Research Article

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Chemical Composition of Essential Oils of Different Hypericum Species Growing Wild in Shouf Biosphere Reserve, Lebanon

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ABSTRACT

This study aims to assess the chemical composition of six Hypericum species growing wild in Shouf Biosphere Reserve (SBR) of Lebanon and discuss therapeutic potential of their different constituents. Fresh aerial parts were randomly collected from different locations during the flowering stage of the plant in 2022. Using hydrodistillation by Clevenger apparatus, essential oils were isolated and then analysed by gas chromatography (GC) and GC-Mass spectrometry. A total of 134 different chemical compounds were identified. H. triquetrifolium illustrated the highest diversity of compounds (55) followed by H. lanuginosum (51), H. perforatum (38) and H. thymifolium (30), H. libanoticum (23) and H. hircinum (15). While hydrogenated sesquiterpenes constituted the highest percentages of the overall composition of H. triquetrifolium, H. hircinum and H. perforatum (45.08 %), 31.88 % and 67.97%, respectively, oxygenated monoterpenes and hydrogenated monoterpenes were dominant in H. lanuginosum and H. thymifolium, respectively. Major compounds included β-Caryophyllene, γ-Terpinen, Ylangene, and α-Elemene found in the literature to have a wide range of therapeutic effects in pharmacological research. Findings confirm the traditional use of studied Hypericum species and the high potential of essential oils as rich source of many bioactive compounds of wide applications in pharmaceutical, cosmetics and food industries.

Keywords

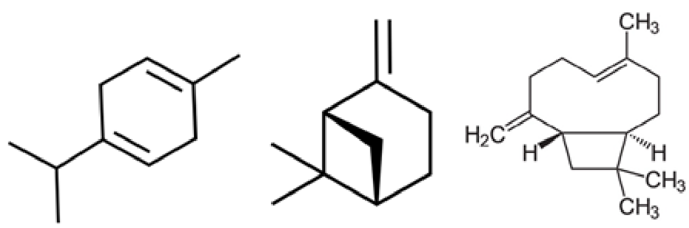
Hypericum, Essential oil, Chemical composition, Gas chromatography (GC), GC-Mass spectrometry (MS), Shouf Biosphere Reserve, Lebanon.

Introduction

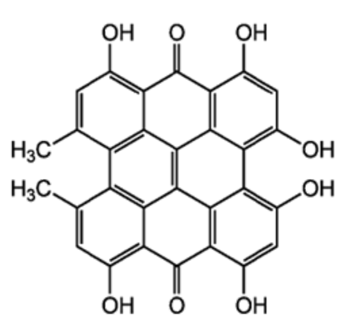
There are more than 500 taxa of the genus *Hypericum* L. (Hypericaceae), classified into 36 taxonomic sections [1], that exist almost everywhere in the world. Many *Hypericum* species are used in traditional medicines of different countries in the treatment of skin conditions such as wounds, eczema and burns [2]. They are also used in the treatment of rheumatism, gastroenteritis, ulcers, and several neurological conditions (i.e. hysteria and depression). Recent scientific research on pharmacological properties of *Hypericum* species by different bioassays reports a wide range of bioactivities including anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral and antiseptic effects [3-5], anticancer [6] and cytotoxic [7], and antidepressant [8].

Primary compounds of the essential oils of *Hypericum* species include hypericin (Figure 1), tannins, flavonoids, phenolic acids, quercitrin, hyperoside, isoquercitrin, chlorogenic acid and rutin [3,9,10]. Some of these constituents are found to demonstrate promising pharmacological properties by several studies. For example, hypericin and its derivatives have been found to have antidepressant activity [11,12]. Consequently, the European Union Commission approved its internal use for psychovegetative disturbances, depressive moods, anxiety and nervous unrest. Hypericin is also used in treatment of viral infections [13] and as a major photosensitizing agent in photodynamic cancer therapy [14]. It suppresses the proliferation of alloreactive T cells, inhibits the growth of breast carcinoma in immunocompetent Wistar rats, and activates mitochondria-mediated apoptosis in MT-450 cells [15]. Further, *Hypericum* species are among the high selling natural supplements. *H. perforatum,* especially, is registered in Europe under the THR scheme (Traditional Herbal Registration) and is considered as one of the main herbal products in USA with a total sale of USD 6-8 million dollars [1,16]. Primary production areas for *H. perforatum* in Europe include Germany, Italy and Romania, and the majority of the harvest goes toward the production of crude drugs [4]. In 2004, the plant represented nearly 13% valuing at around €70 million of all European herbal product sales in Germany [1].

The chemical composition of essential oils from many *Hypericum* species growing in different regions of the world have been extensively examined. Among these are *H. scabrum* L. and *H. perforatum* L. from Turkey [17,18], Uzbekistan [19] and Iran [3]. Other studied species include *H. olympicum* L. from Serbia [20]. Typical constituents of *Hypericum* species essential oils include the monoterpenes α- and β-pinene (Figure 1), limonene and myrcene, the sesquiterpenes β-caryophyllene (Figure 1), and caryophyllene oxide, and hydrocarbons such as *n*-decane, C_{16}- and C_{29} alkanes and C_{24}, C_{26} and C_{28} alkanols [3,4]. More specifically, flavonols (catechins), naphthodianthrons (hypericin, pseudohypericin), xanthones, coumarins, lycosides, anthraquinones, phloroglucinols (hyperforin, adhyperforin), flavonoids (rutin, hyperoside, quercitrin), flavonol glycosides, lactones, pyrones, lipids, triterpenes, and tannins represent the main constituents in *H. perforatum* [21]. Whereas, α-pinene, β-pinene, (*E*)-β-ocimene, β-caryophyllene and germacrene-D dominate constituents of *H. helianthemoides, H. scabrum* and *H. perforatum* from Iran [3]. Main constituents in *H. scabrum* from other regions of Iran are α-pinene, while the major representatives in *H. helianthemoides* (Spach) Boiss. were β-Caryophyllene and spathulenol [22] and α-pinene and β-Caryophyllene in the essential oils of *H. scabrum, H. hyssopifolium*, *H. helianthemoides* and *H. perforatum* flowers [23].



