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Phytochemical and Antimicrobial Evaluation of the Essential Oils and Antioxidant Activity of Aqueous Extracts from Flower and Stem of *Sinapis arvensis* L.

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ABSTRACT

The present study has been designed to determine phytochemical and antimicrobial evaluation of the essential oils and antioxidant activity of aqueous extracts from flower and stem of *Sinapis arvensis* L. (Brassicaceae). The essential oils of plants had a pallid yellowish color with a distinctive sulfury odor. GC/MS analysis of the essential oils exposed a complex mixture of compounds involving monoterpenes, sesquiterpenes, nitriles aldehydes and sulfur-containing compounds. the essential oil stem of *S.arvensis* included Benzyl isothiocyante (15.15%), Cubenol (15.12%), dimethyl trisulfide (6.12%), 6,10,14-trimethylpentadecane-2-one (3.85%), indole (1.91%), dimethyl tetrasulfide (2.22%), 1-butenyl isoithiocyanate (18.4%), Thymol (3.44%), octadecane (4.14%), Spathulenol (2.64%), Hexadecane (4.09%), 1-epi–Cubenol (2.41%) and Octadecanal (1.14%) as major constituents. The flower of *S.arvensis* contained Dimethyl trisulfide (5.24%), Dimethyl trisulfide (19.2%), Dimethyl trisulfide (4.03%), Thymol (4.62%), Indole (1.41%), 2-Phyenyl isothiocyanate (7.45%), δ-Cadinene (3.40%), Spathulenol (1.58%), 9-Methylthiononanonitrile (3.21%),

Hexadecane (3.02%), 1-epi–Cubenol (3.01%), Cubenol (14.32%), Octadecane (2.41%), 6,10,14-Trimethylpentadecane-2-one (3.25%), Nanodecane (1.01%) and Octadecanal (1.42%) as major components. In this study, the antibacterial activity of stem and flower essential oils from *S.arvensis* was evaluated on five pathogenic multi-drug resistant bacteria strains by agar disc diffusion method. The most susceptible Gram-positive and Gram-negative bacteria to the stem and flower essential oils Sinapis arvensis were *Staphylococcus aureus* (NCTC7428) and *P.aeruginosa* (MTCC 2453), respectively. According to antioxidant activity resulted, it was highlighted that antioxidant activity of aqueous extract of flower was weaker than stem.

Keywords: *S.arvensis* L.; essential oil, antibacterial activity; GC/MS, Antioxidant activity.

INTRODUCTION

Plants play an important role in providing food for humans. Among plants economic significance medicinal and aromatic plants which played a critical where it used as therapeutic agents to a long time (Cordell, 1995).[1] Natural products of higher plants be possible provide a new source of antimicrobial agents with maybe novel mechanisms of activity (Barbour *et al*., 2004; Hamil *et al*., 2003; Machado *et al*., 2003).[2-4] Many microorganisms caused the infectious are resistant to drugs synthetic, therefore, an optional therapy is much required and pull toward the attention of many researchers the worldwide (Mohanan *et al*., 1998).[5] Plants are very important resource due to produce complex molecular. The plant produce structures such as secondary metabolism and their derivatives have antimicrobial properties, such as alkaloids, phenolic compounds, terpens, tannins, cumarins, flavonoids, isoflavonoids and glycosides (Simoes *et al*., 1999).[6]

On the other hand, major focus of ethnopharmacological research current day is related to the discovery of new antioxidants. Antioxidants prevent the formation of damaging reactive oxygen species in the body. Antioxidants can also prevent the peroxidation of biological molecules by chelating transition metals that produce hydroxyl radicals through the Haber-Weiss and Fenton reactions (Chew *et al*., 2009).[7]

*Sinapis arvensis (*Brassicaceae; syn. *Brassica arvensis* (L), *Brassica sinapis Vis.*, *Brassica sinapistrum Boiss.* Link*)*, commonly known as field mustard, wild mustard or charlock. Brassicaceae is a large family includes 3,700 species spread over 338 genera (Simpson, 2010).[8] Brassicaceae has a worldwide distribution. The difference between family members are in having a pungent flavor and sulfury odor lead to the volatile isothiocyanate derivatives, acquired upon hydrolysis of glucosinolate (Hashem, 1999; Lin, 2000; Afsharypuor, 2005; Al-Qudah, 2010).[9-12] In developing countries, *Sinapis arvensis* L. are used as fodder to livestock, food and folklore medicine (Bendimerad, 2007).[13] *S.arvensis* is native to Europe and grows wildly in western Asia, Iran and North Africa and in every of America (Frankton, 1987).[14]

