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Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils

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ABSTRACT

The chemical composition of essential oils isolated from aerial parts of seven wild sages from Western Canada – *Artemisia absinthium* L., *Artemisia biennis* Willd., *Artemisia cana* Pursh, *Artemisia dracunculus* L., *Artemisia frigida* Willd., *Artemisia longifolia* Nutt. and *Artemisia ludoviciana* Nutt., was investigated by GC–MS. A total of 110 components were identified accounting for 71.0–98.8% of the oil composition. High contents of 1,8-cineole (21.5–27.6%) and camphor (15.9–37.3%) were found in *Artemisia cana*, *A. frigida*, *A. longifolia* and *A. ludoviciana* oils. The oil of *A. ludoviciana* was also characterized by a high content of oxygenated sesquiterpenes with a 5-ethenyltetrahydro-5-methyl-2-furanyl moiety, of which davanone (11.5%) was the main component identified. *A. absinthium* oil was characterized by high amounts of myrcene (10.8%), *trans*-thujone (10.1%) and *trans*-sabinyl acetate (26.4%). *A. biennis* yielded an oil rich in (*Z*)-beta-ocimene (34.7%), (*E*)-beta-farnesene (40.0%) and the acetylenes (11.0%) (*Z*)- and (*E*)-en-yn-dicycloethers. *A. dracunculus* oil contained predominantly phenylpropanoids such as methyl chavicol (16.2%) and methyl eugenol (35.8%). *Artemisia* oils had inhibitory effects on the growth of bacteria (*Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*), yeasts (*Candida albicans*, *Cryptococcus neoformans*), dermatophytes (*Trichophyton rubrum*, *Microsporum canis*, and *Microsporum gypseum*), *Fonsecaea pedrosoi* and *Aspergillus niger*. *A. biennis* oil was the most active against dermatophytes, *Cryptococcus neoformans*, *Fonsecaea pedrosoi* and *Aspergillus niger*, and *A. absinthium* oil the most active against *Staphylococcus* strains. In addition, antioxidant (beta-carotene/linoleate model) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were determined, and weak activities were found for these oils.

1. Introduction

Aromatic plants are frequently used in traditional medicine as antimicrobial agents and their essential oils, mixtures of natural volatile compounds isolated by steam distillation, have been known since antiquity to possess antibacterial and antifungal properties.

Previous works have suggested that several essential oils showed important antimicrobial activity against bacteria, yeasts, dermatophyte and *Aspergillus* strains (Griffin et al., 1999; Rios et al., 1988; Janssen et al., 1987), and have therapeutic potential, mainly in diseases involving mucosal, cutaneous and respiratory tract infections. The major constituents of many of these oils are phenolic compounds (terpenoids and phenylpropanoids) like thymol, carvacrol or eugenol, of which antimicrobial and antioxidant activities are well documented (Lawrence, 2005).

Nevertheless, aromatic plants producing non-phenolic essential oils, like some *Artemisia* species, are also used as spices and in folk remedies as antiseptics. Powdered leaves of *A. absinthium*, *A. biennis*, *A. frigida* and *A. ludoviciana* have been applied externally in salves and washes by North American native people for treating sores and wounds and, internally to treat chest infections (Kershaw, 2000).

Antioxidants retard oxidation and are sometimes added to meat and poultry products to prevent or slow oxidative degradation of fats. Antioxidant agents are effective due to different mechanisms such as free radical scavenging, chelating of pro-oxidant metal ions or quenching singlet-oxygen formation. The aromatic leaves of *A. frigida* and *A. dracunculus* (tarragon) have been also used as spice and to preserve meat (Kershaw, 2000). These species might be a source of natural antioxidants and antimicrobials.

Artemisia genus includes 15 perennial aromatic herbs and shrubs that grow wild in dry or semi-dry habitats in the province of Alberta, Canada (Moss, 1983). In the literature, there are only a few papers dealing with the essential oil composition and properties of some of these species but they are from different geographic origins. Analysis of the chemical composition of *A. absinthium* oils extracted from plants grown in USA showed beta-thujone (17.5–42.3%) and *cis*-sabinyl acetate (15.1-53.4%) as

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the main components (Lawrence, 1992). Recently, the chemical composition, antimicrobial and antioxidant activities of *A. absinthium* and *A. dracunculus* oils from Turkey, were investigated. The results showed chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%), and caryophyllene oxide (4.3%) as the main components in *A. absinthium* oil. *A. dracunculus* oil contained mainly (*Z*)-anethole (81.0%), (*Z*)-beta-ocimene (6.5%), (*E*)-beta-ocimene (3.1%), limonene (3.0%) and methyl eugenol (1.8%). The oils had inhibitory effects on the growth of bacteria and fungi tested and also showed moderate to weak antioxidant activities, respectively (Kordali et al., 2005a,b).