γ-Terpinen, β-pinene, β-Caryophyllene (In order from left to right) and



Hypericin

Figure 1: Chemical structures for four main compounds of hypericum species.

In Lebanon, around 20 *Hypericum* species are found in different parts of the country [24]. Eleven of these species i. e. *H. confertum* Choisy, *H. hircinum* L., *H. lanuginosum* Lam., *H. libanoticum* Robson., *H. montbretii* Spach, *H. pallens* Banks & Sol., *H. scabrum* L., *H. tetrapterum* Fries., *H. perforatum, H. thymifolium* Banks & Sol., and *H. triquetrifolium* Turra are found in the Shouf Biosphere Reserve (SBR), where the plant material of present study is collected [25].

Situated on Mount Lebanon, SBR is the largest nature reserve in Lebanon and the southern part of the Mediterranean. It covers an area of 500 km2 (50,000 hectares), or 5% of the Lebanese territory, at an altitudinal gradient between 800 and 1900 m above sea level. It is characterized by a bio-climatic gradient of the Mediterranean climate: a cold, rainy, and snowy winter and moderate to hot weather in the summer, with an average annual rainfall of around 1200 mm. In addition to its role as a multifunctional ecosystem, SBR hosts a very rich biodiversity of wild plants, with many species used in traditional medicines and the health care systems of local communities [2]. Table 1 provides a list and botanical description, along with the IUCN conservation status, of Hypericum species found in SBR.

In a view of a recent ethnobotanical survey by the authors focusing on the traditional use of SBR medicinal plants by local communities [2], several *Hypericum* species were reported to be of high use value. Among the reported treated ailments and diseases were; inflammatory, burns, wounds, headaches, menstruation, mental-nervous, bruises, insect stings, warts, blood purification, diarrhea, cystitis, hepatitis, rheumatism, tonsillitis, anxiolytic, depression, and cholesterol. The mentioned ethnobotanical study holds a very useful opportunity in 'ethno-directed' strategy for screening and identifying bioactive compounds from *Hypericum* species and other plants in human quest for novel drugs and compounds of pharmaceutical and food industry applications [26]. This strategy has been shown to be effective way in identifying promising initial leads. In this context, medicinal plants of Lebanon and surrounding region form the basis of sophisticated traditional medicine systems, dating from around 2600 BC [27]. Earliest records document the use of approximately 1000 plant-derived substances including oils of *Cedrus libani* (Lebanon cedar), *Cupressus sempevirens* (Cypress), *Crateagus* sp., *Daucus* sp. and *Asparagus* sp. all of which continue to be used today in traditionally Lebanese medicine. To this end, *Hypericum* species of SBR carry a high potential to play important role in improving the wellbeing and the socio-economic status of surrounding communities. Indeed, medicinal plants of SBR are considered the one of the viable ecosystem services of the reserve prompting current research [28].

The aim of this study is to characterize the chemical profile of essential oils of six *Hypericum* species growing wild in SBR and explore their potential as a source of bioactive molecules useful in the innovation of novel drug discovery, food preservation, cosmetics and pharmaceuticals.

Table 1: Botanical and habitat description to the 11 *Hypericum* species that exist in SBR.

Name	Habitat	Botanical description	IUCN status and endemicity
H. confertum Choisy.	Wet sandy	Spreading pubescent plant 20-50 cm; sessile leaves with transparent points; sessile cyme, glandulous and ciliated sepals. Flowering time (Ft): 4-5	Not evaluated.
H. hircinum L.	Near water	A shrub of 1-2 m high. It has opposite leaves, glabrous shrubby tree 1-1.5 m tall, heavy scented; bright yellow petal 2 cm; sepal 4-5 mm. Ft: 6-9.	Not evaluated.
H. lanuginosum Lam.	Shady woodland. Not so common in SBR.	Pubescent plant 40-120 cm; upper leaves are without punctuations, black glands at leaf edge; ample panicle; dentated, glandulous sepals. Ft: 5-7.	Not evaluated.
H. libanoticum Robson.	Rocky woodland. It is available at higher altitude.	Glabrous, suffrutescent, perennial plant ± 60 cm, often much branched at the base. Flowering stems erect or ascending with sparse foliage above. Ft: 5-8.	Vulnerable Endemic to Lebanon and Syria.
H. montbretii Spach,	Shady woodland, sandstones.	A perennial herbaceous plant, 40-50 cm long, stem with 2 prominent strips; leaves with translucent punctuations and black points at edge; terminal corymbs; petals with black glands. Ft: 5-6.	Not evaluated.
H. pallens Banks & Sol.	Rocky	Glabrous shrubby plant 30-60 cm with reddish branches; slightly petiolate cuneate leaves; short racemes; sepals with 2-3 black glands, shorter than petals. Ft: 4- 7.	Not evaluated. Endemic to Lebanon, Syria and Turkey.
H. scabrum L.	Rocky woodland at higher altitude.	Glabrous plant 40-60 cm; linear, sessile leaves with prominent glands, small spines; compact terminal corymb. Ft: 5-7.	Not evaluated.
H. tetrapterum Fries.	Stream side	Glabrous plant 20-40 cm with 4 prominent strips; leaves with prominent veins, transparent and black punctuations; divided calyx, pale yellow corolla. Ft: 6-10.	Not evaluated.
H. thymifolium Banks & Sol.	Woodland	Glabrous subshrub, 50-60 cm., with long, rigid branches, evergreen, elliptical, 5-8 mm., very short stalked leaves. Ft: 1-12.	Not evaluated.
H. triquetrifolium Turraar	Open areas	Glabrous, glaucescent, 50-80 cm high, with leaves strewn with black or translucent dots, cordate at the base, wavy on the margins, gradually decreasing. Ft: 5-9.	Not evaluated.
H. perforatum L.	Open areas and road sides.	Glabrous plant 50-100 cm; stem with 2 prominent strips; sessile leaves with translucent punctuations and black points at edge; ample corymb. Ft: 5-7.	Least concern.

