Natural Product Sciences
25(2) : 81-91 (2019)
https://doi.org/10.20307/nps.2019.25.2.81

<Review>

Essential Oil of *Marrubium vulgare*: Chemical Composition and Biological Activities. A Review

Benalia Yabrir*

Department of Biology, Faculty of Nature and Life Science, Ziane Achour University, Djelfa 17000, Algeria

Abstract – *Marrubium vulgare*, plant species belonging to *Marrubium* genus, is widespread in the Mediterranean areas, introduced elsewhere and also cultivated in many countries. Its oil is recognized to possess a considerable biological activities with varied chemical composition. This paper aims to overview the chemical composition and biological activities of *M. vulgare* essential oil's considered as a medicinal plant, widely used in folk medicine overall the world. In essential oils of *M. vulgare*, germacrene D, β-caryophyllene, β-bisabolene, bicyclogermacrene and carvacrol are generally considered as either mains or minor constituents and each species presents its own composition. Sesquiterpenoids were the dominant fraction while monoterpenoids were present in appreciable or in trace amount. Oxygenated fractions dominated in monoterpenes however, hydrocarbon fraction overpowered in sesquiterpenes. These oils are biologically active, they exhibit an antioxidant and antimicrobial activities and other activities. Due to the variability of composition of essential oil, further studies are necessary, particularly regarding their chemical's which may cause an important change in the biological activities of oils and probably defined different chemotype.
Keywords – Antimicrobial activity, antioxidant activity, chemical constituents, *Marrubium vulgare*

Introduction

Species of labiatae family are almost cosmopolitan, but absent from the coldest regions of high latitude or altitude[1] and with a center of distribution in the Mediterranean region.[2] Although some small groups have a local distribution in Australia, South-west Asia, and South America.[3] Labiatae species produce a great variety of secondary compounds, especially essential oils[1] which are found in prolruding multicelled glandular trichomes[4] and produced in glandular hairs on the surfaces of leaves and inflorescences.[1] This family is well known with two major series of genera: oil-rich and oil-poor species[5] and several members of the family are used as sources of essential oils.[3] *Marrubium* genera belongs to this family which consists of annual or perennial herbs is mostly characterized by grain with tricolpate pollen,[1,6] and so is oil-poor one regarding the Lawrence hypothesis.[7] Belonging to *Marrubium* genus, *Marrubium vulgare* (*M. vulgare*) is the most representative, more widespread in the Mediterranean areas of Europe and North Africa[8], introduced elsewhere[1] and also cultivated in many countries.[9-12]

Essential oils of *M. vulgare* have been subjected to many studies. Some are related to their chemical composition[13-22] and its variability,[10-11,23-25] others described their biological activities: antioxidant,[26-28] antibacterial or antifungal[29-31] and hepatoprotective acitivity.[28] Mosquitocidal and molluscicidal activity were also studied.[32]

This article constitutes the continuation of an article already published[33] and overview the chemical and biological activities of *M. vulgare* essential oil's considered as a medicinal plant, widely used in folk medicine overall the world. For this, databases such as Web of Science, Pub Med, Sciences direct, Scopus and Google Scholar were interrogated. "*Marrubium vulgare*" and "Essential oil of *Marrubium vulgare*" were the key words used without restriction for the research.

Botanical and ecology of *Marrubium vulgare*

Marrubium vulgare, also known as "horehound", is described as following: annual or perennial herbs, with at least some branched hairs and often also with simple hairs. A tough, woody, branched taproot or having numerous fibrous lateral roots and numerous stems, which are quadrangular, erect, very downy and from 20 to

*Author for correspondence
Benalia Yabrir, Department of Biology, Faculty of Nature and Life Science, Ziane Achour University, Djelfa 17000, Algeria
Tel: +00 213 777 44 13 83; E-mail: byabrir@yahoo.fr

100 cm high.[1,34,35]

Leaves are roundish ovate usually toothed, petiolate, veined hoary on surface and arranged opposite along stem in pairs; 1 - 4 cm or up to 6 cm long, 1 - 5 cm wide. Inflorescence in axils of upper leaves, the flowers are white and in crowded axillary whorls, these axillary subglobose multiflowered verticals forming interrupted spikes along stem, often subtended by leaves. The calyx is tubular lobed, veined; short soft-hairy, 10-toothed, each tooth with a small hooked spine/bristle, alternately long and short. Corolla is white to pale lavender, tubular, bilabiate, small, 5 - 10 mm long, tube about 2 - 4 mm longer than calyx; corolla 2-lipped of which the upper lip 2-lobed is bifid and erect medially notched and the lower lip spreading, 3-lobed with middle lobe larger. In corolla-tube are included style, stamens and anther sacs divergent; nutlets rounded or subtruncate at apex, glabrous or with sessile glands at apex.

Calices, flowers and especially the leaves, are covered with different trichomes, amongst them numerous stellate trichomes, peltate glandular trichomes and capitate glandular trichomes.[36] Micromorphological analyses of non-glandular and glandular trichomes of *M. vulgare* were studied by investing the types and sizes of trichomes from the stem, leaf, calyx, and corolla using light microscopy (LM) and scanning electron microscopy (SEM) techniques.[37] According these authors, the surfaces of *M. vulgare* vegetative and reproductive organs are densely clothed with glandular and non-glandular trichomes. The glandular trichomes consists of peltate and capitate however, non-glandular trichomes consists of multicellular uniserrate and multicellular branched. *M. vulgare* is characterized by stellate trichomes;[34] on the upper surface of *M. vulgare*, the long branches of stellate hairs are four-celled and, the number of short branches increases to 14 with altitude. Ecological conditions affect also morphological and density of trichomes.[38]

The pollen and seed morphology of *M. vulgare* was described by Akgul et al.[6] The pollen grains are radially symmetrical and isopolar. The shape is oblate-spheroidal. The pollen grains are tricolpate and the apertural membrane is psilate-perforate.

From an ecological point of view, horehound will establish on infertile soils and it is often the first colonizer of eroded areas, although it rarely persists in competition from annual or perennial pasture species.[39] The plant grows in alkaline and poor soils. It is an early colonizer of sheep camps, rabbit warrens, and other disturbed sites, from which it encroaches into adjacent farmland.[40] According the same authors, it is a drought tolerant plant that is widely distributed in areas with a minimum of 200 mm annual rainfall. Due to its adaptability to different environmental conditions, horehound is considered as a troublesome weed.[41]

Horehound is native to temperate Eurasia, Europe, the Middle East and the Mediterranean region including North Africa,[42] more widespread and introduced elsewhere.[1] It is widely naturalized in North America where it was once cultivated and extracted for use in confections and medicines.[43] Apparently, it is introduced to New Zealand deliberately for its medicinal properties.[40] Horehound appears to have been introduced into Australia from Europe for use as a garden herb and for beer brewing purposes.[42] Due to its medicinal properties, environmental requirements for germination of *M. vulgare* seeds were studied.[39,41,43] Also, many authors sees cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation.[44] Thus, *M. vulgare* is cultivated in Egypt,[9] in Poland[10,11] and in Morocco.[12]

