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**Research Article**

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# Volatile oils composition of *Hypericum amblysepalum* Hochst., *Hypericum spectabile* Jaub. & Spach. and *Hypericum helianthemoides* (Spach) Boiss. growing in Turkey

**Ebru Yüce Babacan**

Munzur University, Pertek Sakine Genç Vocational School, Tunceli, Turkey

**Abstract** The composition of the essential oils of *Hypericum amblysepalum* Hochst., *H. spectabile* Jaub. & Spach. and *H. helianthemoides* (Spach) Boiss. were determined in this study. Fourty eight, twenty three and fifty three components were identified representing 89.8%, 80.5% and 89.5 of the oils, respectively. GC and GC-MS analyses showed that δ-3-carene (11.9%), caryophyllene-oxide (10.3%), *cis*-ocimene (9.6%), β-caryophyllene (9.2%) and α-pinene (9.0%) were the most abundant components of *H. amblysepalum*, whilst β- caryophyllene (13.4%), germacrene D (13.3%), α-cadinol (12.8%) and caryophyllene oxide (8.8%) were the major constituents of *H. spectabile*; those of *H. helianthemoides* oil were α-pinene (25.7%), δ-3-carene (25.1%), d-limonene (6.5%), *cis*-ocimene (4.6%) and undecane (4.0%). Three oils were characterized by the presence of many components which could have numerous applications in food, pharmaceutical and perfume industries.

**Keywords** GC-MS, essential oil, δ-3-carene, β-caryophyllene, α-pinene

## Introduction

The genus *Hypericum* L. includes approximately 350 to 400 species. Its name was given by the Greeks to plants hung above their religious figures to ward off evil spirits. One species is now known as Saint John's Wort *(Hypericum perporatum* L.), and pills made from crude extracts of the whole plant are sold as an antidepressant throughout the world market. This genus is usually characterized by a set of morphological characters, such as its shrubby or perennial herbaceous habits, glabrous or simple hairs with translucent and often opaque black or reddish glands, opposite or whorled leaves, four- or five-fascicled stamens, dorsifixed or basifixed anthers, and short-cylindric seeds with finely to coarsely reticulate seed coats [1]. Because of the difficulty in handling its large numbers of species and predicting the evolutionary directions of those morphological characters, only a few systematic studies have been performed [1-3].

The genus *Hypericum* L. belongs to the Clusiaceae family and encompasses approximately 460 species accommodated in 36 sections [4]. In Turkey, the genus is represented by 89 species of which 43 are endemic [5,6]. *Hypericum amblysepalum*, *H. spectabile* and *H. helianthemoides* belongs to Section *Drosanthe* Robson, which includes 23 taxa, distributed mostly in the East Anatolia of Turkey [7]. In the monograph study done by Robson, the number of taxa in this section was 26 [8].

The flowering plant genus *Hypericum* (Hypericaceae) contains the well-known medicinally valuable species *Hypericum perforatum* (common St. John's wort). Species of *Hypericum* contain many bioactive constituents,

including proanthocyanins, flavonoids, biflavonoids, xanthones, phenylpropanes and naphthodianthrones that are characterized by their relative hydrophilicity, as well as acylphloroglucinols and essential oil components that are more hydrophobic in nature [9].

Inter and ıntraspecific variations in the essential oil composition of many species of this genus were previously reported, and depending on genetic and environmental factors, seasonal variation, plant organs and analytical methods used [10-12].

*Hypericum amblysepalum* Hochst., *H. spectabile* Jaub. & Spach. and *H. helianthemoides* (Spach) Boiss are three *Hypericum* species whose oils have been subjected to analysis in this article. *Hypericum spectabile* have not been subjected to any previous essential oil analysis. It is aimed that to compare the major constituents of the *Hypericum* species with the genus patterns.

## Experimental

### Plant Material

The specimens of *H. amblysepalum* and *H. spectabile* were collected in Gaziantep (Southeastern Anatolia, Turkey) in 2008 and *H. helianthemoides* specimens were collected Van (Eastern Anatolia, Turkey) in 2008. Voucher specimens (FUH-9860, FUH-9865 and FUH- 9875) are kept at the Fırat University Herbarium (FUH), Elazig, Turkey.

### Isolation of the essential oils

Air-dried aerial parts of the plant materials (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h.