Table 2: The *Hypericum* species, date of collection and localities.

Botanical Name Speciman Number - IPNI ID	Common Names	Distribution	Location	GPS	Collection Date	Fresh weight (gr)	Yield of essential oil (ml)
Hyperucim hircinum L. 433491-1	Common Name Stinking St John's-wart	Baleares, Corse, Cyprus, East Aegean Is., France, Greece, Italy, Kriti, Lebanon-Syria, Morocco, Palestine, Sardegna, Saudi Arabia, Sicily, Spain, Turkey	Ain Quani	33°39'11.88"N 35°36'30.17"E 701 m	June 12, 2021	130	0.1
Hypericum lanuginosum L. 433546-1	Common Name Woolly St John 's-wort	Cyprus, Lebanon-Syria, Palestine, Sinai, Turkey	Maasser Village	33°39'30.00"N 35°38'56.94"E 1112 m	July 13, 2021	160	0.1
Hypericum libanoticum N.Robson 433564-1	Lebanon St John's-wart Common Name	Lebanon-Syria	Barouk top mountian	33°40'55.64"N 35°41'52.11"E 1833	June 9, 2021	175	0.2
Hypericum perforatum L. 433719-1	Common Name John's-wort	Very large area, started in Afganistan to China to Lebanon to Yugoslavia and introduced to a very large area too Argentina, USA, Australia, etc.	Ana main road West Bekaa	33°41'43.04"N 35°45'38.72"E 893 m	June 2, 2021	155	0.2
Hypericum thymifolium Banks & Sol. 433912-1	Common Name Curled-leaved St John's-wort	Lebanon-Syria, Palestine, Turkey	Baadaran	33°38'14.8"N 35°37'35.6"E 1052 m	July 1, 2021	90	0.2
Hypericum triquetrifolium Turra 433931-1	Common Name Curled-leaved St John's-wart	Albania, Algeria, Baleares, Cyprus, East Aegean Is., France, Greece, Iran, Iraq, Italy, Lebanon-Syria, Libya, Palestine, Sicily, Sinai, Spain, Tunisia, Turkey, Turkey-in-Europe, Yugoslavia	Maasser- Ain Massaad	33°40'29.64"N 35°40'33.09"E 1263 m	June 17, 2021	167	0.1

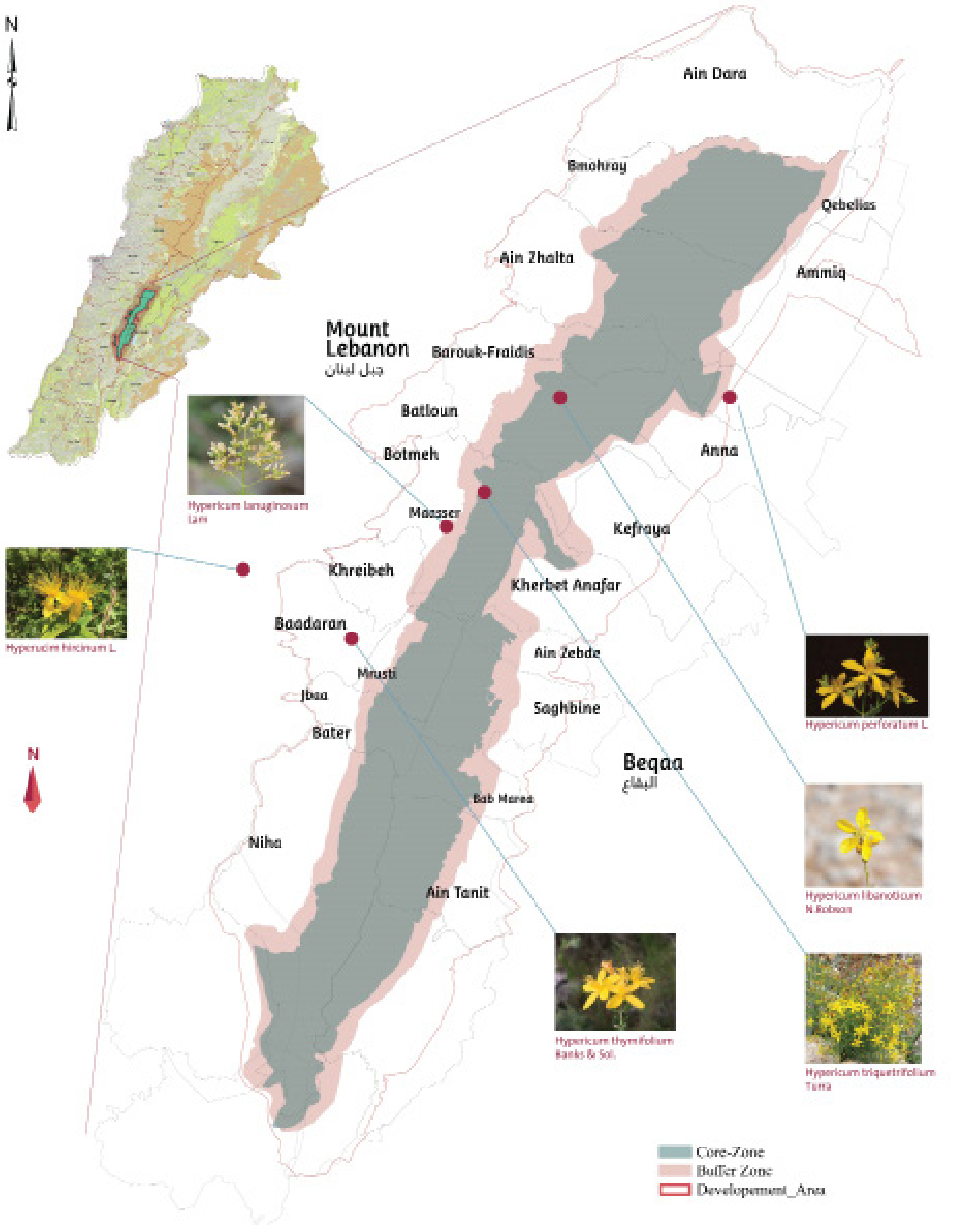


Figure 2: Map showing the Shouf Biosphere Reserve and collection sites of studied *Hypericum* species.

