SUPPLEMENTARY MATERIAL

The genus *Artemisia* L. in the Northern region of Saudi Arabia: Essential oil variability and antibacterial activities.

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Abstract

Four species of the genus *Artemisia* L. (*A. monosperma*, *A. scoparia*, *A. judaica* and *A. sieberi*) growing in the Northern region of Saudi Arabia were investigated with respect to their volatile oil contents. The yield of oil varied between 0.30% and 0.41 %, % (w/w). *A. monosperma* showed the highest number of compounds with 30 components representing 93.78% of the oil composition. However, *A. judaica* showed the lowest number of compounds with only 16 components representing 87.47 % of the essential oil. *A. scoparia* and *A. sieberi* are both composed of 17 components, representing 97.14% and 94.2% of the total oil composition. *A. sieberi* and *A. judaica* were dominated by spathulenol (30.42% and 28.41 % respectively). For *A. monosperma*, butanoic acid (17.87%) was a major component. However, *A. scoparia* was a chemotype of acenaphthene. (83.23%). Essential oil of studied species showed high antibacterial activities against common human pathogens.

Key words: Chemical diversity, *Artemisia* L., Saudi Arabia, Spathulenol, Butanoic acid, acenaphthene, antibacterial activities.

Appendix S1.

Material and methods

Plant material

Artemisia specimens were collected during the full bloom stage (between May and June 2015) from wild plants growing in Wadi Arar (Wadi of the Anizah tribe: 30° 55' 13'' N, 41° 0' 3''E) in the Northern region of Saudi Arabia. The specimens were identified in the department of Biology, College of Sciences, Northern Border University. Voucher specimens were deposited (AS823, AS824, AS825, AS826) in the herbarium of the college of Science.

Isolation of essential oil

The air-dried aerial parts (100 g) were subjected to hydro-distillation during four hours using a Clevenger apparatus. The oil obtained, was dried over anhydrous sodium sulfate yielding 0.3 to 0.41 % (w/w) on the dry weight basis, and stored in dark vial at 4°C before analysis.

Gas chromatography

The gas chromatography (GC) analyses of the oil samples were carried out using Agilent (HP7890 GC) gas chromatograph equipped with a Flame Ionization Detector (FID) and a HP-5MS fused silica column (30 m × 0.32 mm, 0.25 µm film thickness). The sample was injected directly into the column and Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed from 60° to 220° with an increase in rate of 3°/min. The injection volume was 0.2 µL.

Gas chromatography-mass spectrometry

Analyses of the oils were performed out on Agilent mass spectrometer (Model HP 5975 C) coupled to an Agilent gas chromatograph with a HP-5MS capillary column (30 m×250 µm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, and 0.25 µm film thickness). The sample was injected directly in split less mode. Helium was used as the carrier gas (flow rate 0.8 ml/min). The oven temperature was programmed to rise from 60 to 220°C at a rate of 4°C/min; the transfer line temperature was 230°C.

GC (FID) Analyses

Quantitative analyses of the essential oils was carried out using an Agilent 6890 N gas chromatograph equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. Nonpolar HP-5 MS column (30 m x 250 µm, 0.25 µm film phenyl methyl siloxane) was used. The carrier gas was helium with a flow rate of 1mL/min. The split ratio was 200:1. All analyses were performed using the following temperature ramp: oven kept isothermally at 50 °C for 2 min, increased from 50 to 280°C at the rate of 5°C/min and then kept at 280 °C for 3 min. Injector and detector temperatures were held at 270 and 320°C, respectively.

Antimicrobial activities

Essential oils from the 4 studied species were tested against a panel of microorganisms, including Gram-positive and Gram-negative bacteria. Microorganisms were provided from the culture collection of the Laboratory of Natural Substances, at the National Institute of Research and Physico-Chemical Analyses.

The antimicrobial activities of essential oil were tested against 5 bacterial strains, including two Gram-negative bacteria; *Escherichia coli* (strain ATCC 8739) and *Salmonella typhimurium* (strain ATCC 14028) and three Gram-positive bacteria; *staphylococcus aureus* (strain ATCC 6538), *Streptococcus agalactiae*; and *Enterococcus feacium* (strain ATCC 19434).