Pharmacological properties of *Marrubium vulgare*

Marrubium vulgare is recognized to possess many effects on human body. It is widely used in folk medicine in all over the world to treat a variety of ailment. In Algeria, it is used in folk medicine to cure several diseases of the digestive tract, such as diarrhoea, as well as diabetes, rheumatism, cold and respiratory pains.[24] In Iran, it is used as a neurosedative, hypotensive, anti-spasmodic, antinociceptive, antidiabetic, anti-inflammatory agent and a protective effect against Ischemia-Reperfusion injury.[45] In Morocco, *M. vulgare* is used as antidiabetic, febrifuge, emmenagogue, tonic, expectorant, hypoglycemic, antiseptic, antipyretic, anti-diarrheal, diuretic, anti-icteric.[46] In India, it is regards to possesses tonic, aromatic, stimulant, expectorant, diaphoretic and diuretic activities, laxative in large doses and as an emmenagogue.[47] In Jordan, leaves and flowers of *M. vulgare* are used in the treatment of cough, tuberculosis, asthma, analgetic, liver cirrhosisa.[48] In the United States of America (New Mexico), leaves are used to treat cold and fever.[49] In Mexico, it is used as a traditional medicine to aid digestion, soothe a sore throat, relieve inflammation and treat diabetes.[50] In Germany, extracts of aerial parts from *M. vulgare* are used in treatment of stomach diseases and coughs.[51] In Brazil, it is employed in folk medicine against a variety of diseases, such as gastrointestinal disorders, intestinal infections, inflammatories processes, among others.[52] In Egypt, the plant is frequently employed as folk medicine to treat a variety of ailments, exhibits antispasmodic and

antinociceptive effects and which possesses tonic, aromatic, stimulant, expectorant, diaphoretic and diuretic properties.[53] In Libya, this plant is traditionally used as expectorant and antispasmodic in acute or chronic bronchitis, coughs, asthma and in general for respiratory infections.[28] In Tunisian folk medicine, it is used as hypotensive, hypoglycemic and cardiotonic.[26] In Pakistan, young leaves of *M. vulgare* are used to treat cough.[54] Some of traditionally use assigned for *M. vulgare* are confirmed and demonstrated in many modern studies and research by several authors in all over the world.

Extraction and analysis of essential oils

The isolation of aromatic materials from natural plant products and in particular details of essential oil production are well discussed and illustrated.[55] Recently, techniques for extraction of essential oils from plants were highlighted by the literature review of Rassem et al. both for conventional methods and innovative techniques,[56] and the advantages and disadvantages of some of these methods were discussed.[57]

Conventional methods consists of hydrodistillation, steam distillation and from the epicarp of *Citrus* fruits by a mechanical process, or by dry distillation as defined by Lawrence.[4] A true essential oil is not a CO_2 extract, a halohydrocarbon solvent extract or an empyreumatic distillate according the same author. While, innovative techniques consists of many microwave methods which are proposed and were patented for extraction of essential oils. The first was that of Craveiro et al. concerning the Compressed Air Microwave Distillation (CAMD).[58] Microwave-assisted extraction (MAE) was patented since 1991.[59] Microwave hydrodistillation (MWHD) was developed by Stashenko et al.[60] Solvent-free microwave-assisted extraction (SFME) was patented by Chemat et al.[61] Microwave hydrodiffusion and gravity extraction (MHG) were also patented by Chemat et al.[62] In 1997, Pallado et al. prove the effectiveness of supercritical fluid extraction in aroma chemistry in comparison with the same essential oils extracted by means of more classical techniques.[63] Recently, and based on the electroporation of cell membranes by high-level electrical field pulses enhancing the mass transfer of the intracellular content, Miloudi et al. applied the Pulsed Electric Field (PEF) pretreatment to induce permeabilization by electroporation of the biological membranes and enhance the extraction process, with special regard to yield increase and reduction of processing time on *M. vulgare* plant.[64]

Generally, the obtained essential oils from *M. vulgare* were dried over anhydrous sodium sulphate and stored in dark at low temperature until requirement. The exploitation of modern analytical methodologies, such as gas chromatography (GC) and related hyphenated techniques, is practically unavoidable for identification and quantification of the essential oil components and possible adulteration.[65] Essential oils which consist of complex mixtures of chemical compounds and many oils contain over 50 individual compounds which can generally be identified using gas chromatography.[66] Essential oils obtained from various *M. vulgare* plants were submitted to GC and GC-MS analysis, widely employed in this field.[63]

Chemical composition and yields

Yields/Identified compounds – The yields of isolated essential oils from *M. vulgare* ranged from 0.02% (Tunisian area[26]) to 1% (Libyan country[28]) both from dread areal parts (Table 1). Constituents identified varied from 11(Turkey[18]) to 50 (Algeria[27]). Detected compounds amount to 57.50% (Egypt[32]) and 100% (Tunisia[26] and Iran[29]) of the total oil composition.

The low values of essential oils is specific of species of this genre who present tricolpate pollen grains. Thus, according to Lawrence,[7] Labiatae genera with tricolpate pollen grains are oil-poor. Because of the low oil content of *M. vulgare*, some authors think that this species is not normally used as an important source of essential oil and its therapeutic application is often neglected.[23] The relatively low values may be due to environmental condition as well as the hot and dry North and East African growing conditions as stipulate the same authors. However, by applying Pulsed Electric Field (PEF), the extraction process is much more accelerated and is significantly improved, the oil mass obtained with the treated samples of *M. vulgare* increases up to three times.[64]

Main constituents – Some compounds are majority (generally between 2 and 6) because they are present at high levels, others are minority because they are present at low levels, while others are present at levels generally less than 0.05% and are in the form of traces. In *Marrubium vulgare* essential oils, germacrene D, β-caryophyllene, β-bisabolene, bicyclogermacrene and carvacrol are generally considered as either mains or minor constituents (Table 2).