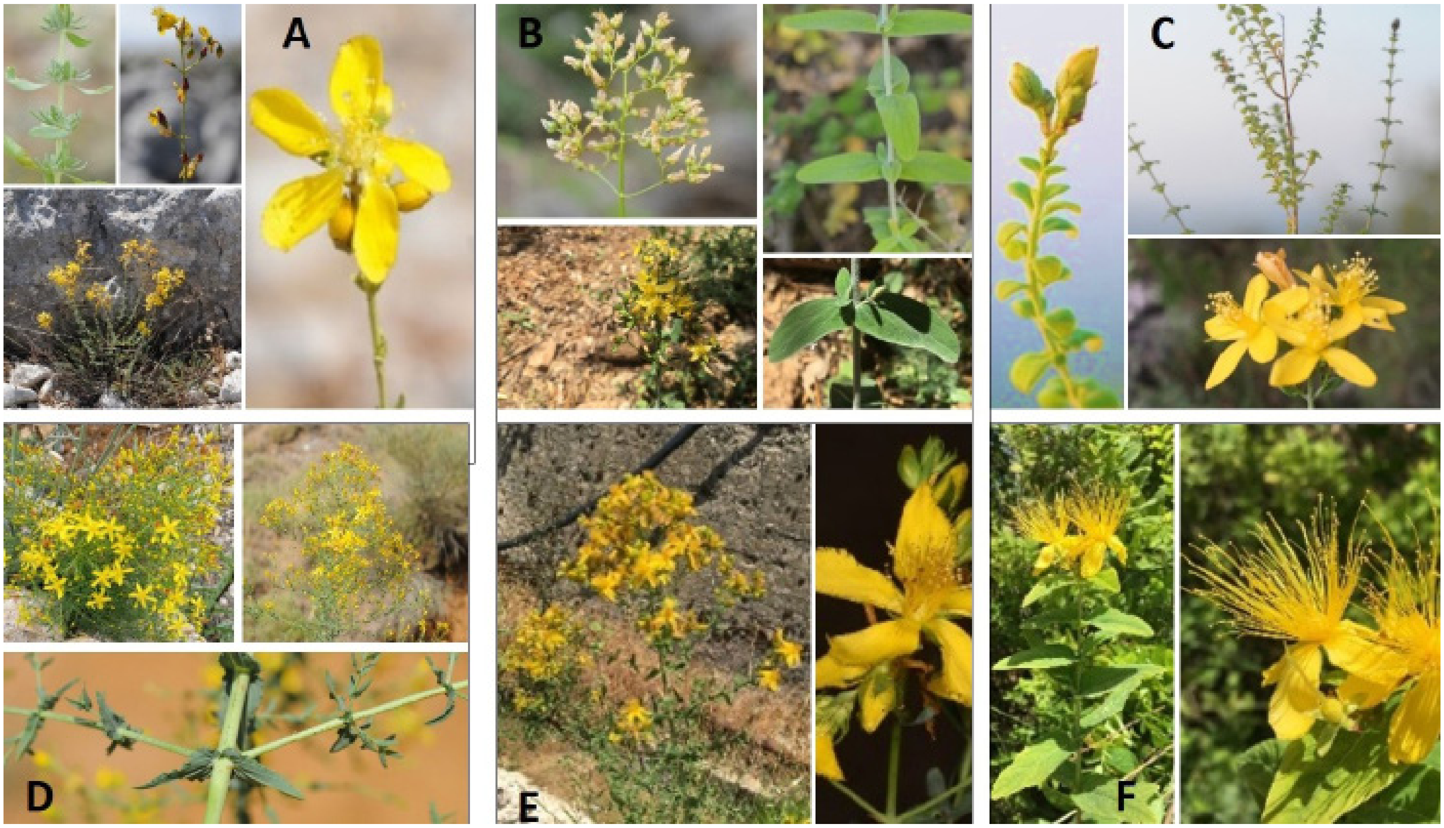


Figure 3: The morphological characterization of the studied *Hypericum* species, *H. libanoticum (A)*, *H. lanuginosum (B)*, *H. thymifolium (C)*, *H. triquetrifolium (D), H. perforatum (E)*, and *H. hircinum (F)*.

Materials and Methods

Plant materials

Composite samples of about 100-200 gr fresh weight of aerial parts of *Hypericum* species at full flowering stage were collected at random from SBR different localities during summer 2021. Plant identification was confirmed by Professor Dr. Nelly Arnold Apostolides using the determination keys of the "*New Flora of Lebanon and Syria*" [24].

Species collected, sites of collection and coordinates along with other details are presented in Table 2 and Figure 3. Voucher specimens were collected and deposited at the Herbarium of SBR.

Essential oils extraction

Essential oils were obtained of plant material mixed with distilled water by hydro-distillation Clevenger type apparatus following the standard procedure of the 6th Edition of European Pharmacopeia (2007). The extracted essential oils were stored at 4^{o}C until chemical analysis was performed.

