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Chemical Composition and Antimicrobial Activity of the Essential Oil from Sinapis alba L. and Sinapis arvensis L. (Brassicaceae) growing wild in Jordan

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ABSTRACT

The chemical composition and antimicrobial activity of essential oils from the shoots of S. alba L. and S. arvensis L. (Brassicaceae) were examined. The essential oils of both plants had a pale yellowish color with a characteristic sulfury odor. GC/MS analysis of the essential oils revealed a complex mixture of compounds including aldehydes, nitriles, sulfur-containing compounds, mono- and sesquiterpenes. S. alba was dominated by benzyl isothiocyanate (64.89%), benzyl nitrile (12.05%), thymol (7.20%), 2-phenyl isothiocyante (6.50%), limonene (4.73%) and 1-butenyl isoithiocyanate (0.59%), while S. arvensis contained 1-butenyl isoithiocyanate (36.42%), cubenol (14.27%), dimethyl trisulfide (10.39%), dimethyl tetrasulfide (6.3%), octadecane (2.55%), 6,10,14-trimethylpentadecane - 2-one (2.2%) and indole (1.88%) as major constituents. The antibacterial activity of essential oils from S. alba and S. arvensis was evaluated on seven bacterial strains by agar disc diffusion and agar well diffusion methods. The most susceptible Gram-positive and Gram-negative bacteria to the oil of S. alba were Staphylococcus epidermidis (ATCC 12228) and Escherichia coli (ATCC 25922), respectively. The oil of S. arvensis exhibited antimicrobial activity only towards Proteus vulgaris (ATCC 29905).

Key words: Sinapis alba L.; S. arvensis L; Brassicaceae; essential oil; antibacterial activity; GC/MS.

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INTRODUCTION

Brassicaceae (the mustard family) is a large family comprising 3,700 species spread over 338 genera [1]. The family has a worldwide distribution, but mostly in the Northern temperate regions and strikingly diverse around the Mediterranean. Economic importance includes numerous vegetable and flavoring plants that are of major interest for human health and nutrition [2]. Members of the family are distinctive in having a pungent flavor and sulfury odor due to the volatile isothiocyanate derivatives, obtained upon hydrolysis of glucosinolates [3-6].

Sinapis (mustard), a genus of Brassicaceae, has a long history of use as condiments and as herbal medicines. In many developing countries, Sinapis species are used as food, fodder to livestock, and in folklore medicine [7]. This genus comprises six annual species: Sinapis alba L. (white mustard), S. arvensis L. (wild mustard), S. flexuosa Poir., S. pubescens L., S. recurvata All., and S. setigera J. Gay ex Lange [8]. Literature survey revealed that only S. alba (white mustard) and S. arvensis (wild mustard), have been screened for their secondary metabolites [9, 10]. The two species are annual herbs distinguished by their yellow flowers with crossly-arranged four petals, and silique-type fruits [1, 11]. S. arvensis is native to Europe and grows wildly in western Asia, North Africa and throughout much of North America [12]. Several flavonoids have been previously isolated and identified by Durkee and Harborne from flower and leaves extracts of S. arvensis of Egyptian origin [13]. In addition, Ali et al. reported the presence of glucosiolates such as glucobrassicin, neoglucobrassicin and sinapin in the seeds of this species [14].

S. alba is widely distributed around the Mediterranean region, where it has most likely originated [15,16]. The plant is extensively grown for its mustard and as fodder crop. In addition, this species is known to be of great medicinal importance due to its antineoplastic, antimicrobial, and insecticidal activities [17,18]. However, there is limited published research on essential oil composition and activity of both species in spite of the historical and traditional knowledge of both oils' medicinal importance. The essential oil composition of the aerial parts of S. arvensis, growing wild in Algeria was investigated [7].On the other hand, Sefidkon et al. studied the chemical composition of the essential oil of S. alba grown in Iran [19].

