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RESEARCH ARTICLE

Characterization of *Sideritis trojana* Bornm. essential oil and its antimicrobial activity

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ABSTRACT

The components of the essential oil obtained from the dried aerial parts of *Sideritis trojana* Bornm. by hydrodistillation was analyzed both by GC-FID and GC-MS, simultaneously. Overall 57 compounds were identified representing 83.8% of the oil. The major components of the oil were identified as valeranone (11.3%), α-bisabolol (10.9%) and β-caryophyllene (8.8%), respectively. The composition of the oil showed quantitative chemical variation from previously studied material in terms of its major components. Moreover, the essential oil was evaluated for its *in vitro* antibacterial and anticandidal activities using a broth microdilution method. A selected panel of standard strains of Gram (+) and Gram (-) human pathogens as well as *Candida albicans* were used in the assay. As a preliminary result, it was observed that the oil displayed relatively moderate antibacterial activity against *Helicobacter pylori* with MIC value of 250 μg/mL when compared to standard antimicrobials. As a conclusion, it is worthwhile to evaluate the plant material against a broader spectrum of activities.

Key words: *Sideritis trojana*; Lamiaceae; essential oil; GC-FID and GC-MS; antimicrobial; *Helicobacter pylori*.

1. Introduction

The genus *Sideritis* L. contains over 150 species distributed in the temperate and tropical regions of the world especially in the Mediterranean basin [1]. *Sideritis trojana* Bornm. (Lamiaceae) is known as "kazdağı çayı" in Turkey and its aerial parts are sold in local bazaars. It is widely used as a popular herbal tea for the treatment of stomach ache, abdominal pain, kidney disease as well as against sore throat in the Balıkesir and Çanakkale provinces of Turkey where the plant species grow wild [2]. This species is endemic to Ida Mountains and constitutes one of the 46th *Sideritis* species in the flora of Turkey [3, 4]. Previous phytochemical studies reported the occurrence of diterpenes [5], essential oil [6], iridoids, phenylethanoid glycosides and flavonoids [7] in the aerial and underground parts of this species. Moreover, the *in vitro* antimicrobial [8] and antioxidant activities [7] of different extracts and/or the secondary metabolites purified thereof were reported. The different solvent extracts as well as the essential oils obtained from various *Sideritis* species were also reported to possess antimicrobial activities [1, 6]. In the course of our ongoing phytochemical and bioactivity studies on Lamiaceae plants, we herein report the chemical characterization of *S. trojana* essential oil distilled from the

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aerial parts as well as its *in vitro* antimicrobial activity against five bacteria and a *Candida* standard strain.

2. Results and Discussion

2.1. Essential oil composition

Analysis of the essential oil was carried out by GC-FID and GC-MS, simultaneously. Totally, 57 compounds were identified representing 83.8% of the essential oil (Table 1). The essential oil was mainly represented by oxygenated sesquiterpenes (28.7%) and sesquiterpene hydrocarbons (19.1%) including valeranone (11.3%), α-bisabolol (10.9%), β-caryophyllene (8.9%) and germacrene D (4.4%) and caryophyllene oxide (4.2%). The other major compounds of the oil were as follows: α-pinene (5.2%) and 9-geranyl-*p*-cymene (4.8%). *S. trojana* belongs to the section *Empedoclia* (Rafin.) Bentham. which constitutes one of the two sections of *Sideritis* species growing in Turkey [3]. Kırımer *et al.* reported the chemical composition of the essential oil of two *Sideritis* species including *S. trojana* [6]. The essential was reported to be dominated by monoterpene hydrocarbons (α/β-pinenes; 31.6%). When the overall chemical composition of the essential oil obtained in this study is compared to that of the same species reported previously [6], the major differences were quantitative rather than qualitative. In other words, the relative abundance of the volatile principles of *S. trojana* showed variation between these two studies. As the plant materials were collected from very close locations, the variations might arise due to the possible different collection periods. It is also noteworthy that 9-geranyl-*p*-cymene, a volatile diterpene that was found in the oil at 4.8% could be a useful chemotaxonomic marker for *S. trojana* within *Sideritis* genus. When the oil yield is compared to those of other *Sideritis* species, it was found rather low (0.08%) in the current study and it also seems to be in line with the hypothesis "the lower the oil yield the higher the sesquiterpene yield" of Kırımer *et al.* for the essential oils of *Sideritis* species in the section *Empedoclia* [9]. The same authors also categorized *Sideritis* species growing in Turkey into six groups according to their major compounds of the essential oil as "monoterpene hydrocarbon rich", "oxygenated monoterpene rich", "sesquiterpene hydrocarbon rich", "oxygenated sesquiterpene rich", "diterpene rich" and "others" [9, 10]. As inferred from Table 1, the essential oil of *S. trojana* is rich in oxygenated sesquiterpenes. It is stated that *S. trojana* is morphologically close to *S. taurica* and *S. dichotoma* [3]. When the essential oil composition of *S. trojana* is compared to those of *S. taurica* and *S. dichotoma*, the oil of *S. trojana* seems to be closer to that of *S. taurica* as both essential oils contain α-bisabolol and germacrene D as the major sesquiterpenes whereas *S. dichotoma* is reported to be rich in diterpenes mainly geraterpinene [9].

