Progress in Nutrition 2022; Vol. 24, N. 2: e2022035 DOI 10.23751/pn.v24i2.12172 © Mattioli 1885

Original article

Fixed and essential oil composition of *Hypericum venustum*, *Hypericum scabrum* and *Hypericum spectabile* species

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Abstract. This study attempts to determine the oil compositions of *H. venustum* Fenzl, *H. scabrum* L., and endemic *H. spectabile* Jaub. & Spach species by extracting the essential oil from the samples taken during the flowering period, the essential oil and fixed oils from the fruits taken during the seed period. Plant samples of these species were collected from the natural flora of Kahramanmaras in 2020. The essential oil content of these species was identified as 0.20- 0.27% in the flowering period, and 1.25%-2.62% in the fruits taken during the seed period. Essential oils were extracted in Neo-clevenger apparatus, fixed oils were extracted in Soxhlet device. The components of the extracted oils were analyzed by GC/MS in the West Mediterranean Agricultural Research Institute (BATEM) laboratory. The essential oil samples collected during the flowering period consisted of 35 components, and the main component was α-pinene. This component (α-pinene) was 15.06% in *H. venustum*, 73.77% in *H. scabrum* and 60.21% in *H. spectabile*. The essential oil obtained from the fruits in the seed period held a total of 13 different components, and the main component was similarly α-pinene. This component (α-pinene) was determined to be 90.55% in *H. venustum*, 89.96% in *H. scabrum* and 82.88% in *H. spectabile*. The fixed oil rate in the fruits taken during the seed period varied between 16.89% and 24.43%. In fruit samples, six of the fixed oil components had a ratio of over 91%. The rate of unsaturated fat (81.30-91.23%) in the fruits of the *Hypericum* plant was considerably higher than the saturated fat rate (7.74-9.78%). The main fixed oil component was unsaturated fatty acids linolenic and linoleic acid.

Key words: essential oil, fixed oil, *H. scabrum*, *H. venustum*, *H. spectabile*

Introduction

Hypericaceae family members are evergreen, deciduous shrubs or herbaceous plants. They have black or red glandular hair that contains essential oil and mostly hypericin as an active ingredient. Leaves are simple with reciprocal or rarely circular formation. The flower cluster is located at the tip or the plant has a single flower. The fruits are in berry or capsule form. This family includes 9 genera and about 540 species. *Hypericum* L. genus has 420 species worldwide and is represented in Turkey by 96 species, 9 subspecies, and 11 varieties, out of which 48 taxa are endemic. Although it is prevalent in the northern hemisphere, it also grows in temperate zones (1). *Hypericum* L. taxa are employed in various medical applications in various diseases globally. The secondary metabolites that certain rare and endemic *Hypericum* species contain in very small amounts are important in pharmaceutics (2). They are mainly used in traditional medicine in the treatment of wounds, eczema and burns (3). Certain *Hypericum* species are also used in ulcer and rheumatism, as a sedative, and due to their anti-inflammatory and antiseptic properties (4-7). Naphthodianthrones are typical compounds in *Hypericum* species, and the most important components in this group include hypericin and pseudohypericin. They also contain phloroglucinol-derivatives hyperforin and quercetin, quercitrin, hyperoside and

routine flavonoids (8). Hypericin and pseudo-hypericin have antiretroviral properties. Hyperforin extracts of *Hypericum* species also have antidepressant effects (9). Various medicinal properties of several *Hypericum* species were investigated and their effects were demonstrated. *Hypericum scabrum* L. is a herbaceous perennial species, which is 15-45 cm tall with an erect stem, 7-20 mm long elliptical or lanceolate leaves. The flower cluster includes 15 or more flowers. It grows in arid regions, reef slopes and forest glades in Turkey (10). *H. scabrum* flowers contain flavonoids, phenolic acids, vitamins and phytosterols, dominated by catechin, vanillic acid, vitamin K and ergosterol (11). *H. scabrum* L. has antimicrobial and antiulcerogenic properties and is also used as a sedative, antiseptic, analgesic, anthelmintic, and antifungal, and in the treatment of diarrhea and hemorrhoids (12-16). *H. scabrum* essential oil includes high α-pinene content, as well as spathulenol, p-cymene, acetophenone, carvacrol, (E)-caryophyllene, myrcene, cadalene, β-pinene, n-nonane, and thymol (13, 17, 18). *Hypericum spectabile* is a perennial herbaceous endemic species, with 35-60 cm height and erect stem, 20-45 mm long triangular leaves that encircle the stem. The flower cluster is pyramidal. It grows in field borders and slopes in Turkey. *H. spectabile* species contains hyperoside, isoquercitrin, kaemferol, quercitrin, quercetin, amentoflavone, and hyperforin, and has antioxidant, anti-inflammatory and antimicrobial properties (19). *H. venustum* is a perennial herbaceous 25-75 cm tall plant with a hairless stem that shoots from the ground and 13-32 mm long, ovoid and elliptical leaves. The flower cluster is narrow and pyramidal or cylindrical. It grows in wetlands or along rivers. *H. venustum* species has antioxidant properties due to the phenolic content (20). In this study, essential oils of three different *Hypericum* species in two different periods and fixed oil components in the mature seed period were determined.