GC and GC/MS analysis

Chemical profiles of obtained essential oils were determined by GC and GC/MS using Agilent Technologies 7890 gas chromatography equipped with a Flame Ionization Detector and a HP-5MS 5% capillary column (30m x 0.25mm x 0.25 µm film thickness). Mass spectra were recorded at 70 e-v of electron energy and a mass range of 50-550 m/z. The carrier gas was Helium of 99.99 % purity at a flow of 0.8 mL/min. The initial column temperature was 60^{o}C programed to increase to 280^{o}C at a rate of 4^{o}C/min. The split ratio was 1:40 and injection temperature was set at 300^{o}C. Essential oil samples of 1 µL were injected manually in the split mode.

Identification of chemical components was based on retention indices and comparison with mass spectral data of authentic standards and computer matching with Wiley 229, Nist 107, and Nist 21 libraries. Comparisons with fragmentation patterns of the mass spectra reported in literature were also used.

Results and Discussion

A total of 134 different chemical compounds were found in the *Hypericum* species under study (Table 3). *H. triquetrifolium Turra* ***(H. tri)*** has the highest number of chemical compounds (55), followed by *H. lanuginosum (**H. lan**)* (51), *H. perforatum (**H. per)*** (38), *H. thymifolium (**H. thy)*** (30), *H. libanoticum (**H. lib**)* (23) and *H. hircinum (**H. hir**)* (15).

In terms of chemical compounds, nine chemical compounds such as Heneicosane, n-Hexadecanoic acid, Caryophyllene oxide, α-Calacorene, and α-Amorphene, etc. were common among four *Hypericum* species. Thirteen other chemical compounds i.e. Heptacosane, Phytol, Platambin, and Copaen compounds, were common among three *Hypericum* species. In addition, 21 compounds were common among two *Hypericum* species, with the remaining 90 chemical compounds being found in one *Hypericum* species only. For example, β-Caryophyllene was revealed as common among *H. hircinum, H. libanoticum, H. lanuginosum,*

Table 3: Chemical composition of studied *Hypericum* species, highlighting in bold the top 5 compounds in each.

Nu.	Library/ID	*H. tri* %	*H. hir* %	*H. lib* %	*H. lan* %	*H. per* %	*H. thy* %
1	2,6,10,14-Tetramethylheptadecane						0.97
2	(-)-4-Terpineol				1.27		
3	(-)-Allospathulenol				**3.31**		
4	(-)-Spathulenol		1.76	3.85			
5	(4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide				0.45		
6	(E)-Longipinane			1.95			
7	(Z)-beta-Ionone				1.63		
8	1,2,3,4,5-Pentamethyl-1,3-cyclopentadiene	4.18					
9	1,5,5-Trimethyl-6-methylene-cyclohexene	0.32					
10	10s,11s-Himachala-3(12),4-diene			**5.42**	**5.94**	0.66	
11	15-Hydroxy-α-muurolene	0.65					
12	1-Dodecanol					1.28	
13	2-Isopropyl-5-methyl-9-methylene[4.4.0]dec-1-ene						1.25
14	2-methylpropyl benzoate	0.33					
15	3,4-Dimethyl-3-cyclohexenylmethanal						0.94
16	3-Methyl-4-isopropylphenol			3.82			
17	5-butyl-Docosane	0.18					
18	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione			0.97			
19	Acorenone				1.36		
20	Aromadendr-1-ene				1.11		
21	Ascabiol				2.90	0.69	
22	Benzene, 1-methyl-3-(1-methylethyl)						0.48
23	Benzyl salicylate			2.14			
24	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl				0.70		
25	Boronia butenal	0.47					
26	Cadalene						1.04
27	Cadina-1,4-diene						0.89
28	Cadine-1,4-diene	0.34					
29	Camphene		3.96				
30	Camphor	1.10	**5.79**		1.26		
31	Carvacrol			2.49	2.20		
32	β -Caryophyllene		**20.98**	2.13	2.66	0.56	**3.17**
33	Caryophyllene oxide		**9.36**	**8.74**	1.18	1.11	
34	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene			2.00			
35	cis,cis,cis-1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene					1.26	
36	Cis,trans-Nepetalactone		3.61				
37	Cis-3-Hexen-1-ol, benzoate			4.40			
38	cis-9,10-dihydrocapsenone	0.33					
39	Cis-caryophyllene	0.49					
40	cis-β-Ocimene					0.56	
41	cis-β-Terpineol	0.21			1.28		
42	Copaene				1.57	0.69	2.41
43	Cyclodecane					**6.09**	
44	Cyclohexadecane					0.84	
45	Cyclosativene	0.37				**19.87**	
46	Cyclotridecane				1.54		
47	Docosane				0.57		
48	Dodecane	0.54				0.78	
49	Epiglobulol						0.36
50	Eucalyptol	1.48	**5.83**		2.58		
51	Eudesma-4,11-dien-2-ol				0.82		
52	Guaia-3,9-diene					1.44	
53	Hendecane					2.37	
54	Heneicosane	0.07		1.17	1.15	0.24	
55	Heptacosane	0.40		1.47		0.26	
56	Heptadecane	0.08					
57	Hexacosane	0.09			0.95		
58	Hexadecane, 2,6,10,14-tetramethyl-						0.24
59	Hexahydrofarnesyl acetone					0.82	**2.75**
60	Isohomogenol	0.24					
61	Isoledene	0.53					
62	Isononane					1.06	
63	Limonene						1.89
64	Longicyclene				**3.30**		
65	Longifolenaldehyde						0.28

