

# Chemical compositions of aerial parts and root of *Hypericum olympicum* L. essential oils, and their antimicrobial activity screening

Damla KIRCI<sup>1\*</sup> , Yavuz Bülent KÖSE<sup>2</sup> , Betül DEMİRCİ<sup>3</sup> 

<sup>1</sup> Department of Pharmacognosy, Faculty of Pharmacy, Selçuk University, 42000 Konya, Turkey.

<sup>2</sup> Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, 26000 Eskişehir, Turkey.

<sup>3</sup> Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26000 Eskişehir, Turkey.

\* Corresponding Author. E-mail: [damla.kirci@selcuk.edu.tr](mailto:damla.kirci@selcuk.edu.tr) (D.K.); Tel. +09-332-241 00 41.

Received: 27 February 2023 / Revised: 12 May 2023 / Accepted: 14 May 2023

**ABSTRACT:** *Hypericum olympicum* L. (Hypericaceae) thrives in Turkey, S. Balkans, Greece, and Serbia. While the decoction prepared is internally used for stomachache; used externally for inflamed wounds and cuts in traditional. This research aims to examine the in vitro antimicrobial of the essential oil (EO) obtained using *H. olympicum* aerial parts. The EOs from *H. olympicum* aerial parts and roots were distilled and characterized by GC-FID and GC/MS systems, respectively. Germacrene D (26.4%) and  $\alpha$ -pinene (8.5%) were found as the main components of the aerial parts of *H. olympicum* EO. The EO of *H. olympicum* root were characterized with undecane (38.9%),  $\alpha$ -pinene (14.2%), and 3-methyl nonane (9.2%). The antimicrobial activity of EO of *H. olympicum* aerial parts was observed *S. pyogenes* (219  $\mu\text{g}/\text{mL}$ ), *E. coli* (8.75  $\text{mg}/\text{mL}$ ), *S. aureus* (109  $\mu\text{g}/\text{mL}$ ) and *C. albicans* (55  $\mu\text{g}/\text{mL}$ ). Essential oil of *H. olympicum* aerial parts is differently reported in the literature while the chemical composition of EO of *H. olympicum* root is identified for the first time. According to our findings, the essential oil of the *H. olympicum* aerial parts showed a strong effect against skin wound pathogens.

**KEYWORDS:** *Hypericum olympicum*; hypericaceae; essential oil; antimicrobial activity.

## 1. INTRODUCTION

The genus *Hypericum* L., Hypericaceae, includes more than 450 species divided into 36 sections of herbs, shrubs and trees growing widely in Europe, America and West Asia [1,2]. It is distributed in subtropical, warm temperate, Mediterranean and mountainous tropical regions and is constantly transplanted from its wild environment and cultivate [3]. Eighty-nine species represent the *Hypericum* L. genus, and 43 of these are endemic to Turkey [4,5]. *H. olympicum*, which grows in sparse populations on dry spots and rocky grounds, naturally are perennial in Turkey [6].

The *Hypericum* species are used as traditional treatments in many countries of the World. The different parts of *H. perforatum* (St. John's Wort) are commonly used as an anti-depressant, wound healer, and anti-inflammatory. Also, *H. perforatum* traditionally consume due to an anti-depressant in several parts of European countries [7].

The different parts of *Hypericum* species, the leaves, flowers, and fruits, were traditionally prepared as infusion due to medicinal effects such as vermifuge, wound healing, antihysterical, diuretic, anti-depressive and sedative agent in the Canary Islands [8].

In Turkey, different parts of *Hypericum* species are traditionally used as appetizer, sedative, antidiarrheic, antispasmodic, anti-helminthic and diuretic [9]. Also, *Hypericum olympicum* is widespread in Macedonia, Greece, and Serbia [4,6,10,11].

The *Hypericum* species is called "goat St John's wort", the solid goat-like smell of the leaves, while *H. olympicum* is called "Sarı kantaron" in Turkish folk medicine. Aerial parts of *H. olympicum* traditionally have been prepared with the decoction method. At the same time, the decoction prepared is traditionally used internally for stomachache and externally for inflamed wounds and cuts [8,12].

