Journal of Pharmaceutical and Health Sciences 2014;2(3), 115-123.

Original Article

Screening of Flowers Essential Oil and Hexane Extract of Rheum ribes L. from Iran- Chemical Composition, Antioxidant and Antimicrobial Activities

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Abstract

The hexane extract of the flowers of Rheum ribes L. which was collected from north-western Iran, was obtained by Soxhlet apparatus and the essential oil composition was obtained by hydro distillation and analyzed by using GC-GC/MS. The extract from the flowers was characterized by a high amount of unsaturated fatty acids (UFA) (66.0%) and some of long chain hydrocarbon compounds. The main components of the hexane extract were 9-octadecenoic acid(ω-9) (42.8%), 9, 12- octadecadienoic acid (linoleic acid or ω- 6) (19.6%), hexadecanoic acid, (palmitic acid) (8.6%), 1,2-benzenedicarboxylic acid diisooctyl (5.7%), dodecane (3.7%) and γ- linolenic acid (3.6%). The hexane extract from Rheum ribes flowers detected as an important source of ω-6 and ω-9 compounds. This study reveals that the flowers of this plant are attractive sources of oily components, especially the essential ones, as well as of effective natural sources of unsaturated fatty acids. Twenty three compounds representing 97.5% of the distilled oil of R. ribes flowers were identified, among them germacrene-d (22.3%), α-pinene (13.5%), terpinolene (12.4%), p-cymene (10.6%), bicyclogermacrene (9.6%) and limonene (8.6%) were the major constituents. The antimicrobial activity of the essential oil and hexane extract were determined against some bacteria and both samples had a moderate effect on the some Gram-positive and Gram-negative bacteria. The antioxidant activity of the extract and essential oil were evaluated by DPPH method. The results indicate that hexane extract of this plant flowers possess considerable antioxidant activity.

Keywords: Rheum ribes, Polygonaceae, antibacterial, essential oil, fatty acid, germacrene-d, ω- 6 and ω- 9.

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INTRODUCTION

The genus Rheum is represented in Iranian flora by three species, among which R. persicum is endemic[1]. Rheum ribes is locally known as "Rivas" in Persian and found mostly in Western and North-western Iran, Eastern Turkey, Northern Iraq and Lebanon. It is one of the wild rhubarb species belonging to Polygonaceae family and utilized as vegetable in various countries. R. ribes is a perennial vegetable species spread from North and Central Asia to the other continents. It has also wild forms in Iran and Anatolia[2]. Rheum ribes is the only rhubarb species grown from 1800 to 2800 m high rocky countryside of Iran and has thick perennial rhizomes, annual large bean-like brown-green leave with stalks, edible flower stalks, yellowish small paniculated flowers, three sided red winged seeds. It has been reported that its stems have been consumed either cooked or raw and a brown dye has been isolated from its roots for the local carpet industry[3]. The fruit in Polygonaceae family is a single-seeded achene with
the pericarp tightly attached to the testa. This fruit is commonly referred to as the seed. Freshly harvested seeds exhibit different degrees of dormancy. This presents a problem when they are to be germinated shortly after harvesting for progeny evolution, physiological studies or quality assessment[4]. Rheum ribes has some medicinal properties such as preventing of stomach upset, vomiting, hemorrhoids, lessening the symptoms of diabetes, measles and smallpox and increasing appetite. The roots of Rheum ribes, collected from Bingol, contain tannins (8%) and anthracene derivatives (0.025%)[5]. Four anthraquinone derivatives, two anthraquinone glucosides, one dianthron glucoside and one stilbene glucoside have been reported from subterranean parts of the plant[6]. The anthraquinones chrysophanol, physcion and emodin, the flavonoids quercetin, 5- desoxyquercetin, quercetin 3-0-rhamnoside, quercetin 3-0- galactoside and auercetin 3-0-rutinoside were isolated from the shoots of Rheum ribes and reported from Turkey[7]. Plant materials always remain an important source of proteins and essential fatty acids for their nutritional, industrial and pharmaceutical applications[8]. 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. No oil from any single source has been found to be suitable for all purposes because oil from different sources generally differ in their fatty acid composition. 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[9-12]. 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[13-17]. 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[18, 19].