Materials and Methods

Collection of plant material

H. venustum and *H. scabrum* were collected at 1903 m elevation in Nurhak Mountain Kocaseki locality in Kahramanmaras province Nurhak district. Identification of plants was made by an expert in the field, using the flora of Turkey (Volume 2). The field studies were conducted in early June for flowering period sampling and in mid-August for fruiting period sampling. The endemic *H. spectabile* species was collected from its natural habitat at Kahramanmaras Sutcu Imam University, Avsar campus.

Essential and fixed oil isolation

Essential oil samples were extracted from the dried aboveground sections of the plants and ripe and ready-to-dry fruits with water distillation method in a neo-Clevenger device for three hours. A hundred grams of dried and ground plant samples were used for essential oil production. Fruit fixed oil samples were distilled within 6-8 hours with petroleum ether in the Soxhlet device. Fixed oil samples were obtained from ripe and dried fruits using 15 grams ground sample. The essential and fixed oil samples obtained with distillation were analysed by GC/MS in the West Mediterranean Agricultural Research Institute (BATEM) laboratory to determine the essential and fixed oil components. Chromatograms of fixed and essential oils are given in Figure 1.

Essential oil composition analysis

The essential oil composition of samples was analysed by gas chromatography (Agilent 5975C) coupled to flame ionization detector and mass spectrometry (Agilent 5975C) using capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 μm). Essential oils were diluted 1:50 ratio with hexane. GC-MS/FID analysis was carried out at split mode of 50:1. Injection volume and temperature were adjusted as 1 μl and 250°C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 0,8 ml/ min. The oven temperature was programmed as follows: 60°C for 10 min, increased at 20°C/min to 250°C, and held at 250°C for 8 min. MS spectra were monitored between 35 and 450 amu and the ionization mode used was an electronic impact at 70 eV (21, 22).