Sinapis is a dominant element of the spring vegetation in the Mediterranean region of Jordan. The two species, S. arvensis and S. alba grow wildly and abundantly in this region, especially along roadsides and waste places [20].

To the best of our knowledge, the chemical composition and the biological activities of the volatile oils of both Sinapis species, growing wild in Jordan, have not been investigated earlier. The current investigation aims at determining the chemical composition of the essential oils of both species and evaluating the antimicrobial activity of these oils.

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MATERIALS AND METHODS

Plant material

Aerial shots of S. alba and S. arvensis were sampled during March 2010, from natural populations around the town Irbid (120 km North Amman, Jordan). The plants were identified by Dr. Riyadh Muhaidat (Department of Biological Sciences, Yarmouk University, Jordan). Voucher specimens were deposited at the herbarium of the Biology Department at Yarmouk University.

Isolation of Essential Oil

Fresh shoots (450 g) of S.alba and S. arvensis were finely chopped and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus, yielding ca. 0.067% and 0.043% (w/w), pale yellow fragrant oils, respectively. Oils were then dried over anhydrous sodium sulfate and immediately stored in GC-grade n-hexane at 4°C until use for further analysis by gas chromatography/mass spectrometry (GC/MS).

GC-FID analysis

Quantitative analysis of the essential oils from both species was carried out using a Hewlett Packard HP-8590 gas chromatography equipped with a split-splitless injector (split ratio, 1:50) and an FID detector. An OPTIMA-5 fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used. The oil was analyzed under a linear temperature program applied at 3°C/min starting from 60 °C through 246 °C. Temperatures of the injector and detector (FID) were maintained at 250°C and 300 °C, respectively. Concentrations (% contents) of oil ingredients were calculated using their relative area percentages, obtained by FID, assuming a unity response by all components.

GC-MS analysis

The chemical analysis of the essential oils was carried out using GC-MS (Varian chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands). The chromatographic conditions were as follows: column oven program, 60 ºC (1 min, isothermal) to 246 ºC (3 min, isothermal) at 3 ºC/min; the injector and detector temperatures were 250 and 300 ºC, respectively. Helium was the carrier gas (flow rate 0.90 mL/min). A HP-5 MS capillary column (30 m ×0.25 mm i.d., 0.25 µm film thicknesses) was used. The actual temperatures in MS source reached approximately 180 ºC and the ionization voltage was 70 eV. A hydrocarbon mixture of n-alkanes (C_8-C_{20}) was analyzed separately by GC/MS under same chromatographic conditions using the same HP-5 column.

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Compound identification

Identification of the compounds was based on the built in libraries (Nist Co and Wiley Co, USA) and by comparing their calculated retention indices relative to (C_8-C_{20}) n-alkanes literature values measured with columns of identical polarity (Adams, 2004; McLafferty, 1962; Miller and Bruno, 2003) or with authentic samples.

Antimicrobial study

Microorganisms and culture conditions

In vitro antimicrobial activity of essential oils from S. alba and S. arvensis was determined against seven different bacterial strains (obtained from the Department of Biological Sciences, Yarmouk University, Jordan) by agar disc diffusion and agar well diffusion methods. The investigated microorganisms included three Gram-positive bacteria (Bacillus cereus ATCC11778, Staphylococcus epidermidis ATCC 12228**,** and Enterococcus faecalis ATCC 29212) and four Gram-negative bacteria (Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 10031, Serratia marcescens ATCC 27117, and Proteus vulgaris ATCC 29905). Bacterial strains were cultured overnight at 37 ºC in Trypton Soy broth (TSA).

