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ACTIVITIES OF SOME MARRUBIUM SPECIES
ESSENTIAL OIL: A REVIEW**

Benalia Yabrir

*Department of Biology, Ziane Achour University, PB 3117,
Djelfa 17000, Algeria
e-mail: byabrir@yahoo.fr; phone: +(213) 7 77 44 13 83*

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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SOME MARRUBIUM SPECIES ESSENTIAL OIL: A REVIEW

Benalia Yabrir

Department of Biology, Ziane Achour University, PB 3117, Djelfa 17000, Algeria

e-mail: byabrir@yahoo.fr; phone: +(213) 7 77 44 13 83

Abstract. Due to their efficiency and safety, synthetic products raise several questions as for their use; thus, medicinal plants regain interest as potential source of bioactive natural compounds. *Marrubium* species are recognized to possess many beneficial effects on the human body. They are widely used in folk medicine all over the world to treat a variety of ailments. This paper reviews information on the essential oil of *Marrubium* species (except *M. vulgare*) described until now regarding extraction, chemical composition and biological activities. *Marrubium* essential oils, although quantitatively poor, are rich in chemical composition. This composition consists especially of sesquiterpenoids and a little amount of monoterpenes. *Marrubium* essential oils exhibit antioxidant and antimicrobial activities. However, because of the lack of literature concerning essential oil of these species, further studies are necessary, particularly regarding their activities.

Keywords: antimicrobial activity, antioxidant activity, chemical composition, essential oil, *Marrubium* sp.

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List of abbreviations:

ABTS	2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
AchE	acetylcholinesterase
ATCC	American Type Culture Collection
BchE	butyrylcholinesterase
BHA	butylatedhydroxyanisole
BHT	butylated hydroxytoluene
CNCTC	Czech National Collection of Type Cultures
CUPRAC	cupric reducing antioxidant capacity
DPPH	1,1-diphenyl-2-picrylhydrazyl
EC50	half maximal effective concentration
EO	essential oil
HD	hydro-distillation
HS-SPME	head-space solid-phase micro extraction
IC50	half maximal inhibitory concentration
IPP	isopentenyl diphosphate
ISO	International Organization for Standardization
MAE	microwave-assisted extraction
MAHD	microwave assisted hydro-distillation
MH	monoterpene hydrocarbon
MIC	minimum inhibitory concentration
MMC	minimum microbiocidal concentration
NCIMB	National Collection of Industrial, Food and Marine Bacteria
NRRL	Northern Regional Research Laboratory Culture Collection
OM	oxygenated monoterpene
OS	oxygenated sesquiterpene
PSE	pressurized solvent extraction
RSKK	Refik Saydam National Type Culture Collection
SD	steam-distillation
SFE	supercritical fluid extraction
SFME	solvent-free microwave extraction
SH	sesquiterpene hydrocarbon
XOD	xanthine-oxidase activity

Introduction

Essential oils (EOs) are obtained from a variety of aromatic plant materials including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots. These aromatic compounds are formed by plants as by-products or final metabolic products [1] and stored in one or several plant organs [2]. They are contained in leaves and/or reproductive structures and sometimes in the stem and roots of plants [3]. According to Chemat, F. and Cravotto, G., the EOs extracted from different organs in the same plants are variable for their names and uses because of their odours [1]. It consists of a mixture of many volatile compounds, and it can be classified into two main groups: hydrocarbons, which consist of terpenes, such as monoterpenes, sesquiterpenes, and diterpenes; and oxygenated compounds, such as esters, aldehydes, ketones, alcohols, phenols, oxides, acids, and lactones [4]. Nitrogen and sulphur compounds also occasionally exist [1]. This composition varies very much due to many factors including genetic and environmental conditions [3,5,6]. The experimental (methods of isolation) act too [7]. The chemical composition of essential oil is in strict direct relation with biological activities and this relation may be attributed both to their major components and to the minor ones [8]. As reported by the same author, the essential oil, in its totality, acted less than the major constituents. The biological activity of essential oils and their constituents was reviewed by Nakatsu, T. *et al.* [9].

According to El-Gazzar, A. and Watson, L., *Labiatae* family have for centuries been acknowledged as a group of considerable pharmaceutical and culinary interest [10]. This family does not contain any dangerous plants; all are aromatic, stimulating or bitter and tonic [11]. The *Labiatae* family contains 236 genera and about 7173 species, almost cosmopolitan, but absent from the coldest regions of high latitude or altitude [12]. This family is well known with two major series of genera: oil-rich and oil-poor species [10]. *Marrubium* genera belong to this family which consists of annual or perennial herbs, and is an oil-poor one. Species of this genus are characterized by grains with tricolpate pollen [13,14] and so oil-poor, according to Lawrence hypothesis [15]. The essential oil yield is an irrefutable evidence.

The exact number of species of the genus *Marrubium* is not well known. Generally, about forty species are mentioned, mainly distributed throughout Asia, North Africa and Europe

[12,14]. In Turkey, *Marrubium* genus comprises twenty two species [16-18]; eleven species occur in Iran [19-26]; five to ten species exist in Tunisia [27]; it includes six species and one hybrid in Algeria [28,29] and five species in Greece [30]. Some of *Marrubium* species are described or known as endemic as well as *M. aschersonii* to Tunisia [27]; *M. duabense*, *M. crassidense*, *M. procerum* and nine other species to Iran [24,25,31]; *M. thessalum*, *M. velutinum* and *M. cylleneum* to Greece [30,32,33]; *M. deserti* to Algeria [34]; *M. persicum* to Armenia, Turkey and Iran [35]; *M. bourgaei*, *M. trachyticum*, *M. cephalanthum*, *M. globosum* and thirteen other species to Turkey [16-18].

There are a few studies concerning the essential oil isolated from *Marrubium* species, seventeen of which are elucidated. Those that exist are much more related to chemical characterization and identification of essential oils components [16-43]. The literature regarding the biological activity of EO from *Marrubium* genus is scarce and includes only few studies concerning *M. duabense* [31], *M. globosum* [36,37], *M. cuneatum* [36], *M. peregrinum* [38], *M. deserti* [28, 29], *M. cylleneum* [32] and *M. incanum* [39]. The biological activity is essentially related to antimicrobial [28,29,31,36,39] or antioxidant [28,29,37,38] properties. Anticholinesterase [29] and ocular allergy [32] of *Marrubium* EOs are also investigated.

Because is the most representative among the genus *Marrubium*, more widespread and introduced elsewhere [12], *M. vulgare* will be discussed in a separate paper. Its chemical composition and biological activity are well documented overall the world and widely studied by several researchers.