Artemisia dracunculus ethanolic extract significantly reduced hyperglycemia in mice with chemically induced insulin deficiency and diabetes and, an activity-guided fractionation revealed six active polyphenolic compounds (Schmidt et al., 2007). The essential oil was screened in guinea pig and rat plasma in order to assess antiplatelet activity and inhibition of clot retraction. *A. dracunculus* oil showed high antiplatelet activity against ADP, arachidonic acid and the thromboxane A [2] agonist U46619 and a good ability to destabilize clot retraction, which was attributed to the presence of phenylpropanoids (Tognolini et al., 2006).

Volatiles from the leaves of *A. ludoviciana* from USA and Mexico were previously identified by gas chromatography–mass spectrometry as alpha-pinene, camphene, 1,8-cineole, camphor, borneol, nonanal, linalool, carvacrol and *para*-alpha-dimethylbenzyl alcohol. Nonanal isolated from the oil showed symptomatic relief of induced diarrhea and a delay of the intestinal transit activity in mice (Zavala-Sánchez et al., 2002; Blust and Hopkins, 1987; Domínguez and Cárdenas, 1975). Previous research showed that alpha-pinene (10.2%), 1,8-cineole (10.1%), artemisia ketone (11.4%) and camphor (24.6%) were the main components of the essential oil of *A. biennis* grown in Iran (Nematollahi et al., 2006).

The neutral pentane extract from ground leaves and stems of *A. cana* collected in USA was vacuum short path distilled and yielded 0.4% (dry basis) of a fragrant yellow oil. The oil was fractionated by chromatography and the monoterpenes, santolina triene, artemisia triene, artemiseole, 1,8-cineole, camphor, alpha-pinene, lyratal, isolyratol, lyratol, chrysanthemol, *trans*-chrysanthemal, chrysanthemyl acetate, fraganyl acetate, fraganol, 2-isopropenyl-5-methyl-hexa-*trans*-3,5-dien-1-ol, 2,2-dimethyl-6-isopropenyl-2*H*-pyran, 2,3-dimethyl-6-isopropyl-4*H*-pyran, 2,4-diisopropenyl-5*H*-furan and rothrockene, were identified by ^{1}H NMR, ^{13}C NMR and IR spectroscopy (Gunawardena et al., 2002).

The essential oil of *Artemisia frigida* aerial parts was obtained by steam distillation of plants collected in Russia, Kazakhstan and Mongolia, and ranged from 0.1% to 0.7% (dry basis). The chemical composition of the oils was very similar, camphor, 1,8-cineole and borneol being the main components. The essential oil obtained from plants grown in Kazakhstan also contained a significant amount of *trans*-thujone and thujanols. The oil yield of different plant parts was also determined: flowers (0.6%), leaves (0.1%), stems (0.001%) and roots (0.1%) (Bodoev et al., 2000; Atazhanova et al., 1999; Satar, 1986). The composition of *A. longifolia* essential oil has not been investigated previously.

To the best of the author's knowledge, a detailed investigation of the aroma volatiles of *Artemisia* species grown in Western Canada has not yet been undertaken. Also, the antimicrobial and antioxidant effects of *A. biennis*, *A. cana*, *A. frigida*, *A. longifolia* and *A. ludoviciana* oils have not been reported to date. Therefore, the aim of the present work is to determine the chemical profile of *Artemisia* oils obtained from seven wild sages grown in Alberta, Canada by gas chromatography–mass spectrometry (GC–MS), and describe their antimicrobial and antioxidant activities.

2. Results and discussion

One hundred-ten volatile components were identified on the basis of their mass spectra characteristics, retention indices and co-chromatography with available standards using two columns of different polarities. The percentage of each component within the essential oils was determined on HP-5 and DB-Wax columns and is presented in Table 1.