The phytochemical composition of *Hypericum* species was exposed, and these species produce a broad spectrum of valuable components, mainly phloroglucinols (adhyperforin and hyperforin), naphthodianthrones (pseudohypericin and hypericin), xanthenes /benzophenones, flavonoids (kaempferol,

**How to cite this article:** Kirci D, Köse YB, Demirci B. Chemical compositions of aerial parts and root of *Hypericum olympicum* L. essential oils, antimicrobial activity. J Res Pharm. 2023; 27(5): 2153-2159.

quercetin), bioflavonoids, and tannins (proanthocyanidins) [2,13]. These metabolites exhibit a broad range of biological effects, including cytotoxic, antibacterial, and anti-inflammatory activities, in addition to their well-known antidepressant activity [14,15].

Essential oils (EOs), in particular, are an interesting mixture of volatile components that were shown to have a strong antimicrobial, antiangiogenic, and antioxidant effects, even though *Hypericum* species are generally known to have low EO yields < 1%. The EO combinations mainly consist of mono- and sesquiterpenes, in the form of hydrocarbons or oxygenated derivatives. The EOs of *Hypericum* species are included monoterpenes as major components (*a*-pinene, limonene, and (*E*)-caryophyllene) [15,16].

When *H. perforatum* has been subjected to many chemical compositions and biological activities in recent years, the biological activities of *H. olympicum* essential oil has not yet been thoroughly investigated.

This research includes the chemical components of aerial parts and roots of *H. olympicum* EOs. Also, the antimicrobial effects of *H. olympicum* aerial parts were evaluated against skin pathogens.

## 2. RESULTS and DISCUSSION

### 2.1. Essential oil composition

The current study aimed at the determination of the chemical components of essential oils of *H. olympicum* aerial parts and root. The essential oils were subjected to hydrodistillation to obtain EOs, then EOs were examined by GC/MS and GC/FID simultaneously. The yield of *H. olympicum* aerial parts was calculated as 0.23% while a small quantity of EO of *H. olympicum* root is trapped in *n*-hexane. The chemical compositions of the EOs are listed in Table 1.

A total of 51 volatile compounds were identified in the essential oil components of *H. olympicum* root, representing 93.2% of the total oil. The EO of *H. olympicum* root were characterized with undecane (38.9%), *a*-pinene (14.2%), 3-methyl nonane (9.2%), 2-methyl-decane (5.8%) and *a*-cadinol (2.1%).

The EO of *H. olympicum* root was included total monoterpenes (75.6%), total sesquiterpenes (13.0%), fatty acid (1.0%) and the others (3.6%). Previously, there have been no studies on the essential oil composition of *H. olympicum* root.

Eighty-four volatile compounds were identified in the essential oil composition of *H. olympicum* aerial parts, representing 92.2% of the total oil. The EO of *H. olympicum* aerial parts were characterized with germacrene D (26.4%), *a*-pinene (8.5%),  $\delta$ -cadinene (5.5%),  $\gamma$ -muurolene (4.0%), 3-methyl nonane (2.8%), (*Z*)- $\beta$ -farnesene (2.8%),  $\gamma$ -cadinene (2.5%) and *a*-cadinol (2.4%).

**Table 1.** Chemical composition of essential oils of *H. olympicum* root and aerial parts

RRI	Compound	Root %	Aerial parts %	Identification Method
965	3-Methyl nonane	9.2	2.8	MS
1000	Decane	0.3	0.1	RRI, MS
1032	<i>a</i> -Pinene	14.2	8.5	RRI, MS
1035	<i>a</i> -Thujene	-	0.1	MS
1065	2-Methyl decane	5.8	1.3	MS
1076	Camphene	-	0.2	RRI, MS
1100	Undecane	38.9	0.5	RRI, MS
1118	$\beta$ -Pinene	1.5	1.9	RRI, MS
1151	$\delta$ -4-Carene	0.1	0.2	MS
1159	$\delta$ -3-Carene	0.3	-	MS
1174	Myrcene	0.8	0.6	RRI, MS
1176	<i>a</i> -Phellandrene	tr	0.1	RRI, MS
1200	Dodecane	0.4	0.1	MS
1203	Limonene	1.1	1.3	RRI, MS
1218	$\beta$ -Phellandrene	0.2	0.1	RRI, MS
1220	<i>cis</i> -Anhydro linalool oxide	-	0.1	MS
1244	2-Pentyl furan	0.4	tr	MS
1246	( <i>Z</i> )- $\beta$ -Ocimene	0.1	0.5	MS
1255	$\gamma$ -Terpinene	0.4	0.5	RRI, MS
1266	( <i>E</i> )- $\beta$ -Ocimene	tr	0.5	MS
1280	<i>p</i> -Cymene	1.1	0.2	RRI, MS
1290	Terpinolene	0.2	0.3	RRI, MS
1300	Tridecane	0.6	tr	RRI, MS
1400	Nonanal	0.3	0.1	MS

