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Chemical Composition and General Toxicity of Essential Oil Extracted from the Stalks and Flowers of *Rheum Ribes* L. Growing in Iran

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The essential oil of *Rheum ribes* L. stalks and flowers, growing in Iran, were extracted with hydro-distillation and analyzed by GC-MS. Thirty constituents representing 93.84% of Rheum oil were identified. The oil was found to be rich in hydrocarbons especially long-chain n-alkanes (80.81%). The most abundant components in the oil included tricosane (26.29%), heneicosane (26.07%), pentacosane (10.63%), heptacosane (10.37%) and palmitic acid (3.64%). The essential oil was also evaluated for general toxicity bioassay using brine shrimp lethality method. The toxicity profile of the oil indicated some degree of toxicity in comparison with podophyllotoxin.

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Introduction

The genus *Rheum* (Polygonaceae) comprises about 103 species and is widespread mainly in North America, Europe and the east regions of the Mediterranean including Iran, Turkey, Afghanistan, Pakistan, Russia and China [1,2]. This genus is documented in Iranian flora by four species including *R. ribes*, *R. turkestanicum*, *R. persicum* and *R. Khorasanicum,* in which the last two species are endemic [2,3]. In Iran, the genus *Rheum* is mainly represented by *R. ribes* L., a hardy perennial herb that grows in mountainous areas with the common Persian name "Rivas" [4,5]. In traditional medicine, the roots of *R. ribes* have been used in the treatment of ulcers, hemorrhoids [6,7], hypertension, obesity, diarrhoea, diabetes [8,9], high cholesterol, constipation and psoriasis [10,11]. Several reports on this plant have revealed that it exhibited various pharmacological properties such as antioxidant [8,12], antibacterial [13,14], antifungal [15] and antiviral [16] effects. Moreover, the hypoglycaemic effect of aqueous extract of *R. ribes* has been reported in different *in vivo* and *in vitro* assays [1, 17-19]. Phytochemical studies on different extracts of *R. ribes* indicated the presence of several anthraquinones, a dianthron glucoside, sennoside A, rhaponticin, a stilbene glucoside and flavonoids [1,20,21,22] but to the best of our knowledge, the volatile constituents of this plant have not been previously investigated. Therefore, regarding the prevalent food and medicinal uses of *R. ribes*, we focused on the chemical composition of the volatile oil of this plant extracted by hydro-distillation of the stalks and flowers and to investigate its general toxicity effect against brine shrimp.

Materials and Methods

Plant Material

Flowers and stalks of *R. ribes* L. were purchased from local market of Tabriz (East Azarbaijan province, Iran) in May, 2013. The plant materials were taxonomically identified by the herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran and voucher specimen (no: Tbz-Fph-734) was deposited in the herbarium.

Isolation of the essential oil

The essential oil from air-dried stalks and flowers of *R. ribes* (50 g) were obtained by hydro-distillation for 3h using a Clevenger-type apparatus [23]. The oil was dried over anhydrous sodium sulphate and kept at 4°C until analyses. The extraction yield was 0.024 % (w/w), based on the dry weight.

GC-MS analysis

The analysis of the volatile oil was performed using a Shimadzu GCMS-QP5050A gas chromatograph-mass spectrometer (GC-MS) equipped with a fused methyl silicon DB-1 column (60 m × 0.25 mm i.d., 0.25 µm film thickness) and coupled to a mass selective detector. Helium was used as carrier gas at a flow rate of 1.3 mL/min. The column temperature was kept three min at 50°C, increased to 265°C at a rate of 5.0°C/min increase, and finally 5 min at 265°C. The injector temperature was 240°C and split ratio was adjusted at 1:5. The injection volume was 1 µL. The mass operating parameters were as follow: ionization potential 70 eV; ion source temperature 260°C; quadrupole temperature 100°C; solvent delay 7.0 min; resolution 2000amu/s and scan range 30-600 amu; EM voltage 3000 volts. The components of the oil were identified by matching their fragmentation pattern of mass spectra with those of the spectrophotometer database using the NIST10, NIST 21, NIST 69 and Wiley 229 library data and comparing their Kovats retention indices relative to the series of n-hydrocarbons (C_8-C_{20}) with literature data [24].