The volatile components identified accounted for 71.0–98.8% of the oils composition. The analyzed oils can be classified in four groups according to their chemical make-up. *A. cana*, *A. frigida*, *A. longifolia*, and *A. ludoviciana* oils contained high amounts of oxygenated monoterpenes (48.8–75.6%), with 1,8-cineole and camphor being the most representative. Camphene and alpha-pinene were the dominant components in the monoterpene hydrocarbon fractions. The latter oil was also characterized by a high content of the oxygenated sesquiterpenes (17.8%). Davanone along with other sesquiterpenes containing a 5-ethenyltetrahydro-5-methyl-2-furanyl moiety were present in this fraction. Approximately 75.0% of *A. ludoviciana* oil composition was characterized. The remaining unidentified components were mainly sesquiterpenes.

Previous research showed that bornane derivatives (camphor, borneol and bornyl acetate) and 1,8-cineole are major characteristic components of many species of *Artemisia* genus, such as: *A. annua*, *A. vulgares*, *A. diffusa*, *A. santonicum*, *A. spicigera*, *A. afra*, *A. asiatica*, *A. austriaca* and *A. pedemontana* (Perez-Alonso et al., 2003; Kordali et al., 2005a). Borneol (2.3-8.1%) and bornyl acetate (0.1-2.8%) were also identified in the four *Artemisia* oils previously mentioned.

Artemisia absinthium oil was characterized by high amounts of myrcene (10.8%), *trans*-thujone (10.1%) and *trans*-sabinyl acetate (26.4%). Approximately 71.0% of *A. absinthium* oil composition was identified. The remaining unidentified components were monoterpene esters and sesquiterpenes. Phenylpropanoid compounds comprised 52.2% of *A. dracunculus* oil, with methyl chavicol and methyl eugenol being the most representative constituents. *A. biennis* yielded an oil rich in (*Z*)-beta-ocimene (34.7%), (*E*)-beta-farnesene (40.0%) and the acetylenes (11.0%) (*Z*)- and (*E*)-en-yn-dicycloethers.

The results of the quantitative analysis for the recovery of essential oil from the plant material are also presented in Table 1. The highest oil contents were found for *A. cana* (1.3%) and *A. frigida* (1.5%). The oil yield from the aerial parts of *A. absinthium*, *A. biennis, A. dracunculus, A. longifolia* and *A. ludoviciana* were variable and ranged from 0.3% to 0.5%.

A susceptibility screening test using the agar-well diffusion method was employed to evaluate the activity of *Artemisia* oils against 10 microorganisms of clinical importance. The results are presented in Table 2.

The diffusion method is generally used as a preliminary screening for antimicrobial activity prior to more detailed studies (Hammer et al., 1999). The usefulness of this method is limited to the generation of preliminary quantitative data only, as the hydrophobic nature of most essential oils and plant extracts components prevents their uniform diffusion through the agar medium. Based on this, it is recommended to use an emulsifier such as DMSO, to assure contact between the microorganism and the possible antimicrobial agent (Hili et al., 1997).

The results of antimicrobial activity showed that the oils had varying degrees of growth inhibition against the microorganisms tested. All essential oils were active against the microorganisms assayed, however, *Escherichia coli* and *Candida albicans* showed less susceptibility to the essential oils. *A. biennis* oil was the most active against dermatophytes, *Cryptococcus neoformans*, *Fonsecaea*