1466	<i>α</i> -Cubebene	-	0.4	MS
1493	<i>α</i> -Ylangene	-	0.3	MS
1497	<i>α</i> -Copaene	tr	0.7	MS
1535	<i>β</i> -Bourbonone	-	0.2	MS
1544	<i>α</i> -Gurjunene	-	0.1	MS
1553	Linalool	-	0.9	RRI, MS
1589	<i>β</i> -Ylangene	-	0.4	MS
1597	<i>β</i> -Copaene	-	0.8	MS
1600	<i>β</i> -Elemene	-	0.2	MS
1611	Terpinen-4-ol	-	0.1	RRI, MS
1612	<i>β</i> -Caryophyllene	tr	1.4	RRI, MS
1628	Aromadendrene	0.4	0.4	MS
1639	Cadina-3,5-diene	-	0.2	MS
1661	Alloaromadendrene	-	1.4	MS
1668	( <i>Z</i> )- <i>β</i> -Farnesene	0.4	2.8	MS
1677	<i>epi</i> -Zonarene	-	0.4	MS
1687	<i>α</i> -Humulene	0.4	1.8	RRI, MS
1688	Selina-4,11-dien	0.3	0.2	MS
1704	<i>γ</i> -Muurolene	1.6	4.0	MS
1706	<i>α</i> -Terpineol	-	0.5	RRI, MS
1719	Borneol	-	0.2	RRI, MS
1726	Germacrene D	1.2	26.4	MS
1740	<i>α</i> -Muurolene	0.3	1.3	MS
1755	Bicyclogermacrene	-	1.9	MS
1773	<i>δ</i> -Cadinene	1.7	5.5	MS
1776	<i>γ</i> -Cadinene	1.0	2.5	MS
1779	( <i>E,Z</i> )-2,4-Decadienal	tr	-	MS
1799	Cadina-1,4-diene	tr	0.5	MS
1807	<i>α</i> -Cadinene	0.5	0.7	MS
1827	( <i>E,E</i> )-2,4-Decadienal	0.7	tr	MS
1849	Calamenene	0.4	0.3	MS
1849	Dihydro- <i>β</i> -ionon	1.2	-	MS
1857	Geraniol	-	0.8	RRI, MS
1868	( <i>E</i> )-Geranyl acetate	0.3	0.1	MS
1929	2-Methyl butyl benzoate	-	0.1	MS
1941	<i>α</i> -Calacorene	-	0.2	MS
1945	1,5-Epoxy-salvial-4(14)-ene	-	0.2	MS
1958	( <i>E</i> )- <i>β</i> -Ionone	-	0.2	MS
1973	1-Dodecanol	0.1	-	RRI, MS
1977	Dihydro- <i>β</i> -ionol	0.4	-	MS
2037	Salvial-4(14)-en-1-one	-	0.3	MS
2048	Cubeban-11-ol	-	0.1	MS
2050	( <i>E</i> )-Nerolidol	-	0.5	MS
2057	Ledol	-	0.3	MS
2071	Humulen epoxide II	-	0.2	MS
2080	Cubenol	-	0.4	MS
2080	Junenol	0.8	0.3	MS
2088	1- <i>epi</i> -Cubenol	0.3	0.6	MS
2098	Globulol	-	0.4	MS
2104	Viridifurolol	-	0.2	MS
2130	Salviadienol	-	0.3	MS
2144	Spathulenol	0.4	1.9	MS
2173	6- <i>epi</i> -Cubenol	0.2	-	MS
2187	<i>T</i> -Cadinol	-	1.2	MS
2209	<i>T</i> -Muurolol	0.6	1.1	MS
2214	<i>ar</i> -Turmerol	0.2	-	RRI, MS
2219	Torreyol	-	0.4	MS
2247	<i>trans-α</i> -Bergamatol	-	0.2	MS
2255	<i>α</i> -Cadinol	2.1	2.4	MS
2256	Cadalene	0.2	-	MS
2278	Torilenol	-	0.6	MS
2300	Tricosane	-	0.1	MS

