

Chemical Composition of Essential Oil of *Haplophyllum tuberculatum* (Rutaceae) Grow Wild in Different Habitats of Egypt

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Abstract: Essential oils isolated from the aerial parts of *Haplophyllum tuberculatum* (Forsskal) A. Juss collected from five different locations of Egypt showed significant differences in their yield and chemical composition among the different locations. Estragole, Myrtenal, Spathulenol and a homologous series of medicinally important monoterpene and sesquiterpenes are succinctly viewed in the analyzed essential oils. The extensive disparity in the abundance and existence of both the major and minor detected bio active compounds reflects a considerable chemo-diversity that can favor the medicinal use of each group towards certain medical conditions the fact that could explain its wide range of different traditional uses reported in literature.

Key words: GC-MS • Estragole • *Haplophyllum tuberculatum* • Myrtenal • Rutaceae • Spathulenol • Terpenoids • Tricyclene

INTRODUCTION

Rutaceae is a large family comprising about 161 genera and 1815 species [1-5]. *Haplophyllum tuberculatum* (Forsskal) A. Juss is one of the most species-rich, but poorly known genera of Rutaceae [6-10]. It occupies a wide range of habitats and generally shows striking interspecific differences in many morphological, anatomical and molecular characteristics [11-17]. A review of literature revealed some reports on its wide range of extensive traditional uses. The plant is collected and sold in the shops in the west Mediterranean coastal region under the name 'shajarah'. In north Sinai, it is known as dharret rieh, or um-jeneinah. Flowering and fruiting branches are used in the Egyptian folk medicine as febrifuge and for local antipoin, vomiting, nausea, constipation, malaria, difficult childbirth, anemia, rheumatism, gastric pains, intestinal worms, eye and ear

troubles, aphrodisiac, decoction and for rheumatic pains [18]. In the north of Oman, the juice expressed from the leaves is used as a remedy for headaches and arthritis and also used to remove warts and freckles from the skin and also to treat skin discoloration, infections and parasitic diseases [19]. In Saudi Arabia, *H. tuberculatum* is used to treat malaria, rheumatoid arthritis and gynecological disorders [20]. While, in Sudan the herb is used as an antispasmodic, to treat allergic rhinitis and gynecological disorders, asthma and breathing difficulties [21] and so on, indicating a large degree of variability in its traditional uses as a function of geographic and ecological location. The main objective of the present study was analyzing the essential oil obtained by hydro distillation of *H. tuberculatum* aerial parts collected from five different habitats of Egypt using GC and GC- MS to illustrate the main differences in their yield and chemical composition.

MATERIALS AND METHODS

Plant Material: Fresh aerial parts (the top 25 cm) of the *H. tuberculatum* plant were collected during the period of last June (2010) from five different Egyptian locations, Group A (Ras Al-Hekma), Group B (Zawet El-Hawala), Group C (Garawlah), Group D (Samala) and Group E (El-Sadat). Group A-D is distributed along the north western coastal region of Egypt while group E is located at Minufiya Governorate. The plant specimens were authenticated then identified according to Boulos [22] and Täckholm [23]. The voucher specimens have been deposited in the herbarium of the Environmental Studies and Research Institute, Minufiya University, Sadat City, Egypt (ESRI Acc. No. 3012 - 3017).

Extraction of Oil: 50 g of each of the collected samples of *H. tuberculatum* fresh aerial parts was subjected to hydro distillation separately for 3 hours, using a Clevenger-type apparatus, according to the European Pharmacopoeia (Council of Europe, 2007). The oil yield was estimated as a dry weight (v/w) of plant materials. The collected oils were dried using anhydrous sodium sulfate and stored at -84 °C in glass vials until analysis.

Sample Preparation for GC and GC/MS Analysis: One ml of hexane was added to each sample and mixed well. After phase separation upper layers were taken to autosampler vials with no treatment and 1 µl was directly injected to GC and GC-MS.

