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Full Length Research Paper

Chemical composition and antimicrobial activity of the flower and root hexane extracts of *Althaea officinalis* in Northwest Iran

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The hexane extracts of flower and root of *Althaea officinalis* which were collected from northwestern Iran (Khalkhal) were obtained by Soxhlet apparatus. The fatty acids were derived from methyl esters and determined by gas chromatography/flame ionization detector (GC/FID) and gas chromatograph/mass spectrometry (GC/MS) systems. The hexane extract from the flower and root contained omega-3 (20.5 and 14.9%, respectively). The other main compounds of the flower extract were palmitic acid (13.0%), heptacosane (9.3%) and nonacosane (11.2%). In the root extract, palmitic acid (16.8%), linoleic acid (omega-6) (28.0%) and naphthalene decahydro 2, 6- dimethyl (16.4%) were the main components. The antimicrobial activity of the hexane extracts of those samples were determined against some Gram-positive and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*), as well as three fungi (*Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae*). The bioassay showed that the both extracts exhibited good antimicrobial activity. This study reveals that all the parts of this plant are attractive sources of fatty acid components.

Key word: *Althaea officinalis*, Malvaceae, fatty acid, omega-3, omega-6, palmitic acid, antimicrobial activity.

INTRODUCTION

The genus *Althaea* is represented in the flora of Iran by five species (Mozaffarian, 2007). *Althaea officinalis* L. is a valuable medicinal plant belonging to the Malvaceae family. This plant commonly known as marshmallow, (Khatmi in Persian) is a downy, perennial herb, distributed in the northwest to northeast of Iran. The seeds of this plant are demulcent, diuretic and febrifuge (Mhaskar et al., 2000). The roots of the plant are the most frequently used pharmaceutical raw material, but the flowers are also significant, particularly in folk medicine. A pale pink, five- petaled flower, up to 5 cm in diameter, grow individually or in bunches from the corners of the leaves in the upper part of the stalks.

The roots counteract excess of stomach acid, and are prescribed to treat peptic ulcer, gastritis and intestinal problems including ileitis, colitis, diverticulitis and irritable bowel syndrome. Proven medicinal properties are primarily attributed to the mucilage which is present in the flowers and roots and has antitussive, softening and coating effects (Hojden, 1996; Kozlowski et al., 1989; Strzelecka and Kaminski, 2000). In recent years much effort has been made to search for natural active plant components with diminished adverse effects. Plant polysaccharides are ranked among compounds, which shield and protect the nerve endings and alleviate pain. A study on the aqueous extracts and polysaccharides from *A. officinalis* roots and those effects on cellular internalization and stimulation of cell physiology of human epithelial cells *in vitro* have been reported. Based on the results of the identification, the authors suggest that the

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Abbreviations: GC/FID, chromatography/flame ionization detector; **GC/MS,** gas chromatograph/mass spectrometry; **IOOC,** International Olive Oil Council; **DDM,** disc diffusion method; **PUFAs,** polyunsaturated fatty acids; **ALA or ω-3,** α-linolenic acid; **LA,** linoleic acid; **EFAs,** essential fatty acids; **UFA,** unsaturated fatty acid; **SFA,** saturated fatty acid.

aqueous extracts and polysaccharides from the roots of *A. officinalis* are effective stimulators of cell physiology of epithelial cells which can prove the traditional use of Marshmallow preparations for treatment of irritated mucous membranes within tissue regeneration (Deters et al., 2010).

An ointment prepared from the roots is applied to cure inflammatory tumors, burns, boils and abscesses. A root decoction is utilized as mouth wash to relieve inflammation, bruises, sprain, as an expectorant in cough, hoarseness of voice, bronchitis and whooping cough (Prajapati et al., 2003). Marshmallow preparations belong to the group of agents used for the treatment of respiratory tract infections, stomatitis, pharyngitis and esophagitis as well as digestive tract disorders and metabolism disorders (Buchwald and Szczyglewska, 1999; Kozlowski, 1995). The phytochemical investigations carried out so far have revealed herniarin and umbelliferone. From the roots of *A. officinalis* two flavonoid glycosides were separated. Phenolic acids and coumarins were identified as hypolaetin-8-glucoside and the new flavonoid sulphate isoscutellarein-4-metyl ether-8-glucoside. Besides the mucilage, the flowers contain flavonoids, the most important of which is tiliroside, a compound with a proven anti-inflammatory effect (Gudej, 1990). Kaempferol, chlorogenic acid, caffeic acid, p-coumaric acid, tiliroside and diostin-8-hydroxy-8-O-β-D glycoside were isolated from the dried roots of *A. officinalis* var. Russalka (Gudej, 1991).

