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Original Article

Composition, Antioxidant and Antimicrobial Activities of Hexanic Extract from Prunus armeniaca L. Kernel from North-West Iran.

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Abstract

Chemical characteristics, fatty acid composition, antioxidant and antimicrobial activities of Prunus armeniaca kernel oil were evaluated in this study. The hexane extracts of kernel of P. armeniaca, 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 oil from kernel extract contained ω-6 (51.6%). The other main components were hexadecanoic acid (palmitic acid) (23.0%), octadecanoic acid (stearic acid) (10.6%) and 9-hexadecenoic acid (2.7%). Hexane extract of kernels from P. armeniaca detected as an important source of ω-6 compound. The antioxidant activity of the extract was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate that hexane extract from P. armeniaca kernels possess considerable antioxidant activity. The IC50 values in the DPPH assay was 165μg/mL. The antimicrobial activity of the extract was determined against some Gram-positive and Gram-negative bacteria (Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, S. 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 oil exhibited moderate antimicrobial activity. This study reveals that the hexanic extract from kernel of this fruit is attractive sources of ω-6.

Keywords: Prunus armeniaca, Rosaceae, kernel, ω- 6, fatty acid, antioxidant activity, antimicrobial activity.

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INTRODUCTION

Plant seeds and kernels always remain an important source of proteins and essential fatty acids for their nutritional, industrial and pharmaceutical applications [1]. The human body needs essential fatty acids to construct and repair cell membranes enabling the cells to obtain optimum nutrition and expel harmful waste products. A primary function of essential fatty acids, which support the cardiovascular, reproductive, immune and nervous systems, is the production of prostaglandins. These regulate body functions such as heart rate, blood pressure, blood clotting, fertility and play a role in immune system by regulating inflammation [2-5]. The seeds and kernels are important sources of oils being of nutritional, industrial and pharmaceutical importance. Even though an ideal intake ratio of omega-6 to omega-3 fatty acids is between 1:1 and 4:1, most of the Americans intake a ratio between 10:1 and 25:1 (6-8). The minimum healthy intake per adult for both
linolenic (omega-3) and linoleic (omega-6) acid via diet is 1.5 gram/day of each. Fatty acids deficiency and omega 6/3 imbalance is linked with serious health conditions, such as heart attack, cancer, insulin resistance, asthma, lupus, schizophrenia, depression, postpartum depression, accelerated aging, stroke, obesity, diabetes, arthritis, and Alzheimer's disease, among others [9-13]. α- Linolenic acid (ALA) is the principal omega-3 fatty acid, which is metabolized into eicosapentaenoic acid (EPA), and later into docosahexaenoic acid (DHA) by a healthy human being [14]. EPA and the gamma-linolenic acid (GLA) are synthesized from linoleic (omega-6) acid, which are later converted into hormone- like compounds known as eicosanoids that aid in many body functions in animals including vital organ function and intracellular activity [15]. Numerous species of the plants which are rich fatty acids especially in seeds are of great importance as herbs and spices. During recent years, plant compounds have come more into the focus of phytomedicine. Their widespread use has raised the interest of scientists in basic research of fatty acids. Especially, the antimicrobial and antioxidant activities of fatty acids have been investigated in recent years [16,17].

The genus Prunus (family: Rosaceae) is represented in Iran by one species [18]. Prunus armeniaca L. (syn: Armeniaca vulgaris Lam.) (Apricot) is a member of this genus and is widely distributed in most countries of the world. Some fruit seeds such as cherry, apricot, citrus and apple can be used as sources of oils. Some seed oils are already used for several purposes: blending with highly saturated edible oils to provide new oils with modified nutritional values as ingredients in paint and varnish formulations, surface coatings and oleo-chemicals, and as oils for cosmetic purposes (Helmy, 1990). The fatty acid analysis of some Turkish apricot seed oils by GC and GC-MS techniques have been studied in Turkey previously. It was reported that linoleic, palmitic, stearic, α -linolenic and oleic acids represent the major constituents of the hexanic extract of this fruit [20]. To the best of our knowledge, this is the first report on the hexanic extract of P. armeniaca kernel and its antioxidant and antimicrobial activities from North- West Iran.

EXPERIMENTAL

Plant materials:

Kernels of P. armeniaca were collected in the Meshkinshahr garden (Ardabil province) area at an altitude of 1850 m in July 2011. A voucher specimen (P- 114) is kept at the Herbarium of Agriculture Research in Ardabil Center, Iran.

Extraction:

Dried and powdered materials (kernel) were extracted with hexane (Merck 98%) using

a Soxhlet apparatus (70ºC, 2.5h) to obtain the fatty acids and the other apolar constituents. The extracts were concentrated by rotary evaporator under vacuum at 45ºC. The extraction yields are presented in Table 2.

Methylation of hexane extract:

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

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). N2 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 3 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.

GC/MS analysis:

GC/MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25μm). The column temperature was kept at 60°C for 3 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 were identified by comparing their retention times and mass peaks with those of standard methyl ester 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 of Cheung et al. [21]. Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of hexane extract in different concentrations (0.1-1.6 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 IC50 values (μg extract/mL) for comparison. IC50 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 [22]. Discs containing 30 μL of the hexanic extracts were used and growth inhibition zones were measured after 24 h and 48 h of incubation at 37°C 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 Prunus armeniaca kernel are listed in Table 1, in which the percentage and retention time of components are given. According to the results, the hexane extract yield of the studied kernel oil of P. armeniaca was found 7.9% on the basis of dry weight of the sample material. The major saturated and unsaturated components including linoleic (ω-6), palmitic and stearic acids are shown in the Table 1. The major polyunsaturated fatty acid (PUFA) was linoleic acid. As can be seen in Table 1, about 93.0% (9 components) of the extract from kernel were identified. The main components of the hexane extract were 9, 12-octadecadienoic acid (linoleic acid or ω-6) (51.6%), hexadecanoic acid (palmitic acid) (23.0%) and octadecanoic acid (stearic acid) (10.6%).