MATERIALS AND METHODS

Plant Material

Sinapis arvensis var. orientalis (L.) was collected in June 2012 from area of Hamoon international wetland of Zabol (Iran) province in during the flowering stage. Taxonomic determination of the plant was approved by the Department of Botany of Shahid Beheshti University of Medical Sciences, Iran. Collected plant materials were having moisture removed in the shade and the flowers and aerial parts were separated from the roots. The voucher specimen has been deposited at the herbarium of Zabol University, Iran.

Isolation of the Essential Oil

The flower and stem *Sinapis arvensis var. orientalis* were dried and milled into a fine powder and 40 g was subjected for 2 h of hydrodistillation using a Clevenger-type apparatus. The acquired essential oil was collected, and having moisture removed over anhydrous sodium sulphate and kept at 4ºC until analysis.

GC-MS Analysis

The Analyses of the essential oils was carried out using a varian gas chromatography 3600 with DB5 (methyl phenyl siloxane, 30mm × 0.25mm i.d.); the carrier gas was helium; split ratio 1:15 and flame ionization detector. Temperature program was from 60ºC (2min) to 240ºC at 5ºC/min, injector temperature of 250ºC and detector temperature of 260ºC. GC-MS (Shimadzu GC/MS model QP5050) was used on a cross-linked 5% methyl phenylsiloxane (HP-5, 30m × 0.25mm id, 0.25µm film thickness. Carrier gas was helium, split ratio 1:15 with quadrupole mass spectrometer operating at 70ev ionization energy. The retention indices for every component were calculated by using retention time of n-alkenes (C8-C25) which were injected after the essential oil under the same condition. The components were recognized by comparing retention indices (RRI, DB-5) with those of standards. The results were also confirmed by comparing their mass spectra with the published mass spectra or Wiley library or with the published mass spectra data (Massada, 1976; Adams, 2001; Ramaswami, 1988).[15-17]

Microorganisms and Antibacterial Activity

The two Gram-positive include *Bacillus subtilis (*MTCC 441), *Staphylococcus aureus* (NCTC7428) and three Gram-negative include *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* **(**MGH 78578) and *Pseudomonas aeruginosa* (MTCC 2453) pathogenic multi-drug resistant bacteria, were obtained from the microbiological laboratory of the Falsafi hospital. Golestan, Iran. The microorganisms were inoculated on to nutrient agar slants at 37°C and maintained at -80°C. Microorganisms and culture conditions *in-vitro* antimicrobial activity of essential oils from *S.arvensis* was concluded against all bacteria by agar disc diffusion methods.

Antibacterial activity testing was carried out according to Parekh *et al* (2005) and Vuddhakul *et al*. (2007) with slight modifications.[18,19] The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 × 108 CFU/ml) and poured into Petri plates. For agar disc diffusion method, the disc (0.6 cm) was saturated with 200 µl of the test oils, dried, and introduced on the upper layer of the seeded agar plate. For agar well diffusion method, six equidistant wells (6 mm in diameter) were cut from the agar with the help of a cork-borer (0.6 cm). 50,100,150,200,250 µl of the test oil was introduced into the well. The plates were incubated overnight at 37 ºC for 24 h. Microbial growth was determined by measuring the zones of bacterial inhibition minus the discs diameter were recorded for each bacterial strain in millimeters (Aureli et al. 1992; Ilçim *et al*. 1998).[20,21] Discs with

hexane and cephalosporin (Padtan Teb Co., Iran) were used as positive controls. Cephalosporin is an antibiotic with antibacterial activity that inhibits cell wall synthesis although some bacteria like *Klebsiella* spp., *Proteus* spp and *Pseudomonas* sp. are resistance to its (Pfeifer *et al*. 2010; Wright *et al*. 2010).[22,23] All tests were performed in duplicate and the results were shown as mean values.

Preparation of Aqueous Extract

20 gram of flower and stem of *S.arvensis* was finely ground utilizing a homogenizer and was extracted with distilled water at room temperature for 24 hours. This mixture was then filtered using Whatman No.1 filter paper to remove debris and a volatile extract was then evaporated at 35ºC using a rotary evaporator.

