Journal of Medicinal Plants Research Vol. 5(20), pp. 5127-5131, 30 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Chemical constituents, antimicrobial and antioxidant activity of the hexane extract from root and seed of *Levisticum persicum* Freyn and Bornm.

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Accepted 5 July, 2011

The hexane extracts from root and seed of *Levisticum persicum* Freyn and Bornm, which were collected from northwestern Iran were obtained by Soxhlet apparatus. The fatty acids were derived to methyl esters and determined by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) systems. The extracts from the root and seed were characterized by a high amount of unsaturated fatty acids (UFA) (45.9 and 58.6%, respectively) and some of sesquiterpenoid compounds. The main components of the root and seed extracts were ω-3 (29.2 and 48.1%), acorenone B (12.6 and 8.3%), ω-6 (9.9 and 7.6%), germacrene D (8.8 and 9.6%) and β-farnesene (8.7 and 8.3%), respectively. The hexane extract from seed of *L. persicum* detected as an important source of ω-3 compound. The antioxidant activity of both hexane extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate that extracts from different parts of *L. persicum* possess considerable antioxidant activity. The highest radical scavenging activity was detected in seed (IC_{50} = 95 µg/mL). The antimicrobial activity of the extracts of those samples were determined against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The bioassay showed that the both oils exhibited good antimicrobial activity. This study reveals that all the parts of this plant are attractive sources of fatty acid components, especially the essential ones, as well as of effective natural antioxidants.

Key words: *Levisticum persicum*, Apiaceae (Umbelliferae), fatty acid, antioxidant activity, antimicrobial activity, ω- 3.

INTRODUCTION

The genus Levisticum is represented in the flora of Iran by only one species, *Levisticum persicum* Freyn et Bornm. (syn. *Levisticum officinale* Koch.) (Rechinger, 1987; Mozaffarian, 2007). *L. persicum* (Garden lovage) is a perennial aromatic plant from the Apiaceae (Umbelliferae) family cultivated in most European countries. All parts of the *L. persicum* exhibit a strong flavor which generally characterizes celery. The root extract has a warm-spicy note, but the seeds and leaf flavor are more diffusive and penetrating (Toulemonde et al., 1987, 1988). The essential oil from the roots, leaves and seeds of *L. persicum* are used in the food, beverage, perfumery and tobacco industries. The root of *L. persicum* has also been known for centuries as a traditional medicine possessing carminative and spasmolytic activity (Cu et al., 1990; Lawrence, 1987; Gijbels et al., 1982; Segebrecht and Schilcher, 1989). *L. persicum* has long been cultivated in Europe and North America.

The chemical composition of the herb (leaf) oil has been the subject of previous study (Cisowski, 1988; Lawrence, 1980; Tibori et al., 1974; De Pooter et al., 1985). The phthalides are believed to play a major role in the aroma of *L. persicum* (Lawrence, 1987; Gijbels et al., 1982; Majchrzak et al., 2001). The phthalide ligustilide has been found to be appropriate for phytochemical identification and quality monitoring of the drug (Segebrecht and Schilcher, 1989). The published results revealed that major volatile constituents obtained from *L. persicum* were α-terpinyl acetate (40.5%) and

β-phellandrene (16.7%) (Samiee et al., 2006). In a previous investigation on composition of the essential oil of *L. persicum* root from some European countries, Raal et al. reported that the principal components were β-phellandrene (0.1 to 48.9%), pentylcyclohexadiene (0 to 12.3%), trans-sabinyl acetate (0 to 12.1%), α-terpinyl acetate (0 to 26.1%), (Z)-3-butylidene phthalide (0.1 to 31.2%) and (Z)-ligustilide (0.2 to 70.9%). Phthalide isomers were predominant (73.2 to 82.6%) in the oils from Estonia, France and Belgium (Raal et al., 2008).To the best of our knowledge, there is no previous report on the fatty acid composition of the root and seed extracts from the *L. persicum* and those biological activities. Therefore, it is important and necessary to investigate further the composition of the root and seed hexane extracts and biological activities.

MATERIALS AND METHODS

Plant materials

Root and seed of *L. persicum* were collected separately in the Givi-Ardabil road (Ardabil province in northwest Iran) area at an altitude of 1750 m in August 2010 A voucher specimen (L-325) is kept at the Herbarium of Agriculture Research in Ardabil Center (HARAC), Iran.

Extraction

Dried and powdered materials (root and seed) were extracted with hexane using a Soxhlet apparatus (70ºC, 4 h) to obtain the fatty acids, sesquiterpene compounds and the other apolar constituents. During extraction procedures, hexane (95%) was used. The extracts were concentrated by rotary evaporator under vacuum at 40ºC. The extraction yields were presented in Table 2.

