Trends Phytochem. Res. 1(1) 2017 9-14

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

Chemical constituents, antioxidant and antibacterial activities of the hexane extract of *Alchemilla sericata* Reichenb

Ali Shafaghat[1, ✉], Akram Panahi[2] and Masoud Shafaghatlonbar[3]

[1]Department of Chemistry, Khalkhal Branch, Islamic Azad University, Khalkhal, Iran
[2]Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[3]Department of Chemistry, College of Sciences, University of Birjand, Birjand, Iran

ABSTRACT

The hexane extract from the aerial parts of *Alchemilla sericata* Reichenb. which was collected from Khalkhal-Asalem road, Ardabil Province, northwestern Iran, was obtained using a commercially available Soxhlet apparatus. The fatty acids were derived to methyl esters and determined through gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) systems. The extract was characterized by high amounts of saturated fatty acids (SFA) (66.0%) along with lower quantities of some other terpenoid compounds. Accordingly, the main components of the hexane extract were found to be hexadecanoic acid (41.4%), 9,12-octadecadienoic acid (21.0%), octadecanoic acid (18.0%) and dioctylphthalate (7.7%), respectively. The hexane extract from *A. sericata* was also detected as a rich source of an important source of palmitic acid compound. In addition, its antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The results indicate that extracts from the aerial parts of *A. sericata* possess considerable antioxidant activity. The highest radical scavenging activity was detected in this plant oil (IC_{50}=185 µg/mL). The antimicrobial activity of the hexane extract sample 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 bioassays used showed that the hexane extract exhibited a moderate antimicrobial activity. This study revealed that the extracts from the aerial parts of this plant are potential sources of fatty acid components, as well as effective natural antioxidants.

ARTICLE HISTORY

Received: 18 January 2017
Revised: 02 February 2017
Accepted: 07 February 2017
ePublished: 01 March 2017

KEYWORDS

Alchemilla sericata
Rosaceae
Fatty acid
Antioxidant activity
Antimicrobial activity
Palmitic acid

1. Introduction

Organic fatty acids are naturally found in vegetables and fruits and may be formed during some processes like fermentation or may be added into food products during the manufacturing process. Numerous species of the plants which are rich fatty acids especially in seeds are of great importance as herbs and spices. During the recent years, plant compounds have come more into the focus of phytomedicine discipline (Buckle, 1999; Sylvestre et al., 2006). Their widespread use has raised the interest of scientists in basic research on fatty acids, as well. Specifically, the biological activities of fatty acids, essential oils and hydroalcoholic extracts have been recently investigated (Karlova et al., 2010; Shafaghat, 2011; Mohammadhosseini et al., 2016; Chang et al., 2016).

The genus *Alchemilla* is represented in Iranian flora by twenty four species, among which fourteen ones are endemic to the country. These include *A. amardica* Rothm, *A. farinosa* Frohner, *A. rechingeri* Rothm, *A. hessii* Rothm, *A. fluminea* Frohner, *A. melancholica* Frohner, *A. microscopica* Frohner, *A. hyrcana* (Buser)

✉ Corresponding author: Ali Shafaghat
Tel: +98-452-4251220-22, Fax: +98-451-7742659
E-mail address: shafaghata@yahoo.com

Juz. *A. surculosa* Frohner, *A. pectiniloba* Frohner, *A. citrina* Frohner, *A. gigantodus* Frohner, *A. condensa* Frohner and *A. plicatissima* Frohner (Mozaffarian, 2007).

In the course of phytochemical studies of the North-West medicinal plants from Iran in particular *Alchemilla* species, the Iranian *Alchemilla sericata* (Rosaceae) (Paye Shir-e-Kork Abrishami in Persian) was investigated. To date, no phytochemical studies on *Alchemilla sericata* have been reported. However, the species from the genus *Alchemilla* have been studied mainly for the contents of flavonoids, triterpenes, tannins, phenolic acids and total phenols (Olafsdottir et al., 2001; Felser and Schimmer, 1999; Ivancheva and Stantcheva, 2000; Ayaz and Hayirlioglu-Ayaz, 2001; Condrat et al., 2010). Phenolic compounds are considered as potent free-radical scavengers in different media (Karamac et al., 2005; Fernandez-Pachon et al., 2006).

In a previous investigation on phenolic acids and free radical scavenging activity of *A. jumrukczalica* from Bulgaria, Nikolova et al. (2012) reported that the principal phenolic acids components being gentisic, protocatechuic, salicylic and caffeic acids. Salicylic, protocatechuic, caffeic, *trans*-cinnamic, gentisic and vanilic acids were the major bonded phenolic acids in this plant extract.