Study of Antioxidant activity by DPPH method

The DPPH radical scavenging activity of the extracts from *S.arvensis* flowers and stem were measured according to the procedure described by Brand-Williams (Brand-Williams, 1995).[24] Briefly, 2 ml samples of various concentrations of the extracts in aqueous were separately added to a 1 ml solution of DPPH radical in aqueous l (final concentration of DPPH was 0.1 mM). The mixture was shaken forcefully and permitted to stand in the dark at room temperature for 30 minutes. Then, the absorbance of the resulting solution was measured at 517 nm using a UV-Vis, Specords 100 spectrophotometer (model 6715 UV/VIS Split Double beam model, JENWAY co. England). Inhibition of free radical DPPH was computed as percentage [IP (%)] as follows: IP (%) = 100 × (A blank - A sample) / A blank

Where a blank is the absorbance of the control (containing all reagents exclude the test compound) and a sample is the absorbance of the test compound. The antioxidant activity of the extracts was expressed as the IC_{50}. IC_{50} values (µg/ml) mark the concentration of sample, which is required to scavenge 50% of DPPH free radicals. This was obtained by interpolation and applying linear regression analysis. For the calculation of these values, Microsoft Excel was used. Percent inhibition after 25 minutes was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity. In this test, BHT was used as positive control and the sample solution without DPPH was used as blank.

RESULTS

Chemical Composition of the Essential Oil

The yield of volatile oils of *Sinapis arvensis* acquired by hydrodistillation of the finely powdered flower and stem were 0.4 and 0.3 % (v/w) respectively. The oils were light yellow and with a perfumery odor. The major constituents of essential oil stem of *S.arvensis* included Benzyl isothiocyante (15.15%), Cubenol (15.12%), dimethyl trisulfide (6.12%), 6,10,14-trimethylpentadecane-2-one (3.85%), indole (1.91%), dimethyl tetrasulfide (2.22%), 1-butenyl isoithiocyanate (18.4%), Thymol (3.44%), octadecane (4.14%), Spathulenol (2.64%), Hexadecane (4.09%), 1-epi–Cubenol (2.41%) and Octadecanal (1.14%). The flower essential oil stem of *S.arvensis* contained Dimethyl trisulfide (5.24%), Dimethyl trisulfide (19.2%), Dimethyl trisulfide (4.03%), Thymol (4.62%), Indole (1.41%), 2-Phyenyl isothiocyanate (7.45%), δ-Cadinene (3.40%), Spathulenol (1.58%), 9-Methylthiononanonitrile (3.21%), Hexadecane (3.02%), 1-epi–Cubenol (3.01%), Cubenol (14.32%), Octadecane (2.41%), 6,10,14-Trimethylpentadecane-2-one (3.25%), Nanodecane (1.01%) and Octadecanal (1.42%) as major components.

The chemical constituents of the essential oils, their percentages and retention indices are summarized in Table 1. The compounds were separated into seven classes including monoterpenes, oxygenated monoterpenoids, sesquiterpenes, sesquiterpenoids, nitrogen-containing compounds (nitrile), sulfur-containing compounds and others. Analysis of essential oil from *S.arvensis* has shown that it contains two nitriles, thirteen terpenoids, seven sulfur-containing compounds and sixteen others compounds.

In a study is conducted on *S.arvensis* that grown in Algeria showed that the essential oil composition 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 methylpenta decane (9.1%), heptadecane (10.5%), dimethyl tetrasulfide (7.3%) and 6,10,14-trimethylpentadecane-2-one (8.6%) (Bendimerad *et al*., 2007).[13] The current investigation revealed great qualitative and quantitative differences in the oil composition between the plants grown in Algerian origins. This could be attributed to many factors involving the, altitude, location, methods and time of harvest and drying, type of soil, climatic and seasonal fluctuations and the proportion of plant parts being used for distillation. Nevertheless, the major determinant for the oil composition is definitely the genetic factors determining the chemotype of the species (Al-Jaber *et al*., 2011).[25] All these factors together, in turn, affect the biological activities of the essential oils. However, glucosinolate found to occur in all examined species of Sinapis of different origins.

