SHORT REPORT

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Chemical Composition of a New Taxon, *Seseli gummiferum* subsp. *ilgazense*, and its Larvicidal Activity against *Aedes aegypti*

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Abstract: Mosquitoes are vectors for many pathogens and parasites that cause human diseases including dengue, yellow fever, West Nile, chikungunya, filariasis and malaria which cause high rates of human morbidity and mortality under extreme conditions. Plants are an excellent source for mosquito control agents because they constitute rich sources of bioactive chemicals. They are also biodegradable and environment-friendly. The present study reports on the larvicidal activity of the essential oil of *Seseli gummiferum*. subsp. *ilgazense* (Apiaceae) against *Aedes aegypti* larvae. Essential oil showed 100 and 70% mortality at 125 and 62.6 ppm, respectively, with no mortality at 31.25 ppm. Aerial parts of *S. gummiferum* subsp. *ilgazense* were subjected to hydrodistillation to yield 0.6% oil. The essential oil was analyzed by GC-FID and GC-MS techniques. The main constituents in the oil were sabinene (28.8%), germacrene D (9.5%) and α-pinene (7.2%).

Keywords: Asteraceae; *Seseli gummiferum*; essential oil; sabinene; germacrene D; α-pinene; *Aedes aegypti*; larvicidal activity.

1. Plant Source

Seseli L. is one of the largest genera in the family Apiaceae with 125 to 140 taxa and distributed in Europe, Asia, Africa, North America and Australia [1]. In Turkey, *Seseli* is represented by 13 taxa and *S. gummiferum* Pall. ex Sm. subsp. *ilgazense* A. Duran, O. Cetin & M. Ozturk was recently reported as a new taxon [1]. In Turkish folk medicine, a few *Seseli* species such as *S. tortuosum* L. fruits is used as an emmenagogue and antiflatulence agent and *S. libanotis* Koch leaves are consumed as a vegetable in the

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eastern part of Turkey [2]. Essential oils of *Seseli* taxa of Turkey have been reviewed [3].

Seseli gummiferum Pall. ex Sm. subsp. *ilgazense* A. Duran, O. Cetin & M. Ozturk was collected from Kastamonu: Ilgaz Mountain National Park, Kastamonu road, from Catoren village to Buyuk Hacet Hill, Turkey, and identified by Prof. Dr. Ahmet Duran. A voucher specimen was deposited at the Faculty of Education, Department of Biology, Konya, Turkey (Voucher specimen code: 8135).

2. Previous Studies

Previous phytochemical studies on *Seseli* species have reported the presence of coumarins, phenolic acids, phenylpropanoids and terpenoids [2]. Biological activities of *Seseli* essential oils have particularly focused on the antimicrobial, insect repellent and anti-inflammatory effects [2, 4, 5].

Mosquitoes are vectors for many pathogens that cause human diseases including dengue fever, yellow fever, and malaria [6]. These illnesses can result in high rates of human morbidity and mortality in environments where appropriate medical resources are not available [7]. The primary method of mosquito control relies on the use of biological and/or synthetic insecticides [6]. Due to long term repeated chemical use, mosquito species have acquired resistance to commonly used insecticides, and especially against commercial pyrethroids [8-11]. Therefore, new and alternative mosquito control agents are emerging. Plant-derived products including essential oils (EOs) may offer an alternative and effective means of managing populations of mosquitoes [8-11]. As a part of our ongoing investigation of Turkish medicinal and aromatic plants, we report on the chemical composition of the aerial parts of *S. gummiferum* subsp. *ilgazense*, an endemic species from Turkey, and its larvicidal activity against 1st instar *Aedes aegypti* (L).

3. Present Study

Isolation of the Essential Oil: Essential oil of *S. gummiferum*. subsp. *ilgazense* was hydrodistilled from dried aerial parts for 3 h using a Clevenger apparatus to yield 0.6% (w/w).