This paper is not intended to be an exhaustive review, but rather to present an overview of scientific knowledge on chemical composition and biological activities of *Marrubium* essential oils (except *M. vulgare*) described until now. Because methods of extraction of essential oils from medicinal plants affect substantially both chemical compounds and composition of the essential oil, the extraction procedures applied for their isolation will be discussed as well as conventional methods and innovative techniques; followed by the quantification of oil separated from various species of *Marrubium* in term of yields, this constitutes the first section of the review. The second section discusses the chemical composition focusing on the main constituents. Generally, these components are grouped in five

groups namely monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon, oxygenated sesquiterpene, the other constituents are combined in a single class. The last section deals with biological activities, including (i) antioxidant activity by considering assays used, (ii) antimicrobial activity with particular attention to microorganism testing and antimicrobial testing methods, and (iii) other activities were also presented according to available data such as allergic conjunctivitis and anticholinesterase activity. In addition, further investigations and studies are suggested.

Progress on the extraction methods of essential oils

Isolation of essential oils

Essential oils isolation is performed in accordance with International Organization for Standardization (ISO 9235) that defines them as “Product obtained from vegetable raw material—either by distillation with water or steam, or from the epicarp of fruits by a mechanical process, or by dry distillation” [44]. Also, according to ISO 9235 “Essential oils may undergo physical treatments (e.g., re-distillation, aeration) that do not involve significant changes in their composition”.

The amount of essential oil produced depends on four main criteria: the length of distillation time, temperature, operating pressure,

and, most importantly, the type and quality of the plant material [1]. Due to the cost of energy and time and environmental impact of essential oils extraction by conventional methods, new technologies were developed with more efficient extraction processes (reduction of extraction time and energy consumption, increase of extraction yield, improvement of essential oils quality [1]). Thus, many microwave methods were proposed and patented. Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and pressurized solvent extraction (PSE) are fast and efficient unconventional extraction methods developed for extracting analytes from solid matrixes, summarize Kaufmann, B. and Christen, P. [45]. Advantages and disadvantages of some of conventional methods and innovative techniques were discussed by Scheffer, J.J.C. [7].

Since the description of Clevenger apparatus for the determination of volatile oil, all *Marrubium* species studied were subjected for the distillation method (Table 1). In this way, dried areal parts of plants were water (hydrodistillation “HD”) or steam (steam distillation “SD”) distilled for 1 to 5 hours.

Extraction yield

The very low yield (0.01%) was obtained from *M. parviflorum* Fish. et Mey. Subsp. *Oligodon* (Boiss.) Seybold in Turkey [41] and *M. aschersonii* in Tunisia [27]; the highest (0.91%) from *M. astracanicum* Jacq. in Iran [20] (Table 1).

Table 1

Extraction of <i>Marrubium</i> EOs.					
<i>Marrubium</i> specie	Plant part	Extraction method	Yield (%)	Country	Ref.
<i>M. bourgaei</i> ssp. <i>caricum</i> P.H. Davis	Air-dried	Hydrodistillation	-	Turkey	[16]
	Aerial parts	Clevenger type (3h)			
<i>M. bourgaei</i> Boiss. ssp. <i>bourgaei</i>	Powdered	Water distillation	0.4	Turkey	[17]
	Aerial parts	Clevenger type (3h)			
<i>M. anisodon</i> C. Koch	Air-dried	Hydrodistillation	-	Turkey	[18]
	Aerial parts	Clevenger type (3h)			
<i>M. cuneatum</i> Russel	Air-dried	Water steam distillation All-glass apparatus	0.15	Iran	[19]
	Aerial parts Flowering stage				
<i>M. astracanicum</i> Jacq.	Dried	Hydrodistillation Clevenger type (5h)	0.91	Iran	[20]
	Aerial parts Flowering stage				
<i>M. parviflorum</i> Fish. & C. A. Mey.	Air-dried	Hydrodistillation Clevenger type (3h)	0.08	Iran	[21]
	Aerial parts Flowering stage				
<i>M. astracanicum</i> Jacq.	Air-dried	Hydrodistillation Clevenger type (4h)	0.33	Iran	[22]
	Aerial parts				
<i>M. anisodon</i> C. Koch	Dried	Hydrodistillation Clevenger type (3-4h)	0.7	Iran	[23]
	Aerial parts				
<i>M. propinquum</i> Fisch. and C.A.Mey.	Dried	Hydrodistillation Clevenger type (3-4h)	0.8	Iran	[23]
	Aerial parts				

Continuation of Table 1

<i>Marrubium specie</i>	Plant part	Extraction method	Yield (%)	Country	Ref.
<i>M. crassidens</i> Bioos.	Air-dried Aerial parts Flowering stage	Hydrodistillation Clevenger type modified (4h)	0.26	Iran	[24]
<i>M. astracanicum</i> Jacq.	Air-dried Aerial parts Flowering stage	Hydrodistillation Clevenger type modified (4h)	0.19	Iran	[24]
<i>M. crassidens</i> Boiss.	Air-dried Aerial parts Flowering stage	Hydrodistillation Clevenger type (3h)	0.2	Iran	[25]
<i>M. persicum</i> C.A. Mey (HD)	Air-dried Aerial parts Flowering stage	Hydrodistillation Clevenger type (4h)	0.23	Iran	[26]
<i>M. persicum</i> C.A. Mey (HS-SPME)	Powdered Aerial parts Flowering stage	Headspace Solid-Phase Microextraction	-	Iran	[26]
<i>M. persicum</i> C.A. Mey (SFME)	Dried Ground Aerial parts	Solvent-Free Microwave Extraction	0.23	Iran	[26]
<i>M. persicum</i> C.A. Mey (MAHD)	Dried Aerial parts	Microwave Assisted Hydrodistillation Microwave oven	0.30	Iran	[26]
<i>M. aschersonii</i> Magnus	Dried aerial parts	Hydrodistillation Clevenger type (2h)	0.01	Tunisia	[27]
<i>M. deserti</i>	Dried Aerial parts	Hydrodistillation Clevenger type (4h)	0.02	Algeria	[28]
<i>M. deserti de Noé</i>	Dried Aerial parts Flowering stage	Hydrodistillation Clevenger type (4h)	0.15	Algeria	[29]
<i>M. thessalum</i> Boiss. and Heldr.	Air-dried Aerial parts Flowering stage	Hydrodistillation Clevenger type modified (4h)	0.03	Greece	[30]
<i>M. duabense</i> Murata	Air-drying Floral and leaves	Hydrodistillation Clevenger type (4h)	0.1	Iran	[31]
<i>M. cylleneum</i> Boiss. and Heldr.	Aerial parts	Steam distillation (3h)	0.16	Greece	[32]
<i>M. velutinum</i> Sm.	Air dried whole flowering stems	Steam distillation (3h)	0.03	Greece	[33]
<i>M. peregrinum</i> L. (1)	Air dried whole flowering stems	Steam distillation (3h)	0.07	Greece	[33]
<i>M. peregrinum</i> L. (2)	Air dried whole flowering stems	Steam distillation (3h)	0.07	Greece	[33]
<i>M. deserti</i>	Floral and leaves	Steam distillation Laboratory apparatus (4h)	-	Algeria	[34]
<i>M. persicum</i> C. A. Mey.	Air-dried Aerial parts Flowering stage	Hydrodistillation Clevenger type (5h)	-	Iran	[35]
<i>M. cuneatum</i> Banks and Solander	Air-dried Aerial parts	Hydrodistillation European pharmac (3h)	0.19	Lebanon	[36]
<i>M. globosum</i> Montbr. and Auch.	Air-dried Aerial parts	Hydrodistillation European pharmac (3h)	0.13	Lebanon	[36]
<i>M. globosum</i> subsp. <i>globosum</i>	Air-dried Ground plant	Water distillation British-type Clevenger (3h)	0.02	Turkey	[37]
<i>M. peregrinum</i> L. (no. 1)	Air-dried plant	Hydrodistillation European pharmac (3h)	0.11	Serbia	[38]
<i>M. peregrinum</i> L. (no. 2)	Air-dried plant	Hydrodistillation European pharmac (3h)	0.09	Serbia	[38]