2369	Eudesma-4(15)7-dien-1- $\beta$ -ol	-	1.0	MS
2503	Dodecanoic acid	-	0.1	RRI, MS
2622	Phytol	-	0.6	MS
2670	Tetradecanoic acid	0.3	tr	RRI, MS
2900	Nonacosane	0.6	0.4	RRI, MS
2931	Hexadecanoic acid	0.7	0.5	RRI, MS
	Monoterpene hydrocarbons	75.6	19.8	
	Oxygenated monoterpenes	-	2.6	
	Sesquiterpene hydrocarbons	8.1	54.8	
	Oxygenated sesquiterpenes	4.9	12.8	
	Fatty acid	1	0.6	
	Diterpenes	-	0.6	
	Others	3.6	1	
	<b>Total</b>	<b>93.2</b>	<b>92.2</b>	

**RRI:** Relative retention indices calculated against n-alkanes;

% calculated from Flame Ionization Detector (FID);

**tr:** Trace (<0.1 %);

Identification method based on the relative retention indices (RRI) of authentic compounds on a HP Innowax column;

**MS:** identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries

The EO of *H. olympicum* aerial parts was found total monoterpenes (22.4%), total sesquiterpenes (67.6%), fatty acid (0.6%), diterpenes (0.6%) and the others (1.0%).

In previous studies, the aerial parts of *H. olympicum* were collected from Greece and Serbia [6,10,20]. Gudžić and co-workers reported that the main components of *H. olympicum* essential oil from Serbia were (*E*)-anethole (30.7%),  $\beta$ -farnesene (12.4%) and  $\delta$ -cadinene (8.7%) [6]. However, (*E*)-anethole was not detected in the *H. olympicum* essential oils from Greece, which were published 2001 and 2003 years [20]. In other study, Pavlović and co-workers reported *H. olympicum* EO was found germacrene D (16.0%) and (*E*)-caryophyllene (7.4%) as major components and were identified total 41 volatile compounds [10]. While the component with the highest content in *H. olympicum* from Serbia was (*E*)-anethole (30.7%), it was not detected in the EOs from Turkey and Greece. According to Serbia, the aerial parts of the *H. olympicum* EO from Turkey are similar to the essential oil of the *H. olympicum* from Greece. There are significant differences in the chemical components of EOs of *H. olympicum* due to the location difference. Also, the other parameters affecting the composition of *Hypericum* EOs could be linked to variables such as developmental stages, seasonal variation, genetic factors, phenological cycle, and environmental conditions [15].

The chemical composition of essential oil of *H. olympicum* aerial parts was compared to other *Hypericum* species essential oil of *H. saturejifolium* Jaub. and Spach. from Turkey (germacrene D: 30.2%) and essential oil of *H. perforatum* leaves from the USA (germacrene D: 25.7%) [21,22]. According to the results, the chemical compositions of the three EOs were close.

## 2.2. Antimicrobial activity

The antimicrobial activity of the essential oil of *H. olympicum* aerial parts was tested against reference. The strains were the gram-negative (*E. coli*), gram-positive bacteria (*S. aureus*, *S. pyogenes*) and the yeast (*C. albicans*) strains. The results of the antimicrobial activity of the EO are given in Table 2.

**Table 2.** MIC values of the essential oil of *H. olympicum* aerial parts ( $\mu\text{g/mL}$ )

Microorganisms	EO	Ciprofloxacin
<i>Streptococcus pyogenes</i> ATCC 13615	219	0.0312
<i>Escherichia coli</i> NRRL B-3008	8750	0.0625
<i>Staphylococcus aureus</i> ATCC 6538	109	0.125
<i>Candida albicans</i> ATCC 90028	55	1*

\*Ketoconazole

While the EO is demonstrated the highest antimicrobial activity against *C. albicans* (55 µg/mL), it recorded the highest antibacterial activity against *S. aureus* (109 µg/mL). The EO showed moderate antibacterial activity against *E. coli* (8.75 mg/mL). Among the tested microorganism, *S. aureus* and *C. albicans* were more sensitive to the EO of *H. olympicum* aerial parts. From the results obtained, the EO observed a more potent activity against Gram-positive bacteria than Gram-negative bacteria. Also, the EO was found as effective against skin pathogens (*S. aureus* and *S. pyogenes*).