Table 1: Chemical composition (%) of the hexanic extract from seed of P. armeniaca.

Compound * (Related Fatty acid)	Rt (min)	(%)
9-Hexadecenoic acid, methyl ester (9-Hexadecenoic acid)	11.3	2.7
Hexadecanoic acid, methyl ester (palmitic acid)	11.4	23.0
9-Octadecenoic acid methyl ester (9-Octadecenoic acid)	11.7	0.5
9,12-Octadecadienoic acid, methyl ester (linoleic acid, or ω- 6)	12.7	51.6
Octadecanoic acid, methyl ester (stearic acid)	13.0	10.6
11-Eicosenoic acid, methyl ester (11-Eicosenoic acid)	14.2	1.4
Eicosanoic acid, methyl ester (arachidic acid)	14.4	1.6
1,2-Benzenedicarboxylic acid, diisooctyl ester (Diisooctyl phthalate)	15.9	1.1
Squalene	17.5	0.5
Total	-	93.0%

**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.*

The unsaturated fatty acid contents (56.2%) were higher than saturated ones (35.2%). In fact, the kernel extract mainly include unsaturated fatty acids, with a clear predominance of linoleic acid (LA). One of the essential fatty acids (EFAs), LA was a predominant component in kernel of P. armeniaca. Linoleic acid is an omega-6 fatty acid, detected in this work. The ratios of unsaturated fatty acid (UFA)/ SFA (saturated fatty acid) were 1.6 (Table 2). The literature survey on this plant showed presence of some other reports on the same subject.

Table 2: Class compositions and yield of the hexanic extract from P. armeniaca kernel.

Class composition	(%)
Saturated fatty acid	35.2
Unsaturated fatty acid	56.2
Other compounds	1.6
Yield(V/W)	7.9
UFA/SFA*	1.6

** UFA= Unsaturated fatty acid; SFA= Saturated fatty acid.*

For instance, the apricot kernel oil from Egyptian origin was also shown to contain oleic, linoleic, and palmitic acids as the principal fatty acids [23]. In another study on the fatty acid composition of the seed oil of wild apricot growing in India, which was reported to consist of 94% unsaturated fatty acids, it was found to compose of palmitic acid (3.9%), oleic acid (66.2%), linoleic acid (28.2%), and arachidic acid (0.1%), while it did not have stearic and linolenic acid at all, which appeared to be different from our results [24]. In Femenia et al.'s study, the bitter and sweet varieties of P. armeniaca growing in Spain were also shown to contain oleic and linoleic as the principal fatty acids [25].

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 kernel oil obtained from P. armeniaca scavenged the DPPH radical in a dose-dependent manner and the DPPH radical scavenging activity (IC50) are shown in Table 3. According to this data, hexane extract of kernel was the most efficient free radical scavenger by the lowest IC50 value of 165 μg/mL. The activity of the reference antioxidant (vitamin C) was much higher than that of kernel oil.

Table 3. DPPH free radical scavenging activity of hexane extracts from P. armeniaca kernel and standard antioxidant, vitamin C.

No	Sample	IC50 (μg/mL)
1	hexanic extract	165
2	Vitamin C(Ref.)	28

The hexane extract from P. armeniaca 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 moderate biological activity against all tested fungi and bacteria except for two resistant bacteria, Staphylococcus aureus and Pseudomonas aeruginosa, as well as a fungi, Aspergillus niger. The most sensitive microorganisms were Staphylococcus epidermidis, Escherichia coli and Klebsiella pneumoniae with inhibition zones of 14.6, 15.1 and 16.3mm, respectively. Other microorganisms were found to be less sensitive to the extracts with inhibition zones ranged from 7 to 12 mm. It is conceivable that the antimicrobial property of the hexane extracts from P. armeniaca might be ascribed to its high content of fatty acids.

Table 4. Antimicrobial activity of the hexane extract from P. armeniaca kernel.

Tested microorganism	Zone of inhibition (mm) *			
	Sample		Antibiotics	
	Hexane extract	Gentamicin	Nystatin	Tetracycline
B. subtilis	12.5	NTb	NT	22.4
S. epidermidis	16.3	NT	NT	34.3
E. faecalis	7.9	NT	NT	9.6
S. aureus	**NA	NT	NT	21.4
K. pneumoniae	14.6	20.4	NT	NT
P. aeruginosa	NA	12.8	NT	NT
E. coli	15.1	23.9	NT	NT
A. niger	NA	NT	16.7	NT
C.albicans	8.2	NT	18.5	NT
S. cerevisiae	11.6	NT	18.4	NT

**Inhibition zone diameter (mm), including diameter of sterile disk 6 mm.*
***NA = Not Active, NT= Not Tested.*

In conclusion, there were some differences in the fatty acid profiles of another origin of this fruit kernel. Our study on the fatty acid analysis of the apricot showed that the kernel oil of apricot growing in Northwest Iran have variances in their fatty acid contents, which may be due to soil properties, climate, and additional environmental conditions. As mentioned in above studies, apricot has also beneficial health effects as well as its rich unsaturated fatty acid content and therefore, this oil could be considered a good alternative for essential fatty acids for cosmetics and nutraceutical industries.

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