Continuation of Table 1

<i>Marrubium specie</i>	Plant part	Extraction method	Yield (%)	Country	Ref.
<i>M. peregrinum</i> L. (no. 3)	Air-dried plant	Hydrodistillation European pharmac (3h)	0.14	Serbia	[38]
<i>M. incanum</i> Desr.	Air-dried Flowering stage	Hydrodistillation European pharmacopoeia	0.05	Serbia	[39]
<i>M. parviflorum</i> Fisch. et Mey. <i>subsp. oligodon</i> (Boiss.) Seybold	Dried Aerial parts	Water distillation Clevenger type (3h)	0.01	Turkey	[41]
<i>M. astracanicum</i> Jacq	Air-dried leaves Flowering stage	Steam distillation Glass apparatus (1h)	0.25	Iran	[42]
<i>M. incanum</i> Desr.	Dried in oven (30°C) Aerial parts Flowering stage	Steam distillation (3h) Deryng apparatus	0.04	Poland	[43]

Hamedeyazdan, S. *et al.* [35] obtained a very small quantity of essential oil from the aerial parts of *M. persicum* by hydrodistillation which made the authors increase the distillation time until 5h and use xylene as an absorbing medium. Also, Demirci, B. *et al.* [16] and Kirimer, K. *et al.* [18] resort to the use of n-hexane to recover the essential oil by trapping, due to the poor yield of oil from air-dried aerial parts of *M. bourgaei* ssp. *carcicum* P.H. Davis and *M. anisodon* respectively, were all subjected to hydrodistillation for 3h.

These values confirm the hypothesis of Lawrence, according to which *Labiatae* genera with tricolpate pollen grains are oil-poor [15]. First of all, there are two main pollen types in *Labiatae*: tri- and hexacolpate [46], secondly, the pollen grains of subfamily *Lamioideae* are inoperculate and usually tricolpate [13], and then most species of *Marrubium* genera belonging to *Lamiaceae* (*Labiatae*) family are tricolpate pollen [13,14]. Mohammadhosseini, F. did not find any difference between the EO's yield extracted from *M. persicum* by using classical or advanced methods [26]. In a review of Kaufmann, B. and Christen, P., they demonstrated that extraction yields of the analytes by using techniques involving microwave assisted extraction and pressurized are equivalent to or even higher than those obtained with conventional methods [45]. On the other hand, Chemat, F. and Cravotto, G. state that, the yield of EOs from plants is between 0.005% and 10% [1].

Chemical composition

Essential oils are considered as secondary metabolites which occur in some species but not others. Like all substances, they are characterized by an analytic and highly variable chemical composition. Most components isolated within essential oils are poly-molecular (*i.e.* composed of a wide variety of compounds). In addition to the

major compounds (generally between 2 and 6), there are minor compounds and a certain number of constituents in the form of traces. These constituents belong, almost exclusively, to two groups characterized by distinct biogenetic origins: the terpenoids group (terpenic compounds) and the group of aromatic compounds (phenylpropanoids) derived from phenylpropene, much less common [3]. They may also contain various products derived from degradative processes involving non-volatile constituents [4].

The structure of terpenoid compounds consists of several "isoprene" units, the universal five-carbon building block, on which is often present one or more similar or different functional groups, mostly being oxygenated sites with one or more oxygen atoms (O), and some nitrogen-N or sulphur-S functional groups. This isoprene is the basis of the concept of the "isoprene rule" enunciated in 1953 by Ruzicka, L. [47]. This rule considers isopentenyl diphosphate (IPP), referred to as active isoprene, as the true precursor of the terpene molecule.

Essential oil phenylpropanoids are derived from phenylalanine; the phenylpropane skeletal compounds are derived from the latter, synthesized *via* the shikimate pathway [3]. According to the same authors, this group provides indispensable and significant flavour and odour to the oil, when present.

Chemical composition related to the main constituents

Between fourteen (*M. crassidens* [25]) and one hundred thirty nine (*M. parviflorum* [41]) components of the essential oil of *Marrubium* species were identified and those identified account for about 99.81% (*M. incanum* [43]) to 86.33% (*M. cylleneum* [32]) of total oil contents. Some of them (germacrene D, β -caryophyllene, caryophyllene oxide, bicyclo-germacrene and spathulenol) are generally considered as either

main or minor constituents in EO of *Marrubium* species (Table 2). A major constituent of one species may not be for another species.

Germacrene D is one of the most common sesquiterpenes found in the oils of *Marrubium* genus except for some species as well as *M. bourgaei* [17], *M. duabense* [31], *M. velutinum* Sm and *M. peregrinum* [33], *M. astracanicum* Jacq [42] and *M. astracanicum* [22]. It is considered as the main component of *M. deserti* [28], *M. anisodon* [23], *M. incanum* [43], *M. cuneatum* [36], and one of the main constituents of *M. incanum* [39], *M. peregrinum* [38,40], *M. parviflorum* [21,41], *M. propinquum* [23], *M. astracanicum* [24], *M. crassidens*

[24,25], *M. thessalum* [30], *M. persicum* [26,35], *M. cuneatum* Russel [19] and *M. deserti* [29,34]. In other species, the amount of this compound is small and/or in the form of trace. A very low percentage (0.1%) was found in the EO of *M. persicum* C.A. Mey obtained by Headspace Solid-Phase Microextraction Method (HS-SPME) [26] and in *M. globosum* Montbr. et Auch. (0.3%) [36]. Low quantities were found in *M. persicum* C.A. Mey obtained by Microwave Assisted Hydrodistillation technique (MAHD) (6.1%) [26], *M. peregrinum* (2) (4.81%) [33], *M. cylleneum* (3.68%) [32], *M. astracanicum* (1.4%) [20] and *M. anisodon* (1%) [18].

Table 2

Main constituents (in %) of *Marrubium* species EO.