66	Longifolene	**5.80**			1.33		
67	Neodihydrocarveol	0.62					
68	Nerolidol	2.07		1.52		3.20	
69	n-Hexadecanoic acid	0.64			0.85	0.67	2.17
70	Nonacosane	2.47		2.20	1.24	0.70	
71	Nonadecane				1.21		
72	Nonane					0.66	
73	Octadecane				1.21		
74	Palustrol		1.90				
75	Patchulane	0.48					
76	Pentacosane						0.58
77	Phytane	0.23					
78	Phytol	2.07			2.13	0.47	
79	Platambin	2.83			2.49		1.92
80	p-Thymol	0.38					
81	Sabinene	0.30					
82	Safranal	0.34					
83	Santolina triene	0.22			1.21		
84	Spathulenol	**8.89**			**3.60**	2.95	**9.32**
85	tau-Muurolol	**6.40**					
86	Tetracosane	0.07			1.07		
87	Tetradecanoic acid	0.89					0.42
88	Thujone	3.62		5.40	**5.06**		
89	trans-Pinocarveol						0.92
90	Undecane	0.15					2.24
91	Valencene	**4.58**			2.36		
92	Valeranone		3.04				
93	Verbenene						0.44
94	Viridiflorol	0.99			1.71		**3.16**
95	Ylangene					**20.28**	
96	Ylangene				1.92		
97	Zingiberene	2.17					1.52
98	α-Amorphene	1.31	2.16	2.39		1.80	
99	α-Bisabolol			**7.61**			
100	α-Cadinol					3.01	0.99
101	α-Calacorene	1.29					
102	α-Caryophyllene						0.90
103	α-Cedrene					**5.38**	
104	α-Cubebene	0.56					
105	α-Curcumene		1.99				
106	α-Elemene	**13.10**					
107	α-Eudesmol		2.57				
108	α-Farnesene					0.80	
109	α-Fenchene				2.03		
110	α-Himachalene			2.02	1.15		
111	α-Humulene	2.31					
112	α-Longifolene				1.47		
113	α-Longipinene					1.91	
114	α-Pinene			3.82		2.42	
115	α-Selinene					**4.24**	
116	α-Terpineol	4.20					
117	α-Thujene				1.28		
118	β-Bourbonene	0.39			1.96	0.67	
119	β-Cedrene					1.13	
120	β-Cubebene	1.25			0.97	1.29	0.34
121	β-Cymene			4.95			
122	β-Elemene				1.44	0.47	
123	β-Farnesene					1.07	
124	β-Himachalene			**5.53**			
125	β-Myrcene	0.36			1.29		
126	β-Neoclovene				1.21		
127	β-Pinene	0.73	4.57		1.64		**35.91**
128	β-Selinene				3.29		
129	β-Thujone	3.71			3.02		
130	γ-Cadinene	0.97			1.73		0.43
131	γ-Elemene		2.42				
132	γ-Terpinen	2.82	**20.81**	**9.31**	3.26		
133	δ-3-Carene						1.96
134	δ-Cadinene				3.03	2.41	0.98

H. perforatum and *H. thymifolium.* While hydrogenated sesquiterpenes constituted the highest percentages of the overall composition of *H. triquetrifolium* (45.08 %), *H. hircinum* (31.88 %) and *H. perforatum* (67.97%), oxygenated monoterpenes and hydrogenated monoterpenes dominated in *H. lanigunosum* and *H. thymifolium*, respectively (Table 4).

Based on their contents of sesquiterpenes and monoterpenes, the studied *H.* species may be assembled into two groups. While five species are dominated by sesquiterpenes consisting of four hydrogenated forms, and one of the oxygenated type, hydrogenated monoterpenes were featured in one species only (Table 4, Figure 4).

Among the major compounds, α-Elemene, β -Caryophyllene, and 10s, 11s-Himachala-3(12), 4-diene represented the highest contents in *H. triquetrifolium* (13.1%), *H. hircinum* (20.98%), and *H. lanuginosum* (5.94%) respectively. Moreover, Ylangene, o-Cresol, β-Pinene were the major compounds in *H. perforatum* (20.28%), and *H. thymifolium* (35.91%) respectively, whereas γ-Terpinen (9.31) and caryophyllene oxide (8.74%) were dominant in *H. libanoticum.*

Table 4: Number of chemical compounds and percentages of monoterpenes and sesquiterpenes.

	H. tri	*H. hir*	*H. lib*	*H. lan*	*H. per*	*H. thy*
Number of compounds	55	15	23	51	38	30
Hydrogenated monoterpenes	9.61%	29.34%	22.82%	10.71%	13.94%	**49.38%**
Oxygenated monoterpenes	17.46%	15%	21.86%	22.39%	1.97%	2.08%
Hydrogenated sesquiterpenes	**45.09%**	**31.88%**	26.44%	**47.26%**	**67.97%**	15.24%
Oxygenated sesquiterpenes	25.00%	18.63%	**27.42%**	16.72%	12.23%	27.98%
Compounds of highest percentage Peak Area	$C_{15}H_{24}$ α-Elemene (13.10%)	$C_{15}H_{24}$ β -Caryophyllene (20.98%)	$C_{10}H_{16}$ γ-Terpinen (9.31) $C_{15}H_{24}O$ β -Caryophyllene oxide (8.74%)	$C_{15}H_{24}$ 10s,11s-Himachala-3(12),4-diene (5.94%)	$C_{15}H_{24}$ Ylangene (20.28%)	$C_{10}H_{16}$ β-Pinene (35.91%)
Total	97.15%	94.85%	98.54%	97.08%	96.11%	94.68%