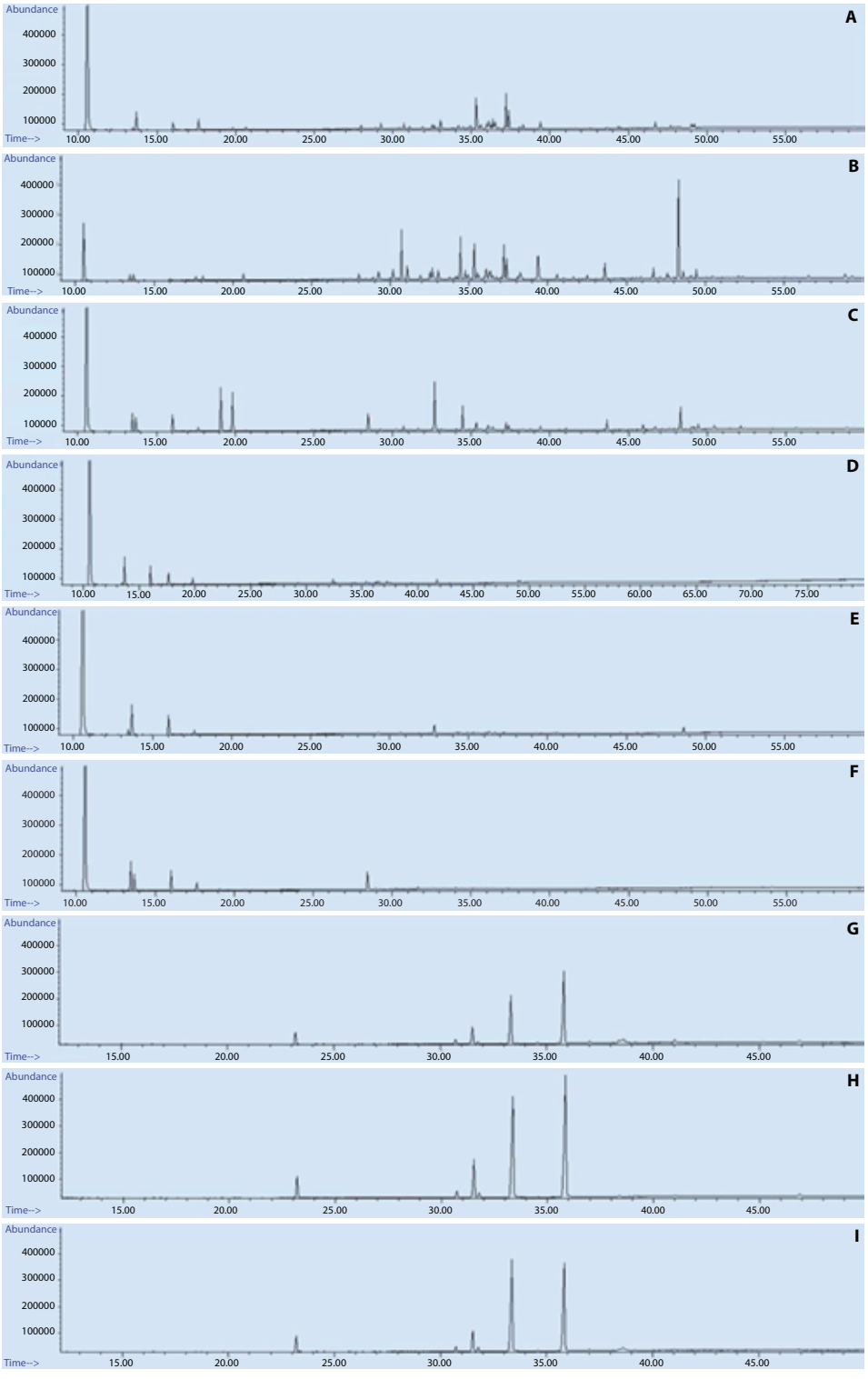

Figure 1. Chromatograms of the examined essential and fixed oil samples; A: *H. scabrum* flower essential oil, B: *H. venustum* flower essential oil, C: *H. spectabile* flower essential oil, D: *H. scabrum* fruit essential oil, E: *H. venustum* fruit essential oil, F: *H. spectabile* fruit essential oil, G: *H. scabrum* fruit fixed oil, H: *H. venustum* fruit fixed oil, and I: *H. spectabile* fruit fixed oil.

Fatty acid composition analyses

The fatty acid composition of the samples was analyzed by gas chromatography (Agilent 5975C) coupled to flame ionization detector and mass spectrometry (Agilent 5975C) (GC-MS-FID). As a beginning, fatty acid methyl esters (FAMEs) were prepared (23) and then injected into GCMS/FID. Separations were carry out using an HP innowax capillary column (60 m, 0.25-mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 0.8 mL min-1. Injector and detector temperatures were 250 and 260 °C, respectively. The temperature programming for the column was performed as follow; started from 150 °C and raised to 200 °C with an increment of 10 °C/minute, hold at 200 °C for 5 minutes, then increased to 250 °C with 5 °C/minute increments and hold 250 °C at 10 minutes. A sample of 1 μL was injected by an auto sampler with a split mode (1:50). The content (percentage by weight) of fatty acids was calculated from their corresponding integration data. MS spectra were monitored between 35-450 amu and the ionization mode used was an electronic impact at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas. FAMEs were identified by comparison of their retention times with those of the reference standards. FAMEs were further identified by using WILEY and NIST libraries of the GC-MS system (24).

