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Short Communication

Chemical composition and antibacterial properties of essential oil and fatty acids of different parts of *Ligularia persica* Boiss

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Abstract

Objective: The objective of this research was to investigate the chemical composition and antibacterial activities of the fatty acids and essential oil from various parts of *Ligularia persica* Boiss (*L. persica*) growing wild in north of Iran.

Materials and Methods: Essential oils were extracted by using Clevenger-type apparatus. Antibacterial activity was tested on two Gram-positive and two Gram-negative bacteria by using micro dilution method.

Results: GC and GC⁄MS analysis of the oils resulted in detection of 94%, 96%, 93%, 99% of the total essential oil of flowers, stems, roots and leaves, respectively. The main components of flowers oil were cis-ocimene (15.4%), β-myrcene (4.4%), β-ocimene (3.9%), and γ-terpinene (5.0%). The major constituents of stems oil were β-phellandrene (5.4%), β-cymene (7.0%), valencene (3.9%). The main compounds of root oil were fukinanolid (17.0%), α-phellandrene (11.5%) and B-selinene (5.0%) and in the case of leaves oil were cis-ocimene (4.8%), β-ocimene (4.9%), and linolenic acid methyl ester (4.7%). An analysis by GC-FID and GC-MS on the fatty-acid composition of the different parts of *L. persica* showed that major components were linoleic acid (11.3-31.6%), linolenic acid (4.7-21.8%) and palmitic acid (7.2-23.2%). Saturated fatty acids were found in lower amounts than unsaturated ones. The least minimum inhibition concentration (MIC) of the *L. persica* was 7.16 µg/ml against *Pseudomonas aeruginosa*.

Conclusion: Our study indicated that the essential oil from *L. persica* stems and flowers showed high inhibitory effect on the Gram negative bacteria. The results also showed that fatty acids from the stems and leaves contained a high amount of poly-unsaturated fatty acids (PUFAs).

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Introduction

For centuries, essential oils have been used for the treatment of infections and diseases, in different parts of the world (Rios and Recio, 2005). Nowadays, the use of essential oils is growing and there is a

noticeable range application for them (e.g. in food and beverages industry, as fragrances in perfumes and cosmetics) but the oils also cover a broad spectrum of biological activities which has aroused the researchers' interest. In the past two decades, there has been a lot of research to study the antimicrobial activity of essential oils. The main constituents of some plant essential oils are thymol, carvacrol, linalool and eugenol that have been shown to have a wide spectrum of antimicrobial activities (Kalemba and Kunicke, 2003; Dorman and Deans, 2000). Recently, the antibacterial properties and potential use of essential oils in foods have been investigated (Burt, 2004).

Antimicrobial activities of spices and herbs have been known for several centuries (Bagamboula et al., 2003). Essential oils and their components are becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purposes, including food preservation, complementary medicine and natural therapeutics. At present, essential oils are used by the flavoring industry for flavor enhancement and for their antioxidant effects (Cosentino et al., 2003). Fatty acids have also a wide range of functions (Elias, 1983). For example, some polyunsaturated fatty acids such as nervonic acid, linoleic acid and arachidic acid are vital for human growth (Carvalho et al., 2006).

Ligularia persica Boiss (*L. persica*). is an important species of Compositae family. According to Flora Iranica, there is only one species of *Ligularia* in Iran that is endemic of north of Iran. The local names of this genus are ″Zabantala″ and ″Pirsonbol″ (Rechinger, 1989). *Ligularia* species are used in traditional medicines such as treatment of coughs, inflammations, jaundice, scarlet fever, rheumatoid arthritis, and hepatic diseases (Xie et al., 2010). Up to now, several phytochemical studies have identified various compounds such as steroids, alkaloids, flavonoids, lignans, sesquiterpenoids, and terpenoids in *ligularia* species (Yang et al., 2011).

The secondary metabolites reported from *L. persica* have anti-bacterial, anti-lung cancer, anti- stomach cancer, anti-hepatotoxicity, anti-thrombotic, anti-coagulation and anti-insect activity (Yang et al., 2011). Extraction of roots of *L. persica* and chromatographic separation revealed one new derivative of tovarol, four new derivatives of shiromodiol, α - and β - eudesmol, bakkenolide A and four known eremophilane derivatives (Marco et al., 1991). There is a report on chemical composition and antimicrobial activities of aerial parts of *L. persica* in the literature (Mirjalili and Yousefzadi, 2012). However, no previous work has been conducted on different part of this plant. Also, there is no report on the fatty acids composition and antibacterial activity of the different parts of *L. persica* essential oils. Therefore, the aim of this research is to analyze the chemical constituents and fatty acids of different parts of *L. persica* and antibacterial activity of the essential oils of different parts of *L. persica* was then investigated and discussed.