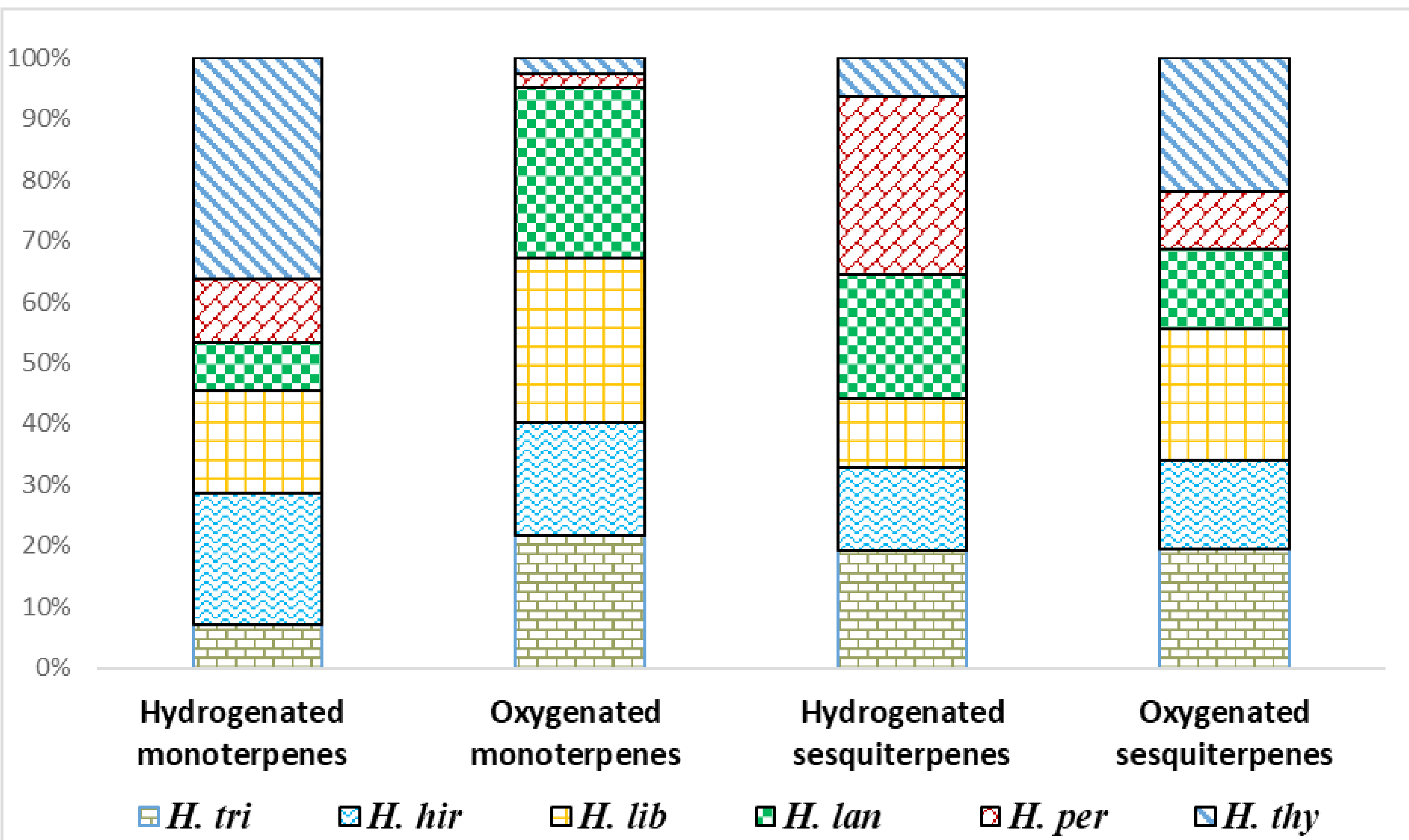


Figure 4: Percentages of monoterpenes and sesquiterpenes for the *H.* species.

Table 5: Comparison of chemical constituents of *Hypericum* species under the present and other similar studies from different countries.

No.	Botanical Name	Present study	Chemical composition in literature	Ref
1	*Hypericum lanuginosum* Lam.	**SBR - Lebanon**: 10s,11s-Himachala-3(12),4-diene(5.94%), Thujone (5.06 %), Spathulenol (3.60 %), (-)-Allospathulenol (3.31 %), Longicyclene (3.30%)	**Turkey:** spathulenol (17.3%), caryophyllene oxide (13.1%), α-pinene (11.7%) and undecane (6.2%)	[29]
2	*Hypericum libanoticum* N.Robson	**SBR - Lebanon**: γ-Terpinen (9.31%), β-Caryophyllene oxide (8.74%), α-Bisabolol (7.61%), β-Himachalene (5.53%)	First time herein reported	None
3	*Hypericum perforatum* L.	**SBR - Lebanon:** Ylangene (20.28%), Cyclosativene (19.87%), Cyclodecane (6.1%), α-Cedrene (5.38%), α-Selinene (4.24%)	**Turkey:** Germacrene D (23%), β-caryophyllene (14%), bicyclogermacrene (5%), caryophyllene oxide (4%) and spathulenol (4 %)	[30]
			Syria: exo 6-carbonyl methoxy tricycle [2.2.1.0(2,7)] heptane, by 21.89%	[31]
			France: α-pinene (15.3%) caryophyllene oxide (10.4%)	[32]
4	*Hypericum thymifolium* Banks & Sol.	**SBR - Lebanon**: β-Pinene (35.91%), Spathulenol (9.32%), β -Caryophyllene (3.17%), Viridiflorol (3.16%)	**Syria:** Isooctyl phthalate (30.39%), Tetracosane (28.18%) and Nonacosane (9.12%)	[33]
5	*Hypericum triquetrifolium* Turra	**SBR - Lebanon**: α-Elemene (13.1%), Spathulenol (8.89%), tau-Muurolol (6.40 %), Valencene (6.29%), Longifolene (5.8%)	**Turkey:** 1-hexanal (18.8%), 3-methylnonane (12.5%), α-pinene (12.3%)	[34]
			Iran: Germacrene D (21.7%), β -caryophyllene (18.3%), γ- -cadinene (6.4%), trans- β -farnesene (4.3%), α -humulene (3.8%), β -selinene (3.7%), γ- cadinene (3.3%), trans-phytol (3.2%)	[35]
			Greece: (E)- β -caryophyllene (27.9%), caryophyllene oxide (15.7%)	[36]
6	*Hyperucim hircinum* L.	**SBR - Lebanon:** β -Caryophyllene (20.98 %), γ-Terpinene (20.81 %), Caryophyllene oxide (9.36 %), Eucalyptol (5.83 %)	**Turkey:** -pinene (88.3%)	[37]
			Greece: (E)-caryophyllene (65.87%)	[38]