Results and Discussion

The essential oil composition of *H. venustum, H. scabrum*, and endemic *H. spectabile* species samples collected during the flowering period is presented in Table 1. It was observed that there were 35 components in essential oils extracted from the samples that were collected during the flowering period of the three *Hypericum* species (Table 1). The color of the oil obtained from the samples during the flowering period was light green. It was determined that *H. venustum species* yielded 0.20% essential oil content during flowering, and the analysis results revealed 25 components. The main component was α-pinene (15.06%), followed by linalool (10.41%), trans-β-farnesene (8.69%), cis-calamenene (8.66%), ɣ-murolene (7.98%), δ-cadinene (7.55%), α-amorphene (4.47%), caryophyllene oxide (3.55%), and β-caryophyllene and ɣ-amorphene (3.46%). It was determined that *H. scabrum* species essential oil content was 0.24% during the flowering period. The essential oil obtained from this species included 21 components, where the main component was α-pinene (73.77%), followed by δ-cadinene (3.95%), ɣ-murolene (3.73%), beta-pinene (2.57%), and alpha-amorphene (2.09%). Baser et al. (25) reported that the major component in essential oil obtained with the aboveground *H. scabrum* fractions collected in Uzbekistan was α-pinene (11.2%), followed by spathulenol (7.2%), β -cymene (6.1%), acetophenone (4.8%), and carvacrol (4.7%). Cakir et al. (13) reported that in *H. scabrum*, α-pinene content was 71.6%, b-caryophyllene content was 4.8%, myrcene content was 3.8%, cadalene content was 3.4% and β-pinene content was 2.9%. Pirbalouti et al. (26) reported that *H. scabrum* α-pinene content was 49.96%, β-pinene content was 9.70%, limonene content was 6.66%, and β-ocimene content was 5.64%. In a study conducted to determine the essential oil composition of *H. scabrum*, prevalent in Tajikistan flora, Sharopov et al. (27), reported that the main components were α-pinene (44.8%), spathulenol (7.1%), trans-verbenol (3.9%), verbenone (6.0%), γ-murolene (3.5%), β-pinene (2.8%), trans-pinocarveol (2.3%), unidentified content (2.3%), trans-carveol and caryophyllene oxide (2.1%). α-pinene content (73.77%), which was the main component in *H. scabrum* in the present study, was higher than the levels reported by Baser (25), Pirbalouti et al. (26), Sharopov et al. (27) and similar to those reported by Cakir et al. (13) (71.6%). The essential oil content was 0.20-0.27% in flowering period, which was consistent with the figures reported by Baser (25), Cakir et al. (13), and Pirbalouti et al. (26), while lower than the figure reported by Sharopov et al. (27) (0.40%).

Endemic *H. spectabile* species included 0.27% essential oil content during the flowering period. In this essential oil, alpha-pinene content was 60.21%, β-ocimene content was 12.49%, β-caryophyllene content was 7.05%, trans-β-farnesene content was 3.08%, alpha-longipinene and undecane content was 2.61%, β-myrcene content was 2.59%. It included a total of 15 components.

Table 1. Composition of essential oils of *H. venustum*, *H. scabrum* and *H. spectabile* species taken during flowering

No	RI	Compound	*H. venustum* (%)	*H. scabrum* (%)	*H. spectabile* (%)
1	1022	α-pinene	15.06	73.77	60.21
2	1101	undecane	1.45	-	2.61
3	1106	β-pinene	1.56	2.57	2.23
4	1160	β -myrcene	-	0.96	2.59
5	1160	myrcene	-	-	-
6	1197	limonene	-	1.59	-
7	1231	β -ocimene	-	-	12.49
8	1268	cymene	1.57	-	-
9	1459	α -cubebene	-	0.50	-
10	1472	α -longipinene	-	-	2.61
11	1495	α -copaene	2.08	0.74	-
12	1524	camphor	2.67	-	-
13	1540	linalool	10.41	0.73	0.52
14	1552	linalyl acetate	2.69	-	-
15	1597	β -copaene	1.64	0.45	-
16	1602	β -caryophyllene	3.46	-	7.05
17	1615	aromadendrene	2.13	1.05	-
18	1662	trans- β -farnesene	8.69	-	3.08
19	1672	p-allylanisole	1.96	-	-
20	1692	γ-muurolene	7.98	3.73	1.14
21	1699	α -terpineol	0.99	-	-
22	1702	viridiflorene	-	0.65	-
23	1714	epizonarene	-	0.62	-
24	1720	γ -amorphene	3.46	1.40	1.10
25	1729	δ-guaiene	1.49	1.20	-
26	1734	α -selinene	-	1.15	-
27	1760	δ -cadinene	7.55	3.95	1.02
28	1766	α -amorphene	4.47	2.09	0.66
29	1796	cuminal	0.70	-	-
30	1840	cis-calamenene	8.66	1.00	0.72
31	2007	caryophyllene oxide	3.55	-	1.34
32	2137	spathulenol	2.42	0.91	
33	2220	α -bisabolol	1.48	-	
34	2238	α -eudesmol	-	0.43	
35	2242	α -cadinene	-	0.51	-
Defined %			98.14	100	99.37
Unidentified %			1.86	0	0.63
Essential oil ratio %			0.20	0.24	0.27

The components and their ratios in the essential oil obtained from the fruits collected during the seeding period are presented in Table 2. Fruit essential oil content was 1.28% in *H. venustum*, 1.25% in *H. scabrum*, and 2.64% in *H. spectabile*. The color of the essential oil was very light yellow and nearly transparent in the fruiting period. The main fruit essential oil component was α-pinene and α-pinene content was the highest in *H. venustum* (90.55%), followed by 7 components, including β-pinene (% 4.05), r-myrcene (% 2.49), and alpha-cedrene (%1.04), totaling to 100%. The content of *H. scabrum* included eight components; 89.96% α-pinene, 3.86% β-pinene, 2.36% β-myrcene, 1.69% limonene, totaling 92.42%. In *H. spectabile*, seven components constituted 98.11% of the whole, mainly 82.88% α-pinene, 4.85% undecane, 3.35% β-pinene, 3.55% β-myrcene, 3.40% alpha-longipinene, and 1.61% limonene. Tanker (28) reported that *H. scabrum* fruit branch essential oil content was 0.72% v/w with volumetric calculations. The fruit essential oil content of the studied species was above this figure and it was suggested that this difference was due to the plant sampling period or different ecological environments.

