Volume 56(2):151-154, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Chemical composition and antimicrobial activity of the essential oil of *Ligularia persica* Boiss. (Asteraceae)

Mohammad Hossein Mirjalili[1]*, Morteza Yousefzadi[2]

[1]Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Evin, Tehran, Iran, [2]Department of Marine Biology, Faculty of Basic Sciences, Hormozgan University, Bandar Abbas, Iran

ABSTRACT The objective of this study was to investigate chemical composition and *in vitro* antimicrobial activity of the essential oil of *Ligularia persica* growing wild in Iran. The essential oil was obtained by hydro-distillation and analyzed by GC-FID and GC/MS. Sixty-one components representing 99.0% of the total oil were characterized. The major identified components were (*Z*)-β-ocimene (12.5%), *cis*-meta-mentha-2,8-diene (8.8%), α-eudesmol (8.7%), valencene (5.9%) and 14-hydroxy-δ-cadinene (5.7%). Oxygenated sesquiterpenes (39.5%) was the main group of the plant essential oil. The essential oil exhibited moderate activity against three bacteria and a yeast, *Candida albicans*, with minimum inhibitory concentration (MIC) values ranging from 1.87 to 7.50 mg/ml. The best inhibitory effects were against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus.*
Acta Biol Szeged 56(2):151-154 (2012)

KEY WORDS

Ligularia persica Boiss.
Asteraceae
essential oil
antimicrobial activity
oxygenated sesquiterpenes

The Asteraceae is the largest family of flowering plants, comprise 1,600–1,700 genera and about 24,000 species distributed all over the world except Antarctica (Funk et al. 2009). The species are a source of many biologically active compounds like essential oils, polyphenolic compounds, flavonoids, phenolic acids and many others (Larocque et al. 1999; Setzer et al. 2004; Zidorn et al. 2006; Wang et al. 2006). Due to their bio-active properties plants from Asteraceae family are commonly used in treatment of various diseases. Antimicrobial activity of the essential oils and their chemical constituents have been extensively studied and shown good fighting potential against drug resistant pathogens (Chavan et al. 2006; Sonboli et al. 2006; Mirjalili et al. 2012). The genus *Ligularia* Cass. belongs to the Asteraceae family (tribe Senecioneae), and comprises ca. 150 species native to Central and East Asia and some parts of Europe (Joharchi 2008). *Ligularia* species are chemotaxonomically characteristic due to the presence of typical pyrrolizidine alkaloids and sesquiterpenoids (Tan et al. 2007). This genus, with the local names of "Zaban tala" and "Pir sonbol", is represented in the flora of Iran by L. *persica* Boiss. as an endemic species (Mozaffarian 1996). Various members of the genus have long been used as folk remedies for their antibiotic, antiphlogistic, and antitumor activities (Chen et al. 1997). The essential oil composition of *Ligularia* species has been extensively studied from different distribution regions especially from China and our literature survey showed that the oil of *Ligularia* species has been found to be rich in sesquiterpenes (Nagano et al. 2007; Kuroda et al. 2007; Tori et al. 2008). The chemistry of *L. persica* has been previously studied and some eremophilane derivatives and sesquiterpenes have been reported from the plant roots (Marco et al. 1991a and 1991b), but so far chemical composition and antimicrobial activity of *L. persica* essential oil have not been studied. The present study describes the chemical composition and *in vitro* antimicrobial activity of the essential oil from the aerial parts of *L. persica*, which have not been studied previously.

Accepted Dec 6, 2012
*Corresponding author. E-mail: m-mirjalili@sbu.ac.ir

Materials and methods

Plant material

The aerial parts of *L. persica* were collected at full flowering stage from Dizin (36° 01´ 36" N, 51° 23´ 58" E at an altitude of 1012 m), Karaj, Alborz Province, Iran. A Voucher specimen (HAPH-90125) has been deposited at the Herbarium of Biology Department, Hormozgan University, Bandar Abbas, Iran.

Essential oil isolation

The essential oil of air-dried sample (100g) was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus, recommended by the British Pharmacopeia. The essential oil was dried over anhydrous sodium sulfate (Na_2SO_4) and kept at 4°C in dark vial until analyzed and tested.