Gas Chromatography: GC analysis was carried out with Hewlett-Packard 7890A apparatus equipped with two flame ionization detectors and fused-silica capillary columns (50 m × 0.22 mm i.d. film thickness 0.25 µm), BP-1 (polydimethylsiloxane) and BP-20 (polyethyleneglycol). The temperature program was as follows: Injection port temperature 260°C (injection mode, split), column oven temperature 40°C for 4 min with a gradient increasing rate of 4°C/min up to 270°C and then hold constant for 2.5 min (isothermal) with a final run time of 64 min. The carrier gas was helium.

Gas Chromatography-Mass Spectrometry Analysis: The identification of volatile constituents was conducted by gas-chromatography (GC-MS) technique - GC 7890A apparatus with Mass spectrometer-MS 5975C VL MSD with Triple-Axis Detector-Automatic sampler- 7683B Series injector and split/split less injection system operating in electronic ionization mode achieved by electron impact at 70 eV. Equipped with HP-Chemstation

data processor and fitted with Varian CP9103 capillary column (60m x 0, 25 mm and 1,4µm film thickness). Helium was used as carrier gas (purity 99.999%). The inlet port temperature 260°C, pressure 151.63 kPa, total flow 141.26 ml/min, septum purge 3ml/min, split ratio 1:100. Vacuum: Flow 1, 3689 ml/min, Pressure 151,63kPa, Average velocity 30cm/sec. The mass spectrometer had a vacuum compensation ON, solvent delay time 5 min to avoid the solvent peak, split ratio 1:100 and electronic pressure control on. The mass spectrum of each peak was recorded in the total ion current mode of the mass spectrometer within a mass scan range of 29-250 m/z. The temperature program was as follows: Injection port temperature, 260°C, Temperature of MS detector 280°C, column oven temperature 40°C for 4 min with a gradient increasing rate of 4°C/min up to 270°C, kept constant for 2.5 min hold time, 64 min was the final time, transfer line temperature was 280°C. The analysis was performed three times and relative percentages of components were calculated as mean values.

Identification of Components: The components of the oils were identified by matching their mass spectral fragmentation patterns with those reported in computerized MS-data bank spectral libraries (NIST 98 and WILEY 138). The compounds identified are reported to have match quality > 90% with respect to the experimental spectrum. The compounds are arranged in order to GC elution on Varian CP9103 capillary column. For quantification purpose, relative area percentages were obtained by electronic integration on total ion current without the use of correction factors. The molecular weights of the compounds were confirmed by comparing their retention indices (RI) on apolar and polar columns with those reported in literature data [24-31]. The RIs were calculated according to the RI Van den Dool and Kratz equation relative to the retention times of a series of n-alkanes [32].

RESULTS AND DISCUSSION

The essential oils obtained by hydro distillation from the aerial parts of *H. tuberculatum* collected from five diverse agro-ecological regions in Egypt showed significant variations in both the total oil yield and chemical composition. On the basis of plant dry weight (v/w) the essential oils yield for the Groups A, B, C, D and E gave an average yield of 0.65%, 0.31%, 0.6%, 0.52% and 0.36% respectively. The overall oil yield, collection site and the geographical information of each group is listed in Table 1. The difference in high oil yield obtained from

Table 1: Location, some geographical information and characteristics of essential oils obtained from *H. tuberculatum* from five diverse habitats in Egypt.

Group	Location	Latitude N	Longitude E	Elevation	Oil yield (%)	Color
A	Ras Al-Hekma	31°06'1.44"	27°54'38.88"	30 m	0.65	Light yellow
B	Zawet El-Hawala	31°12'4.32"	27°28'4.8"	5m	0.31	Pale yellow
C	Garawlah	31°14'13.92"	27°23'6.72"	15m	0.60	Pale yellow
D	Samala	31°20'16.8"	27°15'20.16"	33m	0.52	Light yellow
E	El-Sadate	30°31'53.61"	30°24'42.50"	30m	0.36	Light yellow

Table 2: The relative percentage, chemical composition and the retention indices of identified compounds in the essential oils obtained from the aerial parts of *H. tuberculatum* collected from five diverse habitats in Egypt using GC and GC-MS.