Previously, the antimicrobial activity of *H. olympicum* aerial parts extracts was investigated using an agar disk diffusion assay. This study showed very strong antifungal activity for the methanolic extract of *H. olympicum* [11]. To the best of our knowledge, no antimicrobial activity of the essential oil of *H. olympicum* has been reported in the literature.

Several microorganisms, such as *P. aeruginosa*, and *S. aureus*, are often responsible for skin wounds in animals and humans. Therefore, the well-documented antimicrobial activities from the EOs of *Hypericum* could play a crucial role in figuring out the wound-healing effects of *Hypericum* species. The other EOs of *Hypericum* species (*H. perforatum*, *H. empetrifolium*, and *H. triquetrifolium*) were shown the most significant healing properties. *C. krusei* and *C. tropicalis* were more sensitive to the EOs from *H. empetrifolium*. However, *Staphylococcus* strains were shown mainly as resistant to *H. triquetrifolium* EOs. Also, the EOs of *H. perforatum* were found effective against the other skin pathogens. [14,15].

### 3. CONCLUSION

Essential oil represents an important source of bioactive prepared for human well-being, including antimicrobial treatments—several reports of traditional uses of *Hypericum* species for skin wound problems.

In research, volatile components of EOs of *H. olympicum* root and aerial parts were reported in the present study. Essential oil of *H. olympicum* aerial parts is differently reported in the literature, while the chemical composition of EO of *H. olympicum* root is identified for the first time.

According to our findings, the essential oil of the *H. olympicum* aerial parts showed considerable effect against pathogens. It is especially noteworthy that the efficacy in treating skin-wound is exceptionally high. Therefore, *In vivo* and clinical skin wound healing studies should proceed with the essential oil of *H. olympicum* in the future.

### 4. MATERIALS AND METHODS

#### 4.1. Plant material and essential oil

The aerial parts and root of *H. olympicum* were collected from Kirazlı Waterfall, Eskisehir (Turkey) (Figure 1). The plant is identified by Dr. Yavuz Bülent Köse, and voucher specimens had at the herbarium of Anadolu University, Turkey (ESSE 15428).



Figure 1. *Hypericum olympicum* L.

The EOs were taken by hydro-distillation using a Clevenger-type apparatus for 3h. The EOs were stored in an amber vial and analysed by Gas Chromatography-Mass Spectrometry (GC/MS) and Gas Chromatography-Flame Ionization Dedector (GC/FID), simultaneously.

#### 4.2. Essential oils isolation with GC/MS and GC/FID methods

The hydrodistilled essential oils of *H. olympicum* aerial parts and root were analysed by GC/FID and GC/MS (17). The GC-MS analysis was performed with an Agilent 5975 GC-MSD system. Innowax FSC column (0.25  $\mu\text{m}$ , 60 m  $\times$  0.25 mm film thickness) was run with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV, and the mass range was from m/z 35 to 450.

The GC analysis was carried out using an Agilent 6890N GC system. The FID detector temperature was 300°C. Simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions to obtain the same elution order with GC-MS. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of the analysis are listed in Table 1.

The volatile compounds were identified by comparing their relative retention times (RRT) with those of authentic samples or by comparing their relative retention index (RRI) to the n-alkanes series. Computer matching against in-house "Başer Library of Essential Oil Constituents" and commercial (MassFinder 4 Library, Wiley GC/MS Library) built up by volatile compounds and components of known oils were used.

#### 4.3. Microbial cultures

The test microorganisms used in the research were as follows: *Streptococcus pyogenes* American Type Culture Collection (ATCC) 13615, *Escherichia coli* Northern Regional Research Laboratory (NRRL) B-3008, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 90028.