Materials and methods

Plant Material

L. persica was collected during the flowering stage in July 2012 from Pole Zangule located in central Alborz Mountains (Mazandaran province, North of Iran). The specimen was identified and authenticated by a taxonomist, Dr Alireza Naqinezhad, and a voucher herbarium specimen was deposited in the herbarium of the Department of Biology, University of Mazandaran (No. 1505). The plant material was air-dried at room temperature and protected from light for one week.

Isolation of essential oil

Different parts of *L. persica* (50 g) were subjected to hydro-distillation for 2 hours using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate, filtered and stored at +4 °C until analysis.

Oil extraction and fatty acid methylation preparation

Dried ground plant materials (different parts of *L. persica*) were extracted with hexane using a Soxhlet apparatus (70 °C, 8 hours) to obtain the fatty components. After removing hexane using rotary evaporator, the oily mixtures were derivatized to produce their methyl esters by trans-esterification process with 2 M methanolic KOH at 70°C for 15 minutes (Tavakoli et al., 2012; Paquat, 1992). The organic phases were analyzed by GC-FID and GC-MS systems.

Analysis of the essential oil and fatty acids

GC-FID analysis

The GC analysis of the essential oil and fatty acids was performed using an Agilent Technology 7890A Network gas chromatographic (GC) system, equipped with an FID detector. Compounds were separated on a DB- 5 Fused-silica capillary column (60 m long, 250 µm i.d. with 0.25µm film thickness, Agilent Technology). A sample of 1.0 µL was injected in the split mode with a split ratio of 1:5. The oven temperature was programmed to rise from 50 to 240°C at a rate of 4°C/min.

GC-MS analysis

The GC-MS analysis was performed with an Agilent Technology 5975C mass-selective detector coupled to anAgilent Technology 7890A gas chromatographic. For GC–MS detection, an electron ionization system, with ionization energy of 70 eV, was used. Column oven temperature program was the same as in GC analysis. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Mass range was 30 - 600 *m/z*, while injector and MS transfer line temperatures were set at 220 °C and 250 °C, respectively.

Compounds identification

The oil components were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6- C_{23}) and the oil on DB-5 column under the same conditions. Identification of individual compounds was done by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0 n,NIST 08) or with authentic compounds and confirmed by comparing their retention indices with authentic compounds or with those reported in the literature (Davies, 1990; Shibamoto, 1987; Adams,2007).

Antimicrobial activity

Microbial strains

The essential oils were tested against two Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, and *Streptococcus sobrinus* ATCC 27609 and two Gram-negative bacteria including *Escherichia coli* ATCC 25922,and *Pseudomonas aeruginosa* ATCC 27853.

Micro dilution broth method

Micro-dilution susceptibility assay was performed using the NCCLS method for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Wayne, 1999). Dilutions were prepared in 96-well microtiter plates to get final concentrations ranging from 0 to 4,000 µg/ml. All tests were performed in BHI broth medium. Bacterial cell numbers were adjusted to approximately 1×10^8 CFU (colony forming units)/ml. The 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculums into each well. The final volume in each well was 200 µl. The plates were incubated at 37 °C for 24 hours. Gentamicin was used as positive standard in order to control the

sensitivity of the microorganisms. The growth was indicated by the presence of a white 'pellet' on the well bottom. The MIC was calculated as the highest dilution showing complete inhibition of the tested strains.

Results

Chemical Composition of the Essential Oil

The yields of essential oils of leaf, flower, stem and root of *L. persica* were 0.32%, 1.48%, 0.65%, 0.61% (w/w % based on dry matter weight), respectively . The essential oils of different parts of *L. persica* were obtained by hydro-distillation method and examined by GC-FID and GC–MS. The colors of essential oils were yellow to green. The results obtained from GC-FID and GC–MS analysis of the essential oils of *L. persica* were shown in Table 1. GC and GC⁄MS analysis of the oils were resulted in detection 94%, 96%, 93%, 99% of the total essential oil of flowers, stems, roots and leaves, respectively. The main components of flower oil were cis-ocimene (15.4%), β-myrcene (4.4%), β-ocimene (3.9%), and γ-terpinene (5.0%). The major constituents of stem oil were β-phellandrene (5.4%), β-cymene (6.9%), and valencene (3.9%). The main compounds of roots oil were fukinanolid (17.0%), α-phellandrene (11.5%), and β-selinene (5.0%) and in the case of leaves oil were cis-ocimene (4.8%), β-ocimene (4.9%), linolenic acid, and methyl ester (4.7%).