No.	Class	Compound name	RI expBP-1	RI lit(apolar)	RI expBP-20	RI lit(polar)	Relative percentage (%)				
							GroupA	GroupB	Group C	GroupD	GroupE
1.	MH	Tricyclene	922	923 [24,25]	1029	1032 [29]	n.d.	n.d.	n.d.	n.d.	0.7±0.2
2.	MH	a-Thujene	924	924 [28]	1028	1028[24,28]	1.1±0.3	2.5±0.3	1.5±0.3	1.5±0.3	n.d.
3.	MH	a-Pinene	934	932 [30,25]	1027	1028 [28]	5.1±0.3	6.1±0.3	5.3±0.3	5.4±0.3	7.2±0.2
4.	MH	Camphene	945	946 [25]	1070	1076 [29]	2.5±0.2	3.6±0.2	2.6±0.2	2.5±0.2	6.6±0.3
5.	MH	Sabinene	968	970 [25]	1121	1122 [28]	Tr	3.2±0.3	1.7±0.3	0.8±0.2	0.8±0.2
6.	MH	β-Pinene	973	973 [25]	1111	1112 [28]	2.8±0.2	5.6±0.2	3.8±0.2	3.2±0.2	7.1±0.3
7.	MH	Myrcene	982	984 [25]	1163	1161[24,28, 31]	1.6±0.2	3±0.2	2.4±0.2	1.9±0.2	1.5±0.2
8.	MH	2-Carene	1001	999 [28]	1131	1131 [28]	0.5±0.2	n.d.	0.5±0.2	Tr	n.d.
9.	MH	a-Phellandrene	998	999 [25, 28]	1167	1167 [28]	1.9±0.2	Tr	0.5±0.2	0.9±0.2	1.0±0.2
10.	MH	d-3-Carene	1006	1007 [25, 28]	1147	1147 [28]	2.9±0.1	12.6±0.2	6.8±0.3	4.9±0.2	0.7±0.2
11.	MH	a- Terpinene	1011	1012 [25,28]	1182	1189[24,28, 29]	1.1±0.1	n.d.	Tr	0.5±0.2	0.5±0.2
12.	MH	1,4-Cineole	1016	1016 [24]	1186	--	0.5±0.3	0.7±0.3	0.5±0.3	0.6±0.3	n.d.
13.	MH	Limonene	1031	1032 [31]	1128	1128 [31]	4.6±0.2	5.4±0.2	4.3±0.2	6.2±0.2	1.4±0.2
14.	MH	O-Cymene	1021	1022 [24]	1266	1266 [24]	6.4±0.1	3.7±0.2	4.6±0.3	5.6±0.2	1.9±0.2
15.	MH	β-Phellandrene	1025	1023 [25,28]	1215	1218 [29,28]	5.1±0.2	5±0.2	4.7±0.2	3.1±0.2	2.9±0.3
16.	MH	Eucalyptol	1033	1034 [27, 31]	1200	1213 [31]	1.6±0.2	2±0.2	1.5±0.2	1.8±0.2	Tr
17.	MH	γ-Terpinene	1050	1052 [25,28]	1245	1245 [28]	0.5±0.2	Tr	n.d.	0.5±0.2	Tr
18.	OM	Linalool	1085	1086 [25]	1542	1542 [28]	0.7±0.3	0.7±0.2	0.9±0.2	0.7±0.2	Tr
19.	OM	Cis-p- menth-2-en-1-ol	1109	1108[28]	1558	1558 [28]	8.7±0.1	8±0.2	10.4±0.3	7±0.4	12.3±0.5
20.	OM	Trans-p-Menth-2-ene-1-ol	1125	1126 [24,28]	1622	1622 [28]	10.1±0.2	9.7±0.2	11.1±0.3	8.1±0.4	14.1±0.4
21.	OM	Terpinen-4-ol	1162	1164 [24, 25,28]	1599	1598 [28]	3.4±0.3	2±0.3	2.2±0.3	3.4±0.2	0.6±0.2