The component concentration was obtained by semi-quantification by peak area integration from GC peaks and by applying the correction factors.

Brine shrimp lethality assay (general toxicity)

The general toxicity of the essential oils was evaluated by the brine shrimp lethality bioassay describes by Meyer *et al*. [25] with some modifications [26, 27]. Brine shrimp eggs (*Artemia salina*, Sera brand, Aquarium and Fish shop, Khaghani Avenue, Tabriz, Iran) were hatched in a conical flask with 200 mL artificial seawater (Prepared from commercial sea salt, 40g/L, Aqua Marine, Thailand).The flask was aerated with an air pump and kept in a water bath (29-30°C) under a bright light. After 48 h the napulii hatched. Samples were prepared by dissolving the

essential oils in DMSO to obtain a concentration of 10 µg/mL. The final DMSO concentration did not exceed 1%, which has shown not to have any harmful effects on the napulii. Ten different concentrations were prepared by serial dilution. Then 1 mL of each concentration was transferred into clean sterile vial with a pipette and seawater was added. About 10 napulii were transferred into each test and control (containing DMSO and sea water) vials. Surviving larvae were counted after 24 h of incubation and the mortality rate at each dose and control was determined. Podophyllotoxin was used as a positive control.

Statistical analysis

The percentage lethality was calculated from the mean survival of larvae in oil-treated tubes and controls. The data were reported as mean ± standard deviation. Assess LC_{50} value using non-linear regression, where mortality is related to natural logarithm of concentration. LC_{50} values cannot differ more than 30 %.

Results and Discussion

The hydro-distillation of the edible parts (stalks and flowers) of *R. ribes* exuded greenish yellow oil with a yield of 0.024 % w/w, based on the dry mass. The list of the compounds, in order to elution on DB-1 column, the percentage of individual components and the Kovats indices are summarized in Table 1. A total of 30 compounds were identified, accounting for 93.84% of the total oil while 6.16% of the essential oil remained unidentified. Hydrocarbons, especially long chain n-alkanes had the highest contribution and represented 80.81% of the oil (Fig.1).