There are no previous studies on the fatty acids, essential oil and antibacterial activity of this plant, but there are some reports indicating the antibacterial activity of Rheum ribes methanol extract[20] and other species of Polygonaceae family[21,22]. However, antiviral effects of extracts from different parts of this plant have been reported previously[23]. Also there are reports on the ethnomedical use of different parts of R. ribes in Turkey [24] and Iran[25]. As a continuation of our phytochem-

ical studies on medicinal plants, this article is the first report on the isolation and structural elucidation of essential oil and fatty acid in hexane extract from flowers of R. ribes from Iran.

EXPERIMENTAL

Plant materials: The flower part of Rheum ribes was collected in July 2012 from Sanandaj (Kurdistan province) in northwest of Iran at an altitude of 1950m. A voucher specimen (No: 017) has been deposited at the Herbarium of the Agriculture Research Centre (A.R.C.) Ardabil, Iran.

Extraction of Essential Oil:

The plant flowers (200 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The yield of the oil was 0.1 %(V/W). The essential oil was dried over anhydrous sodium sulphate and stored at 2-3 °C.

Extraction of n- hexane sample extract:

Dried and powdered materials (flowers) were extracted with n-hexane using a Soxhlet apparatus (70ºC, 3.5h) to obtain the apolar components. During extraction procedures, Merck hexane (99 %) was used as an extractor solvent. The extracts were concentrated by rotary evaporator under vacuum at 45ºC. The extraction yield was presented in Table II.

Transesterification:

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 6 min. The upper phases were analyzed by GC/FID and GC/MS systems.

GC analysis:

GC analysis was performed on an Agilent 7890A gas chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). Helium was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (30m × 0.2mm, film thickness 0.32 μm). The column temperature was kept at 40°C for 5 min and then heated to 230°C with a 6°C/min rate and kept constant at 230°C for 5 min. The relative percentages of the characterized components are given in Tables I and II.

GC/MS analysis:

GC/MS analysis was performed using an Agilent 5975 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.32μm). The column temperature was kept at 40°C for 5 min and programmed to 230°C at a rate of 6°C/min and kept constant at 230°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. Identification of the constituents of essential oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples[26]. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Determination of antioxidant activity:

The electron or hydrogen atom donation abilities of the samples were measured from the bleaching of a purple-colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as a reagent[27]. An aliquot of the each sample (100μL) was mixed with ethanol (1.4 mL) separately, and then added to 0.004% DPPH (1 mL, Sigma-Aldrich) in ethanol. The mixture was shaken vigorously and

then immediately placed in a UV-Vis spectrophotometer (Perkin- Elmer Lambda 25) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Vitamin C, a stable antioxidant, was used as a standard reference. The radical-scavenging activity of samples, expressed as percentage inhibition of DPPH, was calculated according to the formula:

$$I\% = [(A_{blank} - A_{sample})/ A_{blank}] \times 100$$

Where Asample is the absorbance of the sample, and Ablank is the absorbance of blank solution. DPPH scavenging activity was expressed as IC50 values (μg extract/ ml) for comparison. IC50 value of the sample defined as the concentration of sample required for the 50% decrease in absorbance of the blank was calculated.

Antimicrobial activity:

The antibacterial activities of the hexane extract and essential oil were evaluated by the disc diffusion method (DDM) using Mueller-Hinton agar for bacteria and Sabouraud Dextrose agar for fungi[28]. Discs containing 30 μL of the hexanic extract and essential oil were used and growth inhibition zones were measured after 24 h of incubation at 37°C for bacteria. Ampicillin and Ciprofloxacin were used as positive controls. The microorganisms used were: Staphylococcus aureus ATCC 25923, Streptococcus pneumoniae ATCC 49619, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Neisseria gonorrhoeae ATCC 49226, Pseudomonas aeruginosa ATCC 27853, salmonella typhimurium ATCC 14028.