22.	OM	Borneol	1151	1151 [28]	1700	1701 [28]	0.5±0.1	0.5±0.2	Tr	0.5±0.2	1.2±0.3
23.	OM	Estragole	1193	1183 [30]	1656	1655 [30]	2.5±0.2	n.d.	n.d.	7.6±0.3	n.d.
24.	OM	p-Cymene-8-ol	1183	1183 [24]	1887	--	0.8±0.1	Tr	0.7±0.2	1±	n.d.
25.	OM	Trans-Piperitol	1191	1191 [28]	1741	1741[28, 29]	4±0.2	4.2±0.2	5.3±	3.5±0.1	5.2±0.3
26.	OM	Cis-Piperitol	1179	1180 [28]	1674	1674 [28]	7±0.4	6.5±0.2	9.3±0.2	5.9±0.3	10.4±0.3
27.	OM	Myrtenal	1170	1170 [28]	1635	1634 [28]	0.5±0.1	n.d.	Tr	0.5±0.1	0.5±0.2
28.	OM	Cryptone	1159	1160 [25, 28]	1667	1665 [28]	3.6±0.3	0.7±0.1	2±0.2	2.9±0.2	Tr
29.	OM	Bornyl formate	1285	--	1580	--	Tr	1.1±0.2	0.5±0.1	0.6±0.1	4.2±0.3
30.	OM	Cuminal	1215	1217 [26]	1780	--	0.7±0.1	n.d.	0.5±0.1	0.7±0.2	n.d.
31.	OM	Bornyl acetate	1270	1270 [25,28]	1578	1571[28,29]	4.8±0.4	5±0.2	4±0.2	4.1±0.3	12.5±0.4
32.	OM	Piperitone	1228	1216 [30]	1722	1673 [30]	2.6±0.3	1.7±0.2	1.7±0.1	2.2±0.3	2.1±0.2
33.	OM	p-Cymen-7-ol	1288	1285 [26]	2099	--	0.9±0.2	n.d.	1±0.3	0.7±0.1	n.d.
34.	Other	Eucarvone	1223	1222 [27]	1721	--	n.d.	0.6±0.1	0.5±0.1	0.8±0.1	Tr
35.	Other	Octyl isovalerate	1301	--	--	--	0.5±0.1	Tr	0.5±0.2	n.d.	n.d.
36.	SH	α-Curcumene	1472	1472 [28]	1770	1768 [28]	0.9±0.2	1.6±0.2	0.8±0.1	0.7±0.1	n.d.
37.	SH	β-Sesquiphellandrene	1503	1513 [25]	1768	1768 [28,29]	n.d.	0.7±0.2	n.d.	n.d.	n.d.
38.	SH	δ-Cadinene	1517	1519 [25]	1756	1756 [28]	n.d.	n.d.	n.d.	n.d.	0.5±0.1
39.	Other	Lauric acid	1568	1571 [27]	2517	--	n.d.	0.5±0.1	0.5±0.1	0.5±0.2	n.d.
40.	OS	Spathulenol	1576	1576 [24, 25]	2129	2124 [28]	n.d.	n.d.	n.d.	n.d.	0.6±0.2
MH	Monoterpene hydrocarbons						38.2	53.4	40.7	39.4	32.3
OM	Oxygenated monoterpenes						50.8	40.1	49.6	49.4	63.1
SH	Sesquiterpene hydrocarbons						0.9	2.3	0.8	0.7	0.5
OS	Oxygenated sesquiterpenes						0	0	0	0	0.6
	Other						0.5	1.1	1.5	1.3	0
Total identified [%]							90.4	96.9	92.6	90.8	96.5
Extraction yields v/w [%]							0.65%	0.31%	0.60%	0.52%	0.36%

