SHORT REPORT

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Essential Oil Constituents and Biological Activities of Leaf Extracts of *Semenovia suffruticosa* from Iran

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Abstract: *Semenovia suffruticosa* (Freyn et Bornm.) Manden. is one of the species of genus *Semenovia* (Apiaceae family). The essential oil of *S. suffruticosa* was obtained by hydrodistillation and analysed by gas chromatography coupled to mass spectrometry (GC/MS). The main components were *cis*-*β*-ocimene (12.9%), linalool (9.5%), *γ*-terpinene (9.0%) and *α*-terpinolene (7.4%), representing the 38.8% of the oil. Antibacterial activity of *S. suffruticosa* ethanol, chloroform, ethyl acetate and aqueous leaf extracts was evaluated for the first time. The various extracts were tested by the disc-diffusion assay for antimicrobial activity against common animal and human infectious bacteria. *Pseudomonas aeruginosa* exhibited the highest sensitivity against the extracts, with a 13-15 mm zone of inhibition. Antiradical activity of *S. suffruticosa* ethanol, chloroform, ethyl acetate and aqueous leaf extracts was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, FRAP (ferric reducing antioxidant power) and *β*-carotene/linoleic acid assays. Ethanol extract was the most powerful free radical scavenger in all these methods. These results, though preliminary, suggest that leaf extracts of *S. suffruticosa* exert promising antioxidant and antibacterial activities.

Keywords: Apiaceae; antiradical activity; antibacterial activity; Iranian traditional medicine. © 2017 ACG Publications. All rights reserved.

1. Plant source

The aerial parts of *S. suffruticosa* were collected during the flowering period in May 2010 from Taftan mountain, Zahedan, Iran (GPS coordinates: 61.20816, 28.22529), at an altitude of ca. 3620 m. Taxonomic identification of the plant materials was confirmed at the Biology Department of University of Sistan and Baluchestan, Zahedan, Iran. The plant also matched with digital herbarium of Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin (http://ww2.bgbm.org/herbarium/(Barcode: B 10 0367207/ImageId: 309161).

2. Previous studies

The genus *Semenovia* (Apiaceae) consists of herbaceous, aromatic, perennial plants growing mostly in Mediterranean, Turkish and Iranian mountainous regions [1,2]. This family has about 300

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genus and 3000 species mainly used as edible vegetables and sources of volatile oils and drugs [3]. Of about 20 species of genus *Semenovia* growing in Asia, 11 are found in Iran and 5 are endemic [4].

The major essential oil components in *S. suffruticosa* were linalool (13.9%) and lavandulyl acetate (11.5%), whereas in *Semenovia tragioides* (Boiss.) were *p*-cymene (20.5%) and *cis*-*β*-ocimene (9.7%) [5,6]. In addition, Sardashti et al. reported *cis*-*β*-ocimene (21.4%) and *γ*-terpinene (7.2%) as the most abundant constituents of *S. suffruticosa* essential oil [7].

Although antioxidant and antimicrobial activities of a few *Semenovia* species have been investigated in a limited number of studies [8,9], to date, no information is available on *S. suffruticosa* biological activity, to the best of our knowledge. Therefore, the aim of this study is to evaluate, for the first time, the antibacterial and free radical scavenging activities of *S. suffruticosa* leaf extracts, besides determining the chemical composition of the essential oil from aerial parts of the plant.