### Gas chromatographic (GC) analysis

The essential oil was analysed using HP 6890 GC equipped with FID detector and HP- 5 MS (30 m x 0.25 mm *i.d.*, film tickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS expressed as below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

### Gas chromatography / mass spectrometry (GC-MS) analysis

The oils were analyzed by GC-MS, using a Hewlett Packard system. HP- Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Res. Lab. (BUBAL) in Firat University. HP-5 MS column (30 m x 0.25 mm *i.d.*, film tickness 0.25 µm) was used with helium as the carrier gas. Injector temperature was 250 $^{o}$C, split flow was 1 ml / min. The GC oven temperature was kept at 70 $^{o}$C for 2 min. and programmed to 150 $^{o}$C at a rate of 10 $^{o}$C / min and then kept constant at 150 $^{o}$C for 15 min to 240 $^{o}$C at a rate of 5 $^{o}$C / min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 eV and a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (WILEY, NIST).

## Results and Discussion

The compounds identified in the oils of *Hypericum* species are listed in Table 1 in order of their elution from the HP-5MS column. The essential oil yields of *H. amblysepalum*, *H. spectabile* and *H. helianthemoides* were 0.15, 0.25, 0.20% v/w, respectively.

Two oils (*H. amblysepalum* and *H. spectabile*) were characterized by a high content of sesquiterpene hydrocarbons (ca.50 and 65%, respectively). The essential oil analysis showed that, in two species studied, sesquiterpenes are present in higher concentrations than monoterpenes. Monoterpenes made up the higher contribution in *H. helianthemoides* species (ca. 75%). *H. perfoliatum*, *H. rumeliacum, H. thymbrifolium*, *H. salsolifolium* and *H. retusum* essential oil were rich in monoterpene hydrocarbons, while the studied species (*H. amblysepalum* and *H. spectabile*) were dominated by sesquiterpene hydrocarbons [10, 13-15].

It is surprising that large qualitative and quantitative differences were found between three *Hypericum* species in view of main compounds. Seventeen compounds are common in studied species of *Hypericum*. Forty-eight components of *H. amblysepalum* were identified, representing 89.8% of the total oil. The most abundant constituents were δ-3-carene (11.9%) and caryophyllene-oxide (10.3%).

Thirty-three components of *H. spectabile* essential oil were identified, representing 80.4% of the total oil. The dominating components were β-caryophyllene (13%) and germacrene D (13.3%). β-Caryophyllene was also among the major constituents of *H. perforatum* [16, *H. triquetrifolium* [11], *H. carinatum* [17], *H. maculatum* [18], *H. foliosum* [19] and *H. brasiliense* [20]. Germacrene D was a characteristic constituent of *H. empetrifolium* (1.5%), *H. perfoliatum* (2.4%), *H. rumeliacum* (3.8%), *H. perforatum* (3.3%), *H. triquetrifolium* (1.5%) [21], *H. perfoliatum* (10.64%) and *H. tomentosum* (2.23%) [22].

Fifty-three components of *H. helianthemoides* essential oil were identified, representing 89.5% of the total oil. The most abundant constituents were α-pinene (25.7%) and δ-3-carene (25.1%). α-pinene was reported as to be the fourth major compound in *H. helianthemoides* oil from Iran [23]. Comparison of the other constituents of this report and ours show that the major compounds are completely different from each other, which could be attributed to different localities where the plant materials were collected.

It is worth mentioning on one hand that other compounds mainly *cis*-ocimene (9.6%), β–caryophyllene (9.2%), α–pinene (9.0%), 3-hexen-1-ol (8.1%) and spathulenol (4.1%) in *H. amblysepalum* and α-cadinol(12.8%), caryophyllene-oxide (8.8%) n-decanoic acid (4.3%), azulene (2.6%), phytol (2.3%) and caryophyllene-II (3.3%) in *H. spectabile* and β-pinene (9.3%), d-limonene (6.5%), *cis*-ocimene (4.6%) and undecane (4.0%) in *H. helianthemoides* oil was determined (Table 1).

From the monoterpenic major component, α-pinene, determined in the essential oils of three *Hypericum* species were reported in the essential oils of, *H. caprifoliatum* (1.5%), *H. myrianthum* (6.5%) [17], *H. perfoliatum* (64.3%), *H. humifosum* (77.2%,), *H. linarifolium* (31.2%) and *H. pulcrum* (46.8%) [24].

The major components, α-pinene, δ-3-carene, *cis*-osimene determined in the essential oils of three *Hypericum* species were reported also in the essential oils of, *H. capitatum* var. *capitatum* (20.3%, 1.1%, 1.2%); *H. capitatum* var. *luteum* (5.1%, 1.2%, 3.9%) [25].