RI exp: Relative retention indices calculated against homologous series of n-alkanes (C9-C20) on both BP-1 (apolar) and BP-20 (polar) columns.

RI lit: Relative retention indices reported in literature.

The components are arranged in order to GC elution on Varian CP9103 capillary column.

The compounds identified using GC/MS are reported to have match quality > 90% with respect to the experimental spectrum.

MH: Monoterpenic hydrocarbons, OM: Oxygenated monoterpenes, SH: Sesquiterpene hydrocarbons, OS: oxygenated sesquiterpenes. Compounds showed relative percentages smaller than 0.1 % was considered as traces (Tr.), n.d.: Not detected.

A: Plants collected from Ras Al-Hekma, B: Plants collected from Zawet El Hawala, C: Plants collected from Garawlah, D: Plants collected from Samala, E: Plants collected from El-Sadate Desert.

H. tuberculatum in the present study (0.31% - 0.65%) and very low content (0.1%) reported in earlier publications on the same species in other countries [11, 13, 15, 17] may explain the major effects of geographical and ecological variations among habitats.

GC and GC/MS analysis the volatile oils of the five groups exhibited a significant qualitative and quantitative difference in the chemical compositions and relative percentages (%) of the identified compounds. Estragole, Myrtenal, Spathulenol, tricyclene and a homologous series of medicinally important oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes and others are succinctly viewed in the analyzed oils (Table 2). Thirty four compounds were identified in the essential oil obtained from Group A, representing 90.4 % of the total oil yield. While, thirty two compounds were identified in Group B, representing 96.9 % of the total oil yields. In group C, thirty four compounds were identified, representing 92.6 % of the total oil yield. The highest number of compounds was obtained from group D; thirty five compounds were identified, representing 90.8% of the total oil yield. However, the lowest number of compounds were identified in group E, twenty nine compounds, representing (96.5 %) of the total oil yield.

In general, oxygenated monoterpenes were identified as the most abundant chemical group among all the studied groups except in group B (Fig.1). The dominance of monoterpene hydrocarbons observed in group B can be contributed to the high percentage of δ -3-carene (12.6%). While the dominance of oxygenated monoterpenes in all the other groups (A, C, D and E) can be contributed to the high percentage of cis-p-Menth-2-en-1-ol (7%-12.3), trans-p-Menth-2-ene-1-ol (9.7%-14.1) and cis-Piperitol (5.9%-10.4%) (Fig. 2). Despite this fact, some other considerable chemical variability of other major and minor detected bioactive compounds was clear in all the studied samples and because of their extensive disparity in the abundance and existence they are considered as a characteristic constituent for each group. This considerable observed chemo-diversity can favor the medicinal importance of each group towards certain medical conditions as each of these compounds has been associated with various therapeutic activities. This fact can explain the wide range of different traditional uses of *H. tuberculatum* reported in literature as a function of different geographical and ecological factors. However, the abundance of oxygenated monoterpenes among the studied groups except in Group B was not in agreement with other several authors who reported monoterpene

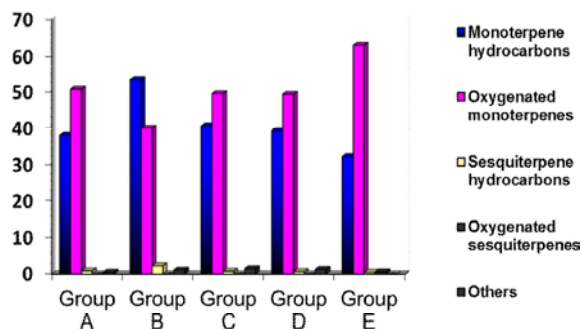


Fig. 1: Percentages of monoterpenes and sesquiterpenes in the essential oils obtained from the aerial parts of *H. tuberculatum* collected from five diverse agro-ecological regions of Egypt.