RESULTS AND DISCUSSION

The essential oil and fatty acid composition of R. ribes flowers was investigated using GC/FID and GC/MS techniques for the first time. The results obtained in the analyses of the essential oil and hexane extract of R. ribes flowers are listed in Table I and II, respectively, in which the percentage, common name, retention time (Rt) and retention indices (RI) of components are given. According to the results, Twenty-three compounds, representing 97.5% of the total components in the oil of R. ribes, were detected and germacrene-d (22.3%), α-pinene (13.5%), terpinolene (12.4%), p-cymene (10.6%), bicyclogermacrene (9.6%) and limonene (8.6%) were the major constituents. Other components were present in amounts less than 5%. The hexane extract yield of the studied flower oil of R. ribes was found 3.3 % on the basis of dry weight of the sample material. The major saturated and unsaturated components including palmitic, stearic, linoleic (ω-6), γ- linolenic and oleic (ω-9) acids are shown in the Table II.

The major polyunsaturated fatty acids (PUFA) were oleic acid (ω-9), γ-linolenic and linoleic. As can be seen in Table II, about 92.5% (12 components) of the extract from flower were identified. The main components of the fatty acid in hexane extract were (C18:1(9c)) or 9-octadecenoic acid (oleic acid or ω-9) (42.8%), 9, 12- octadecadienoic acid (linoleic acid or ω-6) (19.6%), 6, 9, 12 - octadecatrienoic acid (γ-linolenic acid) (3.6%), hexadecanoic acid (palmitic acid) (8.6%) and octadecanoic acid (stearic acid) (1.9%). The unsaturated fatty acid contents (66.0%) were higher than saturated ones (10.5%).

Table 1: Chemical composition (%) from the essential oil of Rheum ribes flowers.

Compound	RI*	Percentage (%)	Fenchyl acetate	1215	0.3
α-Pinene	939	13.5	cis-Isopulegone	1228	2.1
Sabinene	976	tr.	Sabinyl acetate	1291	4.3
β-Pinene	980	0.6	4-Vinyl-2-methoxy		
α –Terpinene	1017	1.3	phenol-	1348	2.1
p-Cymene	1026	10.6	β-Elemene	1391	1.5
Limonene	1031	8.6	Germacrene-d	1480	22.3
trans-β-Ocimene	1050	1.4	β-Selinene	1485	0.6
γ-Terpinene	1062	0.6	Bicyclogermacrene	1495	9.6
Terpinolene	1088	12.4	Myristicin	1532	0.3
Isoterpinolene	1095	1.4	Elemicine	1565	0.2
cis-Epoxy-ocimene	1097	tr.	γ-Dodecadienolactone	1730	3.7
Octyl acetate	1175	0.1	Total		97.5

In fact, the flower extract mainly include unsaturated fatty acids, with a clear predominance of oleic and linoleic acid (LA).

Table 2: Fatty acid compositions in flower oil of R. ribes.

Compound	Common Name	Rt*(min)	%
Cyclohexane,1,2-dimethyl	--	5.1	1.4
n-Decane	Decane	13.0	0.8
Ethanone,1-phenyl	Acetophenone	15.6	0.8
Undecane	--	17.9	1.6
Dodecane	Adakane 12	20.9	3.7
Naphthalene,decahydro-1,5-dimethyl	--	21.8	2.0
C16:0	Palmitic acid	42.4	8.6
C18:3 (6c, 9c, 12c)	γ- Linolenic acid	46.4	3.6
C18:2(9c,12c)	Linoleic acid or ω-6	46.8	19.6
C18:1(9c)	Oleic acid(ω-9)	51.1	42.8
C18:0	Stearic acid	51.4	1.9
Bis(2-ethylhexyl)phthalate	Dioctyl phthalate (DOP)	53.2	5.7
Total	--	--	92.5%

**Rt: Retention time*

Other saturated fatty acids such as palmitic and stearic acid were also present in moderate quantities with a concentration of about 10% of the total fatty acids in the flower oil sample. One of the essential fatty acids (EFAs), LA was a predominant component in flower of R. ribes. Linoleic acid is an omega-6 fatty acid, detected in this work. The ratio of total unsaturated fatty acid (UFA): SFA (saturated fatty acid) was 6.3 (Table III).

Table 3: Saturated and unsaturated fatty acids (FA)in flower oil of R. ribes.