<i>Marrubium</i> specie	Germacrene D	Caryophyllene oxide	β -caryophyllene	Bicyclo-germacrene	Spathulenol	Other major constituents	Ref.
<i>M. bourgaei</i> ssp. <i>caricum</i> P.H. Davis	10.3	3.6	23.2	7.4	1.3	(Z)- β -Farnesene (13.5) Carvacrol (12.5)	[16]
<i>M. bourgaei</i> Boiss. ssp. <i>bourgaei</i>	-	3.8	-	-	-	Hexadecanoic acid (33.3) Hexahydrofarnesyl acetone (6.4) Heptacosane (4.8)	[17]
<i>M. anisodon</i> C. Koch	1.0	1.5	13.3	-	-	(Z)- β -Farnesene (20.2) Nonacosane (18.5)	[18]
<i>M. cuneatum</i> Russel	24.1	-	2.2	37.9	4.8	Limonene (3.7)	[19]
<i>M. astracanicum</i> Jacq.	1.4	1.6	0.7	-	2.5	Methylcyclopentane (15.5) Thymol (10.6) <i>n</i> -Heptane (7.4)	[20]
<i>M. parviflorum</i> Fish. and C.A.Mey	21.5	1.8	15.6	26.3	2.2	-	[21]
<i>M. astracanicum</i> Jacq.	-	2.4	21.2	4.8	3.2	Valeranone (5.4) 6,10,14-Trimethyl-2-pentadecanone (4.2)	[22]
<i>M. anisodon</i> C. Koch	44.2	6.6	10.4	0.4	-	α -Pinene (15) Limonene (5)	[23]
<i>M. propinquum</i> Fisch. and C.A. Mey.	15.8	-	20.1	0.9	-	(E)- β -Farnesene (43.8)	[23]
<i>M. crassidens</i>	14.2	4.6	29.0	14.2	5.6	-	[24]
<i>M. astracanicum</i> Jacq.	23.4	-	0.9	11.9	6.8	α -Humulene (33.7)	[24]
<i>M. crassidens</i> Boiss.	12.9	11.1	20.3	7.5	5.6	Cubenol (11.0) Hexadecanoic acid (8.1)	[25]
<i>M. persicum</i> C.A.Mey (HD)	0.7	2.8	7.4	-	19.5	α -Thujene (17.4) α -Pinene (21.5) δ -Elemene (7.5)	[26]
<i>M. persicum</i> C.A.Mey (HS-SPME)	0.1	8.4	14.0	-	2.1	δ -Elemene (16.9) Eugenol (11.2) Methyl eugenol (10.2)	[26]

Continuation of Table 2

<i>Marrubium</i> <i>specie</i>	Germacrene D	Caryophyllene oxide	β -caryophyllene	Bicyclo- germacrene	Spathulenol	Other major constituents	Ref.
<i>M. persicum</i> C.A.Mey (SFME)	9.5	3.4	6.1	-	25.4	α -Pinene (17.4) δ -Elemene (6.8) Methyl eugenol (6.4)	[26]
<i>M. persicum</i> C.A.Mey (MAHD)	6.1	13.1	8.0	-	8.1	δ -Elemene (12.4) Camphene (8.5) α -Selinene (7.2)	[26]
<i>M. aschersonii</i> magnus	0.5	-	2.4	-	0.4	β -Bisabolene (22.0) α -Thujene (10.3) Eugenol (10.1) α -Humulene (6.2)	[27]
<i>M. deserti</i>	45.7	1.6	-	1.2	-	β -Bourbonene (4.0) α -Terpinolene (3.9) Δ -Cadinene (3.8)	[28]
<i>M. deserti</i> de Noé	7.91	0.90	1.32	2.84	0.96	Tetracosane (31.11) Δ -Cadinene (6.52) α -Cadinol (6.26) <i>t</i> -Cadinol (5.81)	[29]
<i>M. thessalum</i> Boiss. & Heldr.	15.3	21.7	17.6	2.0	-	β -Bisabolene (12.6) trans- β -Farnesene (8.1)	[30]
<i>M. duabense</i> Murata	-	1	-	6.01	1.08	Limonene (33.53) α -Terpineol (10.78) trans-Caryophyllene (8.25) β -Elemene (6.37)	[31]
<i>M. cylleneum</i> Boiss. and Heldr.	3.68	36.54	10.85	-	9.44	2-Pentadecanone-6, 10, 14-trimethyl (5.36) Viridiflorol (4.20)	[32]
<i>M. velutinum</i> Sm	-	-	24.25	3.28	0.79	γ -Muuroleone (27.78) β -Caryophyllene oxide (6.03) (E)- β -Farnesene (4.42)	[33]
<i>M. peregrinum</i> L. (1)	-	-	-	11.03	3.05	(E)- β -Farnesene (24.16) (Z)- β -Farnesene (16.47) epi-Bicyclosesquiphellandrene (12.31)	[33]
<i>M. peregrinum</i> L. (2)	4.81	-	-	4.81	1.52	(E)- β -Farnesene (21.49) (Z)- β -Farnesene (12.04) γ -Muuroleone (9.68) Δ -Cadinene (17.63)	[33]
<i>M. deserti</i>	7.02	-	-	-	-	6,10,14-Trimethylpentadeca-2-one (4.61) 9-Methyl-undec-1-ene (4.17)	[34]
<i>M. persicum</i> C.A. Mey.	10.5	2.1	7.4	1.3	0.5	<i>m</i> -Tolualdehyde (19.2) Acetophenone (14.6) β -Farnesene (6.2)	[35]
<i>M. globosum</i> Montbr. and Auch.	0.3	4.5	12.4	-	5.2	Hexadecanoic acid (7.4) (E)- β -Farnesene (5.8)	[36]
<i>M. cuneatum</i> Banks and Solander	15.6	6.2	5.2	5.2	6.5	Hexadecanoic acid (6.5) <i>p</i> -Methoxyacetophenone (5.4)	[36]
<i>M. globosum</i> subsp. <i>globosum</i>	6.5	7.9	9.0	3.1	15.8	-	[37]
<i>M. peregrinum</i> L. (no. 1)	6.79	4.23	13.20	7.63	5.18	1-Octen-3-ol (4.88) γ -Muuroleone (5.59)	[38]

Continuation of Table 2

<i>Marrubium</i> <i>specie</i>	Germacrene D	Caryophyllene oxide	β -caryophyllene	Bicyclo- germacrene	Spathulenol	Other major constituents	Ref.
<i>M. peregrinum</i> L. (no. 2)	8.56	3.73	14.34	6.42	5.68	γ -Muurolene (5.56) 1-Octen-3-ol (5.08)	[38]
<i>M. peregrinum</i> L. (no. 3)	9.05	4.98	17.99	9.80	3.76	(E)- β - Farnesene (5.08) (Z)- β -Farnesene (5.12) γ -Muurolene (6.26)	[38]
<i>M. incanum</i> Desr.	26.2	3.5	-	11.5	2.0	(E)-Caryophyllene (27.0) α - Humulene (4.4) α -Copaene (4.2)	[39]
<i>M. peregrinum</i> L.	28.1	-	31.3	15.3	1.2	Hexadecanoic acid (10.4)	[40]
<i>M. parviflorum</i> Fish. <i>et</i> Mey. subsp. <i>oligodon</i> (Boiss.) <i>Seybold</i>	11.1	1.0	10.0	-	1.4	Hexadecanoic acid (15.4) (E)- β -Farnesene (7.3)	[41]
<i>M. astracanicum</i> Jacq.	-	35.8	13.1	-	-	Citronellal (16.9) Geranyl acetate (4.9)	[42]
<i>M. incanum</i> Desr.	30.44	0.73	-	1.06	t	E-Caryophyllene (26.37) α -Cadinol (17.21) α -Humulene (10.78)	[43]