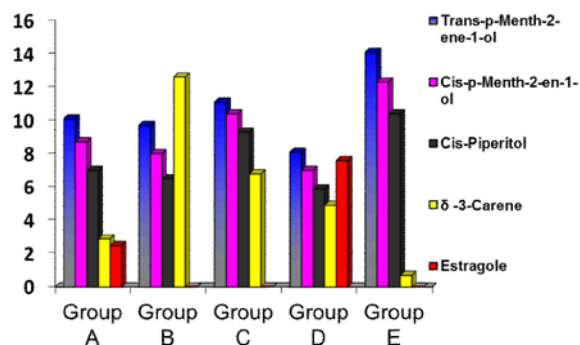


Fig. 2: Percentages of the most dominant major compounds in the essential oils obtained from *H. tuberculatum* collected from five diverse habitats in Egypt.

hydrocarbons as the most dominant group representing up to 72% of the essential oil obtained from *H. tuberculatum* in other countries [13, 14-42]. In Iran [14] linalool (15.5%), alpha-pinene (7.9%) and limonene (5.3%) are reported as the major constituents of *H. tuberculatum*. In the United Arab Emirates [13] alpha-phellandrene (32.9%), beta-caryophyllene (12.8%), beta-pinene (7.6-8.0%), limonene (9.6%) and delta-3-carene (6.0%) are reported as the chief components. While, in Oman β -phellandrene (23.3%), limonene (12.6%), (Z)- β -ocimene (12.3%), β -caryophyllene (11.6%) and α -phellandrene (10.9%) were identified as the major compounds in the essential oil of *H. tuberculatum* [11].

Oxygenated monoterpenes were reported in an earlier study by Kalembe and Kunicka [43] to possess a much stronger antimicrobial activity than that of monoterpenes hydrocarbons. Accordingly the Egyptian *H. tuberculatum* groups under study are expected to possess a

considerably high antimicrobial activity than those reported in other countries. The medicinal importance of the investigated groups as antimicrobial agents have been increased extensively due to the presence of α -terpinene, δ -terpinene, camphene, linalool, borneol and others compounds (Table 2) that are known for their effective antimicrobial activity in addition to other various biological activities [44- 46]. Linalool is also reported to possess an anti-inflammatory, expectorant and stimulant activity [34, 47]. While terpinen-4-ol is known for its anti-asthmatic, bacteriostatic, diuretic and spermicide [45]. Borneol is reported for its effective antimicrobial, antiseptic, tonifying, balancing, spasmolytic, anaesthetic and anti-inflammatory activities [34, 47-50]. The dominance of oxygenated monoterpenes in a plant was also explained to be due to responses to internal factors as histological problems or external factors such as infection, wounds, or defense to invaders [43]. Another layer of control is imposed by environmental conditions, light and water stresses which reduce constitutive monoterpene cyclase activity levels and abolish the wound response [51]. Thus as a rule, the distribution of this terpenes has some value for taxonomy but their occurrence apparently reflects adaptations and particular life strategies embedded in a given phylogenetic framework.

The medical importance of each group towards certain medical conditions depends mainly on the biological activities of its major and minor ingredients that are specific for each group. The presence of α -cymene (6.4%), α -Pinene (5.1%) and β -Phellandrene (5.1%) as major active constituents in Group A had increased the medicinal importance of this group as antiviral, antitumor, anti-pneumonic and antibacterial that are reported to correspond to α -Pinene [33, 37,38, 39] and an expectorant, fungicidal and hepatoprotective activity that corresponds to the reported biological activity of β -Phellandrene [33-39]. While, the presence of δ -3-carene (12.6 %) as the chief compound in group B could increase its medicinal importance in the treatment of metabolic bone disorders and for the maintenance of bone health as it have been recently reported to stimulate significantly the activity and expression of alkaline phosphatase, that promote osteoblastic differentiation [39]. In addition to its reported anti-inflammatory, dermatitogenic and insectifuge activity reported for α -Pinene [41]. However, the presence of delta-3-carene as the major constituent in group B represents the first reported main difference in the chemical composition between the studied groups. This result was consistent with an earlier study obtained from