Media Preparation and Antibacterial Activity

Antibacterial activity testing was carried out according to Parekh et al. and Vuddhakul et al. with slight modifications [24, 25]. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 10^8 CFU/ml) and poured into Petri plates. For agar disc diffusion method, the disc (0.7 cm) was saturated with 100 µl of the test oil, dried, and introduced on the upper layer of the seeded agar plate. For agar well diffusion method, six equidistant wells (7 mm in diameter) were cut from the agar with the help of a cork-borer (0.7 cm). A 100 µl of the test oil was introduced into the well. The plates were incubated overnight at 37 ºC. Microbial growth was determined by measuring the zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used. After incubation, all plates were examined for any zones of growth inhibition and the diameters of these zones were measured in millimeters. All tests were performed in duplicate and the results were shown as mean values.

RESULTS AND DISCUSSION

Hydrodistillation of fresh flowering shoots of S. alba and S. arvensis collected from Jordan yielded 0.08 % and 0.043 % pale yellowish oils for S. alba and S. arvensis, respectively. In the oil of S. alba, 30 components (98.75 %) and in the oil of S. arvensis 39 components (93.44%) were identified. The chemical constituents of the essential oils, their percentages and retention indices are summarized in Table 1.

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Table 1: Essential oil composition (%) of fresh flowering shoots of S. alba L. and S. arvensis L. growing wild in Jordan

No.	Compounds	KI	S. alba [a] L.	S. arvensis [a] .L
1	2-Methypropyl isothiocyanate	930	0.11	-
2	2-Methyl 5-hexenitrile	950	-	0.3
3	Dimethyl trisulfide	965	-	10.39
4	Benzaldeyde[b]	967	0.10	-
5	β-Pinene	978	0.30	-
6	1-Butenyl isothiocyanate[b]	983	0.59	36.42
7	2-Pentylfuran	989	-	0.19
8	3-Butenyl isothiocyanate	990	-	1.26
9	Decane	993	-	0.99
10	3-Hexen1-ol, acetate	1005	0.03	-
11	p-Cymene	1009	-	1.05
12	Limonene	1029	4.73	0.20
13	4-Methylthiobutanenitrile	1037	-	0.61
14	Benzenacetaldehyde	1048	0.02	-
15	Phenlacetonitrile	1089	-	0.25
16	Nonanol	1107	0.06	-
17	Benzyl nitrile[b]	1143	12.05	-
18	4-Methylpentylisothiocyante	1162	0.08	-
19	Terpinen-4-ol	1170	-	0.08
20	dimethyl tetrasulfide	1188	-	6.3
21	Safranal	1199	-	0.09
22	Flamenol	1200	0.10	-
23	Cuminaldehyde[b]	1216	-	0.14
24	Pulegone	1223	0.03	-
25	Benzenepropanenitrile	1245	0.09	-
26	Thymol	1270	7.20	0.16
27	Indole[b]	1296	0.18	1.88
28	4-Vinyl-o-guaiacol	1315	-	0.21
29	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene (ionene)	1353	-	0.32
30	Benzyl isothiocyante	1371	64.89	0.08
31	Dodecanal	1382	0.05	-
32	Tetradecane[b]	1392	-	0.52
33	β-Caryophyllene	1420	0.05	0.28
34	Geranyl acetone	1425	-	0.12
35	2,6,10-Trimethyldodecane	1460	0.05	-
36	5-Methylhexanenitrle	1463	0.22	-
37	2-Phyenyl isothiocyanate	1465	6.50	1.35
38	(E)- β- Ionoe	1469	0.07	0.1
39	Furfural	1482	0.21	-
40	β-Bisabolene	1499	0.02	-
41	δ-Cadinene	1512		0.13
42	5-Methylthiopentyl isothiocyanate	1541	0.09	-
43	Spathulenol	1560	-	1.46