Table 1. Chemical composition of the essential oil of *S. suffruticosa.*

No.	Compound[a]	%	RI[b]	RI*	RI**
1	2-Hexanal	0.01	827	-	-
2	Isobutyl isobutyrate	0.07	904	829	-
3	*α*-Thujone	0.05	927	848	924
4	*α*-Pinene	0.07	934	855	933
5	Propyl isovalerate	0.01	956	-	-
6	Camphene	0.01	959	866	-
7	Sabinene	0.7	974	891	968
8	3-Octanone	0.1	982	895	-
9	*β*-Myrcene	0.3	990	910	-
10	Isobutyl isovalerate	2.5	999	922	-
11	3-Methyl anisole	0.1	1008	924	-
12	*α*-Terpinene	0.9	1012	933	-
13	Cymol	4.7	1016	-	-
14	1,8-Cineole	0.9	1018	942	-
15	Limonene	0.4	1021	944	-
16	*cis*- *β*-Ocimene	12.9	1033	960	-
17	*n*-Butyl isovalerate	0.3	1035	961	-
18	*trans*-*β*-Ocimene	0.8	1038	966	-
19	*γ*-Terpinene	9.0	1050	974	1051
20	*cis*-Sabinene hydrate	0.1	1054	997	-
21	1-Octanol	0.1	1068	-	-
22	*α*-Terpinolene	7.4	1074	1000	1076
23	*β*-Thujone	0.1	1077	-	-
24	Linalool	9.5	1086	1010	1085
25	*n*-Amyl isovalerate	1.5	1093	1016	-
26	Camphor	0.2	1105	-	-
27	Allo-ocimene	0.6	1115	1038	-
28	Epoxy terpinolene	0.2	1121	-	-
29	Lavandulol	0.6	1144	-	1156
30	4-Terpineole	0.5	1150	1073	-
31	*p*-Cimene-8-ol	0.2	1152	1075	-
32	*p*-Alilanisol	0.5	1178	1086	-

33	*z*-2,6-Dimethyl-3,5,7-octatrien-2-ol	0.07	1226	1095	-
34	Hexyl-n-valerate	0.1	1235	1105	-
35	*n*-Octyl acetate	0.3	1241	1109	-
36	Nerol	0.04	1249	1121	-
37	*z*-3-Hexenyl isopentanoat	0.3	1251	-	-
38	Hexyl isovalerate	2.0	1256	1139	-
39	*p*-Methoxy acetophenone	0.2	1263	-	-
40	Lavandulyl acetate	0.1	1272	-	1269
41	*n*-Octyl isobutyrate	2.1	1336	1252	1319
42	Methyl eugenol	0.3	1372	1295	1363
43	*trans*-Caryophyllene	0.1	1407	1320	-
44	Geranyl acetate	0.2	1412	-	-
45	*trans*-*β*-Farnesene	0.2	1422	1349	-
46	Geranyl butyrate	0.4	1429	1352	-
47	Germacrene D	0.1	1471	1361	-
48	Neryl-2-methylpropanoate	0.3	1479	1367	-
59	Bicyclogermacrene	1.0	1487	1372	1497
50	Geranyl isovalerate	1.1	1490	-	-
51	*δ*-Cadinene	0.4	1493	1388	-
52	*trans*-*γ*-Bisabolene	0.03	1497	1396	-
53	*cis*-*α*-Bisabolene	0.1	1501	1406	-
54	Germacrene B	0.05	1520	1415	-
55	Nerolidol	0.1	1536	1426	-
56	Spathulenol	0.6	1551	1432	1580
57	Hydrocinnamyl isobutyrate	0.2	1668	-	-
58	Cinnamyl isovalerate	0.1	1682	-	1644
59	Isospathulenol	0.05	1617	-	-
60	*β*-Eudesmol	0.05	1661	1521	-
61	Cinnamyl ester	1.5	1676	-	-
62	*α*-Bisabolol	0.8	1680	1548	-
63	Phytol	0.02	2091	-	-
	Total	69.3%			

[a]Compounds listed in order of elution from HP-1MS column.
[b]Relative retention indices to C_8–C_{24} *n*-alkanes on HP-1MS column.
RI*: Retention indexes in the study of Sardashti et al. [7]
RI**: Retention indexes in the study of Rustaiyan et al. [5]

3. Present Study

The crushed air-dried plant material (45g) was subjected to hydrodistillation for 2.5 h using a Clevenger-type apparatus [10]. Anhydrous sodium sulphate was used and yellowish oil stored (4^{o}C). GC/MS analysis resulted in the identification of 63 compounds (yield 0.4 % v/w), i.e. the 69.3% of the constituents (Table 1). The main components were *cis*-*β*-ocimene (12.9%), linalool (9.5%), *γ*-terpinene (9.0%) and *α*-terpinolene (7.4%), representing the 38.8% of the oil. In general, the essential oil

consisted mostly of monoterpenes (50.8%) with both non-oxygenated and oxygenated alkene structures.