Among the identified constituents, there are some major compounds that have high potential in food and pharmaceutical industries qualifying their relevant species as viable economic source for many important biomolecules [39-40]. For example, β-Pinene (35.91 %) representing the major compound in *H. thymifolium* has been reported to have antibacterial, antidepressant, cytotoxic, and antimicrobial activities [41]. This compound is also used to treat the bladder, kidney, and urinary stones [42]. Also, β -Caryophyllene (20.98%) which is the major compound in the *H. hircinum* is recently reported to have beneficial effects on obesity, liver diseases, diabetes, cardiovascular diseases, pains and nervous system disorders. γ-Terpinen (9.31%), the major constituent of *H. libanoticum,* is widely used in food, flavours, soaps, cosmetics, pharmaceuticals, tobacco, confectionery, and perfume industries. It also has a strong antioxidant activity and anti-inflammatory benefits [43].

In addition, considerable amount of data on the essential oil content and composition of *Hypericum* species is available in literature providing the basis for some useful comparison with findings of current study (Table 5). In this respect, *H. triquetrifolium* from Iran was found to have 50 compounds with Germacrene D (21.7%), β -caryophyllene (18.3%), γ- -cadinene (6.4%), trans-β -farnesene (4.3%) α -humulene (3.8%), β -selinene (3.7%), γ-cadinene (3.3%), trans-phytol (3.2%) being the major compounds. In Turkey, the major compounds were 1-hexanal (18.8%), 3-methylnonane (12.5%), α- -pinene (12.3%) [29]. Whereas in the case of Greece, (E)-caryophyllene (27.9%), caryophyllene oxide (15.7%) were identified as the major ones [36]. In the current study, 55 compounds were found with α-Elemene (13.1%), spathulenol (8.89%), tau-muurolol (6.40%), valencene (6.29%), and longifolene (5.8 %) representing the top five components.

H. perforatum was found to have 41 compounds in Greece [44]. The major compounds of this species from Turkey were Germacrene D (23%), β -caryophyllene (14%), bicyclogermacrene (5%), caryophyllene oxide (4%) and spathulenol (4%) [30]. In Syria, the major compounds were exo 6-carbonyl methoxy tricycle [2.2.1.0 (2,7)] heptane (21.89%) [31]. In France, α-pinene (15.3%) caryophyllene oxide (10.4%) were the major compounds [32]. In this study, 38 compounds were found with ylangene (20.28%), cyclosativene (19.87 %), cyclodecane (6.1%), α-cedrene (5.38%), α-selinene (4.24%) recorded the highest content.

H. lanuginosum included 41 compounds in Turkey with the major compounds being spathulenol (17.3%), caryophyllene oxide (13.1%), α-pinene (11.7%), and undecane (6.2%) [29]. Whereas, the species is found to include 50 compounds with 10s,11s-Himachala-3(12),4-diene (5.94%), Thujone (5.06 %), Spathulenol (3.60%), (-)-Allospathulenol (3.31%), and Longicyclene (3.30%) being the major ones.

As for the case of *H. thymifolium*, 30 compounds were identified with β-Pinene (35.91%), Spathulenol (9.32%), β-Caryophyllene (3.17%), and Viridiflorol (3.16%) being the major compounds. While in Syria, 32 compounds were identified and Isooctyl phthalate (30.39%), Tetracosane (28.18%) and Nonacosane (9.12%) were found as the major ones [33].

In *H. hircinum*, 39 compounds were found in this study with β-Caryophyllene (20.98 %), γ-Terpinene (20.81%), Caryophyllene oxide (9.36%), and Eucalyptol (5.83%) being the major constituents. In Turkey**,** 18 compounds were found with strong domination of α -pinene (88.3%). In Greece, (E)-caryophyllene (65.87%) was the dominant compound [38]. It is worth noting that although the above mentioned findings were generally restricted to single research work, the obtained compositions can substantially serve more comprehensive future studies. Nevertheless, the aforementioned comparison shows high variations in compositions of same *Hypericum* sp. of different geographic origins. This is no surprise as the production of essential oils from wild plants is influenced by genetic factor together with environmental and growing conditions, such geography, climate, soil, natural pests and enemies, among others [45]. Other factors, such as toxicity of heavy metals and metalloids was also found to impact composition [33,46]. Additionally, extraction methods of essential oils may also have a high influence [47].

Interestingly, the composition of *H. libanoticum,* which is an endemic species to Lebanon and Syria, is herein reported for the first time. The species is found to have 27 compounds with γ-Terpinen (9.31 %), caryophyllene oxide (8.74%), α-Bisabolol (7.61%), and β-Himachalene (5.53%) representing the major constituents.

Conclusion

This study represents the first assessment of the six *Hypericum* species that grow wild in SBR of Lebanon. The GC and GC/MS analysis of essential oils show high levels of monoterpene hydrocarbons (*H. thymifolium*), sesquiterpene hydrocarbons (*H. triquetrifolium*, *H. hircinum, H. lanuginosum*, *H. perforatum)*, and oxygenated sesquiterpenes (*H. libanoticum*). Major constituents were β-Caryophyllene, γ-Terpinen, Ylangene, and α-Elemene. These *compounds* are known to have a wide range of biological activities confirming the ethnobotanical values and traditional use of these species. The present research reports for the first time the chemical composition of *H. libanoticum*. The studied species have a high potential as a good source for novel drug discovery and pharmaceutical, cosmetics and food industries. They would therefore, be ideal targets for future more comprehensive studies on the influence of environmental factors and developmental stages to track any variations on essential oil yield and constitution. Also, it would also be valuable to assess composition of other species available in the SBR aiming at developing propagation projects of *Hypericum* species of high levels of medicinally and economically viable constituents.

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