GC-FID analysis

GC analysis was performed using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out on fused silica capillary DB-5 column

Table 1. Essential oil composition of *Ligularia persica.*

No.	RI	Compounds	%	No.	RI	Compounds	%
1	938	α-Pinene	1.1	36	1576	β-Vetivenene	0.4
2	977	Sabinene	2.3	37	1595	Spathulenol	0.6
3	983	β-Pinene	0.9	38	1603	Caryophyllene oxide	0.3
4	991	*cis*-meta-Mentha-2,8-diene	8.8	39	1613	Salvia-4-(14)-en-1-one	0.2
5	994	dehydro-1,8-Cineol	0.4	40	1633	Selina-1,3,7(11)-trien-8-one	0.3
6	1008	2,3,5-trimethyl Pyrazine	0.8	41	1648	Hinesol	t
7	1020	α-Terpinene	0.2	42	1658	α-Muurolol	0.2
8	1027	*p*-Cymene	0.3	43	1672	α-Cadinol	0.4
9	1037	(*Z*)-β-Ocimene	12.5	44	1678	α-Eudesmol	8.7
10	1047	(*E*)-β-Ocimene	3.5	45	1682	dihydro-Eudesmol	2.1
11	1062	γ-Terpinene	0.4	46	1687	7-epi-α-Eudesmol	5.1
12	1093	meta-Cymenene	0.4	47	1695	4-Cuprenen-1-ol	0.3
13	1099	Linalool	0.3	48	1706	Cyperotundone	t
14	1102	*n*-Nonanal	0.3	49	1712	Acorenone B	1.2
15	1129	allo-Ocimene	0.7	50	1737	5-hydroxy-cis-Calamanene	0.5
16	1133	cis-*p*-Mentha-2,8-dien-1-ol	0.2	51	1770	β-Acoradienol	3.8
17	1174	Borneol	0.8	52	1802	(*Z*)-epi-β-Santalol acetate	t
18	1185	Terpinen-4-ol	0.9	53	1822	14-hydroxy-δ-Cadinene	5.7
19	1196	α-Terpineol	0.6	54	1832	Furanodiene	2.5
20	1206	Verbenone	0.2	55	1838	Neophytadiene	0.2
21	1237	Isobornyl formate	0.2	56	1843	Fukinanolid	3.1
22	1289	Thymol	1.4	57	1850	2 α-acetoxy-11-methoxyAmorpha-4,7-diene	1.9
23	1390	α-Copaene	t	58	1873	dihydro-Columellarin	2.2
24	1403	β-Elemene	1.2	59	2131	Dihydrolinderalactone	0.4
25	1438	(*E*)-Caryophyllene	0.7	60	2172	Ethyl linoleate	0.6
26	1458	(*E*)- β-Farnesene	2.8	61	2294	*n*-Tricosane	1.0
27	1473	9-epi-(*E*)-Caryophyllene	0.7				
28	1480	γ-Gurjunene	0.3				
29	1486	Aristolochene	0.9			Monoterpene hydrocarbons	31.1
30	1489	β-Selinene	1.1			Oxygenated monoterpenes	5.0
31	1498	trans-Muurola-4(15),5-diene	4.7			Sesquiterpene hydrocarbons	20.5
32	1505	Valencene	5.9			Oxygenated Sesquiterpenes	39.5
33	1513	Bicyclogermacrene	0.9			Aliphatic hydrocarbones	2.1
34	1535	δ-Cadinene	0.5			Others	0.8
35	1566	γ-Vetivenene	0.4			Total identified	99.0

RI, retention indices relative to C6–C24 n-alkanes on a DB-5 column; t, trace, less than 0.1 %.

(30 m × 0.25 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250°C and 300°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min; oven temperature program was 60 – 250°C at the rate of 4°C /min and finally held isothermally for 10 min; split ratio was 1:50.

GC/MS analysis

GC/MS analysis was carried out by use of Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (60 m × 0.25 mm i.d.; film thickness 0.25μm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200°C and 250°C, respectively. Mass range was from 35 to 456 amu. Oven temperature program was the same given above for the GC.

Identification and quantification of the oil components

The constituents of essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6–C_{24}) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Adams and Wiley 7.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Adams 1995). For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Microbial strains

Four microbial strains were used: *Bacillus subtilis* (ATCC

Table 2. Antimicrobial activity of the essential oil of *Ligularia persica*.