The powder of air-dried leaves of *S. suffruticosa* (10g) was extracted by ethanol, ethyl acetate, chloroform and water, in a ratio of 1:3, using maceration method for 24h at room temperature. The measurement of antioxidant activities has been carried out at the Department of Chemistry, Faculty of Science, University of Sistan & Baluchestan, Iran. The DPPH scavenging activity of the extracts was measured by the bleaching of the purple methanol solution of DPPH [11]. In DPPH assay, the ethanol extract was the most powerful radical scavenger, with the lowest IC_{50} (μg/mL), followed by *n*-hexane and aqueous extracts. Ethyl acetate and chloroform extracts showed a lower antiradical activity (Supporting information).

The ferric reducing antioxidant power (FRAP) assay was carried out according to Benzie and Strain [13] with some modifications. In FRAP assay, the ethanol and ethyl acetate extracts exhibited the highest antioxidant power, with 40.10 ± 4.02 and 36.88 ± 3.28 mmol Fe^{2+}/mg sample, respectively, followed by chloroform and *n*-hexane extracts (Supporting information).

The antioxidant activity was also determined by β-carotene/linoleic acid assay according to the method described by Miller [12]. In β-carotene/linoleic acid assay, similar to FRAP method, chloroform and ethanol extracts showed the highest antioxidant activity expressed as IC_{50} (μg/mL) (Supporting information).

Antibacterial activity was tested using agar-based disk diffusion assay standardized by the National Committee for Clinical Laboratory NCCLS [14], at the Biochemistry Laboratory of Medicinal and Ornamental Plants Research Institute, University of Sistan & Baluchestan, Zahedan, Iran. Antibacterial activity was assessed against six Gram-positive bacterial strains: *Micrococcus roseus* (ATCC516), *Staphylococcus haemolyticus* (ATCC29970), *Staphylococcus saprophyticus* (ATCC15305), *Staphylococcus aureus* (ATCC6538), *Enterococcus faecalis* (ATCC29212), and *Streptococcus pneumoniae* (ATCC6301); and fourteen Gram-negative bacterial strains: *Escherichia coli* (ATCC25922), *Salmonella paratyphi* A (ATCC9150), *S. paratyphi* B (ATCC8759), *S. paratyphi* C (ATCC13428), *Xanthomonas maltophilia* (ATCC13270), *Enterobacter cloaca* (ATCC13047), *Shigella flexneri* (ATCC9380), *Acinetobacter baumannii* (ATCC17978), *Pseudomonas aeruginosa* (ATCC25619), *Hafnia alvei* (ATCC51873), *Edwardsiella tarda* (ATCC23685), *Klebsiella pneumonia*e (ATCC10031), *Proteus mirabilis* (ATCC29906), and *Yersinia enterocolitica* (ATCC35669). Antibacterial activity was evaluated by measuring the inhibition zone diameter and compared with ciprofloxacin and amoxicillin as positive controls.

Antibacterial activity at 24h of *S. suffruticosa* is reported in Table 2. In general, among the extracts, the ethyl acetate and chloroform exhibited the highest antimicrobial activity, compared with conventional antibiotics, particularly against some Gram-positive bacterial strains, *i.e. Staphylococcus haemolyticus* and *Micrococcus roseus*. Against Gram-negative bacteria, all the extracts exhibited a mild inhibitory activity, in comparison with antibiotics.

The reports on the chemical composition of *S. suffruticosa* essential oil are very scant in the literature. Linalool (13.9%) and lavandulyl acetate (11.5%) were the main essential oil constituents detected by Rustaiyan et al. [5], whereas *cis-β*-ocimene (21.4%) and *γ*-terpinene (7.2%) were among the most abundant essential oil components according to Sardashti et al. [7]. Our results are partly in agreeing with both these studies, since our essential oil was rich in *cis-β*-ocimene (12.9%), linalool (9.5%) and *γ*-terpinene (9.0%).