Centesimal chemical composition could vary according geographical area, indeed for example from Iranian species, germacrene D was one of the major constituent for plants collected from south-eastern of Iran,[14,29] however β-caryophyllene for plants collected from north and center of Iran[15,19] and β-bisabolene for those collected from north

Table 1. Yield, plant part and extraction methods of *Marrubium vulgare* essential oils

Yield	Total identified	Compounds identified	Plant Part	Extraction	Country	Ref
0.05	/	23	Aerial part dried in a drying oven30°C (6 days).	Steam distillation (3h) Deryng apparatus	Poland	(10)
0.05	/	48	Aerial part dried in a drying oven30°C (6 days).	Steam distillation (3h) Deryng apparatus	Poland	(10)
0.07		31	Flowering stage of aerial part dried in a drying chamber at 30°C (4 days)	Hydro-distillaion (3h) Clevenger	Poland	(11)
0.06		98.26	Flowering stage of aerial part dried in a drying chamber at 30°C (4 days)	Hydro-distillaion (3h) Clevenger	Poland	(11)
0.07		96.21	Air dread aerial part	Pharmacopoeial method	Slovak	(13)
0.1	77.08	86.2	Air dread aerial part	Hydrodistillaion (4h) Clevenger (powder)	Iran	(14)
0,09	95.1	34	Air dread Flowering aerial part	Hydro-distillaion (3h) Clevenger	Iran	(15)
0.4	93.5	20	Dread Flowering aerial part	Hydrodistillaion (3h) Clevenger	Iran	(16)
0.02	74.6	35	Air dread aerial part	Hydro-distillaion (2h) Clevenger	Tunisia	(17)
/	/	11	Leaves	Steam distillation (3h) Clevenger	Turkey	(18)
/	99.89	44	Aerial part	Hydro-distillaion (3h) Clevenger	Iran	(19)
/	99.39	32	Flowering fresh herb	Hydro-distillaion (2h) Clevenger	Egypt	(20)
0.05	97.72	28	Leaves dried in a drying oven30°C (6 days).	Hydro-distillaion (3h) Clevenger	Algeria	(21)
/	/	47	Air dread freshly grinded leaves	Hydro-distillaion (3h) Clevenger	Lithuania	(22)
0;05	90	30	Air dread aerial part flowering stage	Hydro-distillaion (3h) Clevenger	Algeria	(24)
0.05	90	30	Air dread aerial part vegetative stage	Hydro-distillaion (3h) Clevenger	Algeria	(24)
/	/	/	Fresh herb (3 developing stages)	Hydro-distillaion Clevenger	Egypt	(25)
0.34	100	34	Air dread aerial part	Hydro-distillaion (4h) Clevenger	Tunisia	(26)
0.05	82.46	50	Air dread aerial part	Hydro-distillaion (4h) Clevenger	Algeria	(27)
1	99.79	36	Air dread aerial part	Hydro-distillaion until constant amount (for at least one hour)	Libya	(28)
0.34	100	34	Air dread leaf	Hydrodistillaion (4h) Clevenger	Iran	(29)
0.75	/	30	Air dread Leaf	Hydro-distillaion (2h) Clevenger (powder)	Iran	(30)
0.2	57.50	19	Fresh aerial part	Hydro-distillaion Clevenger	Egypt	(32)

of Iran.[15,16] Another constituents were found to be major compounds as (E)-β-farnesene, 1,8-cineol and α-pinene;[19] (E)-β-farnesene;[15] δ-cadinene and isocaryophyllene;[16] δ-eudesmol, citronellyl formate, β-citronellol, geranyl tiglate and geranyl formate;[29] caryophyllene oxide and trans-caryophyllene.[14] Carvacrol was absent for all these species while bicyclogermacrene was found at low and same amount for two plants.[14,16]

From Egyptian species, carvacrol[20,25] and thymol[32] were the major compounds. Carvyl acetate,[20] β-phellandrene and carvyl acetate,[25] γ-cadinene and germacrene D-4-ol[32] were other important compounds. All β-caryophyllene, β-bisabolene and bicyclogermacrene were absent while germacrene D was either absent or present in very small quantities.

From Tunisian plants, essential oils of the two species studied present a different composition. So, γ-eudesmol, β-citronellol, citronellyl formate and germacrene D were the major constituent isolated and detected from one[26] while from the other, β-bisabolene, β-caryophyllene, (E)-β-farnesene, 1,8-cineol were the main constituents.[17] Carvacrol and bicyclogermacrene were not detected but germacrene D[17] and β-bisabolene[26] were present at small amount. This difference in composition could be attribute to geographic origin of species. One from north east of Tunisia,[17] the other from south of Tunisia.[26]

From Turkey area, none of the main constituents mentioned before have been found. The major compounds

Table 2. Omnipresent major compounds (in %) of essential oil of *Marrubium vulgare*

Germacrene D	β-Caryophyllene	β-Bisabolene	Bicyclogermacrene	Carvacrol	Other major compounds	Country	Ref
23.85	-	-	20.06	-	E-caryophyllene (44.54) α-Humulene (5.79)	Poland	(10)
43.36	-	-	9.86	9.48	E-Caryophyllene (24.79)	Poland	(10)
27.18	-	-	9.54	6.64	E-Caryophyllene (34.51) δ-Amorphene (8.18)	Poland	(11)
22.45	-	-	11.12	4.71	E-Caryophyllene (36.78) δ-Amorphene (6.15)	Poland	(11)
14.4	45.8	-	-	-	α-Humulene (8.8) δ-Cadinene (5.7) Farnesol (5.3)	Slovak	(13)
10.04	-	1.98	3.38	-	Caryophyllene oxide (18.67) Trans-Caryophyllene (12.77)	Iran	(14)
9.7	11.6	25.4	-	-	(E)-β-Farnesene (8.3)	Iran	(15)
3.1	-	20.4	3.4	-	δ-Cadinene (19.1) isocaryophyllene (14.1)	Iran	(16)
2.4	7.8	28.3	-	-	(E)-β-Farnesene (7.4) 1,8-Cineol (4.8)	Tunisia	(17)
-	-	-	-	-	α-Pinene (28.85) β-Pinene (18.31) β-Phellandrene (17.40) 2-Hexenal (14.80)	Turkey	(18)
0.41	32.19	0.81	-	-	(E)-β-Farnesene (11.39) 1,8-Cineol (8.17) α-Pinene (6.64)	Iran	(19)
-	-	-	-	36.28	β-Phellandrene (15.49) carvyl acetate (11.52)	Egypt	(20)
6.7	11.5	10.3	-	1.8	Eugenol (21.5) γ-Cadinene (9.7) β-Citronellol (9.13)	Algeria	(21)
4.71	8.50	-	1.49	0.84	(Z)-β-Farnesene (9.61) γ-Cadinene (4.96) (E)-Hex-2-enal (4.67)	Lithuania	(22)
0.3	3.9	10.9	-	0.7	Eugenol (50.1)	Algeria	(24)
2.3	3.9	28.8	-	2.1	Eugenol (16.2)	Algeria	(24)
0.06-0.22	-	-	-	31.80-40.75	β-Phellandrene (10.99-15.89) Carvyl acetate (7.57-12.16)	Egypt	(25)
9.37	-	0.86	-	-	γ-Eudesmol (11.93) β-Citronellol (9.90) Citronellyl formate (9.50)	Tunisia	(26)
0.88	-	-	-	-	4,8,12,16-tetramethyl heptadecan-4-olid (16.97) Germacrene D-4-ol (9.61) α-Pinene (9.37	Algeria	(27)
1.69	-	-	-	12.05	Thymol (20.11) (E)-β-Farnesene (15.66) E-Methyl communate (6.18)	Libya	(28)
10	-	0.77	-	-	δ-Eudesmol (11) Citronellyl formate (10) β-Citronellol (8) Geranyl tiglate (7.1) Geranyl formate (6.02)	Iran	(29)
0.74	-	-	-	4.35	Thymol (34.55) γ-Cadinene (17.68) Germacrene D-4-ol (6.37)	Egypt	(32)

of the essential oils were α-pinene, β-pinene, β-phellandrene and 2-hexenal.[18] From Slovakia, *M. vulgare* essential oils exhibit higher concentration of β-caryophyllene and germacrene D.[13] In addition α-humulene, δ-cadinene and farnesol were detected with considerable amounts whereas β-bisabolene, bicyclogermacrene and carvacrol were absent.