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44	Caryophyllene oxide	1576	-	0.16
45	9-Methylthiononanonitrile	1579	-	1.39
46	Hexadecane	1599	-	1.08
47	1-epi-Cubenol	1621	-	1.27
48	Cubenol	1668	-	14.27
49	β-Eudesmol	1670	0.40	0.11
50	Heptadecane	1698	0.08	
51	Octadecane[b]	1783	-	2.55
52	Ethyltetra decanoate	1785	0.28	-
53	6,10,14-Trimethylpentadecane-2-one	1840	0.07	2.20
54	Nanodecane	1899	-	1.07
55	Methyl hexadecanoate	1917	-	1.82
56	Isophytol	1925	0.10	0.09
57	Ethyl hexadecanoate	1987	-	0.94
58	Octadecanal	1999	-	1.32
	Total		(30) 98.75%	(39) 93.44%

[a]Compounds are listed in order of elution. RI values are calculated from retention times relative to those of n-alkanes (C_8-C_{20}) on the non-polar DB-5 column.
[b]Identified with authentic samples.

The essential oil of S. alba was dominated by benzyl isothiocyanate that accounted for 64.89% of the total oil content. In addition, the oil also contained benzyl nitrile (12.05%), thymol (7.20%), 2-phenyl isothiocyante (6.50%) and limonene (4.73%). Interestingly, 1-butenyl isoithiocyanate accounted only for 0.59 % of the total oil content. However, this compound (1-butenyl isoithiocyanate) dominated the essential oil of S. arvensis L. accounting for 36.42 % of the total oil content. Other compounds detected in the essential oil of S. arvensis included cubenol (14.27%), dimethyl trisulfide (10.39%), dimethyl tetrasulfide (6.3%), octadecane (2.55%), 6, 10, 14-trimethylpentadecane - 2-one (2.2%) and indole (1.88%).

Table 2: Classification of the components of the essential oils from the S. alba and S. arvensis L from Jordan.

	Compound	S. alba L.	S. arvensis L.
1	Monoterpene hydrocarbons (%)	(2) 5.03%	(2) 1.25%
2	Oxygenated monoterpenes (%)	(2) 7.27%	(4) 0.53%
3	Sesquiterpene hydrocarbons (%)	(3) 0.1%	(2) 0.41%
4	Oxygenated sesquiterpenes (%)	(1) 0.4%	(5) 17.75%
5	Nitrogen-containing compounds (nitrile) (%)	(3) 12.36%	(3) 2.43%
6	Sulfur-containing compounds (%)	(6) 72.44%	(7) 57.72%
7	Others	(12) 1.15%	(16) 13.34%

The chemical class distributions of the essential oil components of the two plants are listed in Table 2. The compounds were separated into seven classes including monoterpenes, oxygenated monoterpenoids, sesquiterpenes, sesquiterpenoids, nitrogen-containing compounds (nitrile), sulfur-containing compounds and others. The essential oil of S. alba was found to include six sulfur-containing compounds (72.44%), eight terpenoids (12.80%), and twelve others (1.15%). Analysis of essential oil from S. arvensis has shown that it contains seven

sulfur-containing compounds (57.72%), two nitriles (2.43%), 13 terpenoids (19.94%) and 16 others (13.34%).

The essential oil composition of S. alba and S. arvensis were investigated previously in Iran and Algeria. The chemical composition of the essential oil of Iranian S. alba was dominated by different classes of terpenoids [19]. It has been found that the aromatic monoterpene thymol had the highest contribution to the oil of Iranian S. alba, accounting for 53.3 % of the total oil content. The essential oil of the Iranian S. alba also contained limonene (26.7%), β-pinene (3.4%), and β-bisabolene (2.4%) all of which were detected in much lower concentrations in the oil of the Jordanian S. alba. Carvacrol (3.6%) and ionone (3.4%) were not detected in the current investigation.