H. tuberculatum plants collected from Hungarica [42]. In Group C the presence of cis-Piperitol (9.3%) as the major active ingredient together with high percentage of other chemical compounds such as cis-p-Menth-2-en-1-ol (10.4%), trans-p-Menth-2-ene-1-ol (11.1%) and trans-Piperitol (5.3 %) may contributes to the potential insecticidal activities which was reported against three *Anopheles gambiae* spp of mosquitoes inducing 100% of mortality to both sensitive and resistant *Anopheles gambiae* [52]. This synergistic effect has increased the medicinal importance of this group in controlling vectors of the malaria and may favors the medicinal use of this group as a source of natural biocides.

On the other hand, the presence of d-Limonene (6.2 %) which was reported [33-39] to have AchE-inhibitor, acaricide, anticancer, anti-lymphomic, antiviral, antitumor, antimutagenic, antibacterial, sedative, insecticide and insectifuge activity as a major component in group D may favors the medicinal use of this group towards these medical conditions. However the unexpectedly presence of estragole in a concentrations of 7.6% of their total oil yields in group D may abolish or minimize its medicinal importance. The Scientific Committee on Food from the Health & Consumer Protection Directorate-General took a more concerned position and concluded that "Estragole has been demonstrated to be genotoxic and carcinogenic [44- 46]. Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels are indicated especially to sensitive individuals such as young children, pregnant, breastfeeding women and those at risk of breast cancer. Unlike all the studied groups that showed a conspicuous absence of any oxygen containing sesquiterpene compound. Group E was the only group that showed the presence of single oxygenated sesquiterpene compound represented by spathulenol (0.6%). Spathulenol was reported recently to possess an immunomodulatory, antitumor and antimicrobial activity [53, 54, 55]. The presence of other medicinal important monoterpenes hydrocarbon, such as tricyclene (0.7%) and myrtenal (0.5%) have extensively increased the medicinal importance of this group towards several medical conditions. Myrtenal was reported to have anti-zhiemer, antiviral, antimicrobial, vasodilators, hypotensive, calming, antipyretic, sedative, spasmolytic and antitumor activity toward hepatocellular carcinoma [34, 56, 57]. While, tricyclene [53] is reported to be one of the main constituents responsible for the antimicrobial activity of

the essential oil of *Gossypium barbadense* toward *S. aureus*, *Gardinella spp*, *E. coli* and *C. albicans*. To the best of our knowledge saphulenol, tricyclene and myrtenal were not reported to be present in any investigated *Haplophyllum* species before. In addition, the presence of L-bornyl acetate (12.5%) which is reported to possess spasmolytic, sedative, antifungal, anaesthetic and anti-inflammatory activity [34, 47-50] as the major compounds have reported Group E to be the most medicinally important group in this study. This reflects a considerable chemo-diversity that could be correlated to its wide range of traditional uses that differs extensively from one group to another.

Thus this work significantly, reflects considerable extensive variations in the chemical composition among the essential oils obtained not only from the aerial part of *H. tuberculatum*, collected from five different regions of Egypt but also differs significantly from those obtained from other countries of habitat emphasizing the different uses of *H. tuberculatum* in each country.

CONCLUSION

From the present study it can be concluded that the variability in the chemical composition of essential oils of *H. tuberculatum* may be related to different ecological and geographical factors, that could be linked to the varied soil textures [57] and possible adaptation response of different populations, resulting in different chemical products and yields being formed, without morphological differences being observed in the plants [58]. The other factor may respect to the availability of water and height above sea level, additionally, the plant density [59] and the local micro-environment conditions in which the plant grows [60] are also considered. However, a clear correlation between ecological and geographical conditions and the observed chemo diversity of the analyzed essential oils could not be established at least in the present study and other factors may contribute to such variability also the possibility of different varieties and chemical races of the species must also be considered.

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