To the best of our knowledge, there is not any record in the literature on the fatty acid composition of the extract from the *A. sericata* and its biological activities. Therefore, it seems logical and unavoidable to screen the composition of the extract and its corresponding biological activities, in detail.

2. Experimental

2.1. Plant materials

Aerial parts of *Alchemilla sericata* were collected in the Almas height Khalkhal-Asalem road (Ardabil Province in northwest Iran) area at an altitude of 2950 m in July 2015. A voucher specimen (A-121) was deposited at the Herbarium of Agriculture Research in Ardabil Center (HARAC), Iran (Fig. 1).

Fig. 1. Representation of the aerial parts of *Alchemilla sericata*.

2.2. Preparation of the extracts

Dried and powdered materials were extracted using a Soxhlet apparatus (70 ºC, 4 h) to obtain the fatty acids and the other non-polar constituents. To obtain organic extracts from the aerial parts of *A. sericata*, hexane (95%) was used. The obtained extract was then concentrated by a rotary evaporator under the vacuum at 40 ºC. The extraction yields were presented in Table 2.

2.3. Methylation of hexane extract

After removing hexane using a rotary evaporator, the oily mixtures were derived to their methyl esters using the *trans*-esterification process as 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 a methanolic solution of KOH (2.0 M) at room temperature for 60 seconds. The upper phases were subsequently analyzed by GC/FID and GC/MS systems.

2.4. GC analysis

GC analysis was performed on a Shimadzu 15A gas Chromatograph equipped with a split/splitless injector (250 ºC) and a flame ionization detector (250 ºC). 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 ºC for 5 min. and then heated to 220 ºC with a 5 ºC/min. rate and kept constant at 220 ºC for 5 min. The relative percentages of the characterized components are given in Table 1.

2.5. 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 ºC for 5 min. and programmed to 220 ºC at a rate of 5 ºC/min. and kept constant at 220 ºC 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.

2.6. Antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the extract were evaluated by the disc diffusion method (DDM) using Mueller-Hinton agar for bacteria and Sabouraud Dextrose Agar (SDA) for fungi (Baron and Finegold, 1990). 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 ºC and

24 ºC for bacteria and fungi, respectively. Gentamicin and tetracycline were used for bacteria, and nystatin for fungi 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.

2.7. Antioxidant activity tests

The DPPH assay was carried out according to a standard 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 (0.1-1.6 mg/mL) and kept in the dark for 10 min. The mixture was shaken vigorously and then immediately placed in a UV-Vis spectrophotometer (Unico, Shanghai, China) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min. until the reaction reached a plateau. The absorbance of the resulting solution was recorded on a spectrometer against a blank solution (hexane 95%). Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as IC_{50} values (µg extract/mL) for comparison. The IC_{50} value of each sample, defined as the concentration of sample required for the 50% decrease in absorbance of the blank, was calculated.

3. Results and Discussion

3.1. Composition of the hexane extract of *A. sericata*

The results obtained in the analyses of the hexane extract from the aerial parts of *A. sericata* have been summarized in Table 1, in which the percentage and retention time of components are given.

According to the results (see Fig. 2), the mean yield of the hexane extract of the studied aerial part of *A. sericata* was found to be 2.1% in terms of dry weight of the plant material. The major saturated and unsaturated fatty acid including hexadecanoic acid (palmitic acid), 9,12-octadecadienoic acid (linoleic acid or ω-6), octadecanoic acid (stearic acid) and dioctylphthalate are shown in the Table 1. The major polyunsaturated fatty acids (PUFAs) were α-linoleic (ω-6) and 9-hexadecenoic acids. As can be seen in Table 1, about 98.5% (12 components) of the extract profile were identified, while about 1.5% of the profile remained unidentified. Our investigation revealed that the main components of the hexane extract were hexadecanoic acid (41.4%), 9,12-octadecadienoic acid (21.0%), octadecanoic acid (18.0%) and dioctylphthalate (7.7%). The saturated fatty acid contents were higher than unsaturated ones, and some of the phthalic acid derivatives were observed in this plant. In fact, the hexane extract of this plant mainly include saturated and unsaturated fatty acids, with a clear predominance of palmitic acid and linoleic acid (LA). One of the essential fatty acids (EFAs), ω-6 (LA) was a predominant component in this plant. Linoleic acid is an omega-6 fatty acid detected in this work. The main phthalic acid derivative compounds as an ester in the studied extract sample were diisooctyl phthalate (7.7%) and butyl methyl phthalate (0.3%). Other saturated fatty acids such as 9-octadecenoic acid, heptadecanoic acid, 1,2-benzenedicarboxylic acid, butyl methyl and docosanoic acid were also present in small quantities (<1%) of the total fatty acids in the oil sample. The ratios of unsaturated fatty acid (UFA)/SFA (saturated fatty acid) were 0.49 (Table 2).