In essential oils isolated from the three Algerian *M. vulgare*, bicyclogermacrene was absent in all plants.[21,24,27] The other constituents were present at low amount except for β-bisabolene which constitute the major compounds with another constituents.[21,24] Eugenol was the most representative compounds of two Algerian *M. vulgare* essential oils.[21,24] All compounds cited before were absent in one of the three *Marrubium* investigated except for germacrene D and its oil demonstrate the following composition: 4,8,12,16-tetramethyl heptadecan-4-olid, germacrene D-4-ol and α-pinene as major components.[27] In Algeria, these species were from three different regions: northern Algeria,[21] east[24] and north east[27] of Algeria which could be the origin of this chemical difference.

In the essential oil of *M. vulgare* grown in Lithuania, the main constituents being (Z)-β-farnesene, β-caryophyllene, γ-cadinene, germacrene D and (E)-hex-2-enal.[22] According this study, thirty nine constituents were not previously reported in *M. vulgare*. In Libyan one, thymol was the main components followed by (E)-β-farnesene and carvacrol.[28] Germacrene was present at low quantity and β-caryophyllene, β-bisabolene and bicyclogermacrene were not identified. E-methyl communate was also detected at considerable amounts.

Essential oil of *M. vulgare* from Poland is characterized by absence total of β-caryophyllene and β-bisabolene.[10,11] Germacrene D is omnipresent and is the second main constituent either in the second year of cultivation or in vegetative cycle of development of plant. E-caryophyllene was the main component in all plants studied. Bicyclogermacrene was generally, the third compounds whereas carvacrol was classified at the fourth or the fifth place. Other major constituents were as follow: δ-amorphene, α-copaene and α-humulene.

As shown previously, it seems that each species presents its own composition. The major elements vary from one plant to another depending on the harvesting areas. Thus, major compounds to some species are minor to others. Due to that, many commercial essential oil vendors standardize their oils by adding a coupage to make the oils from each season much more reproducible.[4]

According to Pengelly, essential oil composition can vary according to geographic and genetic factors, thus the same aromatic plant, botanically defined, synthesizes an essence that can be biochemically different depending on the biotope in which it develops; these chemical varieties are commonly called chemotypes.[66] Recently, El-Hawary et al. declares that it is not clear how such differences could be related to geographical location or ecophysiological conditions; thus the observed variations in oils chemical composition are often ascribed to the existence of specific chemotypes.[28] Biochemically different, two chemotypes will present not only different therapeutic activities[67] but also highly variable toxicities.[68] The chemotype of an essential oil does not mean that the chemical constituent specified is strongly in the majority.[67] It can only be of low intensity, but its mere presence justifies a specific therapeutic indication.

Grouped components – Usually, compounds isolated from essential oils are classified into two groups. Monoterpenes which are a class of terpenes that consist of two isoprene units ($C_{10}H_{16}$) and sesquiterpenes which consist of three isoprene units ($C_{15}H_{24}$). On which is often present one or more similar or different functional sites whose majority are oxygenated sites with one or more oxygen atoms (O). Thus we speak of monoterpenes (hydrocarbon or oxygenated) and of sesquiterpenes (hydrocarbon or oxygenated). Those who do not belong to these two groups are grouped as others.

In *M. vulgare* essential oils sesquiterpenoids were the dominant fraction (Table 3). Monoterpenoids were present in appreciable[16,21,26,27,28] or in trace amount.[32] Oxygenated fractions dominated in monoterpenes however, hydrocarbon fraction overpowered in sesquiterpenes except for essential oil from *M. vulgare* which growing in Eastern Algeria,[27] in this case, hydrocarbon monoterpenes represent 12.61% and oxygenated sesquiterpenes represent 13.17%. On the other hand, other compounds dominated with 41.46% of the total rate of volatile oil. Other compounds were also found in large quantity in essential oil from Algerian species[24] both in flowering and vegetative phases. It consists particularly in phenylpropanoids which quantified 50.1% of total oil in flowering phase but reached only 16.2% in the vegetative phase. Essential oil of *M. vulgare* collected from the North coast of Egypt amounts to 39.11% for other compounds of total oil.[32]

Hydrocarbon monoterpenes were totally absent in *M. vulgare* from Egypt[32] or in very low amount from Libya.[28] The very low amount of oxygenated monoterpenes were found in the essential oil isolated of *M. vulgare* from Iran[15] and Egypt.[32] Hydrocarbon sesquiterpenes ranged from 16.8%[24] to 93.22%[10] without considering the except *M. vulgare* from Algeria[27] however oxygenated sesquiterpenes varies from 0.78%[21] to 18.09%.[32] The *Marrubium*

Table 3. Grouped components of essential oil of *Marrubium vulgare*

Hydrocarbon monoterpenes	Oxygenated monoterpenes	Hydrocarbon sesquiterpenes	Oxygenated sesquiterpenes	Other compounds	Ref
2.82	7.23	88.21	/	/	(11)
2.23	5.13	87.86	0.9	/	(11)
8.3	0.5	73.6	8.9	3.8	(15)
10.9	14.1	68.2	/	0.3	(16)
2.9	11.3	50.5	1.4	8.5	(17)
7.19	19.65	43.28	0.78	26.82	(21)
8.3	2.4	16.8	0.8	61.6	(24)
2.1	4.7	40.3	2.6	40.4	(24)
1.65	40.02	42.70	6.19	/	(26)
12.61	9.46	5.58	13.17	41.46	(27)
0.36	37.74	22.60	10.25	28.84	(28)
0	0.51	32.48	18.09	39.11	(32)

genus seems to produce oils that are rich in hydrocarbon compounds, with sesquiterpenes forming the major part.[69]

Variability of *Marrubium vulgare* essential oil composition and yield – It is well established that yield and composition of essential oil are not stable and varies very much according several factors. Some of them are related to genetic and environmental conditions.[70-72] Other are related to experimental condition (methods of isolation).[21,57,73]

According to Letchamo and Mukhopadhyay, the content of the essential oil was low and varied among different parts of horehound. Leaves contains more essential oil (0.29%) than flowers (0.10%) or stems (0.01%); no essential oil was found in the seeds.[23] On the other hand, sesquiterpene hydrocarbons were recognized as the most frequent groups of natural compounds in the profiles of the advanced approaches, whereas in the traditional one monoterpene hydrocarbons were found to be the dominant constituting group when analyzing chemical composition of essential oils and volatiles extracted from *Marrubium* species using the traditional and advanced methods.[73]