Essential Oil Composition: The GC-MS and GC-FID analysis were carried out with an Agilent 5975 GC-MSD and Agilent 6890N GC systems, respectively. Analysis conditions and identification of the oil 64 components are similar to our earlier study [6]. Sixty-two compounds constituting 91.1% of the essential oil were characterized. Sabinene (28.8%), germacrene D (9.5%) and α-pinene (7.2 %) were the main constituent of essential oil (Table 1).

Larvicidal Activity: *Aedes aegypti* used in this study were from a laboratory colony maintained at USDA-ARS, Gainesville, Floridausing standard procedures [12]. The bioassays were performed as previosuly described [13]. Permethrin and DMSO were added as positive and negative controls, respectively. In larval screening bioassays, the *S. gummiferum* subsp. *ilgazense* EO killed 100% 1st instar *Ae. aegypti* larvae at the concentration of 125 ppm and followed by 70% mortality at 62.5 ppm and no mortality at 31.25 ppm. Based on this moderate larvicidal activity, *S. gummiferum* subsp. *ilgazense* EO was not considered suitable for further dose-response bioassays. We previously investigated some of the major compounds, present in this oil for the larvicidal activity against 1st instar *Ae. aegypti* larvae. For example, sabinene killed 40% of the larvae at 100 ppm [8] and (+)- and (-)-terpinen-4-ol had no mortality at the highest screening dose of 100 ppm [7]. We also found that (-)-α-pinene [LC_{50}=49.5 (43.5-56.1) ppm] was slightly more toxic than (+)-α-pinene [LC_{50}=65.7 (54.4-83.8) ppm] against 1st instar *Ae. aegypti* larvae [6].

A literature survey indicates the occurrence of sesquiterpenes germacrene D and bicyclogermacrene in *S. gummiferum* subsp. *corymbosum* and spathulenol in *S. gummiferum* subsp. *gummiferum* EOs as main constituents [14]. In the current study, *S. gummiferum* subsp. *ilgazense* EO stands out slightly in containing monoterpene hydrocarbons sabinene and α-pinene, though germacrene D is also present as a second major constituent. *Seseli* species, in general, comprise mono- and sesquiterpenes as major constituents in their EOs as a common characteristic group of chemicals (Suplementary material, Table S1).

Table 1. The Composition of the Essential Oil of *S. gummiferum* subsp. *ilgazense*