Table 1

Chemical composition (%) of the hexane extract from aerial parts of *Alchemilla sericata*.

No	Compound* (Related Fatty acid)	Rt (min)	KI	%
1	9-Hexadecenoic acid, methyl ester(9-Hexadecenoic acid)	29.1	1507	4.0
2	Hexadecanoic acid, methyl ester(Hexadecanoic acid)	29.5	1517	41.4
3	9-Octadecenoic acid(z),methyl ester(9-Octadecenoic acid)	30.6	1546	0.9
4	Heptadecanoic acid, methyl ester(Heptadecanoic acid)	31.0	1556	0.5
5	1,2-Benzenedicarboxylic acid, butylmethyl ester	31.2	1562	0.3
6	9,12-Octadecadienoic acid(z, z)-, methyl ester(9, 12-Octadecadienoic acid)	32.1	1582	21.0
7	Octadecanoic acid, methyl ester(Octadecanoic acid)	32.8	1598	18.0
8	11-Eicosenoic acid, methyl ester(11-Eicosenoic acid)	35.1	1659	1.6
9	Eicosanoic acid, methyl ester(Eicosanoic acid)	35.5	1669	1.8
10	Docosanoic acid, methyl ester(Docosanoic acid)	38.1	1740	0.3
11	Bis(2-ethylhexyl) phthalate	38.4	1747	7.7
12	Squalene	42.0	1846	1.0
	Total	--	--	98.5

*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.