The antimicrobial activity of essential oils was assessed by the paper-disk diffusion method (NCCLS, 1997). Briefly, a suspension of the tested microorganisms was spread on the solid Mueller–Hinton media plates. The essential oils were dissolved in dimethylsulfoxide (DMSO) (1:1; v: v). 15µL of each sample were deposited per filter discs individually and incubated with tested microorganisms. Disc with DMSO alone were used as a negative control. Ampicillin (USP grade, BIOMATIK, Germany) (15µg/disc) was used as a positive reference to compare the sensitivity of strain toward this antibiotic. The Petri dishes were kept at 4°C/2h. Then, these plates were incubated at 37°C. Antimicrobial activities were evaluated by measuring the diameter of the growth inhibition zones in mm (including disc diameter of 6mm) for the test organisms. When the inhibitory zone diameter is lower or equal to 6mm, the sample tested was considered as not active.

Identification of components

The identification of constituents was performed on the basis of retention index (RI), relative to the homologous series of n-alkanes, C8-C24 under same analytical conditions and by matching their recorded mass spectra with the MS library (NIST/Pfleger/Wiley) and available literature (Adams, 2007).

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Appendix S2. Table 1:Chemical composition of4 species of genus *Artemisia L.* essential oil from Northern region of Saudi Arabia.

Compounds		RI ^b	<i>A. monosperma</i>	<i>A.scoparia</i>	<i>A.judaica</i>	<i>A. seiberi</i>
1	<u>Butanoic acid</u>	763	<u>17.87</u>	<u>6.14</u>	---	---
2	β-pinene	938	1.1	0.37	---	---
3	Benzene, 1,2,3-trimethyl-	985	---	---	2.5	0.85
4	Benzene, 1-methyl-3-(1-methylethyl)	1010	---	---	---	0.85
5	Benzene, 1,3,5-trimethyl	1013	---	---	---	0.92
6	β-linalool	1101	2.25	---	5.08	0.31
7	3,7-Dimethyl-6-octen-1-ol	1153	0.8	---	---	---
8	trans-Geraniol	1235	---	0.35	---	---
9	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)	1241	0.48	1.33	5.08	5.21
10	2,6-Octadien-1-ol, 3,7-dimthyl	1254	1.08	---	---	---
11	α-citral	1265	1.12	---	---	---
12	Benzene, 2,4-pentadiynyl	1279	---	0.61	1.39	---
13	Thymol	1293	----	----	----	0.64
14	Berkheyaradulen	1377	1.57	0.47	---	---
15	Methyl cinnamate	1379	---	---	0.5	---
16	<u>Geranayl acetate</u>	1382	<u>6.2</u>	---	---	---
17	1-Phenyl 1-(1-propynyl) cyclopropan	1384	----	0.38	---	---
18	Isohomogenol	1401	3.27	---	---	---

Table 1 (continued)

Compounds		RI ^b	<i>A.</i> <i>monosperma</i>	<i>A.scoparia</i>	<i>A.judaica</i>	<i>A. seiberi</i>
19	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	1405	5.8	0.23	1.33	----
20	Benzene, 1,2-dimethoxy-4-(1-propenyl)	1408	4.64	0.73	---	---
21	Longifolene	1416	1.66	0.22	---	---
22	Caryophyllene	1417	2.34	1.6	---	---
23	Isolongifolene	1419	----	---	---	0.38
24	<u>Acenaphthene</u>	1425	2.38	<u>83.23</u>	<u>9.18</u>	<u>11.34</u>
25	γ- Elemene	1433	0.64	---	0.3	---
26	Davana ether	1450	---	---	4.11	5.84
27	α-Caryophyllene	1462	0.66	0.2	---	---
28	<u>Clovene</u>	1465	<u>6.29</u>	---	---	---
29	Aristolene	1472	5.8	---	---	---
30	γ-curcumene	1483	4.76	----	---	---
31	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	1486	5.75	0.64	---	---
32	2-Isopropeny 1-6-isopropyl-3-methyl-3-vinyl cyclohexanone	1489	2.99	---	---	---
33	Methyl trans-Isoeugenol	1492	3.27	---	---	---
34	γ-Selinene	1494	---	0.1	---	---