β -Caryophyllene is the second important sesquiterpenes component in the *Marrubium* EO's. It is considered as the major constituent in *M. peregrinum* [38,40], *M. crassidens* [24,25], *M. bourgaei* [16], *M. astracanicum* [22], *M. globosum* [36]. Highest content was also found in *M. velutinum* [33], *M. propinquum* [23], *M. thessalum* [30], *M. parviflorum* [21,41], *M. persicum* [26,35], *M. anisodon* [18,23], *M. astracanicum* [42], *M. cylleneum* [32], *M. globosum* [37]. A smaller content was found in *M. astracanicum* [20,24]. However, it is absent in some species as well as *M. bourgaei* [17], *M. duabense* [31], *M. incanum* [39,43], *M. peregrinum* [33], *M. deserti* [28,34].

The third representative component found in EO of *Marrubium* was spathulenol, an oxygenated sesquiterpene which arises from the C5-C10 cyclization of the aromadendrane skeleton. The largest amounts of this compound were found in *M. globosum* [37], *M. persicum* C.A.Mey (HD and Solvent-Free Microwave Extraction "SFME") [26], *M. cylleneum* [32], *M. astracanicum* [24], while the weakest percentages were found in *M. incanum* [43], *M. aschersonii* [27], *M. persicum* [35], *M. velutinum* [33], *M. deserti* [29]. This compound was not found in other studied species [17,18,23,28,30,34,42].

Caryophyllene oxide and bicyclo-germacrene were other main constituents of some *Marrubium* EO's. Caryophyllene oxide was identified as the major compound of *M. cylleneum* [32], *M. astracanicum* [42], *M. thessalum* [30], *M. persicum* C.A.Mey (MAHD) [26], while bicyclo-germacrene in *M. cuneatum* [19], *M. parviflorum* [21]. These compounds were all absent in *M. aschersonii* [27] and *M. deserti* [34].

The rule of Lawrence [15], of which β -caryophyllene and germacrene D are the main constituent of oils isolated from *Labiataea* genera with tricolpate pollen grains is not confirmed in some *Marrubium* genera. Kurkcuoğlu, M. *et al.* reported that the major compounds of the essential oil of *M. bourgaei* were hexadecanoic acid (33.3%), hexahydrofarnesyl acetone (6.4%) and heptacosane (4.8%) [17]. The study performed by Hamdaoui, B. *et al.* revealed that β -bisabolene (22.0%), α -thujene (10.3%) and eugenol (10.1%) were the main constituents of the EO isolated from *M. aschersonii* [27]. In the oil of *M. duabense*, limonene (33.53%), α -terpineol (10.78%), *trans*-caryophyllene (8.25%) and β -elemene (6.37%) dominated [31]. Kirimer, K. *et al.* showed that the important compounds of the EO of *M. anisodon* were (Z)- β -farnesene (20.2%), nonacosane (18.5%) and β -caryophyllene (13.3%)

[18]. According to Lazari, D.M. *et al.*, the essential oil of two different populations of *M. peregrinum* contained (E)- β -farnesene (21.49-24.16%) and (Z)- β -farnesene (12.04-16.47%) as main compounds [33]. The GC-MS analysis of the EO of *M. deserti* revealed the presence of tetracosane (31.11%), germacrene D (7.91), Δ -cadinene (6.52%), α -cadinol (6.26%), *t*-cadinol (5.81%) as the main constituents [29]. Methylcyclopentane (15.5%), thymol (10.6%), *n*-heptane (7.4%) were the most representative components in the EO of *M. astracanicum* [20]. In *M. propinquum*, (E)- β -farnesene (43.8%) is by far the most common component [23]. Thus, the main components vary from oil to oil and the variation in occurrence of certain compounds in a plant is a function of any one of, or a combination of, three factors: genetically determined properties, the age of the plant, and the environment [5]. Sarikurkcü, C. *et al.* indicates that the composition of any plant essential oil studied is influenced by the presence of several factors, such as local, climatic, seasonal and experimental conditions [37]. But, according to Franz, K.H.C. and Novak, G., the dissimilarities due to genetically differences are much bigger than by different environmental conditions [6]. Iranian authors attribute the variation of oil components of *Marrubium* species (*M. astracanicum*, *M. crassidens*, *M. persicum*) growing in different provinces of Iran to the collection time, drying conditions, extraction methods, chemotypes, geographic and edaphic and climatic factors [20,24,35]. In Algeria, Chebrouk, F. *et al.* assign the absence of monoterpene in the extracted oil from *M. deserti* from septentrional east Sahara to the abiotic factors such as specific climate of the region and the geographic factors as altitude and soil [34]. According to Hamedeyazdan, S. *et al.*, these factors could influence the biosynthetic pathways of plants, the relative proportion of the main characteristic compounds of the EO as the secondary metabolites would be variable [35].

On the other hand, Mohammadhosseini, F. studied the chemical composition of the essential oils and volatile fractions from *M. persicum* by using classical (hydrodistillation) and advanced (headspace solid-phase microextraction, microwave assisted hydrodistillation and solvent-free microwave extraction) methods [26]. In this study, 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. Scheffer, J.J.C. argued that methods used to isolate essential oils may give rise to varying compositions; thus, for example, a long lasting distillation may influence the composition of the oil isolated, because isomerization, saponification and other reactions may occur under distillation conditions [7].

Some of major compounds of EO of *Marrubium* species are presented (Figure 1). These structures vary according to: number of isoprene units present in the molecule (hemiterpenes "C5", monoterpenes "C10", sesquiterpenes "C15"), rarely diterpenes "C20"; saturated or unsaturated nature of the bonds; their arrangement, linear or cyclic; spatial configuration (chair form, boat form) and nature of the functional groups, terpene alcohols (R - OH), ketones (R1 - CO - R2), phenols (C₆H₆ - OH), aldehydes (R - CHO), esters (R1 - COO - R2), ethers (R1 - O) [4].