2.2. Antimicrobial activity

The isolated essential oil was evaluated for its antimicrobial activity against five bacteria strains including *Helicobacter pylori* ATCC 43504 strain as well as a human pathogenic *Candida* strain. The essential oil displayed a relative moderate activity against *H. pylori* with MIC value of 250 μg/mL. In a previous study by Kırımer *et al.* the essential oil of *S. trojana* was found to possess mild to moderate antimicrobial activity against *E. coli*, *S. epidermidis* and *C. albicans* (MICs: 62.5-125 mg/mL) [6]. However, in the present study the microbial panel was not susceptible to essential oil at the tested concentrations. The difference between the results might be due to the quantitative variations between these samples as discussed above as well as might arise from the different microbial strains tested.

3. Conclusion

The chemical composition of the essential oil isolated from the aerial parts of *S. trojana* was analysed by GC-FID and GC-MS. The essential oil of plant material was found to be rich in oxygenated sesquiterpenes. The essential oil exerted moderate activity against *H. pylori* with MIC value of 250 μg/mL. This is the first report on the *in vitro* anti-helicobacter activity of *S. trojana* essential oil. Despite its relatively low amount, the essential oil of *S. trojana* might contribute to its folkloric usage to some extent against stomach ache. In conclusion it can be suggested that this species should be evaluated in more detail for its chemistry and biological activities.

4. Materials and Methods

4.1. Plant material

Plant material, the dried aerial parts of *Sideritis trojana* Bornm. in flowering stage was purchased from a local bazaar in Altınoluk, Edremit, Balıkesir, Turkey, in August 2016. The plant material was identified by one of us (Dr. H. Kırmızıbekmez) and a sample is deposited at the Pharmacognosy Research Laboratory, Faculty of Pharmacy, Yeditepe University.

Table 1. Chemical composition of the essential oil of *Sideritis trojana*

No	RRI[a]	Compound	(%)	Identification Method[d]
1	1032	α-Pinene	2.73±0.05[b]	RRI, MS
2	1035	α-Thujene	0.10±0	MS
3	1118	β-Pinene	5.17±0.05	RRI, MS
4	1132	Sabinene	0.33±0.05	RRI, MS
5	1159	d-3-Carene	0.50±0	MS
6	1174	Myrcene	0.30±0	RRI, MS
7	1194	Heptanal	0.20±0	RRI, MS
8	1203	Limonene	1.60±0	RRI, MS
9	1218	β-Phellandrene	0.40±0	RRI, MS
10	1225	(*Z*)-3-Hexenal	0.17±0.05	MS
11	1244	2-Pentyl furan	0.10±0	MS
12	1246	(*Z*)-β-Ocimene	0.50±0	MS
13	1255	γ-Terpinene	tr[c]	RRI, MS
14	1266	(*E*)-β-Ocimene	tr	MS
15	1280	*p*-Cymene	1.30±0	RRI, MS
16	1296	Octanal	0.20±0	RRI, MS
17	1393	3-Octanol	0.23±0.05	MS
18	1400	Nonanal	1.67±0.05	RRI, MS
19	1452	1-Octen-3-ol	1.90±0.08	MS
20	1497	α-Copaene	0.27±0.05	RRI, MS
21	1499	α-Campholene aldehyde	0.10±0	MS
22	1541	Benzaldehyde	0.23±0.05	RRI, MS
23	1548	(*E*)-2-Nonenal	0.30±0	MS
24	1553	Linalool	0.87±0.05	RRI, MS
25	1562	Octanol	0.90±0	RRI, MS
26	1586	Pinocarvone	0.33±0.05	RRI, MS
27	1612	β-Caryophyllene	8.87±0.21	RRI, MS
28	1648	Myrtenal	0.70±0	MS
29	1655	(*E*)-2-Decenal	0.10±0	MS
30	1663	*cis*-Verbenol	0.10±0	MS
31	1668	(*Z*)-β-Farnesene	0.90±0	MS
32	1670	*trans*-Pinocarveol	0.90±0	RRI, MS
33	1687	α-Humulene	0.20±0	RRI, MS
34	1683	*trans*-Verbenol	0.77±0.05	MS
35	1706	α-Terpineol	0.50±0	RRI, MS
36	1726	Germacrene D	4.40±0.08	MS
37	1741	β-Bisabolene	2.27±0.05	MS
38	1755	Bicyclogermacrene	1.50±0	MS
39	1773	d-Cadinene	0.27±0.05	MS
40	1784	(*E*)-α-Bisabolene	0.40±0.08	MS
41	1804	Myrtenol	0.63±0.05	MS
42	1864	*p*-Cymen-8-ol	0.30±0	RRI, MS
43	1958	(*E*)-β-Ionone	0.23±0.05	RRI, MS
44	2001	Isocaryophyllene oxide	0.53±0.05	MS
45	2008	Caryophyllene oxide	4.20±0.08	RRI, MS
46	2041	Pentadecanal	0.27±0.05	RRI, MS
47	2071	Humulene epoxide-II	0.10±0	RRI, MS
48	2131	Hexahydrofarnesyl acetone	0.5±0	MS
49	2145	Valeranone	11.37±0.05	MS
50	2232	α-Bisabolol	10.97±0.19	RRI, MS