#	RRI[a]	RRI[b]	Compound	%*	Identification Method**
1	1014	1012 [d]	Tricyclene	t	MS
2	1032	1025 [c,d]	α-Pinene	7.2	RRI, MS
3	1035	1026 [d]	α-Thujene	1.2	MS
4	1076	1077 [d]	Camphene	1.9	RRI, MS
5	1093	1093 [c]	Hexanal	0.3	RRI, MS
6	1118	1117 [c,d]	β-Pinene	2.0	RRI, MS
7	1132	1122 [d]	Sabinene	28.8	RRI, MS
8	1174	1167 [c]	Myrcene	0.9	RRI, MS
9	1188	1177 [d]	α-Terpinene	1.2	RRI, MS
10	1194	1174 [c]	Heptanal	0.1	MS
11	1195	1192 [f]	Dehydro-1,8-cineole	t	MS
12	1203	1212 [c,d]	Limonene	1.8	RRI, MS
13	1218	1209 [d]	β-Phellandrene	0.3	RRI, MS
14	1244	1232 [f]	Amyl furan	0.1	MS
15	1246	1232 [c,d]	(*Z*)-β-Ocimene	0.3	RRI, MS
16	1255	1245 [d]	γ-Terpinene	2.0	RRI, MS
17	1266	1249 [c]	(*E*)-β-Ocimene	0.4	MS
18	1280	1268 [e] 1282 [d]	*p*-Cymene	2.5	RRI, MS
19	1290	1282 [e]	Terpinolene	0.5	RRI, MS
20	1296	1300 [c]	Octanal	0.2	RRI, MS
21	1406	1399 [f]	α-Fenchone	0.1	MS
22	1474	1459 [d]	*trans*-Sabinene hydrate	0.6	MS
23	1497	1488 [d]	α-Copaene	0.4	MS
24	1504	1495 [f]	Daucene	0.3	MS
25	1535	1523 [d]	β-Bourbonene	0.2	RRI, MS
26	1553	1538 [c]	Linalool	1.0	RRI, MS
27	1556	1560 [e]	*cis*-Sabinene hydrate	0.4	MS
28	1570	1584 [f]	*trans*-*p*-Menth-2-en-1-ol	0.4	MS
29	1586	1575 [d]	Pinocarvone	0.2	MS
30	1589	1576 [d]	β-Ylangene	0.1	MS
31	1590	1579 [d]	Bornyl acetate	2.1	RRI, MS
32	1594	1575 [f]	*trans*-β-Bergamotene	0.1	MS
33	1597	1579 [d]	β-Copaene	0.1	MS
34	1600	1590 [d]	β-Elemene	0.1	MS
35	1611	1601 [d]	Terpinen-4-ol	4.6	RRI, MS
36	1612	1608 [c]	β-Caryophyllene	0.4	RRI, MS
37	1683	1680 [d]	*trans*-Verbenol	0.5	MS
38	1687	1670 [c]	α-Humulene	0.2	RRI, MS
39	1704	1689 [d]	γ-Muurolene	1.0	MS
40	1726	1722 [c]	Germacrene D	9.5	MS
41	1740	1723 [d]	α-Muurolene	0.5	MS
42	1755	1734 [d]	Bicyclogermacrene	0.8	MS
43	1772	1763 [f]	Citronellol	0.4	RRI, MS
44	1773	1755 [d]	δ-Cadinene	0.6	MS
45	1776	1763 [d]	γ-Cadinene	0.3	MS
46	1941	1921 [d]	α-Calacorene	0.2	MS
47	1945	1959 [g]	1,5-Epoxy-salvial(4)14-ene	2.5	MS
48	2001	1967 [c]	Isocaryophyllene oxide	0.2	MS
49	2008	1962 [d] 1979 [c]	Caryophyllene oxide	1.0	RRI, MS
50	2045	2027 [e]	Carotol	2.4	MS
51	2065	*2073* [g]	*p*-Mentha-1,4-dien-7-ol	0.5	MS
52	2069	2047 [d]	Humulene epoxide-II	0.6	MS

		2077 [c]			
53	2123	2130 [g]	Salviadienol	0.5	MS
54	2130	2126 [d]	Spathulenol	2.5	RRI, MS
		2129 [c]			
55	2187	2165 [d]	T-Cadinol	0.6	MS
56	2209	2140 [h]	T-Muurolol	0.5	MS
57	2243	2278 [g]	Torilenol	0.6	MS
58	2255	2227 [d]	α-Cadinol	0.6	RRI, MS
59	2369	2366 [d]	Eudesma-4(15), 7-dien-1β-ol	1.3	MS
60	2565	2493 [h]	14-Hydroxy-α-muurolene	0.2	MS
61	2607	2547 [h]	14-Hydroxy-δ-cadinene	0.2	MS
62	2622	2613 [f]	Phytol	0.1	MS
			Monoterpene Hydrocarbons	51.0	
			Oxygenated Monoterpenes	8.7	
			Sesquiterpene Hydrocarbons	14.8	
			Oxygenated Sesquiterpenes	13.7	
			Diterpenes	0.1	
			Others	2.8	
			Total	91.1	

RRI[a]: RRI Relative retention indices experimentally calculated against *n*-alkanes;
RRI[b]: RRI from literature (c [15]; d [16]; e [17]; f [18]; g [19]; h [20]; g [21]) for polar column values;
*% calculated from FID data;
**Identification Method: 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 in-house Baser Library of Essential Oil Constituents, Adams [22], MassFinder [23] and Wiley [24] libraries

In conclusion, the weaker activity of *S. gummiferum* subsp. *ilgazense* EO may arise from its higher monoterpene content (51%). To the best of our knowledge, this is the first report on the chemical composition of *Seseli gummiferum* subsp. *ilgazense* and its larvicidal activity against *Ae. aegypti*. Natural products will continue to be promising in the search for new and effective agents in pharmaceutical and agrochemical discovery.

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Disclosure Statement

No potential conflict of interest was reported by the authors

Supporting Information

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