Regarding the vegetative cycle of *M. vulgare*, some authors did not found any difference in term of yield of isolated oils between flowering (F) and vegetative (V) phases of this species from Algeria[24] and Poland[10] countries respectively. Among others, Zawislak showed that the content of essential oils in the herb of *M. vulgare* in the second year of cultivation ranged between 0.06% and 0.07%, which was the same as in the herb harvested during the first year of cultivation (between 0.05% and 0.06%).[11]

Concerning the chemical composition of essential oils, it varies from cycle to another. In Algeria area, eugenol and β-bisabolene were the major components in both oils from flowering and vegetative phases, but with different degrees of importance; so, when eugenol was the main component in the F oil (50%), it comprised only 16% in the V oil and β-bisabolene was the dominant component of the V oil (29%), it is the second main component in the F oil (11%).[24] Also, according the same authors, phenyl-propanoids fraction (50.1%) dominated in the oil isolated from the aerial parts collected during the flowering phase against 16.2% in the vegetative phase, whereas the sesquiterpene hydrocarbons (40.3%) constituted the major fraction during the vegetative phase of the plant against 16.8% in the flowering phase. In Poland area, E-caryophyllene, germacrene D, bicyclogermacrene, and α-humulene were the main components of the oil.[10] All these compounds are in considerable amount in the vegetative phase (44.54%, 20.06% and 5.79% respectively) compared to flowering phase (27.79%, 9.86% and 3.26 respectively) except for germacrene D (43.36% in the F phase against 23.85% in V phase). It will be noted that the oil from F phase contain more compounds than from V phase (50 against 23) among them carvacrol is found at an amount of about 9.48% but absent in the later one.

On the other hand, the chemical composition of essential oil of *M. vulgare* was studied from a two-year plantation; results showed that the main compounds were as follows: E-caryophyllene (34.51 – 36.78%), germacrene D (22.45 – 27.18%), bicyclogermacrene (9.54 – 11.12%), δ-amorphene (6.15 – 8.18%), and carvacrol (4.71 –6.64%),[11] while in the herb harvested during the first year of cultivation the main components were as follows: E-caryophyllene (25.91 – 32.06%), germacrene D (20.23 –

31.14%) and δ-amorphene (8.38 – 10.22%).[10]

The ratio of solid to liquid effect and the effect of grinding on the essential oils yield and composition of *M. vulgare* were studied.[21] The chemical composition in the experimental conditions giving the high yield (mass ratio, 3 kg m^{-3} and particle size, $0.1 < d < 0.63$ mm) showed that eugenol (21.5%), β-caryophyllene (11.5%), β-bisabolene (10.3%), δ-cadinene (9.7%), β-citronellol (9.13%), and germacrene D (6.7%) were the major constituents found. In this study, particle size and ratio of solid to solvent, have a significant influence on the yield of essential oils, but particle size was found to be the most significant factor influencing the process.

Biological activities

Biological properties of essential oils are highlighted by the review of Adorjan and Buchbauer.[74] Nakatsu et al. review the biological activities of essential oils and their constituents.[75] According to Pengelly, it is often the unique chemical combination rather than a single component that is responsible for any therapeutic activity.[66] Lahlou stipulate that essential oil, in its totality, acted less than the major constituents and suggest in some cases that biological activity of the essences from the aromatic plants studied may be attributable both to their major components and to the minor ones present in these oils.[76]

Antibacterial activity – Zarai et al. evaluated the antibacterial activity of *M. vulgare* L. essential oils grown in Tunisia against eight bacterial strains Gram (+): *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Staphylococcus aureus* 25923, *Bacillus subtilis* and *Bacillus cereus* and four bacterial strains Gram (-): *Pseudomonas aeruginosa* 27853, *Klebsielle pneumoniae* WHO24, *Escherchia coli* 25922, *Salmonella*.[31] Presence or absence of inhibition zones and zone diameter (DD), the medium inhibitory concentration (IC_{50}) and the minimal inhibitory concentration (MIC) values were the techniques employed for this study. The results obtained showed a significant activity against G (+) bacteria but not on all G (-) bacteria strains. According this study, essential oil inhibited the growth of bacterial strains produced a zone diameter of inhibition from 6.6 to 25.2 mm, along with IC_{50} and MIC values ranging from 560 - 1100 μg/mL and 1120 - 2600 μg/mL, respectively. The strongest activity was observed against *Staphylococcus epidermidis* and *Staphylococcus aureus* 25923. However, the lowest one was observed against *Bacillus cereus* and *Enterococcus faecalis*. Authors assign this activities of essential oil of *M. vulgare* to its composition, especially to its high contents of oxygenated compounds (46.21%) and the highest sensitivity of *Staphylococcus epidermidis* and *Staphylococcus aureus* to its cell wall structure and outer membrane.

Klebsiella pneumoniae is an opportunistic pathogenic bacterial associated with nosocomial infections; many strains of it have developed a resistance against current antibiotics, which cause a serious health problem. Due to that, Dehbashi et al. studied the antibacterial potential effects of *M. vulgare* L. essential oils against 30 strains of *K. pneumoniae* isolated from urine culture of hospitalized patients in Iran.[30] To characterize the antimicrobial activities of this essential oil, the minimum inhibitory concentrations (MIC) and minimum bacterial concentrations (MBC) were investigated. The results showed that on the one hand, overall *K. pneumoniae* was resistant to the three tested antibiotics, namely Ampicillin 25 μg, Sulfamethoxazol 23.15 μg and Gentamicin 10 μg and on the other hand the essential oil had preventive effect on the most isolates. The least and the highest MIC values of *M. vulgare* essential oil were 1.25 and 5 mg/mL respectively. According to this study, it was not found a correlation between the concentration of the essential oil and MIC activity while the MBC activity showed a direct relation to the used concentration.

Staphylococcus aureus is another antibiotic resistant and prevalent pathogen worldwide.[29] An increasing proportion of this bacteria developed resistant to antibiotics such as methicillin and are known as methicillin-resistant *S. aureus* (MRSA). Because the use of plants in traditional medicine, Bokaeian et al. studied the antibacterial activity of essential oil of *M. vulgare* collected in south-eastern of Iran against *Staphylococcus aureus* in vitro.[29] The study was conducted between 2010 - 2011 on 160 healthy subjects, hospital staffs and inpatient from which seventeen strains of *S. aureus* were isolated from nose and throat and characterized biochemically. Agar disk diffusion assay was used with different antibiotics as tetracyclin (30 μg), ampicillin (10 μg), trimethoprim-sulfamethoxazol (1.25+23.15 μg), erythromycin (15 μg), ceftazidime (30 μg), penicillin (10 μg), amikacin (10 μg), ceftriaxon (30 μg). MIC and MBC of plant essential oils were determined with broth microdilution method. The results of this study showed that essential oil of *M. vulgare* had inhibitory effect against most isolated plates. The least MIC and MBC values were 0.3 mg/mL and 0.62 and 1.25 mg/mL respectively and the highest MIC and MBC value of essential oil of *M. vulgare* were 2.5 mg/mL and 5 mg/mL respectively. Concerning the antibiotic effect, 70% or

more of *S. aureus* strains are resistant to trimethoprim, ampicillin, erythromycin, penicillin and 94.1% of which are sensitive to amikacin and 59.9% to ceftazidime. Ceftriaxone and tetracyclin exerts an intermediate action.