Analysis of other reports on the *Hypericum* essential oil from Turkey revealed that α-pinene, which is the dominant constituent of *H. scabrum, H. scabroides* [26], *H. thymbrifolium* (51.3%) [14], *H. avicullariifolium* subsp. *depilatum* var. *depilatum* (52.1%) [27], *H. calycinum* (24.1%), *H. cerastoides* (57.7%) [28], *H. perforatum* (61.7%) and *H. scabrum* (71.6%) [29] essential oils, was also the main constituent of *H. pseudolaeve* (5.7%) [14] and *H. capitatum var. luteum* (5.1%) [25] essential oils.

The essential oils of *H. spectabile, H. amblysepalum* and *H. helianthemoides* were characterized by their relatively low yields, albeit they were found in acceptable percentage on comparison with other species. Three oils were complex mixture of volatile compounds of which many are used as flavouring agents in food, pharmaceutical, perfume and some other industries. These compounds includes high ratios in these plants, δ-3-carene (carene has a sweet and pungent odor), *cis*-ocimene, β-caryophyllene, α-pinene (α-pinene enantiomers can be widely found in nature, they were used by the fragrance industry), germacrene D (is used in the creation and/or manufacturing of fragrance and flavor concentrates of all types), α-cadinol, d-limonene (used chemotherapeutic activities of pharmaceutical preparations) and caryophyllene oxide (is flavoring compound that have also been used for production of cosmetic and industrial goods) [30] were particularly found in the oils of these species. This subjective suggestion should be tested in further research.

As a consequence, this study needs to be continued and extended to other Turkey native population samples of the genus *Hypericum* for a better phytochemical characterization and their industrial applications in order to improve their rational uses.

**Table 1:** Constituents of the essential oils from *Hypericum amblysepalum, H. spectabile* and *H. helianthemoides*

| No | Compounds | RRI | *H. amblysepalum* | *H. spectabile* | *H. helianthemoides* |
|---|---|---|---|---|---|
| 1 | Octane | 971 | -- | -- | 0.1 |
| 2 | Nonane | 996 | 0.6 | 0.4 | 1.5 |
| 3 | α-pinene | 1021 | 9.0 | 3.0 | 25.7 |
| 4 | Camphene | 1034 | 0.1 | -- | 0.6 |
| 5 | β-pinene | 1055 | 0.2 | 0.1 | 9.3 |
| 6 | β-myrcene | 1064 | 0.5 | 0.1 | 2.8 |
| 7 | I–phellandrene | 1078 | -- | -- | 0.2 |
| 8 | α–terpinene | 1086 | -- | -- | 0.2 |
| 9 | p-cymene | 1091 | -- | -- | 0.4 |
| 10 | d-Limonene | 1095 | 0.4 | 0.1 | 6.5 |
| 11 | *Cis*-ocimene | 1100 | 9.6 | 1.9 | 4.6 |
| 12 | δ-3-carene | 1108 | 11.9 | 1.8 | 25.1 |
| 13 | γ-terpinene | 1117 | -- | -- | 0.4 |
| 14 | α–terpinolene | 1137 | -- | -- | 0.5 |
| 15 | Undecane | 1148 | 0.7 | 0.5 | 4.0 |
| 16 | Nonanal | 1151 | -- | -- | 0.1 |
| 17 | Crysanthenone | 1163 | -- | -- | 0.2 |
| 18 | *Allo*-ocimene | 1168 | 0.3 | -- | 0.1 |
| 19 | 2-propanon | 1171 | 0.1 | -- | -- |
| 20 | *Neo-allo*-ocimene | 1176 | -- | -- | 0.1 |
| 21 | *Trans*-pinocarveole | 1178 | -- | -- | 0.1 |
| 22 | Camphor | 1182 | 0.3 | -- | -- |
| 23 | 2-nonanal | 1190 | 0.1 | -- | -- |
| 24 | Borneol | 1200 | 0.1 | -- | 0.3 |
| 25 | 3-cyclohexen-1-ol | 1205 | -- | -- | 0.1 |
| 26 | α–terpineol | 1215 | 0.1 | 0.3 | 0.6 |
| 27 | *Trans*-carveole | 1231 | -- | -- | 0.1 |
| 28 | β–citronellol | 1235 | -- | -- | 0.1 |
| 29 | Nerol | 1253 | -- | -- | 0.1 |
| 30 | 1-decanol | 1271 | -- | -- | 0.1 |
| 31 | Cyclohexasiloxane | 1296 | -- | -- | 0.1 |
| 32 | 2,4-decadienal | 1312 | -- | -- | 0.1 |
| 33 | α–cubebene | 1337 | 0.1 | -- | 0.1 |
| 34 | α–longipinene | 1340 | 0.5 | 1.9 | -- |
| 35 | α–ylangene | 1355 | -- | -- | 0.1 |
| 36 | α–copaene | 1360 | 0.2 | 0.2 | 0.3 |
| 37 | β–bourbonene | 1366 | 0.1 | -- | -- |
| 38 | β–elemene | 1370 | -- | -- | 0.1 |
| 39 | 1-decanol | 1384 | 0.1 | -- | -- |
| 40 | Tetradecanal | 1385 | -- | -- | 0.1 |
| 41 | β–caryophyllene | 1393 | 9.2 | 13.4 | 0.1 |
| 42 | β–cubebene | 1400 | 0.1 | -- | 0.1 |
| 43 | Aromadendrene | 1406 | 1.2 | -- | 0.1 |
| 44 | *Trans*-β-farnesene | 1416 | -- | 0.8 | 1.0 |
| 45 | α-humulene | 1418 | 0.4 | 0.9 | -- |