Table 1
Chemical composition of *Artemisia* species oils and their oil yield

No	Identification	R_t	R_t	Area (%)						
				A. absinthium	*A. biennis*	*A. cana*	*A. dracunculus*	*A. frigida*	*A. longifolia*	*A. ludoviciana*
1	3-Methyl-2-buten-1-ol	773	1325				0.1			
2	Hexanal	801	1078	0.2	0.1	t (⩽0.05)	t	t	t	0.1
3	(2*E*)-Hexenal	854	1205	0.3						0.1
4	*n*-Hexanol	868	1359	0.2					t	
5	Heptanal	903	1180	0.1						
6	Santolina triene	909	1031		4.9	0.8				0.2
7	2,5-Diethenyl-2-methyl-tetrahydrofuran	918	1154							0.3
8	Tricyclene	927	1002	t		0.3		0.2	0.2	0.1
9	alpha-Thujene	931	1022	0.1	t	0.1	t	0.1	0.3	0.1
10	alpha-Pinene	938	1016	0.3	0.2	3.5	0.2	1.5	3.2	1.6
11	alpha-Fenchene	952	1049				0.1			
12	Camphene	953	1057	0.4		4.9	t	4.1	3.3	2.3
13	Thuja-2,4(10)-diene	959	1117			t				0.1
14	Benzaldehyde	964	1501		0.2				t	t
15	Sabinene	977	1114	1.6	0.1	0.1	t	0.2	1.2	0.6
16	Artemiseole	978	1179			0.5				0.8
17	beta-Pinene	981	1096	0.1	t	0.9	0.1	0.4	0.7	0.4
18	6-Methyl-5-hepten-2-one	989	1332		t				t	t
19	**Myrcene**	992	1164	**10.8**			0.6	1.2		
20	Mesitylene	996	1269					0.1	0.2	0.1
21	*n*-Octanal	1004	1283	0.1				t		
22	alpha-Phellandrene	1006	1158	0.8			0.9		0.2	
23	delta-3-Carene	1013	1142				0.1			
24	alpha-Terpinene	1020	1172	0.1	0.1	0.5	0.3	0.7	0.5	0.4
25	*para*-Cymene	1028	1261	1.2	0.8	0.9	0.2	0.5	3.2	0.8
26	Limonene	1032	1188	0.2	0.2	0.4	1.1	0.2	0.3	0.2
27	beta-Phellandrene	1032	1195	t	t		3.4			
28	**1,8-Cineole**	1035	1196	1.0		**21.5**		**25.1**	**27.6**	**22.0**
29	(***Z***)-**beta-Ocimene**	1041	1235	1.5	**34.7**		3.1			
30	(*E*)-beta-Ocimene	1051	1250	0.5	0.7		9.3			
31	*cis*-Arbusculone	1055	1436							0.2
32	gamma-Terpinene	1062	1238	0.3	0.8	0.9	0.1	1.3	1.2	0.8
33	*cis*-Sabinene hydrate	1070	1466		0.2			0.2	0.9	
34	Artemisia alcohol	1085	1510		0.1					
35	**Terpinolene**	1091	1274	0.1			**19.1**			
36	*trans*-Sabinene hydrate	1099	1547		0.4				0.7	
37	Linalool	1100	1553	4.6			0.2			
38	*n*-Nonanal	1105	1386	0.2						
39	Filifolone	1105	1424			1.9		3.3	2.0	1.8
40	*cis*-Thujone	1108	1401	0.5						
41	1,3,8-*para*-Menthatriene	1114	1409				0.1			
42	*trans*-Thujone	1120	1423	**10.1**						
43	Isophorone	1123	1570						0.7	0.3
44	*cis*-*para*-Menth-2-en-1-ol	1124	1559	0.3			t		0.8	
45	Chrysanthemone	1128	1487			2.8		7.4	2.0	1.3
46	Allo-ocimene	1132	1368	t	0.8					
47	(*Z*)-Myroxide	1136	1463	2.4	0.1					
48	*trans*-Pinocarveol	1143	1645			0.6			0.8	
49	*trans*-*para*-Menth-2-en-1-ol	1145	1625			0.1	t			
50	(*E*)-Myroxide	1146	1481	0.1						
51	**Camphor**	1149	1490			**37.3**		**20.6**	**18.5**	**15.9**
52	Hexyl isobutanoate	1152	1296	t						
53	Borneol	1168	1692			3.2		8.1	5.5	2.3
54	Terpinen-4-ol	1180	1593	1.7	1.3	2.3		3.5	3.9	2.2
55	*para*-Cymen-8-ol	1187	1844				0.8		0.2	0.2
56	*trans*-Isocarveol	1189	1792			t				
57	alpha-Terpineol	1191	1693		0.2	1.2		1.1	1.1	0.7
58	Myrtenol	1197	1786			0.3			0.3	1.0
59	**Methyl chavicol**	1199	1657				**16.2**			
60	*n*-Decanal	1207	1490	0.2						
61	*trans*-Piperitol	1208	1743			0.2		0.1		t
62	*trans*-Carveol	1220	1831			0.1				0.2
63	Nerol	1230	1799	0.3						
64	Nor-davanone	1233	1640							1.0
65	Carvone	1247	1712			0.1			0.2	t
66	Geraniol	1257	1850	0.1						
67	Piperitone	1257	1702							t
68	*cis*-Chrysanthemyl acetate	1264	1561					0.3		
69	*cis*-Verbenyl acetate	1287	1655		0.2					
70	Bornyl acetate	1287	1567			0.7		2.8	2.8	0.1
71	***trans*-Sabinyl acetate**	1295	1642	**26.4**						
72	Nor-chrysanthemic acid methyl ester	1348	2434			1.7		3.1		
73	Eugenol	1359	2153				0.2	0.2	0.1	
74	*cis*-Carvyl acetate	1366	1727						t	