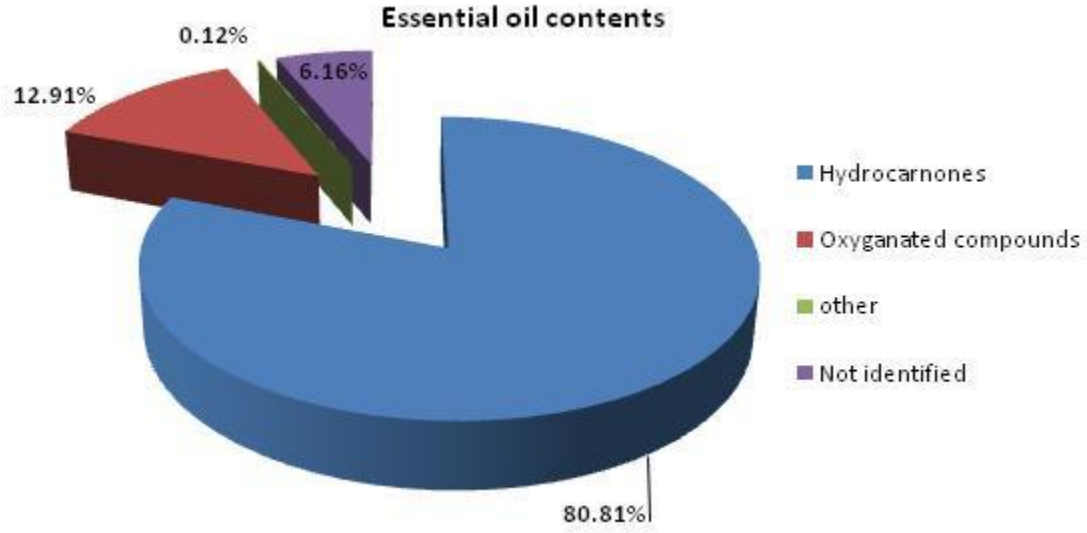

Fig. 1. Identified Chemical groups from the volatile oil of *R. ribes (L)*

The oxygenated compounds were relatively poor and constitute 12.91% of the total oil. The major constituents of the essential oil were tricosane (26.29%), heneicosane (26.07%), pentacosane (10.63%), heptacosane (10.37%) and palmitic acid (3.64%). Other compounds were present in values less than 3% (Table1). Literature survey showed that there is no previous report on the chemical composition of the essential oil of *R. ribes* but there are some for other species of *Rheum*. Previous studies on the essential oils extracted from rhizomes of *R. palmatum* and *R. tanguticum* exhibited that palmitic acid was the main characteristic constituent with percentage of 22.50% and 49.31%, respectively; whereas it was found at a relatively low level (3.64%) in our examined oil. Conversely, Long-chain n-alkanes were present at a low level (< 2%) in the oil composition of *Rheum* species in the previous works [28, 29].The difference might be a result of difference in species,choice of plant part, seasonal factors, geographic location, climatic condition, altitude and extraction method [30-32].

Table 1. Chemical composition of the volatile oil extracted from stalks and flowers of *R. ribes* L.

Compound [a]	KI	%	Identification Method [b]
decane	1000	0.11	GC/MS, I_s
2-methyl-decane	1061	0.15	GC/MS, I_s
tertamethylpyrazine (ligustrazine)	1063	0.12	GC/MS, I_b
nonanal (pelargonaldehyde)	1083	0.63	GC/MS, I_s
nonanol (pelargonic alcohol)	1154	0.12	GC/MS, I_s
decanal (caprinic aldehyde)	1185	0.19	GC/MS, I_s
dodecane	1200	0.23	GC/MS, I_s
1-decanol (capric alcohol)	1255	0.18	GC/MS, I_s
2-methyl-undecane	1285	0.23	GC/MS, I_s
dodecanal (lauraldehyde)	1288	1.36	GC/MS, I_s
1-dodecanol (lauryl alcohol)	1459	2.52	GC/MS, I_s
trans-2-tridecen-1-ol	1492	1.20	GC/MS, I_s
pentadecane	1500	0.25	GC/MS, I_s
2,6,11-trimethyl-dodecane	1503	0.39	GC/MS, I_s
1-tridecanol	1662	0.24	GC/MS, I_s
heptadecane	1700	0.24	GC/MS, I_s
farnesyl alcohol	1704	0.30	GC/MS, I_s
1-heptadecanol	1866	1.13	GC/MS, I_s
nonadecane	1900	1.28	GC/MS, I_s
n-hexadecanoic acid (palmitic acid)	1951	**3.64**	GC/MS, I_s
eicosane	2000	0.31	GC/MS, I_s
heneicosane	2100	**26.07**	GC/MS, I_b
1-nonadecanol	2148	0.15	GC/MS, I_b
docosane	2200	**2.58**	GC/MS, I_b
tricosane	2300	**26.29**	GC/MS, I_b
4-acetoxypentadecane	2349	1.25	GC/MS, I_b
tetracosane	2400	1.19	GC/MS, I_b
pentacosane	2500	**10.63**	GC/MS, I_b
heptacosane	2700	**10.37**	GC/MS, I_b
Total compounds	-	**30**	-
Total Identified	-	**93.84**	-

[a] Compounds listed in order of elution from a DB-1 column, [b] Identification Method (I_s = Kovats retention index according to authentic standard, I_b = Kovats retention index according to bibliography).