Other constituents of essential oils

As shown in Table 3, sesquiterpenoids were the dominant fraction in *Marrubium* EOs, which is in accordance with Lawrence hypothesis [15], except for *M. duabese* [31] and *M. persicum* (HD) [26]. Monoterpenes were present in appreciable [26] or in trace amounts [39]; in some species (*M. thessalum* [30], *M. crassidens* [25] and *M. deserti* [34]) they were totally absent. Both types of terpenoids were characterized by the predominance of hydrocarbon fractions with some differences according to plant species. *Marrubium* genus seems to produce oils that are rich in hydrocarbon compounds, with sesquiterpenes forming the major part [28]. In sesquiterpenoids group components, hydrocarbons related to oxygenated functional groups predominated. Their content varies from 92.8% (*M. propinquum*) [23] to 20.9% (*M. persicum* C.A.Mey "HD") [26], while oxygenated forms varies from 32.4% (*M. crassidens*) [25] to 0.8% (*M. aschersonii*) [27]. In monoterpenoids, hydrocarbons form the totality in *M. crassidens* [24], *M. astracanicum* [24] and *M. deserti* [28]; it ranges from 43.7% (*M. persicum* C.A.Mey "HD") [26] to 0.8% (*M. incanum*) [43], when oxygenates range from 14.0% (*M. aschersonii*) [27] to 0.5% (*M. parviflorum*) [21] or trace amount (*M. incanum*) [39].

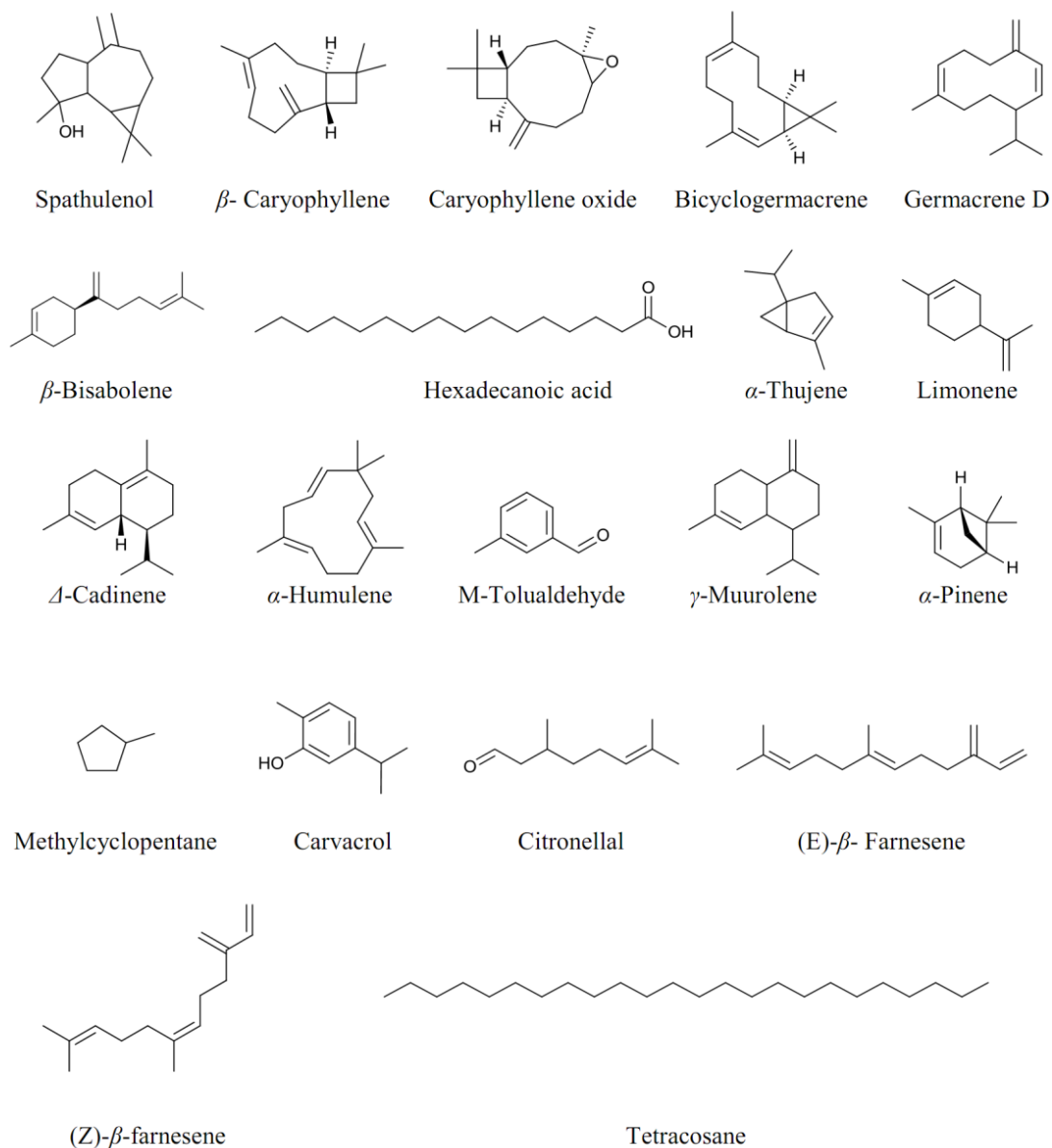


Figure1. Structure of some major compounds of EO of *Marrubium* species.

Table 3

Other components of EO of *Marrubium* species (%).

<i>Marrubium</i> species	MH ^a	OM ^b	SH ^c	OS ^d	Other	Ref.
<i>M. cuneatum</i> Russel	7.8	-	78.9	-	-	[19]
<i>M. parviflorum</i> Fisch. &C.A. Mey.	11.4	0.5	72	5.8	-	[21]
<i>M. astracanicum</i> Jacq	-	-	54.3	-	-	[22]
<i>M. anisodon</i> C. Koch	21.3	3.5	62.2	6.6	-	[23]
<i>M. propinquum</i> Fisch. and C.A. Mey.	2.6	0.6	92.8	-	-	[23]
<i>M. crassidens</i> Bioos.	2.8	0	74.0	13.4	-	[24]
<i>M. astracanicum</i> Jacq.	3	0	72.3	13.4	-	[24]
<i>M. crassidens</i> Boiss.	0	0	44.5	32.4	14.8	[25]
<i>M. persicum</i> C.A.Mey (HD)	43.7	2.2	20.9	26.7	1.1	[26]
<i>M. persicum</i> C.A.Mey (HS-SPME)	13.7	12.8	41.3	10.5	21.4	[26]

Continuation of Table 3

<i>Marrubium species</i>	MH ^a	OM ^b	SH ^c	OS ^d	Other	Ref.
<i>M. persicum</i> C.A.Mey (SFME)	22.0	6.8	33.2	29.6	7.2	[26]
<i>M. persicum</i> C.A.Mey (MAHD)	24.1	4.1	45.1	22.1	3.8	[26]
<i>M. aschersonii</i> Magnus	12.3	14.0	37.3	0.8	15.1	[27]
<i>M. deserti</i>	5.1	0	67.5	0	-	[28]
<i>M. deserti</i> de Noé	4.32	9.26	33.85	16.45	35.84	[29]
<i>M. thessalum</i> Boiss. and Heldr.	0	0	60	29.6	10.4 (miscellaneous)	[30]
<i>M. duabense</i> Murata	33.53	7.9	25.24	2.08	-	[31]
<i>M. velutinum</i> Sm	-	1.06	71.73	10.74	-	[33]
<i>M. peregrinum</i> L. (1)	-	5.58	73.05	6.95	-	[33]
<i>M. peregrinum</i> L. (2)	-	5.34	65.63	6.23	-	[33]
<i>M. deserti</i>	0	0	57.75	0	42.25	[34]
<i>M. persicum</i> C.A. Mey.	9	1.2	27.9	4.8	-	[35]
<i>M. peregrinum</i> L. (1)	3.20	6.68	49.10	11.84	12.84	[38]
<i>M. peregrinum</i> L. (2)	3.37	6.88	52.28	10.94	14.13	[38]
<i>M. peregrinum</i> L.(3)	3.63	7.93	62.71	11.00	10.88	[38]
<i>M. incanum</i> Desr.	-	traces	84.1	9.3	2.9	[39]
<i>M. incanum</i> Desr.	0.8	4.77	76.35	21.16	-	[43]

^a Monoterpene hydrocarbon;^b Oxygenated monoterpene;^c Sesquiterpene hydrocarbon;^d Oxygenated sesquiterpene.