Antifungal activity – The antifungal of essential oil of *M. vulgare* was tested against four fungi strains, namely *Botrytis cinerea*, *Fusarium solan*, *Penicillium digitatum* and *Aspergillus niger* by employing disc agar diffusion for determining disc diameter of zone of inhibition and micro-well dilution method for determining MIC and IC_{50} values.[31] In this study, the disc diameter zones of inhibition ranged from 6.4 - 12.6 mm, along with IC_{50} and MIC values ranging from 2190 - 3220 μg/mL and 1100 - 1190 μg/mL, respectively. The maximal inhibition zones were obtained for *Botrytis cinerea*, however *Penicillium digitatum* and *Aspergillus niger* exhibited weak activity. Germacren D, which is one of the major constituent of *M. vulgare* essential oils, has significant antibacterial and antifungal activities.[31]

Antioxidant activity – Antioxidant effectiveness of *M. vulgare* essential oils from Tunisia were examined by three different methods: The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay, the β-carotene bleaching test and the reducing power assay.[26] Results of this study point out strong protective activity against scavenging of DPPH free radicals and β-carotene bleaching test, and a moderate reducing power effect of the essential oil. In the DPPH assay, this oil exhibited an IC_{50} value of 74 μg/mL, which is about 2 times higher than the synthetic antioxidant (BHT) and is a concentration dependent activity profile. Concerning the β-carotene bleaching method, the IC_{50} of the essential oil was estimated as 36.30 μg/mL compared to BHT (20.30 μg/mL) and at the same concentration (70 μg/mL), the antioxidant activities of the oil were somewhat lower than the BHT ($59.02 \pm 2.00\%$ vs. $77.50 \pm 1.00\%$). The reducing power of *M. vulgare* essential oil at 70 μg/mL was 0.45 ± 0.032, which remained significantly lower than that of BHT at the same concentration, used as positive control (1.05 ± 0.01). According the authors, these activities found are probably in relation with the structure of the hydroxylated compounds, but a possible synergistic effect among oxygen containing compounds can be also suggested.

From Algeria, *M. vulgare* essential oils was studied for its antioxidant activity using the DPPH radical scavenging as unique method employed by authors.[27] The results indicate that this essential oil exhibited an IC_{50} value of 153.84 μg/mL, which is about 2 times higher than the synthetic antioxidant (BHT). According the authors, this oil can be considered an effective source of antioxidants of natural origin.

The in vivo antioxidant activity of essential oil of *M. vulgare* was assessed by measuring the ability of the tested oils to restore glutathione levels in the blood of alloxan induced diabetic rats.[28] The essential oil from the aerial part had restored the levels of glutathione in the diabetic rats and had most powerful antioxidant activity which was comparable to that of vitamin E used as a standard. The increase of blood glutathione (in mg) were from 21.3 ± 0.4 (diabetic untreated) to 35.2 ± 0.8 (diabetic + *M. vulgare* essential oils "50 mg/kg") or to 35.7 ± 0.8 (diabetic + vitamin E "7.6 mg/kg"). The authors attribute this activity to the presence of high percentage of thymol which was reported elsewhere to possess antioxidant activity.

Hepatoprotective activity – The essential oil of *M. vulgare* was tested in adult male albino rats and albino mice for its hepatoprotective activity at a dose of 50 mg/kg body weight.[28] Liver damage was inducted by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride (CCl_4) in liquid paraffin using silymarin 25 mg/kg body weight as a reference drug. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes were measured in the blood of each group of animals at different times. The results showed that the essential oils of *M. vulgare* had potent hepatoprotective activity which was comparable to the effect of silymarin. Therefore, the hepatoprotective activity of this oil may be attributed to its antioxidant activity and especially to the presence of phenolic compounds and monoterpenes in the oil.[28]

Cytotoxicity properties – HeLa cells (cervical cancer line, adherent) were used to investigate the cytotoxicity effect of essential oil of *M. vulgare* grown in Tunisia.[31] The proliferation rates of HeLa cells after treatment with essential oils were determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Results showed that essential oil decrease significantly the viability of HeLa in a dose dependent manner. For a concentration up to 250 μg/mL, essential oil destructed HeLa cells by 27%, however for a concentration higher than 500 μg/mL, all HeLa cells were destructed. According authors, the presence of germacrene D in a good amount can enhances the cytotoxicity of the tested oil. Also the synergistic effects of active chemicals with other constituents of the essential oil should be taken into consideration. Authors conclude that their results are very promising with regards to possible antineoplastic chemotherapy and form a very sound basis for future research.

Mosquitocidal/Molluscicidal activities – Salama et al. studied the mosquitocidal and molluscicidal activity of *M. vulgare* essential oils on *Culex pipiens* and on adult and eggs of *Biomphalaria alexandrina*, respectively.[32] Essential oil of *M. vulgare* was tested at concentrations of 12.5, 25, 50, 100 and 200 ppm (stock solution of the essential oil was prepared in Tween 80).

Mosquitoes were exposed to different concentrations of essential oil for 24 h at room temperature, and were kept under normal laboratory conditions at 26 ± 2 ºC and 60 ± 10% relative humidity with 12:12 D/L photoperiod. Mortality was recorded after 24 h of continuous exposure during which no food was offered to the test organisms. The lethal concentration to 50% (LC_{50}) and 90% (LC_{90}) were determined. Results of larvicidal and pupicidal activities of the essential oils of *M. vulgare* L. against *Culex pipiens* after 12 h exposure period showed that essential oil gave 50% and 90% larvicidal and pupicidal activities at 200 and 400 ppm, respectively for each.

The bioassay of molluscicidal activity against *Biomphalaria alexandrina* was evaluated by immersion technique. Adult snails and egg masses were exposed for 24 h at room temperature. Tested snails and egg masses were examined to assess mortality. Mortality was evaluated using crushing technique (5% sodium hydroxide solution). Egg masses were examined under the microscope for detecting the embryos and its vitality. Snails were considered dead if they remained motionless, did not respond to the presence of food or if the shell looked discolored. Results of this test showed that LC_{50} and LC_{90} of *M. vulgare* essential oil versus adult snails were 50 and 100 ppm/3 h, respectively and 100% snail ovicidal activity against eggs at 200 ppm/24 h.