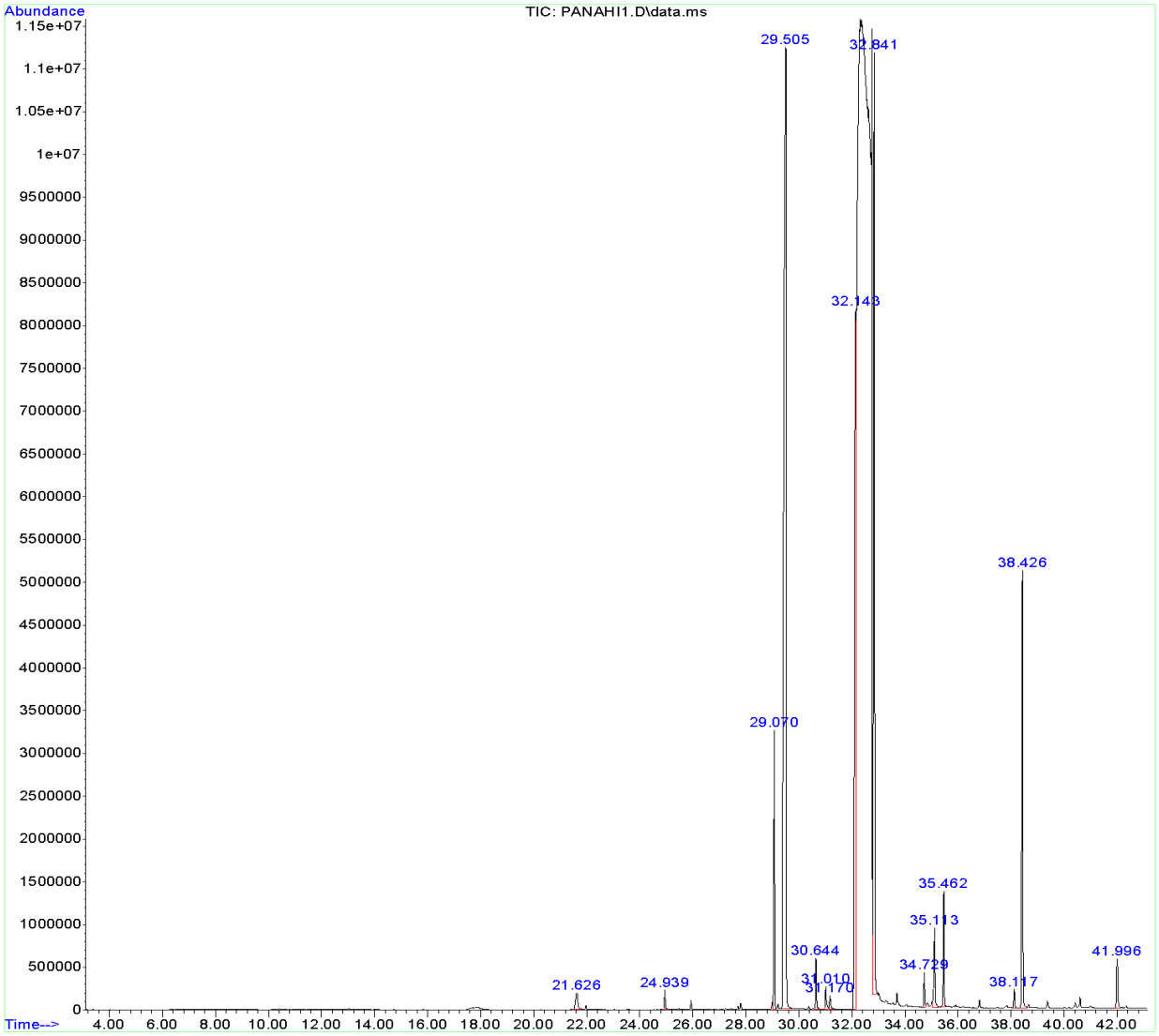

Fig. 2. GC-chromatogram of the hexane extract from the aerial parts of *Alchemilla sericata*.

Table 2
Class compositions and yield of the hexanic extract from *Alchemilla sericata*.

Class composition	(%)
Saturated fatty acid (SFA)	66.0
Unsaturated fatty acid (UFA)	32.5
UFA/SFA	0.49
Yield	2.1

3.2. Antioxidant activity of the hexane extract of *A. sericata*

The antioxidant activity of hexane extract 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 hexane extract from *A. sericata* scavenged the DPPH radical in a dose-dependent manner, and the DPPH radical scavenging activity (IC_{50}) was represented in Table 3. According to this data, hexane extract was the most efficient free radical scavenger by the lowest IC_{50} value of 185 µg/mL. The activity of the reference antioxidant (vitamin C) was much higher than that of plant oil.

Table 3
DPPH free radical scavenging activity of hexanic extract from *Alchemilla sericata* and standard antioxidant, vitamin C.

No	Sample	IC_{50} (µg/mL)
1	hexanic extract	185
2	Vitamin C(Ref.)	27

3.3. Antimicrobial activity of the hexane extract of *A. sericata*

The hexane extract of aerial part from *A. sericata* was tested against four Gram-positive and three

Table 4
Antimicrobial activity of the hexanic extract from *Alchemilla sericata*.

Tested microorganism	Zone of inhibition (mm)*			
	Sample	Antibiotics		
	Hexane extract	Gentamicin	Nystatin	Tetracycline
B. subtilis	12.9±0.15	NT[b]	NT	22.5±0.12
S. epidermidis	11.1±0.11	NT	NT	34.2±0.49
E. faecalis	NA[a]	NT	NT	9.5±0.22
S. aureus	12.2±0.14	NT	NT	21.2±0.31
K. pneumoniae	9.2±0.21	20.2±0.31	NT	NT
P. aeruginosa	10.1±0.12	12.7±0.13	NT	NT
E. coli	NA	24.2±0.9	NT	NT
A. niger	10.7±0.21	NT	16.5±0.42	NT
C.albicans	8.9±0.14	NT	18.6±0.21	NT
S. cerevisiae	10.1±0.11	NT	18.2±0.31	NT

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

Gram-negative bacteria, as well as three fungi strains. The results, presented in Table 4, show that the hexane extracts exhibited a moderate biological activity against all tested fungi and bacteria except for a resistant Gram-negative bacteria, *Klebsiella pneumoniae*, as well as a fungi, *Candida albicans*. A simple perusal of Table 4 represent that the most sensitive microorganisms against extracts were *Bacillus subtilis* with inhibition zones of 12.9 mm and *Staphylococcus aureus* 12.2 mm, respectively. However, the other microorganisms were found to be less sensitive towards the hexane extract of *A. sericata* with inhibition zones ranged from 8.9 to 11.7 mm.