#### 4.4. Antimicrobial activity

The microdilution-broth susceptibility assay was used for the antimicrobial evaluation of the EO of *H. olympicum* aerial parts. The EO was calculated as the minimal inhibitory concentration (MIC,  $\mu\text{g}/\text{mL}$ ).

Microorganism isolates for antimicrobial activity testing were obtained from the American Type Culture Collection (ATCC) and Agricultural Research Service Culture Collection (NRRL) in lyophilized form. Until inoculation and purity testing, the microorganisms were kept in glycerol at -85°C. As reported previously, the potential antibacterial activity of materials was assessed using a broth microdilution test based on a modified Clinical and Laboratory Standards Institute procedure. *S. pyogenes*, *E. coli*, *S. aureus* and *C. albicans* were used as test microorganisms. For the first stock solution, the sample (2 mg/mL) was dissolved in sterile dimethyl sulfoxide (DMSO). 100  $\mu\text{L}$  of samples were placed into 96-well microplates, and fold serial dilutions were carried out. Following the dilutions, 50  $\mu\text{L}$  serial dilutions of turbidometrically adjusted microorganisms (105-106 CFU/mL) were put onto the plates. After a 24-hour incubation period at 37°C, the first well was treated with 20  $\mu\text{L}$  of resazurin, ensuring the Minimum Inhibitory Concentrations (MIC) on all microplates, where the lowest concentration of the samples inhibited visible growth. The standard antibiotics, ciprofloxacin (Merck) (for bacteria) and ketoconazole (Fluka) (for fungi) (128-0.25  $\mu\text{g}/\text{mL}$ ) were used as standard controls. The test plate additionally included solvent and microbiological controls. All the test samples were subjected to antibacterial tests at least three times [18,19] (See Table 2 for the results).

**Author contributions:** Concept and Design -D.K., B.D.; Supervision - B.D.; Resources -B.D., Y.B.K.; Materials - Y.B.K.; Data Collection and/or Processing - D.K.; Analysis and/or Interpretation - B.T., D.K.; Literature Search - D.K.; Writing - D.K., B.D., Y.B.K.; Critical Reviews - D.K., Y.B.K., B.D.

**Conflict of interest statement:** The authors declared no conflict of interest.

#### REFERENCES

- [1] Robson NKB. *Hypericum*. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH (editors). Flora Europea Vol. 2. Cambridge, UK: Cambridge University Press., 1968, pp. 261-269.