Table 1 (Continued)

No	Identification	R_t	R_t	Area (%)						
				A. absinthium	*A. biennis*	*A. cana*	*A. dracunculus*	*A. frigida*	*A. longifolia*	*A. ludoviciana*
75	alpha-Copaene	1377	1476	0.1			0.2		0.2	
76	beta-Elemene	1393	1575	0.2				0.7		
77	(***Z***)-**Jasmone**	1398	1923					0.2		
78	**Methyl eugenol**	1407	2006				**35.8**			
79	*cis*, Threo-davanafuran	1417	1769							0.3
80	beta-Caryophyllene	1420	1576	0.9	0.6		0.1	0.1		t
81	*para*-Menth-1-en-9-ol acetate	1423	1808					t		
82	(*Z*)-beta-Farnesene	1445	1632	0.1						
83	(***E***)-**beta-Farnesene**	1461	1664		**40.0**					
84	Germacrene D	1482	1686		0.4		1.4			
85	ar-Curcumene	1484	1761				0.2			
86	Davana ether (isomer)	1494	1884							1.0
87	Bicyclogermacrene	1496	1710				0.6		0.3	
88	(*E*,*E*)-alpha-Farnesene	1509	1744				0.2			
89	Davana ether (isomer)	1514	1915							2.9
90	Cubebol	1516	1878						0.2	
91	delta-Cadinene	1525	1741				0.2		0.2	
92	Artedouglasia oxide A	1536	1999							0.4
93	Artedouglasia oxide D	1561	2064							0.1
94	Davanone B	1566	1968							0.2
95	(*E*)-Nerolidol	1566	2042	0.4			0.2			
96	Spathulenol	1578	2108			0.1	0.4		0.4	
97	Artedouglasia oxide B	1583	1970							0.4
98	Caryophyllene oxide	1583	1953	0.2	0.2					
99	Neryl isovalerate	1586	1878	1.8						
100	beta-Copaen-4-alpha-ol	1587	2133			t				
101	**Davanone**	1589	2023							**11.5**
102	epi-alpha-Cadinol	1642	2157				0.1			
103	epi-alpha-Muurolol	1643	2173				t		1.9	
104	beta-Eudesmol	1650	2209						1.0	
105	alpha-Cadinol	1655	2217				0.1		0.1	
106	alpha-Bisabolol	1684	2208	0.2						
107	Chamazulene	1728	2352	0.3						
108	(2*Z*,6*E*)-Farnesyl acetate	1819	1715		0.5					
109	(***Z***)-**en-yn-Dicycloether**	1881	2845		**10.0**					
110	(*E*)-en-yn-Dicycloether	1894	2985		1.0					
	Monoterpene hydrocarbons			18.0	43.3	13.3	38.7	10.4	14.3	7.6
	Oxygenated monoterpenes			49.3	2.5	74.5	1.0	75.6	68.0	48.8
	Sesquiterpene hydrocarbons			1.6	41.0		2.9	0.8	0.7	
	Oxygenated sesquiterpenes			0.8	0.7	0.1	0.8		3.6	17.8
	Phenylpropanoid						52.2	0.2	0.1	
	Acetylenes				11.0					
	Other compounds			1.3	0.3	trace	0.1	0.3	0.2	0.8
	Total			71.0	98.8	87.9	95.7	87.3	86.9	75.0
	Oil yield (%, w/w – dry basis)			0.5	0.3	1.3	0.4	1.5	0.5	0.4

R_t = Kovat's retention index; *t* = trace ($\leqslant$0.05%).

Table 2
Growth inhibition zones of microorganisms in millimeters (±standard deviation)