Methylation of hexane extract:

After removing hexane using rotary evaporator, the oily mixtures were derived to their methyl esters using the trans-esterification process as par the reports of the international olive oil council (IOOC) (2001) and IUPAC (1992). In this process, dried hexane extracts were dissolved in hexane and then extracted with 2M methanolic KOH at room temperature for 60 s. The upper phases were analyzed by GC/FID and GC/MS systems.

Gas chromatography (GC) analysis

GC analysis was performed on a Shimadzu 15A gas Chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50m × 0.2mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 5 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table 1.

Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis was performed using a Hewlett Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25µm). The column temperature was kept at 60°C for 5 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 1 mL/min. MS were taken at 70 eV. The fatty acids and terpenoids were identified by comparing their retention times and mass peaks with those of standard compound mixtures and by NIST-Wiley library data search. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Antioxidant activity tests

The DPPH assay was carried out according to the modified method (Cheung et al., 2003). Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of hexane extract in different concentrations (0.1 to 1.6 mg/mL) and kept in the dark for 10 min. The absorbance of the resulting solution was recorded on a spectrometer at 520 nm against a blank of hexane. Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as IC_{50} values (µg extract/mL) for comparison. IC_{50} value of each sample defined as the concentration of sample required for the 50% decrease in absorbance of the blank was calculated.

Antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the extracts were evaluated by the disc diffusion method (DDM) using Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi (Baron and Finegold, 1990). Discs containing 30 µL of the hexanic extracts were used and growth inhibition zones were measured after 24 and 48 h of incubation at 37 and 24°C for bacteria and fungi, respectively. Gentamicin and tetracycline for bacteria and nystatin for fungi were used as positive controls. The microorganisms used were: *Bacillus subtilis* ATCC 9372, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 3583, *Pseudomonas aeruginosa* ATCC 27852, *Escherichia coli* ATCC 25922, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 5027 and *Saccharomyces cerevisiae* ATCC 9763.

RESULTS AND DISCUSSION

The results obtained in the analyses of the hexane extract of *L. persicum* root and seed are listed in Table 1, in which the percentage and retention time of components are given. According to the results, the hexane extract yields studied of the different part of *L. persicum* were found 2.4 (root extract) and 3.2% (seed extract) on the basis of dry weight of the plant materials. The highest total percentage was detected in seed. The total contents of hexane extracts varied from 99.3 to 99.7% (Table 1). The major saturated and unsaturated fatty acid including linolenic (ω-3), linoleic (ω-6) and 6-Octadecenoic acid are shown in the Table. The major polyunsaturated fatty acids (PUFAs) were α-linolenic (ω-3) and linoleic (ω-6) acids. As can be seen in Table 1, about 99.7% (15 components) of the extract from seed and 99.3% (16 components) from root extract were identified.

Table 1. Chemical composition (%) of the hexanic extract from root and seed of *Levisticum persicum*.

Compound* (Related fatty acid)	Rt (min)	r (%)	s (%)
(+)-β-Farnesene	7.6	8.7	8.3
(+)-β-Funebrene	7.7	4.3	1.9
Germacrene- D	7.9	8.8	9.6
β- Selinene	8.1	3.5	1.2
Zingiberene	8.2	4.4	5.7
β-Bisabolen	8.3	1.7	0.5
β- Himachalene	8.4	1.4	0.2
β-Sesquiphellandrene	8.5	2.3	0.1
Germacrene B	8.6	0.7	-
Carotol	8.7	0.7	1.1
Acorenone B	9.6	12.6	8.3
Hexadecanoic acid, methyl ester (palmitic acid)	11.6	2.7	3.5
9,12-Octadecadienoic acid, methyl (linoleic acid) or ω-6	12.8	9.9	7.6
9,12,15-Octadecatrienoic acid, methyl ester(linolenic acid) or ω- 3	12.9	29.2	48.1
6-Octadecenoic acid, methyl ester (6-Octadecenoic acid)	13.0	6.8	2.9
Octadecanoic acid, methyl ester(Stearic acid)	13.1	1.6	0.7
Total (%)	--	99.3	99.7

*The composition of the extracts was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention times (Rt). Rt= Retention time; r= root; s= seed.

Table 2. Class compositions and yield of the hexanic extract from root (r) and seed (s) of *Levisticum persicum*.