4. Concluding remarks

The present study is the first report on the composition of the hexane extract from the aerial parts of *A. sericata* growing wild in Iran. This study indicated that *A. sericata* aerial parts are rich in saturated fatty acids and exhibit free radical scavenging activity in the DPPH style tested and have a good antioxidant activity. It can be seen that the growth of tested bacteria responded differently to the oil and its components, which indicates that different components may have different modes of action or that the metabolism of some bacteria is able to better overcome the effect of the oil or adapt to it. Gram negative bacteria are in general more resistant than Gram positive types. It is conceivable that the antimicrobial property of the hexane extract from *A. sericata* might be ascribed to its high content of fatty acids.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgment

The author wish to thank Ardabil Branch, Islamic Azad University (IAU), for financial and technical supports for this investigation.

References

Ayaz, F.A., Hayirlioglu-Ayaz, S., 2001. Total phenols and condensed tannins in the leaves of some *Alchemilla* species. Biolog. Bratislava. 56(4), 449-453.

Baron, E.J., Finegold, S.M., 1990. Methods for testing antimicrobial effectiveness. In: Diagnostic Microbiology. Stephanie M (Ed.). Baltimore, Mosby, pp. 171-194.

Buckle, J., 1999. Use of aromatherapy as a complementary treatment for chronic pain. Altern. Ther. Health Med. 5(5), 42-45.

Chang, S., Abbaspour, H., Nafchi, A.M., Heydari, A., Mohammadhosseini, M., 2016. Iranian *Foeniculum vulgare* essential oil and alcoholic extracts: Chemical composition, antimicrobial, antioxidant and application in olive oil preservation. J. Essent. Oil-Bear. Plants 19(8), 1920-1931.

Cheung, L.M., Cheung, P.C.K., Ooi, V.E.C., 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 81(2), 249-255.

Condrat, D., Mosoarca, C., Zamfir, A.D., Crişan, F., Szabo, M.R., Lupea, A.X., 2010. Qualitative and quantitative analysis of gallic acid in *Alchemilla vulgaris*, *Allium ursinum*, *Acorus calamus* and *Solidago virga-aurea* by chip-electros pray ionization mass spectrometry and high performance liquid chromatography. Cent. Europ. J. Chem. 8(3), 530-535.

Felser, C., Schimmer, O., 1999. Two new flavonoid glycosides from the leaves of *Alchemilla speciosa*. Planta Med. 65(7), 668-670.

Fernandez-pachon, M.S., Villano, D., Troncoso, A.M., Garcia parrilla, M.C., 2006. Determination of the phenolic composition of sherry and table white wines by liquid chromatography and their relation with antioxidant activity.

Anal. Chim. Acta. 563(1-2), 101-108.

Ivancheva, S., Stantcheva, B., 2000. Ethnobotanical inventory of medicinal plants in Bulgaria. J. Ethnopharm. 69(2), 165-172.

Karamac, M., Kosinska, A., Pegg, R.B., 2005. Comparison of radical scavenging activities for selected phenolic acids. Polish J. Food. Nut. Sci. 14(2), 165-170.

Karlova, T., Polakova, L., Smidrkal, J., Filip, V., 2010. Antimicrobial effects of fatty acid fructose esters. Czech J. Food Sci. 28(2), 146-149.

Mohammadhosseini, M., Akbarzadeh, A., Hashemi-Moghaddam, H., 2016. Gas chromatographic-mass spectrometric analysis of volatiles obtained by HS-SPME-GC-MS technique from *Stachys lavandulifolia* and evaluation for biological activity: A review. J. Essent. Oil-Bear. Plants 19(6), 1300-1327.

Mozaffarian, V. 2007. A Dictionary of Iranian Plant Names. Farhang Moaser Publishers, Tehran, Iran.

Nikolova, M., Dincheva, I., Vitkova, A., Badjakov, I., 2012. Phenolic acids and free radical scavenging activity of *Alchemilla jumrukczalica* Pawl. Inter. J. Pharmac. Sci. Res. 3(3), 802-804.

Olafsdottir, E.S., Omarsdottir, S., Jaroszewski, J.W., 2001. Constituents of three Icelandic *Alchemilla* species. Biochem. System Ecolog. 29(9), 959-962.

Shafaghat, A., 2011. Chemical constituents, antimicrobial and antioxidant activity of the hexane extract from root and seed of *Levisticum persicum* Freyn and Bornm. J. Medic. Plants Res. 5(20), 5127-5131.

Sylvestre, M., Pichette, A., Longtin, A., Nagau, F., Legault, J., 2006. Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. J. Ethnopharmacol. 103(1), 99-102.