Fatty acid Composition

The analysis of fatty acid obtained from different parts of *L. persica* revealed the presence of over 19 compounds as shown in Table 2. The major components were linoleic acid (10.9-31.6%), linolenic acid (4.7-21.8%) and palmitic acid (7.2-23.2%). The results demonstrated that the quantities of unsaturated fatty acids (20.4-54.7%) were higher than saturated fatty acids (9.1-28.9%).

Table 1. Essential Oil composition of the different parts of *L. persica*.

Chemical Componds	RI[a]	%Leaf	%Flower	%Stem	Root %
α –Thujene	927	0.2	trace	0.4	———
α –Pinene	939	1.3	0.6	2.6	1.8
α –Fenchene	952	0.1	0.1	0.4	———
Camphene	954	0.2	0.1	0.6	0.1
Verbenene	959	trace	———	trace	0.1
β –Phellandrene	981	2.6	———	5.4	———
Sabinene	982	2.7	trace	———	———
β – Pinene	984	0.9	0.7	2.5	3.1
β –Myrcene	995	2.0	4.4	2.8	0.5
α –Phellandrene	1007	0.6	0.2	2.7	11.5
δ - 3 Carene	1014	trace	———	0.3	2.4
α-Terpinene	1019	0.3	0.1	0.6	0.2
β –Cymene	1026	0.8	0.2	6.9	2.3
D-Limonene	1030	0.9	0.2	1.6	0.9
cis-Ocimene	1036	4.8	15.4	1.0	———
β –Ocimene	1041	4.9	3.9	3.7	1.0
γ –Terpinene	1053	0.4	5.0	0.8	2.0
trans-Sabinene hydrate	1057	———	———	0.2	———
cis-Sabinene Hydrate	1058	0.09	trace	———	———
E-Citral	1061	0.3	trace	0.5	———
Linalool	1098	0.4	trace	0.3	0.2

Chemical Componds	RI[a]	%Leaf	%Flower	%Stem	Root %
Alloocimene	1132	2.7	4.1	1.5	———
cis-β-Terpineol	1144	0.1	———	trace	———
4-Terpineol	1178	0.4	0.1	0.4	———
α-Terpineol	1189	0.3	trace	0.1	0.1
Myrtenal	1207	0.4	———	0.4	———
Carvacrol	1299	0.2	———	0.5	0.4
4-Decenoic acid, methyl ester	1311	1.8	0.2	0.7	———
Myrtenol	1335	0.3	———	trace	———
(+)-4-Carene	1357	0.4	———	0.5	———
α –Selinene	1363	———	———	0.1	———
trans-Carveol	1375	0.2	———	0.2	———
Geranyl acetate	1383	0.6	———	1.1	———
β –Damascenone	1395	0.2	———	———	———
β –Bourbonene	1403	———	trace	———	———
β – Elemene	1405	———	1.1	0.4	———
Mentha-1,4,8-triene	1407	0.8	———	———	———
trans-Caryophyllene	1426	0.3	0.3	0.8	2.0
α-Cedrene	1437	0.1	trace	0.1	———
α –Amorphene	1446	———	trace	———	———
Germacrene B	1447	0.1	———	———	0.3
α –Gurjunene	1454	———	———	trace	0.6
β –Farnesene	1455	0.2	1.2	0.1	———
Thujopsene	1456	0.7	———	———	0.7
5,9-Undecadien-2-one	1459	0.2	———	———	———
β –Selinene	1463	0.3	0.4	0.4	5.0
Geranyl propionate	1477	———	———	0.4	———
β –Guaiene	1478	0.4	———	———	0.3
1s,Cis-Calamenene	1486	———	———	0.2	0.2
β –Ionone	1430	———	———	0.1	———
(-)-Aristolene	1490	0.2	0.2	0.2	1.3
γ –Curcumene	1492	———	———	1.2	———
Germacrene-D	1505	0.7	1.1	———	———
Valencene	1509	1.9	3.3	4.0	7.1
Vitispirane	1522	———	0.4	———	———
γ–Cadinene	1534	———	0.2	———	———
β –Agarofuran	1537	1.2	1.5	2.3	1.0
δ –Cadinene	1540	0.3	0.1	0.1	1.5
(E,Z)- α-Farnesene	1543	———	0.2	———	———
γ –Gurjunene	1544	———	———	0.1	0.4
Cis-A-Bisabolene	1545	———	trace	———	1.5
α –Agarofuran	1550	1.2	———	2.8	———
β –Vatirenene	1583	0.2	0.1	———	———
γ –Elemene	1598	0.4	———	———	———
(+) Spathulenol	1600	———	———	0.1	———
(-)-Spathulenol	1602	1.4	0.7	———	———
Caryophyllene oxide	1608	0.8	0.3	0.5	0.1
Diepi-.α.-cedrene epoxide	1609	———	———	———	1.1