Biological activity

Synthetic products widely used, both in medication and in the food industry, raise currently several questions as to their efficiency and safety. If in the first case the development of resistance of microorganisms to various antibiotics is of concern to medical specialists, the use of additives such as antioxidants is suspected to have adverse effects on the health of consumers. EOs are beginning to raise a lot of interest as a potential source of bioactive natural molecules. They are being studied for their possible use as an alternative to synthetic products. The effectiveness of EO has been attributed mainly to the presence of bioactive compounds in their composition. According to Nagy, M. and Svajdlenska, E., the difference in chemical composition of EOs of *Marrubium* species may cause an important change in the biological activities of oils [40].

Antioxidant activity

Only three species of *Marrubium* were investigated for their EO's antioxidant activities. The first study on the antioxidant activity of the members of *Marrubium* was conducted on *M. globosum* [37]. The two other species were

carried out on *M. deserti* [28,29] and on *M. peregrinum* [38].

The antioxidant effect of EOs of *M. globosum* was determined by three different *in vitro* assays namely 1,1-diphenyl-2-picrylhydrazyl (DPPH), β -carotene/linoleic acid and reducing power [37]. In this study, the antiradical dose required to reduce 50% of the free radical (EC₅₀ of DPPH) was 1203.38±7.18 µg/mL which was considered very weak compared to the positive control butylatedhydroxytoluene (BHT) (55.48±0.87 µg/mL), also, the EC₅₀ value of reducing power assay, 4315.80±2.54 µg/mL, was very weak compared to BHT (171.26±1.11 µg/mL). In β -carotene/linoleic acid this EOs showed the weakest activity potential, too. In this test system, the % inhibition capacity of the EOs (79.26±1.46%) was found inferior to the inhibition capacity of the positive control BHT (97.44±0.74%). According to this study, the polar sub-fraction of the methanol extract exerted the strongest antioxidant activity, when compared with the EO one, which is almost equal to BHT and could be used in the food industry and other fields which are processing natural products [37].

The antioxidant activity of *M. deserti* EOs from Algerian species were evaluated using three

in vitro assays: scavenging effect on DPPH, the 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) test and the phosphomolybdenum method [28] and β -carotene-linoleic acid, DPPH free radical scavenging, and CUPRAC (cupric reducing antioxidant capacity) assays [29]. The results of these studies showed that the essential oils of *M. deserti* exhibited a remarkable antioxidant activity and significant reducing power compared with the standard antioxidants such as butylated hydroxyanisole (BHA), BHT, α -tocopherol and ascorbic acid. The concentration of *M. deserti* oil resulting in a 50% inhibition of the DPPH free radical scavenging activity (IC₅₀) was 22.3 μ g/mL. In the ABTS assay, the antioxidant activity was 8.93 mM expressed as Trolox equivalents and in the phosphomolybdenum method, the total antioxidant activity of the essential oil was 0.70 mM expressed as α -tocopherol equivalents [28]. In the β -carotene-linoleic acid assay, the oil exhibited good lipid peroxidation inhibition activity, demonstrating 76.81 \pm 0.59% at 200 μ g/mL concentration. In DPPH and CUPRAC assays, however, the essential oil showed weak activity [29]. Authors stipulate that this oil may be suggested for further use as a natural additive in food and pharmaceutical industries.

M. peregrinum EOs from three different localities (Rimski sanac - no.1, Novi Knezevac - no.2 and Senta - no.3) of Serbia were evaluated for their antioxidant properties by Kaurinovic, B. *et al.* [38]. The free radical scavenging capacity (RSC) was assessed measuring the scavenging activity of EOs on DPPH, O₂^{•-}, NO[•] and OH[•] radicals; the xanthine-oxidase activity (XOD) was determined by the nitric method and effects on lipid peroxidation (LP) were evaluated by following the activities of EOs in the Fe²⁺/ascorbate induction system. Results showed that all samples expressed strong antioxidant effects, with the best effect on DPPH radical from that of Senta locality, with IC₅₀ values as follows: 13.48; 13.41 and 11.69 μ g/mL for *M. Peregrinum* no.1, no.2 and no.3, respectively. That of BHT (positive control) was 14.31 μ g/mL. Regarding the O₂^{•-} assay, the greatest ability was from EO from the Senta (no.1) locality (IC₅₀=10.82 μ g/mL), which is a bit weaker compared to BHT (10.46 μ g/mL). Similar results were obtained from the same locality in the NO[•] method, where the EO exhibited the strongest inhibitory effect (IC₅₀= 8.81 μ g/mL compared to BHT, IC₅₀= 8.63 μ g/mL). Concerning the deoxyribose assay, all the examined EOs from all three locations, except those at the lowest

concentration (0.213 μ g/mL), inhibited the degradation of deoxyribose greater than BHT (24.12%). The highest activity was shown by the EO of *M. peregrinum* from Senta (no.3) again, especially at the concentration of 2.130 μ g/mL (67.12%) and 1.598 μ g/mL (55.18%). The antioxidant activities of all three EOs were dose dependent. Results of activities of XOD with *M. peregrinum* EO showed that only the EO from Senta (no.3) expressed a stronger protective effect than BHT (17.63 vs. 19.23 μ g/mL). The examined EOs expressed strong antioxidant capacity in the LP technique. The largest inhibitory activity was exhibited by EO from plants collected at Senta locality, too (from 37.02 to 71.32% of inhibition of LP), greater than BHT (26.15%). In conclusion, the authors of this study state that EOs of this specie could serve as safe antioxidant and antiseptic supplements in preventing deterioration of foodstuffs and beverages and pharmaceuticals.

Antimicrobial activity

Some *Marrubium* EO's have been shown to have moderate antimicrobial activity [29,36,39] or negligible [31,36] while other were ineffective [28,31]. This difference in microbial activity might be attributed to the change of chemical composition of EO [31,36,40] and concentrations used in tests [29,31,39]. Thus, when comparing published data, attention should be drawn to methodological differences: selection of plant extracts, microorganism testing and antimicrobial testing methods. In general, it is the G+ bacteria that are most affected by the *Marrubium* EOs especially at high concentrations [31,36,39].