Class composition	r (%)	s (%)
Terpenoid	49.1	36.9
Saturated fatty acid (SFA)	4.3	4.2
Unsaturated fatty acid (UFA)	45.9	58.6
UFA/SFA	10.7	13.9
Yield	2.4	3.2

There were some differences in the fatty acid profiles of the different part of this plant. The unsaturated fatty acid contents were higher than saturated ones and some of the terpenoids (sesquiterpene compounds) were observed in both parts of this plant. In fact, both fractions mainly include unsaturated fatty acids, with a clear predominance of α-linolenic acid (ALA or ω-3) and linoleic acid (LA). One of the essential fatty acids (EFAs), ω-3 (ALA) was a predominant component in seed of *L. persicum*. Linolenic acid is an omega-3 fatty acid, ranging from 29.2 (in root) to 48.1% (in seed) in this work. The main terpenoid compounds in the *L. persicum* (root and seed) extracts samples studied were acorenone- B (12.6 and 8.3%), β-farnesene (8.7 and 8.3%) and germacrene D (8.8 and 9.6%), respectively. The ratios of unsaturated fatty acid (UFA)/ SFA (saturated fatty acid) were 10.7 and 13.9 in extract from root and seed, respectively (Table 2). The hexanic extract of seed from this plant had a higher proportion of UFA compared to root part (Table 1).

The antioxidant activity of hexane extracts was also reported for the first time. Results obtained in the antioxidant study of the samples are shown in Table 3. Antioxidant activity was tested according to the DPPH (2, 2-diphenyl-1-pycrylhydrazile) radical scavenging method. The both extracts from root and seed obtained from *L. persicum* scavenged the DPPH radical in a dose-dependent manner, and the DPPH radical scavenging activity (IC_{50}) was decreased in the following order: seed > root (Table 3). According to this data, seed was the most efficient free radical scavenger by the lowest IC_{50} value of 95 µg/mL among both the hexane extracts. The activity of the reference antioxidant (vitamin C) was much higher than that of seed oil. Although, seed oil did not differ considerably in compositions, it exhibited the best DPPH scavenging activity.

The extracts of root and seed from *L. persicum* was tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The results, presented in Table 4, show that the hexane extracts exhibited a good biological activity against all tested fungi

Table 3. DPPH free radical scavenging activity of hexane extracts of root, seed from *L. persicum* and standard antioxidant, vitamin C.

No	Sample (hexanic extract)	IC_{50} (µg/mL)
1	Root	235
2	Seed	95
3	Vitamin C(Ref.)	27

Table 4. Antimicrobial activity of the hexane extracts of root (r) and seed (s) of *Levisticum persicum*.

Microorganism	Zone of inhibition (mm) **				
	Hexane Extracts		Antibiotics		
	r*	s*	Gentamicin	Nystatin	Tetracycline
Subtilis	19.7±0.4	19.9±0.2	NT[b]	NT	22.3±0.2
S. epidermidis	18.6±0.3	18.7±0.1	NT	NT	34.1±0.7
E. faecalis	8.8±0.1	9.1±0.3	NT	NT	9.2±0.8
S. aureus	16.5±0.1	16.9±0.2	NT	NT	21.4±0.6
K. pneumoniae	NA[a]	NA	20.1±0.4	NT	NT
P. aeruginosa	9.5±0.2	9.1±0.3	11.4±0.3	NT	NT
E. coli	13.5±0.1	14.0±0.4	24.2±0.8	NT	NT
A. niger	NA	NA	NT	16.2±0.7	NT
C. albicans	14.5±0.5	14.2±0.2	NT	18.5±0.2	NT
S. cerevisiae	13.9±0.4	14.7±0.3	NT	18.3±0.4	NT

*r: root, s: seed ; [a] NA: Not Active; [b] NT: Not Tested. **Inhibition zone diameter (mm), including diameter of sterile disk 6 mm; values are given as mean ±SD.

and bacteria except for a resistant Gram-negative bacteria, *K. pneumoniae*, as well as a fungi, *A. niger.* The most sensitive microorganisms against root and seed extracts were *B. subtilis* with inhibition zones of 19.7 to 19.9 mm, *S. epidermidis* 18.6 to 18.7 mm and *S. aureus* 16.5 to 16.9 mm, respectively. Other microorganisms were found to be less sensitive to the extracts with inhibition zones ranged from 8 to 15 mm. It is conceivable that the antimicrobial property of the hexane extracts from *L. persicum* might be ascribed to its high content of fatty acids and terpenoid compounds.

ACKNOWLEDGEMENT

The author wish to thank Iran National Science Foundation (INSF) for financial support for this investigation.

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