Microorganisms	Inhibition zone diameter (mm)									
	A. absinthium	*A. biennis*	*A. cana*	*A. dracunculus*	*A. frigida*	*A. longifolia*	*A. ludoviciana*	Amphotericin B	Methicilin	Vancomycin
Staphylococcus aureus	25 ± 1.4	20 ± 1.4	12 ± 0.7	10 ± 0.0	11 ± 0.7	14 ± 0.7	13 ± 0.7	–	8 ± 0.5	18 ± 1.0
Staphylococcus epidermidis	20 ± 0.7	5 ± 0.7	8 ± 0.7	10 ± 0.7	10 ± 0.7	11 ± 0.7	10 ± 0.7	–	20 ± 1.0	21 ± 1.0
Escherichia coli	5 ± 0.0	5 ± 0.0	9 ± 0.7	8 ± 0.0	8 ± 0.7	9 ± 0.7	8 ± 0.7	–	–	–
Candida albicans	13 ± 0.7	13 ± 0.7	10 ± 0.0	10 ± 0.7	10 ± 0.7	10 ± 0.7	10 ± 0.7	20 ± 0.5	–	–
Cryptococcus neoformans	14 ± 1.4	20 ± 0.0	14 ± 0.7	13 ± 1.4	13 ± 0.7	12 ± 0.7	12 ± 0.0	20 ±1.0	–	–
Fonsecaea pedrosoi	16 ± 0.7	25 ± 1.4	20 ± 0.0	15 ± 1.4	17 ± 0.7	16 ± 0.7	15 ± 0.7	18 ± 2.0	–	–
Trichophyton rubrum	27 ± 1.4	40 ± 1.4	20 ± 0.0	20 ± 2.1	20 ± 1.4	33 ± 2.1	40 ± 1.4	20 ± 1.0	–	–
Microsporum canis	28 ± 1.4	40 ± 2.1	10 ± 0.0	15 ± 1.4	29 ± 1.4	25 ± 1.4	35 ± 2.1	19 ± 1.0	–	–
Microsporum gypseum	17 ± 0.7	28 ± 1.4	10 ± 0.0	16 ± 1.4	16 ± 0.7	26 ± 1.4	30 ± 1.4	17 ± 0.5	–	–
Aspergillus niger	18 ± 0.7	25 ± 1.4	13 ± 0.7	13 ± 1.4	13 ± 0.7	16 ± 1.4	17 ± 0.7	18 ± 1.0	–	–

pedrosoi and *Aspergillus niger* and, *A. absinthium* oil the most active against *Staphylococcus* strains.

Oxygenated monoterpenes such as 1,8-cineole, camphor, terpinen-4-ol, linalool, alpha-terpineol and borneol, which are representative components in some oils investigated were reported to exhibit antimicrobial activity (Pattnaik et al., 1997; Carson and Riley, 1995). Other *Artemisia* oils rich in camphor and 1,8-cineole were previously demonstrated to have potent antimicrobial activities *in vitro* (Kordali et al., 2005a,b). It has also been demonstrated that (*Z*) and (*E*)-en-yn-dicycloethers exhibited antifungal activity

(Breinlich and Scharnagel, 1968). However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antimicrobial activity exhibited. Possible synergistic and antagonistic effect of compounds in the oil should also be taken into consideration.

The potential antioxidant activity of the oils was determined on the basis of the scavenging activity of the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and inhibition of the coupled oxidation of linoleic acid and beta-carotene (Fig. 1). *Artemisia* essential oils exhibited weak antioxidant abilities for preventing the linoleic acid oxidation and to reduce DPPH radicals. Phenolic compounds such as thymol and carvacrol, both present in Oregano oil, and BHT show potent antioxidant and DPPH radical scavenging activities (Ruberto and Baratta, 2000). *Artemisia* oils are markedly rich in non-phenolic components.

To our knowledge, this is the first study to provide data that these essential oils possess antibacterial and antifungal activities, with weak antioxidant abilities. Also, the chemical composition of these essential oils is described in detail.

The oils obtained from *Artemisia* species investigated are quite interesting from a pharmaceutical standpoint because of their antifungal properties. For instance, *Microsporum gypseum* is known to cause skin, hair and scalp infections in humans and domestic animals (Saikia et al., 2001) and thus *A. biennis* oil can be a good candidate for medicated skin and hair care formulations. Further investigations are in progress to compare the levels of activity in *Artemisia* oils and some of their constituents with the objective of identifying plant substances for future antifungal formulations.

3. Experimental

The wild growing plants of the Central Alberta Prairies – Western Canada, were collected in September 2004, just before the flower-buds open. Voucher specimens have been deposited at the Herbarium of Vascular Plants, University of Alberta, under the voucher numbers: *Artemisia absinthium* L. (No. 72906), *Artemisia biennis* Willd. (No. 16973), *Artemisia cana* Pursh (No. 68872), *Artemisia dracunculus* L. (No. 72907), *Artemisia frigida* Willd. (No. 17075), *Artemisia longifolia* Nutt. (No. 73464) and *Artemisia ludoviciana* Nutt. (No. 75255).