Table 1 (continued)

	Compounds ^a	RI ^b	<i>A. monosperma</i>	<i>A.scoparia</i>	<i>A.judaica</i>	<i>A. seiberi</i>
35	7H-Purine, 2-methoxy-7-methyl-6-(2-propenyloxy)-	1505	0.65	---	---	---
36	12-Norcyercene-B	1558	---	---	---	0.18
37	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-	1567	---	---	0.91	0.94
38	<u>Spathulenol</u>	1578	4.69	---	<u>28.42</u>	<u>30.41</u>
39	Isospathulenol	1628	---	0.23	1.27	2.76
40	Murolol	1644	1.72	---	---	---
41	Dehydroxy-isocalamendiol	1645	1.67	---	---	---
42	α -cadinol	1649	0.82	---	---	---
43	Isoelemicin	1650	---	0.31	---	---
44	Apiol	1677	---	---	0.9	0.76
45	α -bisabolol	1685	0.62	---	---	---
46	α -Bisabolene epoxide	1814	---	----	0.49	0.49
47	Cyercene 4	1832	---	---	1.05	---
48	Isocalamendiol	1853	0.89	---	---	---
49	Longifolenaldehyde	1876	----	---	---	0.27
50	<u>2-Propenoic acid, 3-phenyl-ethyl ester</u>	2149	---	---	<u>22.46</u>	<u>20.71</u>

Table 1 (continued)

	<i>A. monosperma</i>	<i>A.scoparia</i>	<i>A.judaica</i>	<i>A. seiberi</i>
Monoterpenoids (hydrocarbons, Aldehydes, Alcohols....)	6.83	2.05	33.12	26.87
Sesquiterpenoidhydrocarbons	3.95	83.7	9.18	22.68
Oxygenated Sesquiterpenoids	43.62	2.99	34.59	40.15
Phenylpropanoid	7.91	1.04	0	0
Other compounds	31.47	7.36	10.58	4.5
Total	93.78	97.14	87.47	94.2

^a Compounds are listed in order of their elution from HP-5MS capillary column

^b Linear retention index on HP-5MS capillary column, experimentally determined using homologous series of C₈- C₂₄ alkanes.

Appendix S3.

Table 2. Main volatile constituents of the essential oils from *Artemisia* ssp. reported in the literature.

Taxa	Parts and yields	Origin	Compounds	Reference
<i>A. monosperma</i>	Stems	China	1,8-cineole (34.56%) and camphor (16.65%)	Zhu et al., 2013
<i>A. monosperma</i>	Leaves and stems	Saudi Arabia	β -pinene (50.3%), a-terpinolene (10.0%), limonene (5.4%)	Khan et al., 2012
<i>A. monosperma</i>	Arial parts	Saudi Arabia	β- Citronellyl propanoate (21.5%), Geraniol (12.12%), γ cadinene (15.8%)	Sobahi et Abdel-Mogib 2001
<i>A. scoparia</i>	Arial parts	Iran	α-thujone (81.7%), β-thujone (14.5%)	Negahban et al., 2006
<i>A. scoparia</i>	Leaves and roots	India	γ-terpinene (11.1%)	Joshi et al., 2010
<i>A. scoparia</i>	Arial parts	Tajikistan	β-pinene (21.3)	Sharopov and Setzer al., 2011
<i>A. judaica</i>	Arial parts	Egypt	Camphor (34.5)	Sallam et al., 2011
<i>A. judaica</i> L.	Arial parts	Lybia	piperitone (30.21%)	Janačković et al., 2015
<i>A. judaica</i> L.	Arial parts	Sinai Peninsula, Egypt	Piperitone (32.4%), camphor (20.6%)	Putievsky et al., 1992.
<i>A. sieberi</i>	Arial parts	Iran	α-thujone (10.5%), β-thujone (19.8%)	Negahban et al., 2007
<i>A. sieberi</i>	Arial parts	Iran	Camphor (54.7%), camphene (11.7%), 1,8-cineol (9.9%)	Ensieh et al., 2007
<i>A. sieberi</i>	Arial parts	Iran	β -thujone (23%), camphor (19.5%) and α -thujone (15%)	Khosravi et al., 2011