The Antibacterial Activities of Stem and Flower Essential Oils

The antibacterial activities of stem and flower essential oils of *S.arvensis* were assayed in vitro by agar disc diffusion methods gainst five multi-drug resistant bacteria (Table 2, 3). *B.subtilis* (MTCC 441) and *P.aeruginosa (*MTCC 2453) compared other bacteria more resistant to the stem and flower of the essential oils. The most susceptible Gram-positive and Gram-negative bacteria to the stem and flower essential oils *Sinapis arvensis* were *Staphylococcus aureus* (NCTC7428) and *P.aeruginosa* (MTCC 2453), respectively.

Antioxidant Activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997).[26] The scavenging effect of aqueous extract of *S.arvensis* under investigation on DPPH radicals are shown in Fig. 1. The reduction ability of DPPH radicals formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Antioxidants effect on DPPH radical scavenging is seemed to be related to their hydrogen donating ability. Antioxidant activity resulted, it was highlighted that antioxidant activity of aqueous extract of flower was weaker than stem (Fig. 1).

DISCUSSION

The enormous varieties of chemical moiety of *Sinapis arvensis* extract has cyano, isoprene units and disulfide linkages. Combination approach of the mixed effect of all these shown potentiality in bacterial growth inhibition by depilatory action disulfide bonds of cysteine units of amino acids which is the building block of DNA strands of microbial cell. Cyano moiety with thio units of active constituents is soluble in isoprene units of terpenoid which release the constituents slowly in the screening part. (Table-1)

Antioxidant activity has been found in stem is higher in flowers because the radical scavenging on DPPH will be lower in flowers due to the presence of anthocyanin and flavonoids but higher in stem due to the

closed unit stem parenchyma cells. The inhibitory percentage of oxidation is higher in stem rather than stem constituents due to the supramolecular chemistry.[24-26]

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Table 1. Comparison of Chemical Composition of Essential Oils of Flower and Stem in *Sinapis arvensis*

No.	Name of Compounds	Stem	Flower	Oil of Stem%	Oil of Flower%
1	2-Methyl 5-hexenitrile	+	+	0.15	0.15
2	Dimethyl trisulfide	+	+	6.12	5.24
3	1-Butenyl isoithiocyanate	+	+	18.4	19.2
4	2-Pentyl furan	+	-	0.14	-
5	1-Butenyl isothiocyanate	+	+	0.05	0.91
6	Decane	-	+	-	0.17
7	p-Cymene	+	+	0.81	0.36
8	Limonene	+	+	0.14	0.41
9	4-Methyl thiobutanenitrile	+	-	0.79	-
10	Phenyl acetonitrile	+	-	0.32	-
11	Terpinen-4-ol	-	+	-	0.02
12	Dimethyl tetrasulfide	+	+	2.22	4.03
13	Safranal	+	-	0.06	-
14	Cuminaldehyde	+	+	0.07	0.03
15	Thymol	+	+	3.44	4.62
16	Indole	+	+	1.91	1.41
17	4-Vinyl-o-guaiacol	+	+	0.48	0.23
18	Ionene	+	+	0.11	0.04
19	Benzyl isothiocyante	+	+	15.15	0.06
20	Tetradecane	+	+	0.21	0.87
21	β-Caryophyllene	+	+	0.34	0.39
22	Geranyl acetone	-	+	-	0.14
23	2-Phyenyl isothiocyanate	+	+	0.53	7.45
24	(E)-β-Ionoe	+	+	0.63	0.94
25	δ-Cadinene	+	+	0.08	3.40
26	Spathulenol	+	+	2.64	1.58
27	Caryophyllene oxide	+	+	0.19	0.42
28	9-Methylthiononanonitrile	+	+	0.29	3.21
29	Hexadecane	+	+	4.09	3.02
30	1-epi–Cubenol	+	+	2.41	3.01
31	Cubenol	+	+	15.12	14.32
32	β-Eudes mol	+	+	0.31	0.61
33	Octadecane	+	+	4.14	2.41
34	6,10,14-Trimethylpentadecane-2-one	+	+	3.85	3.25
35	Nanodecane	+	+	0.13	1.01
36	Methyl hexadecanoate	+	+	0.18	0.94
37	Isophytol	+	+	0.17	0.19
38	Ethyl hexadecanoate	+	+	0.08	0.04
39	Octadecanal	+	+	1.14	1.42