51	2247	*trans*-α-Bergamotol	0.40±0	MS
52	2255	a-Cadinol	1.10±0	MS
53	2312	9-Geranyl-*p*-cymene	4.80±0.08	MS
54	2337	Kaur-15-ene	0.60±0	MS
55	2384	Hexadecanol α	0.27±0.09	MS
56	2655	Benzyl benzoate	2.43±0.05	MS
57	2931	Hexadecanoic acid	2.83±0.27	RRI, MS
		Monoterpene Hydrocarbons	12.93	
		Oxygenated Monoterpenes	5.2	
		Sesquiterpene Hydrocarbons	19.08	
		Oxygenated Sesquiterpenes	28.67	
		Fatty acid+esters	2.83	
		Diterpenes	5.4	
		Others	9.7	
		Total	83.81±0.12	

[a] RRI: Relative retention indices calculated against *n*-alkanes; % calculated from FID data; [b] mean % calculated from Flame Ionization Detector (FID) data ± SD (*n*=3); [c] tr Trace (< 0.1 %); [d] 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

4.2. Extraction of Essential oil

Plant material was subjected to hydrodistillation in a Clevenger apparatus for 3 h to give an essential oil with a yield of 0.08%. The essential oil was dried over anhydrous Na_2SO_4 and stored at 4°C till use.

4.3. Analysis of the essential oil

4.3.1. Gas Chromatography-FID (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS):

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

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was used 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. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450. The analysis results are expressed as mean ± standard deviation (n=3) as listed in Table 1.

4.3.2. Identification of the compounds:

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) [11, 12] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [13, 14] was used for the identification.

4.4. Antimicrobial activity assays

Escherichia coli NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 14990, *Pseudomonas aeruginosa* ATCC 10145, *Helicobacter pylori* ATCC 43504 and *Candida albicans* ATCC 90028 standard strains were obtained from the American Type Culture Collection (ATCC) and Northern Regional Research Laboratory (NRRL). All microorganisms were stored at -85 °C in 15 % sterile glycerol. The strains were cultured on Mueller-Hinton agar (MHA, Merck, Germany) and Mueller Hinton Broth (MHB, Merck) for *E. coli*, *S. aureus*, *S. epidermidis* and *P. aeruginosa*. Colombia agar containing 5% defibrinated sheep blood and Brucella broth containing 10

Table 2. Antimicrobial activity of *S. trojana* essential oil (MIC, µg/mL)

	Escherichia coli NRRL B-3008	*Staphylococcus aureus* ATCC 6538	*S. epidermidis* ATCC 14990	*Pseudomonas aeruginosa* ATCC 10145	*Helicobacter pylori* ATCC 43504	*C. albicans* ATCC 90028
Essential oil	>1000	>1000	>1000	>1000	250	>1000
Chloramphenicol	8	8	4	>32	16	-
Tetracycline	16	0.25	>32	>32	0.025	-
Amphotericin B	-	-	-	-	-	0.031

% fetal bovine serum were used for H. pylori strain, whereas the Candida strain was refreshed on Potato Dextrose Agar (PDA, Merck) plates and RPMI medium at 37 °C. Thereafter, all microorganisms were standardized versus McFarland No: 0.5 (1 x 108 CFU/mL for bacteria and 1 x 106 CFU/mL for Candida sp.) in sterile saline (% 0.85), turbidimetrically [15, 16]. Modified microdilution assay [17, 18] was used to determine the antimicrobial activity of sample. Stock solution of the test sample was prepared in dimethylsulfoxide (DMSO) and diluted with sterile distilled water. Essential oil dilution series were prepared from 1000 µg/mL to 2 µg/mL in 96 well microtiter plates. 100 µL, 1:100 diluted bacterial suspensions [17] 1:10 diluted Helicobacter suspensions [19, 20] and 1:1000 diluted *Candida* suspensions [15] were then added to each well. After incubation at 37 °C for 18-24 h, for staining of viable microorganisms, 20 µL 0.01 % resazurin solution was added to all of the plate. The first blue well was determined as the minimal inhibitory concentration (MIC, µg/mL). The last row containing medium with microorganism was used as negative control and medium served as a positive growth control. Chloramphenicol, tetracycline and amphotericin B (Sigma, Germany) were used as standard antimicrobial agents at concentration range 0.016-32 µg/mL. All experiments were repeated in triplicate and average MICs are given in Table 2.

Authorship statement

Author contributions: Concept – H.K., B.D.; Design – H.K., F.D.; Supervision – B.D., F.D.; Resource – H.K., B.D.; Materials – H.K., B.D.; Data Collection and/or Processing – N.K., B.D.; Analysis and/or Interpretation - B.D, N.K, F.D., H.K.; Literature Search – H.K., B.D.; Writing – H.K., N.K., B.D., F.D.; Critical Reviews – H.K, B.D., F.D.

Conflict of interest statement

The authors declared no conflict of interest.

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