In this study, authors prove that essential oil of *M. vulgare* is biologically active and effect showed significant time and dose dependent and attribute these activities to thymol or its isomer carvacrol which are in considerable concentration and also to the high sesquiterpene content of *Marrubuim* (50.57%).

Conclusion

In this paper, chemical composition and biological activities of *Marrubium vulgare* essential oil are described. This species is oil poor but rich in composition and possess many biological activities. *Marrubium vulgare* is widespread in the Mediterranean areas and introduced elsewhere, and is widely used in folk medicine in all over the world to treat a variety of ailment. Due to the variability of composition of essential oil, further studies are necessary, particularly regarding their chemicals and factors of variation mainly those related to geographical area, which may cause an important change in the biological activities of oils. This variability can define different chemotype according the bioactive compound present, even at low concentration.

Acknowledgements

This study was supported by the Algerian Ministry of Higher Education and Scientific Research (Project CNEPRU code D04N01UN170120150002).

References

(1) Harley, R. M.; Atkins, S.; Budantsev, A. L.; Cantino, P. D.; Conn, B. J.; Grayer, R.; Harley, M. M.; de Kok, R.; Krestovskaja, T.; Morales, R.; Paton, A. J.; Ryding O.; Upson, T. In Flowering Plants. Dicotyledons: Lamiales (except Acanthaceae including Avicenniaceae); Kadereit, J. W. Ed.; Springer; Germany, **2004**, pp 167-275.

(2) Woodson, R. E. Jr.; Schery, R. W.; Nowicke, J. W.; Epling, C. C. *Ann. Mo. Bot. Gard.* **1969**, *56*, 71-111.

(3) Kokkini, S.; Karousou, R.; Hanlidou, E. *HERBS* **2003**, 3082-3090.

(4) Lawrence, B. M. *Int. J. Aromather.* **2001**, *10*, 82-98.

(5) El-Gazzar, A.; Watson, L. *New Phytol.* **1970**, *69*, 487-492.

(6) Akgül, G.; Ketenoglu, O.; Pinar, N. M.; Kurt, L. *Ann. Bot. Fenn.* **2008**, *45*, 1-10.

(7) Lawrence, B. M. Fragrance and flavours; Oxford & IBH Publishing Company Eds, **1989**, p 71.

(8) Rodriguez Villanueva, J.; Martin Esteban, J. *Phytother. Res.* **2016**, *30*, 1551-1558.

(9) Sabry, R.; Salama, A.; Sharaf-Eldin, M. *Arznei Gewurzpfla.* **2012**, *17*, 164-168.

(10) Zawislak, G. *Farmacia* **2012**, *60*, 287-292.

(11) Zawiślak, G. *Acta Agrobot.* **2015**, *68*, 59-62.

(12) Hmamou, B. D.; Salghi, R.; Zarrouk, A.; Zarrok, H.; Benali, O.; Errami, M.; Hammouti, B. *Res. Chem. Intermed.* **2013**, *39*, 3291-3302.

(13) Nagy, M.; Svajdlenka, E. *J. Essent. Oil Res.* **1998**, *10*, 585-587.

(14) Asadipour, A.; Mehrabani, M.; Nazeri, V.; Tabarraii, M. *Ulum-i-Daroei*, **2005**, *2*, 77-82.

(15) Khanavi, M.; Ghasemian, L.; Motlagh, E. H.; Hadjiakhoondi, A.; Shafiee, A. *Flavour Fragr. J.* **2005**, *20*, 324-326.

(16) Morteza-Semnani, K.; Saeedi, M.; Babanezhad, E. *J. Essent. Oil Res.* **2008**, *20*, 488-490.

(17) Hamdaoui, B.; Wannes, W. A.; Marrakchi, M.; Ben Brahim, N.; Marzouk, B. *J. Essent. Oil Bear. Pl.* **2013**, *16*, 608-612.

(18) Bayir, B.; Gündüz, H.; Usta, T.; Şahin, E.; Özdemir, Z.; Kayır, O.; Şen, O.; Akşit, H.; Elmastaş, M.; Erenler, R. *J. New Res. Sci.* **2014**, *3*, 44-50.

(19) Golparvar, A. R.; Hadipanah, A.; Mehrabi, A. M.; Armin, A. *J. Herb. Drugs* **2015**, *6*, 1-5.

(20) Said-Al Ahl, H. A. H.; Gendy, A. S. H.; Mahmoud, A. A.; Mohamed, H. F. Y. *Intern. J. Plant Sci. Ecol.* **2015**, *1*, 138-141.

(21) Rezazi, S.; Hanini, S.; Si-Moussa, C.; Abdelmalek, S. *J. Agr. Sci. Tech.* **2017**, *19*, 307-322.

(22) Weel, K. G. C.; Venskutonis, P. R.; Pukalskas, A.; Gruzdiene, D.; Linssen, J. P. H. *Fett/Lipid,* **1999**, *10*, S395-S400.

(23) Letchamo, W.; Mukhopadhyay, S. *J. Hort. Sci.* **1997**, *72*, 741-748.

(24) Belhattab, R.; Larous, L.; Figueiredo, A. C.; Santos, P. A. G.;