- [2] Ernst E. *Hypericum*: The genus *Hypericum*. Medicinal and aromatic plants-industrial profiles. 31<sup>st</sup> ed. London: Taylor & Francis Press, 2003. <https://doi.org/10.1201/9780367800963>
- [3] Robson NKB. Studies in the genus *Hypericum* L. (Guttiferae). Bull British Museum – Natural History: Botany. 2001; 8: 55–226.
- [4] Davis PH. *Hypericum* L. In: Davis PH. (editor). Flora of Turkey and the East Aegean Islands, Vol. 2. Edinburgh, UK: Edinburgh University Press, 1967, pp. 392-394.
- [5] Davis PH, Mill RR, Tan K. *Hypericum* L. In: Davis PH, Mill RR, Tan K. (editors). Flora of Turkey and the East Aegean Islands, Vol. 10. 1st ed. Edinburgh, UK: Edinburgh University Press, 1988, pp. 101-102.
- [6] Gudžić B, Dorđević S, Palić R, Stojanović G. Essential oils of *Hypericum olympicum* L. and *Hypericum perforatum* L. Flavour Fragr J. 2001; 16(3): 201-203. <https://doi.org/10.1002/ffj.978>
- [7] Guedes AP, Franklin G, Fernandes-Ferreira M. *Hypericum* sp.: essential oil composition and biological activities. Phytochem Rev. 2012; 11(1): 127-152. <https://doi.org/10.1007/s11101-012-9223-y>.
- [8] Zorzetto C, Sánchez-Mateo CC, Rabanal RM, Lupidi G, Petrelli D, Vitali LA, Bramucci M, Quassinti L, Caprioli G, Papa F, Ricciutelli M, Sagratini G, Vittori S, Maggi F. Phytochemical analysis and in vitro biological activity of three *Hypericum* species from the Canary Islands (*Hypericum reflexum*, *Hypericum canariense* and *Hypericum grandifolium*). Fitoterapia. 2015; 100: 95-109. <https://doi.org/10.1016/j.fitote.2014.11.013>
- [9] Baytop T. Therapy with medicinal plants in Turkey. 2<sup>nd</sup> ed. Istanbul: Nobel Tıp Kitapevleri, 1999, pp. 166.
- [10] Pavlović M, Tzakou O, Petrakis PV, Couladis M. The essential oil of *Hypericum perforatum* L., *Hypericum tetrapterum* Fries and *Hypericum olympicum* L. growing in Greece. Flavour Fragr J. 2006; 21(1): 84-87. <https://doi.org/10.1002/ffj.1521>
- [11] Radulović N, Stankov-Jovanović V, Stojanović G, Šmelcerović A, Spiteller M, Asakawa Y. Screening of *in vitro* antimicrobial and antioxidant activity of nine *Hypericum* species from the Balkans. Food Chem. 2007; 103(1): 15-21. <https://doi.org/10.1016/j.foodchem.2006.05.062>
- [12] Tuzlacı E, Aymaz PE. Turkish folk medicinal plants, part IV: Gönen (Balıkesir). *Fitoterapia*. 2001; 72 (4): 323-343. [https://doi.org/10.1016/S0367-326X\(00\)00277-X](https://doi.org/10.1016/S0367-326X(00)00277-X)
- [13] Mullaicharam AR, Halligudi N. St John's wort (*Hypericum perforatum* L.): A review of its chemistry, pharmacology and clinical properties. Int J Res Phytochem Pharmacol. 2019; 1(1): 5-11. <https://doi.org/10.33974/ijrpps.v1i1.7>
- [14] Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, Prezioso S, Muraro R, Giovanni P. Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-years retrospective study. Antibiotics. 2021; 10(10): 1162. <https://doi.org/10.3390/antibiotics10101162>
- [15] Grafakou ME, Barda C, Karikas GA, Skaltsa H. *Hypericum* essential oils – composition and bioactivities: An update (2012–2022). Molecules. 2022; 27(16): 5246. <https://doi.org/10.3390/molecules27165246>
- [16] Crockett SL. Essential oil and volatile components of the genus *Hypericum* (Hypericaceae). Nat Prod Commun. 2010; 5(9): 1493-1506.
- [17] Demirci B, Dilmaç E, Kırcı D, İlhan G, Demirci F, Kılıç CS, Duman H. Chemical and antimicrobial characterization of essential oils obtained from aerial part, root and fruit of *Ferulago longistylis* Boiss., an endemic species. Nat Volatiles Essent Oils. 2020; 7(1): 18-25. <https://doi.org/10.37929/nveo.703968>
- [18] Amsterdam D. Susceptibility testing of antimicrobials in liquid media. In: Lorian V. (Editor). in antibiotics in laboratory medicine, fifth ed. Philadelphia: USA: Williams and Wilkins Maple Press, 2005, pp. 60-104.
- [19] Işcan G, Kirimer N, Kürkçüoğlu M, Arabacı T, Küpeli E, Başer KHC. Biological activity and composition of the essential oils of *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. subsp. *aleppica*. J Agric Food Chem. 2006; 54, 170-173. <https://doi.org/10.1021/jf051644z>
- [20] Smelcerovic A, Spiteller M, Ligon AP, Smelcerovic Z, Raabe N. Essential oil composition of *Hypericum* L. species from Southeastern Serbia and their chemotaxonomy. Biochem Syst Ecol. 2007; 35(2): 99-113. <https://doi.org/10.1016/j.bse.2006.09.012>
- [21] Kıyan HT, Demirci B, Başer KHC, Demirci F. The *in vivo* evaluation of anti-angiogenic effects of *Hypericum* essential oils using the chorioallantoic membrane assay. Pharm Biol. 2014; 52(1): 44-50. <https://doi.org/10.3109/13880209.2013.810647>
- [22] Schepetkin IA, Özek G, Özek T, Kirpotina LN, Khlebnikov AI, Quinn MT. Chemical composition and immunomodulatory activity of *Hypericum perforatum* essential oils. Biomolecules. 2020; 10(6): 916. <https://doi.org/10.3390/biom10060916>