3.1. Isolation of essential oils

Aerial parts were air-dried in shade at room temperature and the leafless woody stalks discarded. The dried sample was subsequently comminuted using a hammer mill. The moisture contents were kept below 10%. The oils were extracted from 100.0 g of plant material by hydrodistillation using a Clevenger-type apparatus until total recovery of oil (ca. 8 h). The oils were dried over anhydrous sodium sulphate and stored at 5 °C, for further analysis.

3.2. Identification of oil components

The oils were analyzed by GC–MS and GC using two different capillary columns (HP-5 and DB-Wax). The identification of single components was performed by comparison of retention indices on both polar and non-polar columns, mass spectra and co-injection of authentic standards (Adams, 2001). A standard solution of *n*-alkanes (C_7–C_{26}) was used to obtain the retention indices.

Mass spectra were obtained on an Agilent model 5973 MSD mass spectrometer, coupled directly to Agilent 6890 gas chromatograph fitted with a HP-5MS (5%phenyl 95%polydimethylsiloxane, J&W Scientific, USA), 0.25 mm i.d. × 30 m, 0.25 micron film thickness, fused silica capillary column. Complementary analyses were performed on a DB-Wax (polyethylene glycol, J&W Scientific, USA), 0.32 mm i.d. × 30 m, 0.25 micron film thickness, fused silica capillary column. The GC/MSD was operated under the following conditions: HP-5MS column-injector temperature 250 °C; transfer line 260 °C; oven temperature programmed 60–260 °C at 3 °C/min; final hold time 30 min, solvent delay 2 min; DB-Wax column-injector temperature 250 °C; transfer line 240 °C; oven temperature programmed 40 °C for 5 min then 3 °C/min to 220 °C; final hold time 30 min, solvent delay 3 min. Helium was the carrier gas at 1 ml/min; injection volume of 1 μl (1% soln. in CH_2Cl_2), split 1:20. All mass spectra were acquired in EI mode (scan range *m/z* 40–400, ionization energy 70 eV). Ion source and MS quadrupole temperatures were 230 °C and 150 °C, respectively. Quantification was performed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector under the same conditions described above, detector temperatures 280 °C and 250 °C, respectively. The concentration of each component was calculated as

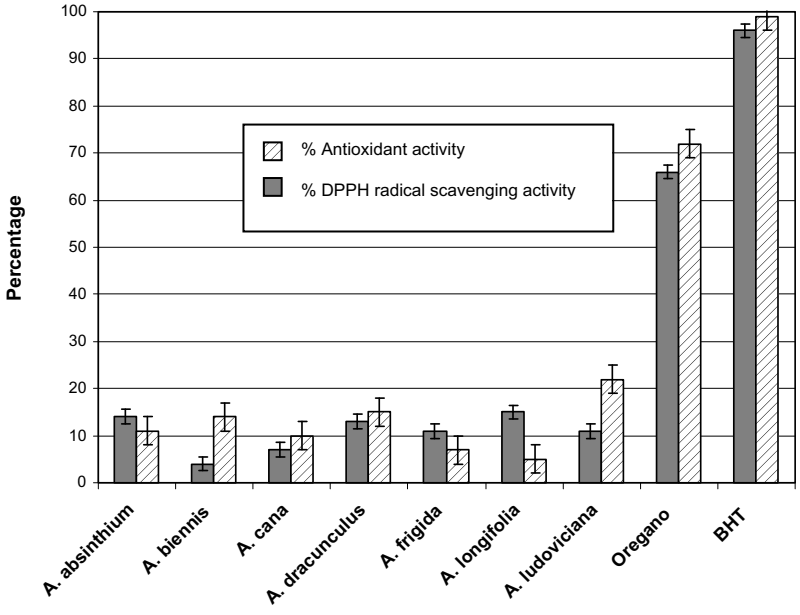

Fig. 1. Comparison of DPPH radical scavenging and antioxidant activities of *Artemisia* and oregano essential oils and BHT.

percentage by internal normalization, assuming identical mass response factor for all compounds.