Costa, M. M.; Barroso, J. G.; Pedro, L. G. *J. Essent. Oil Res.* **2006**, *18*, 369-373.
(25) Said-Al Ahl, H. A. H.; Sabra, A. S. *J. Chem. Pharm. Res.* **2016**, *8*, 863-872.
(26) Kadri, A.; Zarai, Z.; Békir, A.; Gharsallah, N.; Damak, M.; Gdoura, R. *Afr. J. Biotechnol.* **2011**, *10*, 3908-3914.
(27) Abadi, A.; Hassani, A. *Int. lett. chem. phys. astron.* **2013**, *14*, 17-24.
(28) El-Hawary, S.; EL-Shabrawy, A.; Ezzat, S.; EL-Shibany, F. *J. Med. Plants Res.* **2013**, *7*, 1746-1753.
(29) Bokaeian, M.; Saboori, E.; Saeidi, S.; Niazi, A. A.; Amini-Borojeni, N.; Khaje, H.; Bazi, S. *Zahedan J. Res. Med. Sci.* **2014**, *16*, 60-64.
(30) Dehbashi, Z.; Mazaheri, M.; Saeedi, S.; Sabbagh, S. K. *Adv. Herb. Med.* **2016**, *2*, 9-14.
(31) Zarai, Z.; Kadri, A.; Ben Chobba, I.; Ben Mansour, R.; Bekir, A.; Mejdoub, H.; Gharsallah, N. *Lipids Health Dis.* **2011**, *10*, 1-8.
(32) Salama, M. M.; Taher, E. E.; El-Bahy, M. M. *Rev. Inst. Med. Trop. Sao Paulo,* **2012**, *54*, 281-286.
(33) Yabrir, B. *Chem. J. Moldova,* **2018**, *13*, 8-23.
(34) Ahvazi, M.; Balali, G. R.; Jamzad, Z.; Saeidi, H. *J. Med. Plants*, **2018**, *17*, 7-24.
(35) Lodhi, S.; Vadnere, G. P.; Sharma, V. K.; Usman, M. R. *J. Intercult. Ethnopharmacol.* **2017**, *6*, 429-452.
(36) Smolen, K. *Diss. Pharm,* **1960**, *12*, 325-352.
(37) Dmitruk, M.; Haratym, W. *Mod. Phytomorphol.* **2014**, *6*, 85.
(38) Ahvazi, M.; Jamzad, Z.; Balali, G. R.; Saeidi, H. *Iran. J. Bot*. **2016**, *22*, 39-58.
(39) Lippai, A.; Smith, P. A.; Price, T. V.; Weiss, J.; Lloyd, C. *Weed Sci.* **1996**, *44*, 91-99.
(40) Groenteman, R.; Probst, C.; Bellgard, S.; Prebble, J. Manaaki Whenua; Landcare Research: New Zealand, **2017**, p 78.
(41) Benvenuti, S.; Andolfi, L.; Macchia, M. *Seed Tech.* **2001**, *23*, 138-144.
(42) Weiss, J.; Ainsworth, N.; Faithfull, I. CRC for Weed Management Systems, University of Adelaide, Australia, **2000**, p 6.
(43) Young, J. A.; Evans, R. A. *Weed Sci.* **1986**, *34*, 266-270.
(44) Wiersum, K. F.; Dold, A. P.; Husselman, M.; Cocks, M. Frontis: Medicinal and aromatic plants; Bogers, R. J.; Craker, L. E.; Lange, D.; Springer; Netherlands, **2006**, pp 43-57.
(45) Garjani, A.; Tila, D.; Hamedeyazdan, S.; Vaez, H.; Rameshrad, M.; Pashaii, M.; Fathiazad, F. *Folia Morphol.* **2017**, *76*, 361-371.
(46) Tahri, N.; El Basti, A.; Zidane, L.; Rochdi, A.; Douira, A. *Kastamonu Univ., J. Fores. Fac.* **2012**, *12*, 192-208.
(47) Masoodi, M. H.; Ahmad, B. *J. Pharm. Res.* **2012**, *5*, 2668-2671.
(48) Oran, S. A.; Al-Eisawi, D. M. *Dirasat* **1998**, *25*, 84-112.
(49) VanderJagt, T. J.; Ghattas, R.; VanderJagt, D. J.; Crossey, M.; Glew, R. H. *Life Sci.* **2002**, *70*, 1035-1040.
(50) Herrera-Arellano, A.; Aguilar-Santamaria, L.; Garcia-Hernandez, B.; Nicasio-Torres, P.; Tortoriello, J. *Phytomed.* **2004**, *11*, 561-566.
(51) Knoss, W. *Biotechnol. Agric. For.* **1999**, *43*, 274-289.
(52) de Souza, M. M.; de Jesus, R. A.; Cechinel-Filho, V. ; Schlemper, V. *Phytomedicine* **1998**, *5*, 103-107.
(53) Ibrahim, F. M.; Ibrahim, A. Y.; Omer, E. A. *World J. Pharm. Sci.* **2014**, *2*, 1664-1670.
(54) Haq, F.; Ahmad, H.; Alam, M. *J. Med. Plant. Res.* **2011**, *5*, 39-48.
(55) Lawrence, B. M. In Manual on the essential oil industry: The Isolation of Aromatic Materials from Natural Natural Plant Products; De Silva, K.T. Ed; United Nations Industrial Development Organization; Austria, **1996**, pp 57-154.
(56) Rassem, H. H. A.; Nour, A. H.; Yunus, R. M. *Aust. J. Basic Appl. Sci.* **2016**, *10*, 117-127.
(57) Scheffer, J. J. C. *Phytother. Res.* **1996**, *10*, s6-s7.
(58) Craveiro, A. A.; Matos, F. J. A.; Alencar, J. W.; Plumel, M. M. *Flavour Frag. J.* **1989**, *4*, 43-44.
(59) Kumar, S. Analytical technique for natural product research, CABI; Boston, USA, **2015**, p 204.
(60) Stashenko, E. E.; Jaramillo, B. E.; Martinez, J. R. *J. Chromatogr. A.* **2004**, *1025*, 105-113.
(61) Chemat, F.; Lucchesie, M. E. *J. Soc. Ouest-Afr. Chim.* **2005**, *020*, 77-99.
(62) Chemat, F.; Abert-Vian, M.; Fernandez, X. In Microwave-assisted Extraction for Bioactive Compounds Theory and Practice: Microwave-Assisted Extraction of Essential Oils and Aromas; Chemat, F.; Cravotto, G. Ed; Food Engineering Series; USA, **2013**, pp 53-68.
(63) Pallado, P.; Tassinato, G.; D'Alpaos, M.; Traldi, P. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1335-1341.
(64) Miloudi, K.; Hamimed, A.; Benmimoun, Y.; Bellebna, Y.; Taibi, A.; Tilmatine, A. *Carpath. J. Food Sci. Technol.* **2018**, *10*, 104-110.
(65) Zellner, Bd'. A.; Dugo, P.; Dugo, G.; Mondello, L. Analysis of Essential Oils; CRC Press Taylor & Francis Group; Florida, **2010**, pp 151-183.
(66) Pengelly, A. The constituents of medicinal plants: An introduction to the chemistry and therapeutics of herbal medicines; A&U Academic; Australia, **1999**, p 172.
(67) Bruneton, J. Pharmacognosie, Phytochimie, Plantes médicinales; Lavoisier Tec et Doc Ed; France, **1999**, p 1504.
(68) Baudoux, D. *Aroma News* **1997**, *9/97*, 1-6.
(69) Laouer, H.; Yabrir, B.; Djeridane, A.; Yousfi, M.; Beldovini, N.; Lamamra, M. *Nat. Prod. Commun.* **2009**, *4*, 1133-1138.
(70) Lincoln, D. E.; Langeheim, J. H. *Biochem. Syst. Ecol.* **1981**, *9*, 153-160.
(71) Gotsiou, P.; Naxakis, G.; Skoula, M. *Biochem. Syst. Ecol.* **2002**, *30*, 865-879.
(72) Franz, C.; Novak, J. Sources of essential oils; CRC Press Taylor & Francis Group; Florida, **2010**, pp 39-81.
(73) Mohammadhosseini, M. *J. Med. Plants By-prod.* **2016**, *5*, 169-180.
(74) Adorjan, B.; Buchbauer, G. *Flavour Fragr. J.* **2010**, *25*, 407-426.
(75) Nakatsu, T.; Lupo Jr, A. T.; Chinn Jr, J. W.; Kang, R. L. *Studies Nat. Prod. Chem.* **2000**, *21B*, 571-631.
(76) Lahlou, M. *Phytother. Res.* **2004**, *18*, 435-448.

Received October 9, 2018
Revised December 9, 2018
Accepted December 9, 2018