Bendimerad et al. (2007) identified the essential oil composition of S. arvensis grown in Algeria. In addition to dimethyl trisulfide (33.6%) that was identified as the major component in this essential oil , the oil was found to contain also heptadecane (10.5%), methylpentadecane (9.1%), 6,10,14-trimethylpentadecane-2-one (8.6%) and dimethyl tetrasulfide (7.3%). The current investigation revealed great qualitative and quantitative differences in the oil composition between the plants grown in Jordan and those of Iranian and Algerian origins. This could be attributed to many factors including the, location, altitude, methods and time of harvest and drying, type of soil, climatic and seasonal fluctuations and the proportion of plant parts being used for distillation. However, the major determinant for the oil composition is definitely the genetic factors determining the chemotype of the species [26]. All these factors together, in turn, affect the biological activities of the essential oils. Nevertheless, glucosinolates found to occur in all examined species of Sinapis of different origins. Enzymatic breakdown of these secondary metabolites by the action of thioglucosidases or myrosinases produces volatile compounds including nitriles and isothiocyanates that are responsible for the sulfury odor and the strongly pungent taste of various vegetables of the Brassicaceae family [6, 16, 18]. The current investigation revealed that the essential oils of S. alba and S. arvensis of Jordanian origin were rich in isothiocyanates and nitriles.

The antibacterial activities of essential oils of S. alba and S. arvensis were assayed in vitro by agar disc diffusion and agar well diffusion methods against 7 bacterial species. Table 3 summarizes the microbial growth inhibition of essential oil of S. alba. Table 4 summarizes the microbial growth inhibition of essential oil of S. arvensis. The essential oil of S. alba L. showed antibacterial activity for the majority of the tested bacterial strains. On the other hand, the essential oil of S. arvensis showed antibacterial activity only towards P. vulgaris.

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Table 3: Antimicrobial activity of the essential oil obtained from S. alba L. from Jordan.

	Essential oil from S.alba L			
Bacterial Organisms	**25µg**	**50µg**	**100µg**	**150µg**
Escherichia coli ATCC 25922	-	+ 4	+ 4	+ 4
Serratia marcescens ATCC 27117	-	-	-	-
Klebsiella pneumoniae ATCC 10031	+2	+ 3	+ 3	+ 3
Proteus vulgaris ATCC 29905	-	+ 3	+ 4	+ 5
Bacillus cereus ATCC11778	-	-	-	-
Enterococcus faecalis ATCC 29212	-	-	-	-
Staphylococcus epidermidis ATCC 12228	+2	+3	-	-

Classification of results: 0: no inhibition or zone of inhibition of < 0.6 mm; +1: zone of inhibition of 0.6 –10.0 mm; +2:10.1–15.0 mm; +3:15.1–20.0 mm; +4: 20.1–25.0 mm; +5: >26.0mm.

Table 4: Antimicrobial activity of the essential oil from S. arvensis from Jordan

	essential oil from S. arvensis L.			
Bacterial Organisms	**25µg**	**50µg**	**100µg**	**150µg**
Escherichia coli ATCC 25922	-	-	-	-
Serratia marcescens ATCC 27117	-	-	-	-
Klebsiella pneumoniae ATCC 10031	-	-	-	-
Proteus vulgaris ATCC 29905	-	-	+2	+2
Bacillus cereus ATCC11778	-	-	-	-
Enterococcus faecalis ATCC 29212	-	-	-	-
Staphylococcus epidermidis ATCC 12228	-	-	-	-

Classification of results: 0: no inhibition or zone of inhibition of < 0.6 mm; +1: zone of inhibition of 0.6 –10.0 mm; +2:10.1–15.0 mm; +3:15.1–20.0 mm; +4: 20.1–25.0 mm; +5: >26.0mm.

The essential oil of the wild type S. alba showed maximum antibacterial activity against E. coli and P. vulgaris. The essential oils of both plants could not inhibit the Gram-positive B. cereus, and E. faecalis, or the Gram-negative S. marcescens. It is not surprising that differences in the antimicrobial effects between related species of the same genus, such as the Sinapis species used in this study, exist. This is most likely due to additive influence of the different evolutionary selection pressures as well as genetic variation on the chemistry and biological effects of essential oils.

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