Table 2. Antimicrobial activity of the stem essential oil obtained from *Sinapis arvensis* against multi-drug resistant bacteria

Microorganisms	Diameter of the inhibitory zones (mm)						Cephalosporin
	25µg	50µg	100µg	150µg	200µg	250µg	
B.subtilis MTCC 441	11	13	13	13	13	15	24
S.aureus NCTC 7428	12	17	17	17	19	19	18
E.coli MTCC 739	00	15	20	20	21	24	28
K.pneumoniae MGH 78578	11	17	19	21	24	25	26
P.aeruginosa MTCC 2453	05	12	12	14	15	16	12

Table 3. Antimicrobial activity of the flower essential oil obtained from *Sinapis arvensis* against multi-drug resistant bacteria

Microorganisms	Diameter of the inhibitory zones (mm)						Cephalosporin
	25µg	50µg	100µg	150µg	200µg	250µg	
B.subtilis MTCC 441	08	11	11	12	12	13	24
S.aureus NCTC 7428	09	14	16	16	17	20	18
E.coli MTCC 739	00	00	18	19	19	20	28
K.pneumoniae MGH 78578	10	16	18	21	24	24	26
P.aeruginosa MTCC 2453	08	10	13	13	14	14	12

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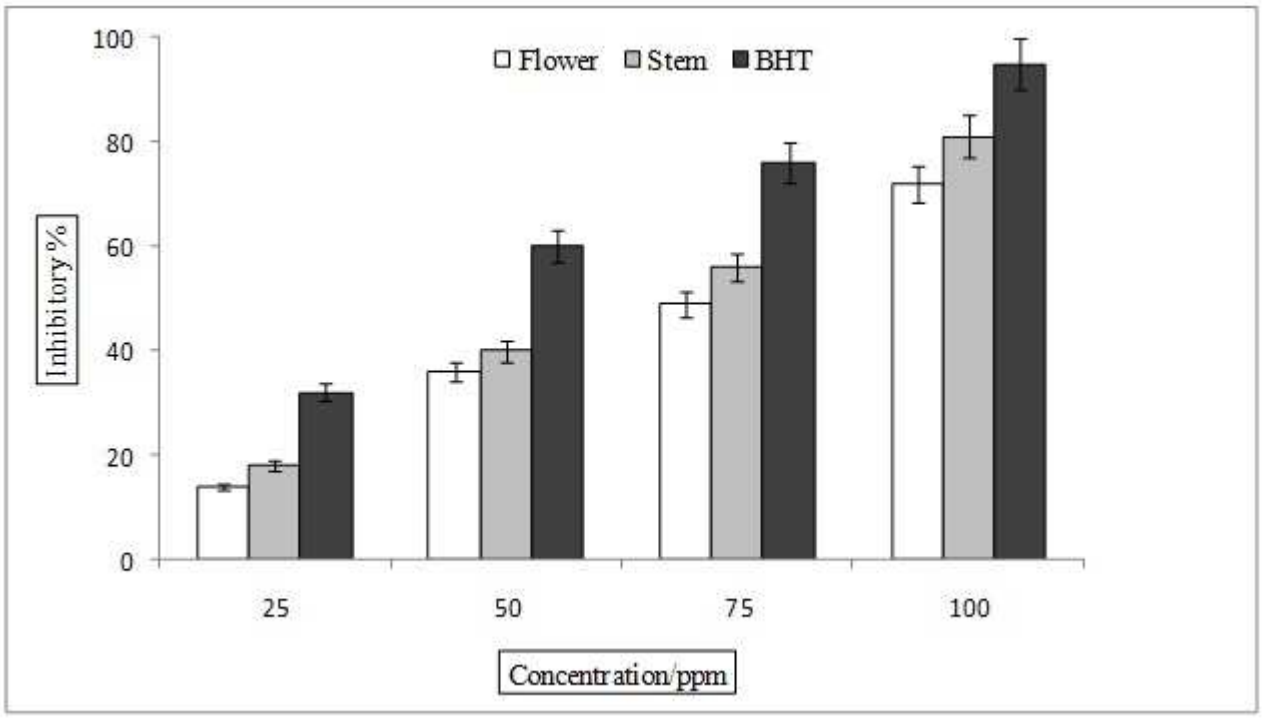

Figure.1. Comparison of Radical Scavenging Effect of BHT and Aqueous Extracts on DPPH Radicals