To the best of our knowledge, there are no previous reports on the fatty acid composition of the flower and root extracts from the *A. officinalis* 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

Flower and root of *A. officinalis* were collected separately in Khalkhal area (Ardabil province in northwest Iran) at an altitude of 1850 m in August 2010. A voucher specimen (A-25) is kept at the Herbarium of Agriculture Research in Ardabil Center (HARAC), Iran.

Extraction

Dried and powdered materials (Flower and root) 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 (93%) 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 per 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 seconds. The upper phases were isolated and dried over anhydrous sodium sulfate. The oils were stored at 2℃ until analysis by gas chromato graph/mass spectrometry (GC/MS).

GC analysis

GC analysis was performed on a Shimadzu 15A gas Chromatograph equipped with a split/splitless injector (250℃) and a flame ionization detector (250℃). N_2 was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60℃ for 5 min and then heated to 220℃ with a 5℃ /min rate and kept constant at 220℃ 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 0.25 µm). The column temperature was kept at 60℃ for 5 min and progra mmed to 220℃ at a rate of 5° C/min and kept constant at 220 ℃ 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.

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℃ 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 *A. officinalis* flower and root are listed in Table 1, in which the percentage and retention time of components are given. According to the results, the hexane extract yields of the studied different part of *A. officinalis* were found 5.1% (flower extract) and 2.7% (root extract) on the basis of dry weight of the plant materials. The highest total percentage was detected in

Table 1. Chemical composition (%) of the hexanic extract from flower and root of *Althaea officinalis*.

Compound * (Related Fatty acid)	Rt	%(f)	%(r)
Undecyne-1	8.8	0.6	-
Nonanoic acid, methyl ester(Nonanoic acid)	11.9	-	0.3
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl	15.9	1.2	0.2
Tetradecanoic acid, methyl ester(Tetradecanoic acid)	18.3	0.4	0.3
Pentadecanoic acid, methyl ester(Pentadecanoic acid)	19.4	-	0.2
9-Hexadecenoic acid, methyl ester(9-Hexadecenoic acid)	20.2	2.5	0.4
Hexadecanoic acid, methyl ester(Hexadecanoic acid)	20.5	13.0	16.8
Cyclopropaneoctanoic acid, 2-hexyl, methyl ester(Cyclopropaneoctanoic acid, 2-hexyl)	21.2	0.7	0.5
Heptadecanoic acid, methyl ester(Heptadecanoic acid)	21.5	-	0.4
Octadecyne-5	21.6	2.2	0.7
7,10-Octadecadienoic acid, methyl ester(7,10-Octadecadienoic acid)	21.9	4.6	-
9,12-Octadecadienoic acid(ω-6), methyl ester(9,12-Octadecadienoic acid)	22.0	-	28.0
8,11-Octadacadienoic acid, methyl ester(8,11-Octadacadienoic acid)	22.2	5.5	7.4
9,12,15-Octadecatrienoic acid (ω-3), methyl ester(9,12,15-Octadecatrienoic acid)	22.3	20.5	14.9
Octadecanoic acid, methyl ester (Octadecanoic acid)	22.4	2.5	2.5
Naphthalene, decahydro-2,6-dimethyl	22.9	2.6	16.4
10-Nonadecenoic acid, methyl ester(10-Nonadecenoic acid)	23.1	-	0.3
Cyclopropaneoctanoic acid, 2-octyl, methyl ester(Cyclopropaneoctanoic acid,2-octyl)	23.2	0.9	1.3
Dihydroionone	23.5	-	1.7
3-Heptadecen-5-yne	23.8	0.3	-
Heneicosane	24.0	9.0	-
Methyl 2-octylcyclopropene-1-heptanoate	24.1	-	0.5
Eicosanoic acid, methyl ester(Eicosanoic acid)	24.2	2.0	0.9
Tetracosan	24.8	0.6	-
Heneicosanoic acid, methyl ester(Heneicosanoic acid)	25.0	-	0.2
Pentacosane	25.6	3.7	-
Docosanoic acid, methyl ester(Docosanoic acid)	25.8	0.9	0.7
Tricosane	26.4	0.6	-
Tricosanoic acid, methyl ester(Tricosanoic acid)	26.6	-	0.3
Heptacosane	27.1	9.3	-
Tetracosanoic acid, methyl ester(Tetracosanoic acid)	27.4	-	0.3
Octacosane	27.9	1.2	-
Squalene	28.2	3.1	-
Nonacosane	28.8	11.2	0.3
γ-Sitosterol	29.1	-	2.4
Total	---	99.1%	97.9%

*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; f= flower; r= root.

flower. The total contents of hexane extracts varied from 97.9 to 99.1% (Table 1). The major saturated and unsaturated fatty acid including linolenic (ω-3), linoleic (ω-6) and hexadecanoic acids are shown in the Table. The major polyunsaturated fatty acids (PUFAs) were α-linolenic (ω-3) and linoleic (ω-6) and 8, 11-octadacadienoic acids. As can be seen in Table 1, about 99.1% (24 components) of the extract from flower, and 97.9% (25 components) from root extract were identified. 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 in root of this plant.

