufacture of products recovered from *Lippia scaberrima* Sond. PhD. thesis, Pretoria, University of Pretoria.

Tisserand, R. and Balacs, T. (1995). Essential oil safety, a guide for health care professionals. Churchill Livingstone. P. 15.

Toroglu S. (2007). In vitro antimicrobial activity and antagonistic effect of essential oils from plant species, *Journal of Environmental Biology* 28, 551-559.

Tuley de Silva, K. (1996) A manual on the essential oil industry. (ed.). United Nations Industrial Development Organization, Vienna.

Ultee e, E. Smid, J. (2001) "Influence of carvacrol on growth and toxin production by *Bacillus cereus", International Journal of Food Microbiology* 64, 373-378.

Uribe-Hernandez, C. J., Hurtado-Ramos, J. B., Olmedo-Arcega, E. R., and Martinez-Sosa, M. A. (1992) The essential oil of *Lippia graveolens* H.B.K. from Jalisco, Mexico, *Journal of Essential Oil Research* 4, 647-649.

Vagionas, K., Graikou, K., Ngassapa, O., Runyoro, D., and Chinou, I. (2007). Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania, *Food Chemistry* 103, 319-332.

Van de Braak, S. A. A. J., and Leijten, G. C. J. J. (1999) Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, P.116.

Van Vuuren, S. F., Viljoen, A. M., Ozek, T., Demirici B., and Baser, K. H. C. (2007) Seasonal and geographical variation of *Heteropyxis natalensis* essential oil and the effect thereof on the antimicrobial activity, *South African Journal of Botany* 73, 441-448.

Vardar-Unlu, G., Candan, F., Sokmen, A., Daferera, D., Polissiou, M., Sokmen, M., Donmez, E., and Tepe, B. (2003) Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. Et Mey. Var. pectinatus (Lamiaceae), *Journal of Agriculture and Food Chemistry* 51, 63-67.

Vilegas, J. H.Y., Lancus, F. M., and Vilegas, W. (1994) composition of the volatile compouds from Anib canelilla (H.B.k) MEZ. Extracted by Co_2 in the supercritical state*, Flavour and Fragance Journal* 4, 39. 82.

Viljoen, A. M., Petkar, S., Van-Vuurenm, S. F., Cristina, A., Figueiredo, L., Pedro, G., and Barroso, J. G. (2006) Chemo-Geographical Variation in Essential Oil Composition and the Antimicrobial Properties of "Wild Mint" - *Mentha longifolia* subsp. Polyadena (Lamiaceae) in Southern Africa, *Journal of Essential Oil Research* 18, 60-65.

Vokou, D., Kokkini, S., and Bessiere, J. M. (1993) Geographic variation of Greek oregano (*Origanum vulgare* ssp. *hirtum*) essential oils, *Biochemical Systematic and Ecology* 21, 287–295.

Vuorelaa, P., Leinonenb, M., Saikkuc, P., Tammelaa, P., Rauhad, J. P., Wennberge, T., and Vuorela, H. (2004) Natural products in the process of fi nding new drug candidates. *Current Medicinal Chemistry 11*, 1375-1389.

Wan, J., Wilcock, A., and Coventry, M. J. (1998) The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*, *Journal of Applied Microbiology* 84, 152-158.

Wang, W., Wu, N., Zu, Y.G. and Fu, Y.J. (2008) Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components, *Food Chemistry* 108, 1019–1022.

Wendakoon, C. N., and Sakaguchi, M. (1995) Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices, *Journal of Food Protection* 58, 280-283.

Weng, X.C., and Wang, W. (2000) Antioxidant activity of compounds isolated from Salvia plebeian, *Food Chemistry* 71, 489-493.

Whish, J. P. M. (1996) A flexible distillation system for the isolation of essential oils, *Journal of Essential Oil Research* 8, 405-410.

Wilkinson, J. M., Hipwell, M., Ryan, T., and Cavanagh, H. M. A. (2003) Bioactivity of *Backhousia citriodora*: Antibacterial and antifungal activity, *Journal of Agriculture of Food Chemistry* 51, 76-81.

Wink, M. (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective, *Journal of Phytochemistry* 64, 3–19.

Wright, J. S., Johnsonm, E. R., and DiLabio, G. A. (2001) Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and

application to major families of antioxidants, *Journal of American Oil Chemists Society* 123, 1173-1183.

Zouari, N., Fakhfakh N., Zouari A., Bougatef A., Karray A., Neffati M., Ayadi, M. A. (2011) Chemical composition, angiotensinI-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut.(Lamiaceae), *Journal of Food and Bioproducts Processing* 89, 257-265.

Прилог 1.

Изјава о ауторству

Потписани-а Abdulhmid Ahmed Giweli

број уписа _____

Изјављујем

да је докторска дисертација под насловом

"Composition, antimicrobial and antioxidant activity of the essential oils of *Thymus algeriensis, Thymus capitatus, Satureja thymbra* and *Salvia fruticosa* from Libya"

- резултат сопственог истраживачког рада,
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Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора : **Abdulhmid Ahmed Giweli**

Број индекса: _____

наслов рада : **"Composition, antimicrobial and antioxidant activity of the essential oils of *Thymus algeriensis*, *Thymus capitatus*, *Satureja thymbra* and *Salvia fruticosa* from Libya"**

Ментор : **Prof. dr Petar Marin**

Потписани/ а __**Abdulhmid Ahmed Giweli**_____

Изјавији да је штампана верзије мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу Дигиталног репозиторијума Универзитета у Београду.

Дозвољавам да се објаве моји лични подаци везани за добијње академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

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Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

“Composition, antimicrobial and antioxidant activity of the essential oils of *Thymus algeriensis, Thymus capitatus, Satureja thymbra* and *Salvia fruticosa* from Libya”

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

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