Microorganisms	*Ligularia persica*		Standard antibiotics	
	IZ[a]	MIC[b]	Ampicillin[c]	Nystatine[d]
Bacillus subtilis	25± 0.2	1.87	14±0.4	-
Staphylococcus aureus	21± 0.9	3.75	13±0.3	-
Escherichia coli	19± 0.3	3.75	12±0.2	-
Candida albicans	16± 0.5	7.50	-	18±0.5

Results were obtained from 3 independent experiments performed in doubly. [a]Inhibition Zone includes diameter of disc (6 mm). [b]Minimum inhibitory concentration values as mg/ml. [c]Tested at 10 µg/disc. [d]Tested at 30 µg/disc. Inactive (-); moderately active (7 - 14); highly active (> 14); nt, not tested. Values are given as mean ± standard deviation.

465), *Staphylococcus aureu*s (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231).

Bioassay

Disc diffusion

The antimicrobial activity of the essential oil was determined by the disk diffusion method (NCCLS, 1997). Briefly, 0.1 ml of a suspension of the test microorganism (10^8cells/ml) was spread on Mueller-Hinton Agar plates for bacteria and Sabouraud Dextrose Agar for the fungi. Sterile 6 mm disks, each containing 10µl of essential oil were placed on the microbial lawns. The plates were incubated at 37°C for 24 h for bacteria and 30°C for 48 h for fungi. The diameters of the zones of inhibition were measured and reported in mm. Triplicate tests were carried out in all experiments.

Determination of minimum inhibitory concentration (MIC)

MIC values were determined by broth microdilution assay recommended by the NCCLS (1999). Serial two-fold dilutions of the essential oil were made in Mueller-Hinton Broth containing 0.5% Tween 80 for bacteria and Sabouraud Dextrose Broth with 0.5% Tween 80 for fungi in 96-well micro titer plates. Fresh microbial suspensions prepared from overnight grown cultures in the same media were added to give a final concentration of 5×10^5 organisms/ml. Controls of medium with microorganisms or the essential oil alone were included. The microplates were incubated at 37°C for 24 h for bacteria and 30°C for 48 h for fungi. The first dilution with no microbial growth was recorded as MIC.

Results and discussion

Essential oil composition

The essential oil yield (w/w%) was 0.3 % based on the dry weight of the plant. The chemical composition of the oil sample was mainly investigated using both GC/FID and GC/MS techniques. Qualitative and quantitative analytical results are listed in Table 1 in the order of their elution on the DB-5 column. In total, 61 constituents were identified and quantified in the essential oil representing 99.0% of the total oil. The major constituents of the oil were (*Z*)-β-ocimene (12.5%), cis-meta-mentha-2,8-diene (8.8%), α-eudesmol (8.7%), valencene (5.9%) and 14-hydroxy-δ-cadinene (5.7%). The classification of the identified compounds, based on functional groups is summarized at the end of Table 1. Oxygenated sesquiterpenes, mono- and sesquiterpene hydrocarbons and oxygenated monoterpenes comprised 39.5%, 31.1%, 20.5% and 5.0% of the oil, respectively. Acorenone B (1.2%) and furanodiene (2.5%) as identified oxygenated sesquiterpene have been recently introduced as powerful antimicrobial and antitumor agents (Sun et al. 2009; Lin et al. 2012).

Antimicrobial assay

Inhibition zones (IZ) and minimum inhibitory concentration (MIC) values of the essential oil showed a variability of inhibition among all the bacteria and fungi tested (Table 2). The results of the antimicrobial activities of the essential oil indicated that the oil exhibited moderate antibacterial and antifungal activity. It was highly effective against Gram positive bacteria *B. subtilis* and *S. aureus* with the inhibition zones 25 and 21 mm and MIC values of 1.87 and 3.75 mg/ml, respectively. Also the oil was effective against Gram negative bacteria *Escherichia coli* with the inhibition zone 19 and MIC value 3.75 mg/ml. Although the essential oil of the present species showed both antifungal and antibacterial activity, but the IZ and MIC values for bacteria were much higher than that of fungi which shows the higher antibacterial potent of the essential oil comparing to antifungal activity. Finally, the essential oil of *L. persica* could be represented as an inexpensive source of natural antibacterial substances for use in pathogenic systems to prevent the growth of bacteria. However, further research is needed to evaluate the effectiveness of the plant essential oil utility as natural antimicrobial agents.

References

Adams RP (1995) Identification of essential oil components by gas chromatography/mass spectroscopy. Allured, Carol Stream, IL.

Chavan MJ, Shinde DB, Nirmal SA (2006) Major volatile constituents of *Annona squamosa* L. bark. Nat Prod Res 20:754-757.