Type and ratio of different FA	% and ratio
Saturated FA	10.5
Monounsaturated FA	42.8
Polyunsaturated FA	23.2
Total unsaturated FA	66.0
Total unsaturated: Saturated	66.0:10.5=6.3
ω- 9:ω-6	42.8:19.6=2.2
Yield	3.3

Overall, the percentage of total unsaturated fatty acids in this oil has an amount of 66.0% while saturated fatty acids have an amount of 10.5%. The high percentage of unsaturated fatty acids is largely attributed to ω- 6 and ω- 9 was the most predominant fatty acids. Unsaturated fatty acid has also been reported to be as effective as Poly Unsaturated Fatty Acids (PUFAs) in the reduction of low density lipoprotein cholesterol in humans[29].

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 IV.

Table 4: DPPH free radical scavenging activity of essential oil and hexane extracts from R. ribes flower and standard

No	Sample	IC50 (μg/mL)
1	Essential oil	565
2	hexanic extract	325
3	Vitamin C (Ref.)	26

Antioxidant activity was tested according to the DPPH (1, 1-diphenyl-2-pycrylhydrazile) radical scavenging method. The flower oils obtained from R. ribes scavenged the DPPH radical in a dose-dependent manner and the DPPH radical scavenging activity (IC50) are shown in Table IV. According to this data, essential oil of flowers was the most efficient free radical scavenger by the lowest IC50 value of 565μg/mL. The IC50 value of hexane extract was higher than the essential oil. The activity of the reference antioxidant (vitamin C) was much higher than that of R. ribes flower essential oil and hexane extract.

The essential oil and hexane extract from R. ribes flower was tested against three Gram-positive and five Gram-negative bacteria. The results, presented in Table V, show that the essential oil and hexane extract exhibited moderate biological activity against some tested bacteria, except for three resistant Gram negative bacteria, Escherichia coli, Neisseria gonorrhoeae, Pseudomonas aeruginosa as well as a Gram positive Staphylococcus aureus. The most sensitive microorganisms against hexane extract and essential oil were Staphylococcus epidermidis, with inhibition zones of 15.0 and 16.2 mm, respectively. Other microorganisms such as Streptococcus pneumoniae, Klebsiella pneumoniae and salmonella typhimurium were found to be less sensitive to the extracts with inhibition zones ranged from 6.5 to 8.0 mm. It is conceivable that the antimicrobial property of the hexane extracts and essential oil from R. ribes might be ascribed to its high content of unsaturated fatty acids and terpenoid components, respectively.

Table 5: Antimicrobial activity of the essential oil and hexane extract from R. ribes flower.

Tested microorg	Gram	Zone of inhibition (mm) *			
		Sample		Antibiotics	
		Essential oil	Hexane extract	Ampicillin	Ciprofloxacin
S. aureus	+	**NA	NA	28.3	NT
S. epidermidis	+	16.2	15.0	25.1	NT
S. pneumoniae	+	7.3	7.8	27.6	NT
E. coli	-	6.3	NA	NTb	21.3
K. pneumoniae	-	6.6	6.5	NT	19.1
N. gonorrhoeae	-	NA	NA	NT	17.5
P. aeruginosa	-	NA	NA	NT	18.9
S. typhimurium	-	8.2	8.0	NT	17.6

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

In conclusion, results in the present study could be an effective introduction to the antioxidant activities of R. ribes flowers, and provided evidence that hexane extract of R. ribes flower may provide potential natural antioxidants for the food industry and other fields. However, further studies are urgently needed for screening for the active components with enhanced antioxidant activity properties in R. ribes flowers. The presence of the bioactive components of R. ribes plant shown in Tables I and II suggests the presence of terpenoid components and fatty acid compounds which have antimicrobial properties. This implies that R. ribes flowers extract and essential oil is a useful antimicrobial plant against the test microorganisms. The hexane extract has also beneficial health effects as well as its rich unsaturated fatty acid content and therefore, it could be considered a good alternative for essential fatty acids for cosmetics and nutraceutical industries.

AKNOWLEDGEMENT

This research project was financed by the Islamic Azad University, Ardabil Branch, Iran.

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