The EO of *M. deserti* collected from Algerian area steppe had no activity on pathogenic bacteria tested (from the American Type Culture Collection (ATCC) and consists of (*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853)) and yeasts and molds (*Candida albicans* and *Aspergillus flavus*), at each of the three concentration used (EO dilutions in ethanol 1/2, 1/5, and 1/10 v/v) [28]. However, EO of *M. deserti* from south Algeria inhibit the growth of microorganisms, but at highest concentrations; the minimum inhibitory concentration was 25 μ L/mL for bacteria, *Staphylococcus epidermidis* MU 30, *Bacillus cereus* RSKK 863(Refik Saydam National Type Culture Collection), *Micrococcus luteus* NRRL B-4375(Northern Regional Research Laboratory Culture Collection), *Streptococcus mutans* CNCTC 8/77 (Czech National Collection of Type Cultures)and for yeasts, *Candida albicans* ATCC

10239; it was 50 µL/mL for *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633, and it was 80 for *Staphylococcus aureus* ATCC6538P [29].

According to the study conducted by Golmakani, H. *et al.* in North Khorassan Province (Iran), the antibacterial bioassay of *M. duabense* Murata EO shows that only *Clostridium perfringens* was affected by a higher concentration while *Staphylococcus aureus*, *Salmonella pullorum* and *Escherichia coli* displayed no significant growth inhibition [31]. This is suspected to be associated with the high contents of oxygenated compounds. Authors suggest introducing *M. duabense* as a medicinal plant against anaerobic bacteria after larger and controlled clinical trials.

Grassia, A. *et al.* demonstrated that EO of *M. cuneatum* collected from Lebanon exhibited a negligible antimicrobial activity, evaluated by the *in vitro* paper-disk diffusion method, against eight selected Gram+ and Gram- bacteria *Bacillus cereus* (PCI 213), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Streptococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphi A* (ATCC 12176), while that of *M. globosum* ssp *libanoticum* showed a low activity, mostly against Gram+ bacteria [36]. The authors attribute this to the content in this oil of a good amount of caryophyllene and the presence of some other components like linalool, α -terpineol, eugenol that, even if present in low amounts, may synergistically increase the action of carvacrol.

On the contrary, the study conducted by Petrovic, S. *et al.* revealed that EO of *M. incanum* exhibited antimicrobial activity against all microorganisms tested but with some differences [39]. These microorganisms consist of four G+ bacteria *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Micrococcus flavus* (ATCC 10240), *Enterococcus faecalis* (ATCC 29212); three G- bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (NCIMB 9111) (National Collection of Industrial, Food and Marine Bacteria), *Pseudomonas aeruginosa* (ATCC 27853) and a yeast *Candida albicans* (ATCC 10259 and ATCC 24433). The antimicrobial activity was assayed using the agar diffusion and broth microdilution methods. Results were carried out by measuring diameters of zone of inhibitions and by determining minimum inhibitory (MIC) and minimum microbiocidal (MMC) concentrations. According

to this study, the best inhibitory and bactericidal effect was detected against *M. flavus* (MIC 6.25 µg/mL; MMC 12.5 µg/mL), followed by *E. coli* and *K. pneumonia* (MIC 12.5 µg/mL; MMC 25 µg/mL, both) while *S. epidermidis* appeared to be the most resistant (MIC 100 µg/mL; MMC 200 µg/mL). The lowest MIC and MMC (6.25 µg/mL and 12.5 µg/mL, respectively) was also found against *C. albicans* (ATCC 24433), whereas *C. albicans* (ATCC 10259) was less sensitive than for the previous one (MIC 12.5 µg/mL; MMC 25 µg/mL). The authors attributed the antimicrobial activity of the oil, to an appreciable degree, to the presence of (E)-caryophyllene.

Allergic conjunctivitis

One third of the world population is affected by some form of allergic disease and ocular involvement is estimated to be present in 40-60% of this population [48]. Pathophysiology of ocular allergies and allergic responses are well discussed [48,49]; it involves sensitization, activation, mast cell degranulation and performed mediators. Histamine is a chemical mediator stored in mast cells; it is released in the early phase reaction with granulocytes degranulation [48]. It plays an important role in inflammatory and allergic reactions and for the treatment of allergic conjunctivitis, antihistamines are used among others [49]. The study conducted by Skaltsa, H. *et al.*, showed that the EO of *M. cylleneum* enhances the amount of histamine present in the rat conjunctiva by acting through a mechanism yet to be identified; this increase in histamine may result from an increase in the rate of synthesis or storage of the amine, which remains to be determined [32].

Anticholinesterase activity

The EO of *M. deserti* was tested for its anticholinesterase activities by measuring the inhibition activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BchE) enzymes using the spectrophotometric method [29]. According to the authors, the EO was inactive against both AChE and BchE, however the methanol extract of this species exhibits weak inhibition against both enzymes when compared to galantamine, used as a standard drug. The importance of acetylcholinesterase and butyrylcholinesterase enzymes for human body is highlighted in the review of Patočka, J. *et al.* [50].

Antibiofilm formation

The effect of *M. deserti* EO's on Bacterial biofilm formation of microorganisms (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC6538P,

Staphylococcus epidermidis MU 30, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* RSKK 863, *Micrococcus luteus* NRRL B-4375, *Streptococcus mutans* CNCTC 8/77 and *Candida albicans* ATCC 10239) was tested with a microplate biofilm assay by Chemsal, A.E. *et al.* [29]. The results showed that EO at the MIC's inhibited biofilm formations of all microorganisms tested in various percentage (1, 1/2, 1/4, 1/8 and 1/16 MIC). The highest antibiofilm was obtained against *C. albicans* at 25 µg/mL (MIC) concentration with 36.31%. On the other hand, the authors found that the antibiofilm activity of EO on tested strains was lower than that of the methanol extract.

Conclusions

The essential oils of seventeen *Marrubium* species have been studied. Characterization and chemical composition analysis identified germacrene D, β-caryophyllene, caryophyllene oxide, bicyclo-germacrene and spathulenol as either main or minor constituents of the *Marrubium* species essential oils. Other identified components included hydrocarbon compounds and sesquiterpenes.

The EOs extracted from only three *Marrubium* species, *M. globosum*, *M. deserti* and *M. peregrinum*, have been investigated for their antioxidant activity. All these oils exhibit a greater activity when compared to standard antioxidants like BHT.

Studies have shown that some *Marrubium* EOs have moderate antimicrobial activity or negligible, while others were ineffective. This difference in antimicrobial activity might be attributed to the change of chemical composition of EOs and concentrations used in tests. The antimicrobial activity of the essential oils has been attributed to the presence of carvacrol and caryophyllene, and some other components like linalool, α-terpineol, eugenol that, even if present in low amounts, may synergistically increase the action of carvacrol.

Species of this genus may be regarded as a potential source of natural chemical compounds and could be used in the fields of food and pharmaceutical industries.

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