The hexane extract of flower was characterized by relatively high contents of the saturated fatty acid compounds. 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 flower of *A. officinalis*. Linolenic acid is an omega-3 fatty acid, ranging from 14.9% (in root) to 20.5% (in flower) in this work. The main

Table 2. Class compositions and yield of the hexanic extract from flower (f) and root (r) of *Althaea officinalis*.

Class composition	f (%)	r (%)
Terpenoid	0.0	0.0
Saturated fatty acid (SFA)	59.7	28.0
Unsaturated fatty acid (UFA)	39.4	68.2
UFA/SFA	0.66	2.44
Yield	5.1	2.7

Table 3. Antimicrobial activity of the hexane extracts of flower (f) and root (r) of *Althaea officinalis*.

	Zone of inhibition (mm) **				
Extracts	**Hexane**		**Antibiotics**		
Microorganism	**f***	**r***	**Gentamicin**	**Nystatin**	**Tetracycline**
B. subtilis	21.7±0.4	18.9±0.2	NT[b]	NT	22.3±0.1
S. epidermidis		17.3±0.1	17.7±0.3 34.6±0.7	NT	NT
E. faecalis	12.3±0.1	11.1±0.2	NT	NT	9.7±0.3
S. aureus	18.4±0.3	16.8±0.1	NT	NT	21.4±0.5
K. pneumoniae		NA[a]	8.5±0.3	21.1±0.4 NT	NT
P. aeruginosa		14.5±0.2	10.9±0.3	11.6±0.2 NT	NT
E. coli	15.9±0.2	16.0±0.4	24.3±0.7	NT	NT
A. niger	7.4±0.2	NA	NT	16.4±0.5	NT
C.albicans	13.5±0.3	14.7±0.1	NT	18.3±0.3	NT
S. cerevisiae	14.9±0.4	14.7±0.2	NT	18.1±0.2	NT

f: flower, r: root ; [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.

saturated fatty acid compounds in the *A. officinalis* (flower and root) extracts samples studied were hexadecanoic acid (13.0 and 16.8%), respectively. Nonacosane (11.2%), heptacosane (9.3%), heneicosane (9.0%), and pentacosane (3.7%) were the major fatty acid components in the flower extract. The ratios of unsaturated fatty acid (UFA)/ saturated fatty acid (SFA) were 0.66 and 2.44 in extract from flower and root, respectively (Table 2). The hexanic extract of root from this plant had a higher proportion of UFA compared to flower part (Table 1). The extracts of flower and root from *A. officinalis* was tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The results, presented in Table 3, show that the hexane extracts exhibited a good biological activity against all tested fungi and bacteria except for a resistant Gram-negative bacteria, *K. pneumoniae*, as well as a fungi, *A. niger.* The most sensitive microorganisms against flower and root extracts were *B. subtilis* with inhibition zones of 21.7-18.9 mm, *S. epidermidis* 17.3-17.7 mm, *S. aureus* 18.4-16.8 mm and *E. coli* 15.9-16.0 mm, respectively.

Other microorganisms were found to be moderate sensitive to the extracts with inhibition zones ranged from 7 to 15 mm. It is conceivable that the antimicrobial property of the hexane extracts from *A. officinalis* might be ascribed to its high content of fatty acid compounds.

Conclusion

This study revealed that *A. officinalis* growing in North west Iran is a suitable source of unsaturated fatty acids that appear to have protective effects for human health. They constituents a good alternative source of chemical composition such as essential fatty acids compared with common vegetable oil. Present results showed that *A. officinalis* flower and root hexanic extracts contain relatively high percentage of important unsaturated fatty acids. These finding may prove the traditional use of *A. officinalis* flowers and roots in preventive and alternative medicine against many illnesses.

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S. epidermidis	17.3±0.1	17.7±0.3	NT	NT	34.6±0.7
E. faecalis	12.3±0.1	11.1±0.2	NT	NT	9.7±0.3
S. aureus	18.4±0.3	16.8±0.1	NT	NT	21.4±0.5
K. pneumoniae	NA[a]	8.5±0.3	21.1±0.4	NT	NT
P. aeruginosa	14.5±0.2	10.9±0.3	11.6±0.2	NT	NT
E. coli	15.9±0.2	16.0±0.4	24.3±0.7	NT	NT
A. niger	7.4±0.2	NA	NT	16.4±0.5	NT
C.albicans	13.5±0.3	14.7±0.1	NT	18.3±0.3	NT
S. cerevisiae	14.9±0.4	14.7±0.2	NT	18.1±0.2	NT

[*] f: flower, r: root ; [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.

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