Chemical Componds	RI[a]	%Leaf	%Flower	%Stem	Root %
Guaiol	1610	———	———	0.2	———
(-)-Lepidozene	1628	———	———	0.2	———
γ –Eudesmol	1633	1.1	2.6	3.5	———
β –Eudesmol	1652	———	———	1.4	———
β –Cadinene	1654	3.3	3.0	0.1	0.6
α –Eudesmol	1656	0.8	———	———	———
(+)-Calarene	1658	2.7	1.7	0.2	2.1
Hinesol	1660	———	———	0.2	———
Cubenol	1663	———	trace	———	———
Elemol	1668	———	———	1.2	———
Veridiflorol	1674	3.0	———	0.3	———
Aromadendrene	1681	2.8	———	———	———
β –Neoclovene	1689	0.4	———	3.1	———
Liguhodgsonal	1714	1.2	———	———	0.6
Cyercene I	1720	3.3	2.2	2.1	1.2
α-Ionene	1745	———	0.1	———	———
β –Thujone	1775	———	———	———	2.9
α –Guaiene	1808	0.1	0.1	———	0.3
Fukinanolid	1836	———	1.0	1.3	17.0
3,5-Dihydroxytoluene	1856	———	3.7	1.7	———
2,5-Furandicarboxaldehyde	1870	2.6	———	———	0.7
Pentadecanoic acid, ethyl ester	1897	0.2	1.2	———	———
Hexadecanoic acid, methyl ester	1930	1.2	1.2	0.5	———
Isophytol	1951	0.1	———	0.1	———
n-Hexadecanoic acid	1969	0.9	0.5	1.8	0.7
Hexadecanoic acid, ethyl ester	1997	0.7	0.3	0.2	trace
Linolenic acid	2105	0.4	2.2	0.4	———
Linolenic acid, methyl ester	2106	4.7	0.6	1.5	———
Phytol	2115	trace	trace	0.1	———
Octadecanoic acid, methyl ester	2126	0.1	trace	trace	———
9-Eicosyne	2132	———	———	0.1	———
1,19-Eicosadiene	2138	———	———	0.1	———
cis-9-Hexadecenal	2155	0.1	———	———	———
Linoleic acid ethyl ester	2163	1.6	0.5	0.2	———
2-Ethylhexyl trans-4-methoxycinnamate	2169	———	———	0.2	———
Octadecanoic acid, ethyl ester	2194	0.1	———	———	———
n-Docosane	2200	trace	trace	trace	———
1-Chloro-Nonadecane,	2201	trace	———	———	———
Bicyclo[10.8.0]eicosane	2224	trace	trace	———	———
Tricosane	2301	0.3	0.8	0.1	———
Monoterpene hydrocarbons		28.6	35.1	34.5	25.9
Oxygenated monoterpenes		4.9	0.4	4.4	3.6
Sesquiterpen hydrocarbons		22	17.3	18.4	26.1
Oxygenated sesquiterpens		7.2	4.6	9.1	18.8
Diterpenoids		0.4	0.9	0.2	———

Chemical Componds	RI[a]	%Leaf	%Flower	%Stem	Root %
Fatty acids		12.3	7.0	4.8	0.75
Aldehydes		2.7	———	———	0.7
Other hydrocarbons		0.6	3.8	1.9	———
Others		20.0	25.0	22.1	17.3
Total		98.7	94.1	95.4	93.1

[a]RI: Retention index relative to n-alkanes (C_6 –C_{23}) on a DB-5 column.