The fixed oil components and their content in the fruits are presented in Table 3. The fixed oil content in fruits collected during the seeding period was determined as 20.10% in *H. venustum*, 16.89% in *H. scabrum*, and 24.43% in *H. spectabile*. The highest fixed oil content was observed in *H. venustum*, where linolenic acid content was 43.32%, linoleic acid content was 35% and oleic acid content was 12.72%. *H. scabrum* yielded 42.72% linolenic acid, 27.41% linoleic acid, and 10.87% oleic acid, while *H. spectabile* yielded 41.98% linoleic acid, 39.26% linolenic acid, and 9.33% oleic acid. Gedik et al. (29) reported that the main fixed oil components of the aboveground sections of *H. venustum* during the flowering period included 42.17% linolenic acid, 7.48% linoleic acid, and 11.20% palmitic acid. While linoleic acid finding was similar to the present study finding with fruit fixed oil, the linoleic acid content in fixed oil during the flowering period was very low. Similarly, palmitic acid was low in fruit fixed oil, while it was higher in samples collected during the flowering period.

Table 2. Essential oil composition of fruits taken at the seed stage of *H. venustum, H. scabrum* and *H. spectabile* species

No	RI	Compound	*H. venustum* (%)	*H. scabrum* (%)	*H. spectabile* (%)
1	1022	α -pinene	90.55	89.96	82.88
2	1101	undecane	0.67	-	4.85
3	1106	β -pinene	4.05	3.86	3.35
4	1160	β -myrcene	2.49	2.36	3.55
5	1197	limonene	0.64	1.69	1.61
6	1231	β -ocimene	-	0.83	-
7	1472	α -longipinene	-	-	3.40
8	1615	aromadendrene	-	-	0.37
9	1592	β -elemene	-	0.50	-
10	1607	α -cedrene	1.04	-	-
11	1692	γ -muurolene	-	0.17	-
12	1759	cis-muurola-3,5-diene	-	0.22	-
13	2220	α -bisabolol	0.57	-	-
Defined %			100	99.42	98.11
Unidentified %			-	0.58	1.89
Essential oil %			1.28	1.25	2.64

Table 3. Composition of fixed oils in the fruit of *H. venustum*, *H. scabrum* and *H. spectabile* species

No	RT	Compound	*H. venustum* (%)	*H. scabrum* (%)	*H. spectabile* S
1	23.201	Palmitic acid	5.51	5.81	5.48
2	30.729	Stearic acid	1.82	2.33	1.90
3	31.537	Oleic acid	12.72	10.87	9.33
4	33.379	Linoleic acid	35.19	27.71	41.98
5	35.868	Linolenic acid	43.32	42.72	39.26
6	38.392	Aracidic acid	0.41	1.64	0.16
Defined %			98.97	91.08	98.11
Unidentified %			1.03	8.92	1.89
Fixed fat ratio %			20.10	16.89	24.43
Saturated fatty acid %			7.74	9.78	7.54
Unsaturated fatty acid %			91.23	81.30	90.57

Conclusion

This study analysed the essential oil of the above-ground parts during the flowering period and the essential and fixed oil components of the fruits in the ripe seed period of three different *Hypericum* species which were naturally distributed in Kahramanmaras flora. The study results revealed that the essential oil rate (1.25-2.62%) in fruits in the ripe seed period was approximately ten times higher than in the flowering period (0.20-0.27%) in *Hypericum*. The main component in the essential oil was noted as α-pinene in both periods. The ratio of α-pinene was found to be higher in the essential oil (90.55%) during the mature seed period than the flowering period (73.77%). The rate of unsaturated fat was found to be much higher than that of saturated fat in terms of fixed fat content. The main fixed oil components were determined to be unsaturated fatty acids linolenic and linoleic acid.

Acknowledgement: The author thanks, Assist. Prof. Dr. Yusuf Ziya Kocabas for the plant identification.

Conflict of interests: No potential conflict of interest relevant to this article was reported by the author.

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