3.3. Antimicrobial analysis

The antimicrobial activities were determined by using the drop agar diffusion method (Hili et al., 1997; Hammer et al., 1999; Cruz et al., 2007). The microorganisms tested were the fungi *Candida albicans* Serotype B ATCC 36802, *Cryptococcus neoformans* T_1-444 Serotype A (fungal collection of Federal University of São Paulo, UNIFESP-SP), *Aspergillus niger*, *Trichophyton rubrum* T544, *Microsporum canis*, *Microsporum gypseum*, *Fonsecaea pedrosoi* 5VPL (fungal collection of Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, UFRJ); and the bacteria *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* MRSA (BMB9393) (Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, UFRJ). The oils were diluted (1:2) in 50% DMSO/sterile H_2O solution. The microorganisms were stored in BHI (Brain Heart Infusion) agar slant tubes at 4 °C. Prior to use, the microorganisms were grown in BHI broth for 24 h (for bacteria and yeast) to 7 days (for conidia of filamentous fungi). Bacteria, yeast and conidia of filamentous fungi (2×10^5 CFU in 100 μl of saline) were spread over Petri dishes containing BHI solid medium and, after 10 min, a 10 μl of each oil solution was placed on the inoculated medium. Likewise, 10 μl of reference antibiotics (1.0 mg/ml, in 50% DMSO/sterile H_2O solution) were used as positive controls: Amphotericin B, Methicillin and Vancomycin. All plates were incubated at 37 °C (for bacteria and yeast) or 28 °C (for conidia of filamentous fungi) and the time of incubation varied from 24 h (for bacteria and yeast) to 7 days (for conidia of filamentous fungi), after which the diameter of the zone of inhibition was measured in mm. The non-toxic effect of 50% DMSO/ sterile H_2O solution was evaluated in BHI agar plates inoculated with all the microorganisms tested. The absence of inhibition zones confirmed the non-toxic effect of the DMSO/water solution. Each assay was repeated three times and the average result and standard deviation calculated.

3.4. Determination of DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured according to the procedures described by Blois (1958) and Kulisic et al. (2004). Briefly, 0.3 mM DPPH (Sigma, St. Louis, MO) in methanol solution was prepared, and a 1.0 ml aliquot was added to 2.5 ml of essential oil solution in MeOH (1.0 mg/ml). After incubation for 30 min in the dark at room temperature, the absorbance was measured at 517 nm, and the percentage of radical scavenging activity was calculated according to the equation $[1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$, where A_{sample} is the absorbance of the test compounds and A_{control} is the absorbance of the control reaction (containing all of the reagents except the test compounds). Oregano oil (commercial sample) and 2,6-di-*tert*-butyl-4-methylphenol (BHT, Sigma, St. Louis, MO) were used as positive controls in a concentration of 1.0 mg/ml. MeOH (1.0 ml) plus oil solution (2.5 ml) were used as blank. DPPH solution (1.0 ml) plus MeOH (2.5 ml) were used as a negative control. Each assay was repeated three times and the average result and standard deviation calculated.

3.5. Determination of antioxidant activity using β-carotene/linoleic acid assay

In this assay, antioxidant activity is determined by measuring the ability of the volatile organic compounds to inhibit the conjugated diene hydroperoxide formation from linoleic acid and β-carotene coupled oxidation in an emulsified aqueous system, which loses its orange color when reacting with the radicals (Miller, 1971; Braca et al., 2001). Quantities of linoleic acid (20 mg, 99% purity, Sigma, St. Louis, MO) and Tween 20 (200 mg, Sigma, St. Louis, MO) were placed in a flask, and a solution of 2 mg of β-carotene (95% purity, Sigma, St. Louis, MO) in 10 ml of $CHCl_3$ was added. Chloroform was completely evaporated using a vacuum evaporator. After removal of $CHCl_3$, 50 ml of distilled water saturated with oxygen by shaking for 30 min was added. Aliquots (200 μl) of each oil sample, dissolved in methanol (1.0 mg/ml), were added to 2.5 ml β-carotene/linoleic acid emulsion. Samples were subjected to oxidation by placing in an oven at 50 °C for 3 h. The absorbance was read at 470 nm, and the relative antioxidant activity was calculated with the equation $AA\% = 100 \times [1 - (A_0 - A_t/A_{00} - A_{0t})]$, where A_0 is the absorbance at the beginning of the incubation, with compound; A_t is the absorbance after 3 h, with compound; A_{00} is the absorbance at beginning of the incubation, without compound (200 μl of MeOH and 2.5 ml of β-carotene emulsion); A_{ot} is the absorbance after 3 h, without compound. Oregano oil (commercial sample) and 2,6-di-*tert*-butyl-4-methylphenol (BHT, Sigma, St. Louis, MO) were used as positive controls. Samples were read against a blank containing the emulsion minus β-carotene. Each assay was repeated three times and the average result and standard deviation calculated.

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