Table 2. Fatty-acid composition of the different parts of *L. persica.*

Methyl esters	RT(min)	%Leaf	%Flower	%Stem	%Root
Butanoic acid, 3-methyl-, methyl ester	9.2	0.3	0.2	———	———
2-Butenoic acid, 2-methyl-, methyl ester	11.01	3.6	0.8	———	2.1
Octanoic acid, methyl ester	23.09	———	5.9	———	———
Dodecanoic acid, methyl ester	36.8	———	6.1	———	0.1
Tetradecanoic acid, methyl ester	42.6	1.7	4.7	0.6	———
Methyl 9-methyltetradecanoate	45.3	———	0.2	———	———
Pentadecanoic acid, methyl ester	45.4	———	———	———	0.6
Pentadecanoic acid, 14-methyl-, methyl ester	46.9	———	———	———	0.3
9-Hexadecenoic acid, methyl ester	47.3	———	———	———	0.6
Hexadecanoic acid, methyl ester	47.9	9.5	11.7	23.2	7.2
Hexadecanoic acid, 14-methyl-, methyl ester	50.3	———	———	———	0.1
Heptadecanoic acid, methyl ester	49.4	0.7	———	1.6	0.71
9-Octadecenoic acid, methyl ester	51.2	———	———	———	0.3
9,12-Octadecadienoic acid, methyl ester	51.9	11.3	10.9	31.6	12.4
9,12,15-Octadecatrienoic acid, methyl ester	52.1	21.8	9.1	21.2	4.7
Octadecanoic acid, methyl ester	52.6	7.7	7.2	2.0	0.3
Eicosanoic acid, methyl ester	58.5	1.0	———	1.1	———
Docosanoic acid, methyl ester	67.6	———	0.1	———	———
other hydrocarbon compounds identified		42.3	43.2	18.8	70.5
∑Saturated fatty acids		13.2	28.9	26.4	9.1
∑Unsaturated fatty acids		44.4	27.1	54.7	20.4

Inhibition of Bacterial Growth

The anti-bacterial activity of the essential oil from *L. persica* against a panel of pathogenic microorganisms was assessed by measurement of minimum inhibitory concentration (MIC). The results are presented in Table 3. It can be concluded that the essential oil of root has the highest anti-bacterial activity and the oil of the leaves has the least efficient antibacterial activity among other parts. The Gram-negative bacterium that exhibited a higher sensitivity to the tested oils was *Pseudomonas aeruginosa*. The essential oil from stems showed the highest anti-bacterial effect against *Pseudomonas aeruginosa* (7.16 μg/ml in terms of MIC) and the least anti-bacterial activity was seen for leaves essential oil against *Staphylococcus aureus* (375 μg/ml in terms of MIC).

Table3. MIC (µg/ml) of the *Ligulari apersica* essential oils.

Types of bacteria	**Gram negative**		**Gram positive**	
Microorganism:	*Pseudomonas aeruginosa*	*Escherichia coli*	*Streptococcus sobrinus*	*Staphylococcus aureus*
ATCC number:	27853	25922	27609	25923
Flowers essential oil	7.8	250	15.6	62.5
Leaves essential oil	23.4	187.5	187.5	375
Stems essential oil	7.2	114.7	114.7	28.7
Roots essential oil	15.6	62.5	31.2	31.2
gentamicin	1	1	0.2	0.5

MIC= Minimum Inhibitory Concentration

Discussion

A comparison between reported chemical composition of the aerial parts of *L. persica* showed that the similar composition were obtained (Mirjalili and Yousefzadi, 2012). In general, monoterpenes and sesquiterpenes were more abundant as compared to the other compounds. In addition, the presence of significant amounts of various bioactive constituents indicates a possible industrial use of these plants. Fukinanolid or bakkenolide A (17.0%), as the most abundant sesquiterpene in roots, α-pinen have been recently introduced as a powerful anti-microbial and anti-tumor agent (Rustaiyan et al., 1999). Cis-ocimen that was the most abundant chemical in flowers (15.4%) is used as raw material in perfumes and cosmetics. Therefore, the essential oils of *L. persica* are suitable as natural supplement sources for food, cosmetic and pharmaceutical industries.

In addition, the amounts of the unsaturated fatty acids in the leaves and stems were higher than of the flowers and roots. Unsaturated fatty acids play a crucial role in human nutrition and health. Polyunsaturated fatty acids (PUFAs) have been considered as health-promoting nutrients in recent years. A growing body of studies illustrates the benefits of PUFAs in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finley, 2001).

Our study reported the secondary metabolites in essential oil and fatty acids extracted from different parts of *Ligularia persica*, as well as their antibacterial activities. These results indicate that *L. persica* may be a rich source of natural products with biological activities.

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Conflict of interest

The authors declare that they have no conflict of interest.

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