In living plants, waxy coatings on leaves and other plant organs are rich in hydrocarbons [33]. Based on previous researches, two patterns of n-alkane distribution might be found in plant tissues with the relative proportions dependency on species. The type A pattern shows a Gaussian-like distribution of even and odd n-alkanes at equivalent quantities, frequently around C22-C28, and generated by paranchymatic tissues, and the type B pattern sharing an alternation in chain length distribution that the odd n-alkanes appears in complete dominance (mostly C25, C27, C29, C31, C33) and produced by epidermis tissues and located in the cuticular waxes [34, 35]. The n-alkanes identified in hydro-distillate of *R. ribes* leaves and stalks correspond mostly to a type B pattern, indicating their probable origin in the cuticular waxes. The brine shrimp lethality bioassay represents a rapid, simple and inexpensive test for screening plant materials toxicity which in most cases correlates reasonably well with cytotoxic and anti-tumor activities [36]. In the current study, the obtained essential oil exhibited moderate toxicity effect against *Artemia salina* with LC_{50} value of 20.02 µg/mL compared to positive control (Podophyllotoxin, LC_{50}= 2.79 µg/mL). The toxicity increased with the increase in the dose of volatile oil and exposure time (Fig.2). As observed here, the Rheum oil is markedly rich in hydrocarbons especially long-chain n-alkanes so the moderate toxicity effect of this oil might be attributed to the presence of these compounds especially odd numbered members of the alkanes. As previously reported, lipophilic hydrocarbons affect the functional and structural properties of membrane lipid bilayer by accumulating in these membranes. As

a result of accumulated hydrocarbon molecules, the integrity of the membranes loses, and an increase in permeability to protons and ions has been observed in several cases. Consequently, dissipation of the proton motive force and impairment of intracellular pH homeostasis arise [37].

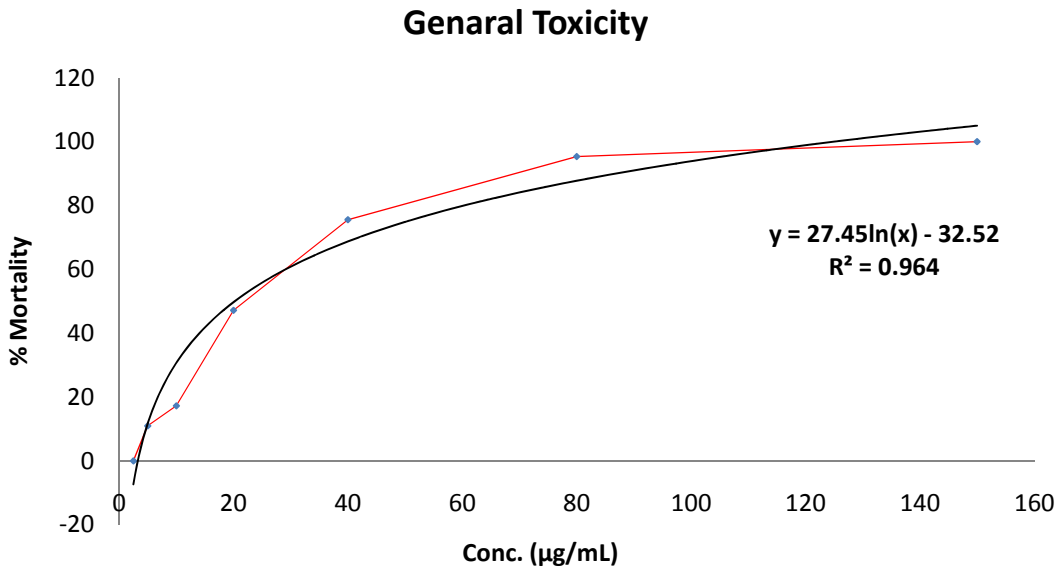

Fig. 2. Brine shrimp lethality assay of essential oil from *R. ribes* against *Artemia salina*

Conclusion

In summary, the present study reported the chemical composition of the essential oil from the edible parts of *R. ribes* for the first time, and also evaluated the general toxicity activity of the oil.

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Conflict of interests

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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