In other species of *Semenovia*, high levels of p-cymene (20.5%) and *cis*-β-ocimene (9.7%) were measured in the essential oil of *S. tragioides* collected from the south east of Iran [6]. In addition, caryophyllene oxide (25.5%) was reported as the major compound in the essential oil of *Semenovia dichotoma* Boiss. [8], as well as geranyl acetate in *Semenovia lasiocarpa* Ashraf & Bhatty [15].

The results obtained by DPPH, FRAP and β-carotene/linoleic acid assays indicated ethanol and ethyl acetate fractions as the most powerful antioxidant leaf extracts of *S. suffruticosa*. Noteworthy, ethyl acetate fraction was also one of the extracts showing the highest antibacterial activity, thus suggesting the presence of highly bioactive (and still unknown) compounds in this fraction. In addition, chloroform and aqueous extracts were active too, thus indicating that components with different polarity may be responsible for the observed antimicrobial activity.

Table 2. Antibacterial activity of different leaf extracts of *S. suffruticosa* against selected Gram-positive and Gram-negative bacterial strains.

Microorganisms	Zone of inhibition diameter (mm)*					
	Plant extract				Antibiotic	
Gram-positive bacteria	**Et**	**E-A**	**Ch**	**Aq**	**C**	**A**
Micrococcus roseus	8	12	10	-	ND	14
Staphylococcus haemolyticus	8	14	15	10	ND	10
Staphylococcus saprophyticus	-	-	-	-	ND	12
Staphylococcus aureus	7	12	10	8	ND	22
Enterococcus faecalis	-	-	-	-	20	ND
Stroptococcus pneumoniae	-	-	-	-	32	ND
Gram-negative bacteria						
Escherichia coli	8	10	-	12	35	ND
Salmonella paratyphi A	-	10	12	7	35	ND
Salmonella paratyphi B	-	-	8	-	27	ND
Salmonella paratyphi C	6	10	10	6	32	ND
Xanthomonas maltophilia	-	-	-	-	32	ND
Enterobacter cloaca	7	7	-	-	40	ND
Shigella flexneri	12	14	12	15	24	ND
Acinetobacter baumannii	-	7	9	-	22	ND
Pseudomonas aeruginosa	13	15	15	14	36	ND
Hafnia alvei	-	-	-	-	32	ND
Edwardsiella tarda	8	-	9	-	42	ND
Klebsiella pneumoniae	7	-	9	-	28	ND
Proteus mirabilis	-	7	8	-	14	ND
Yersinia enterocolitica	8	7	-	-	45	ND

*Et, ethanol extract; E-A, ethyl-acetate extract, Ch, chloroform extract; Aq, aqueous extract; C, ciprofloxacin; A, amoxicillin; -, no inhibition; ND, not determined.

According to our knowledge, no information is currently available on antioxidant and antibacterial activities of *S. suffrutticosa* solvent and aqueous extracts, as well as essential oil.
The methanol extract of *S. tragioides* aerial parts highly inhibited linoleic acid oxidation in the β-carotene/linoleic acid test, though it did not exhibit significant antioxidant activity in DPPH assay. In addition, this fraction only weakly inhibited *Bacillus subtilis* and *Shigella dysenteriae* [9].

S. dichotoma essential oil was active against the Gram-positive bacteria *S. aureus* and *S. saprophyticus*, but showed only moderate inhibitory activity against the Gram-negative bacteria *S. flexnery* and *E. coli* [8]. In another study, the essential oil of *S. frigida* (Boiss.& Hausskn.) Manden. exhibited significant activity against all Gram-positive bacteria, especially *Streptococcus pyogenes* and *Bacillus anthracis*, and moderate inhibitory activity against the Gram-negative bacteria *E. coli* and *K. pneumonia*. Noteworthy, *Pseudomonas aeruginosa* was insensitive to this oil, differently from our extracts [16].

In conclusion, our results have indicated that the extracts of *S. suffruticosa* possess promising antioxidant and antibacterial activities, thus deserving attention and further investigation. In the future, the main constituents of the most active extracts will be characterized, as well as their toxicological profile on eukaryotic cells will be investigated. Not least, a nanotechnology-based drug delivery system will be developed in order to improve efficacy.

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