| | | | | | |
|---|---|---|---|---|---|
| 46 | α–amorphene | 1430 | 1.2 | -- | -- |
| 47 | Germacrene D | 1436 | 0.3 | 13.3 | -- |
| 48 | β–selinene | 1441 | 0.8 | -- | -- |
| 49 | Bicyclogermacrene | 1445 | -- | 1.1 | -- |
| 50 | α–farnesene | 1449 | 0.2 | -- | -- |
| 51 | Naphthalene | 1456 | 0.6 | -- | -- |
| 52 | δ–cadinene | 1458 | 1.1 | 1.6 | -- |
| 53 | α-cadinene | 1470 | -- | -- | 0.1 |
| 54 | Calacorene | 1473 | 0.3 | -- | -- |
| 55 | Dodecanoic acid | 1486 | -- | -- | 0.1 |
| 56 | 3-hexen-1-ol | 1491 | 8.1 | -- | -- |
| 57 | Spathulenol | 1495 | 4.1 | -- | 1.0 |
| 58 | Caryophyllene oxide | 1498 | 10.3 | 8.8 | -- |
| 59 | Salvial-4(14)-en-1-one | 1504 | -- | -- | 0.3 |
| 60 | Cyclododecane | 1512 | -- | -- | 0.4 |
| 61 | *Trans*-β–caryophyllene | 1521 | 0.9 | 1.2 | -- |
| 62 | Azulene | 1526 | -- | 2.6 | -- |
| 63 | α–cadinol | 1539 | 2.6 | 12.8 | 0.3 |
| 64 | Caryophyllene-II | 1547 | 2.7 | 3.3 | -- |
| 65 | 12-norcyrene-B | 1558 | 0.4 | -- | -- |
| 66 | Tetradecanoicacid | 1591 | 0.1 | 0.3 | -- |
| 67 | Benzylbenzoate | 1596 | 1.5 | 0.3 | 0.1 |
| 68 | Benzoic acid | 1605 | 0.1 | -- | -- |
| 69 | 2-pentadecanone | 1631 | 0.7 | 0.5 | 0.3 |
| 70 | Cyclotetradecane | 1650 | 0.1 | 0.2 | 0.1 |
| 71 | Nonadecane | 1660 | -- | 0.2 | 0.1 |
| 72 | *n*-decanoic acid | 1692 | 0.6 | 4.3 | 0.1 |
| 73 | Hexadecanal | 1732 | -- | 0.5 | -- |
| 74 | Heneicosane | 1789 | -- | 0.5 | 0.1 |
| 75 | Phytol | 1794 | -- | 2.3 | -- |
| 76 | Ethyllinoleolate | 1810 | 0.1 | -- | -- |
| 77 | Tricosane | 1903 | 0.2 | 0.8 | 0.1 |
| 78 | Nonacosane | 1942 | 6.9 | -- | 0.2 |
| 79 | Tetracosane | 1949 | -- | 0.4 | 0.1 |
| | Total | | 89.8 | 80.4 | 89.5 |

RRI: Relative Retention Indices

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