Chen HM, Wang YY, Mao JM, Cai MS, Jia ZJ (1997) Syntheses and pharmacological activities of the derivatives of furanosesquiterpenes from *Ligularia virgaurea*. Palnta Med 63:299-302.

Funk V, Susanna A, Stuessy TF, Bayer R (eds) (2009) Systematics, evolution, and biogeography of the compositae. IAPT, Vienna

Joharchi M (2008) Flora of Iran. Research Institute of Forests and Rangelands Press, Tehran, p. 20.

Kuroda C, Kiuchi K, Torihata A, Takeshita K, Gong X, Shen Y, Hirota H, Onuki H, Hanai R (2007) Chemical and genetic diversity of *Ligularia latihastata* and *Ligularia villosa* in Yunnan Province of China. Chem Biodiv 4:2210-2217.

Larocque N, Vincent C, Belanger A, Bourassa JP (1999) Effects of tansy essential oil from *Tanacetum vulgare* on biology of oblique-banded leafroller, *Chorisoneura rosaceana*. J Chem Ecol 25:1319-1330.

Lin J, Dou J, Xu J, Aisa HA (2012) Chemical composition, antimicrobial and antitumor activities of the essential oils and crude extracts of *Euphorbia macrorrhiza*. Molecules 3:5030-5039.

Marco AA, Sanz JF, Garcia-Sarrion A, Rustaiyan A (1991a) Germacrene esters from roots of *Ligularia persica*. Phytochemistry 30:2325-2328.

Marco AA, Sanz JF, Yuste A, Rustaiyan A (1991b) New umbelliferone sesquiterpene ethers from roots of *Ligularia persica*. Liebigs Ann Chem 1991:929-931.

Mirjalili MH, Hadian J, Aliahmadi A, Kanani MR, Sonboli A (2012) Chemical composition and in vitro antimicrobial activity of the essential oil of *Cyclotrichium leucotrichum* from Iran. Nat Prod Res in press.

Mozaffarian V (1996) A Dictionary of Iranian plant names. Farhang Moaser, Tehran, p. 671.

Nagano H, Iwazaki Y, Matsushima M, Sato M, Gong X, Shen Y, Hirota H, Kuroda C, Hanai R (2007) High diversity of *Ligularia dictyoneura* in chemical composition and DNA sequence. Chem Biodiv 4:2874-2888.

National Committee for Clinical Laboratory Standard (NCCLS) (1999) Performance standards for antimicrobial susceptibility testing. 9th International Supplement. M100-S9. Wayne, Pensylvania, USA.

National Committee for Clinical Laboratory Standards (NCCLS) (1997) Performance standards for antimicrobial disk susceptibility test (6th ed.). Approved Standard. M100-A6. Wayne, Pensylvania, USA.

Setzer WN, Vogler B, Schmidt JM, Leahy JG, Rives R (2004) Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. Fitoterapia 75:192-200.

Sonboli A, Babakhani B, Mehrabian AR (2006) Antimicrobial activity of six constituents of essential oil from *Salvia*. Z Naturforsch C 61:160-164.

Sun XY, Zheng YP, Lin DH, Zhang H, Zhao F, Yuan CS (2009) Potential anti-cancer activities of Furanodiene, a Sesquiterpene from *Curcuma wenyujin*. Am J Chin Med 37:589-596.

Tan AM, Li YS, Yang H, Wang ZT, He HP, Zhang M, Hao XJ. 2004. Two new pyrrolizidines from *Liqularia lankongensis*. Chin Chem Lett 15:68–70.

Tang YL, Deng YR, Wang HQ (2003) Chemical components of essential oils from the herb of *Ligularia virgaurea*. Zhongguo Zhong Yao ZaZhi 28:627-629.

Tori M, Watanabe A, Matsuo S, Okamoto Y, Tachikawa K, Takaoka S, Gong X, Kuroda C, Hanai R (2008) Diversity of *Ligularia kanaitzensis* in sesquiterpenoid composition and neutral DNA sequences. Tetrahedron 64:4486-4495.

Wang BS, Yen GC, Chang LW, Yen WJ, Duh PD (2006) Protective effects of burdock (*Arctium lappa* Linne) on oxidation of low-density lipoprotein and oxidative stress in RAW 264.7 macrophages. Food Chem 101:729-738.

Zidorn Ch, Grass S, Ellmerer EP, Ongania KH, Stuppner H (2006) Stilbenoids from *Tragopogon orientalis*. Phytochemistry 67:2182-2188.