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Research Article

Chemical Constituents of *Rheum ribes* L.

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ABSTRACT

Chemical investigation of the dichloromethane extract of *Rheum ribes* has led to the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters (**1**), β-sitosterol (**2**), phytyl fatty acid esters (**3**), triacylglycerols (**4**) and chlorophyllide a (**5**). The structures of **1**-**5** were identified by comparison of their NMR data with literature data.

Keywords: *Rheum ribes* L., Polygonaceae, β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters, β-sitosterol, phytyl fatty acid esters, triacylglycerols, chlorophyllide a

INTRODUCTION

Rheum ribes L. of the family *Polygonaceae*, locally known as “Rivas” is a native plant of Iran which grows in several provinces including Khorasan[1] and in the Middle East[2]. A study reported that palmitic acid [27.08%], n-eicosane [9.9%], n-tetracosane [7.34%], linoleic acid [6.56%], and ethyl linoleate [4.76%] were the main components of the oil of *Rheum ribes.* Extracts and fractions from the plant inhibited the growth of the protozoan, *Trichomonas vaginalis*[3]. Through GC-GC/MS, the constituents of the extract from the flowers of *R. ribes* were characterized as possessing a high quantity of unsaturated fatty acids (66.0 %) and some long chain hydrocarbons. 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 major constituents of the distilled oil were germacrene D (22.3 %), α-pinene (13.5 %), terpinolene (12.4%), p-cymene (10.6 %), bicyclogermacrene (9.6 %) and limonene (8.6 %). The essential oil and hexane extract exhibited a moderate effect on some Gram-positive and Gram-negative bacteria. The hexane extract of this plant‘s flowers possessed considerable antioxidant activity[4]. The essential oil of the stalks and flowers of *R. ribes* L., growing in Iran, were extracted via 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 using a bioassay brine shrimp lethality method. The toxicity profile of the oil indicated some degree of toxicity in comparison with podophyllotoxin[5]. *R. ribes* was also reported to contain the anthraquinones, physcion and rhein and the stilbene; rhaponticin or rhapontin[6]. The anthraquinones, chrysophanol, parietin and emodin, the flavonoids quercetin, fisetin, quercetin 3-0-rhamnoside, quercetin 3-0-galactoside and quercetin 3-0-rutinoside were isolated from the shoots of *R. ribes*[7]. Another study reported the isolation of four anthraquinone derivatives (chrysophanol, physcion, rhein and aloe-emodin), two anthraquinone glucosides (physcion-8-*O*-glucoside and aloe-emodin-8-*O*-glucoside), the dianthron glucoside, sennoside A, and the stilbene glucoside, rhaponticin from the roots and rhizomes[8]. The ethyl acetate extracts of *R. ribes* shoot and root dry powder were shown to be potential scavengers of DPPH radicals (IC_{50} value of 206.28 µg/mL for the shoots and 10.92 µg/mL for the roots). *R. ribes* inhibited the survival of HL-60 cells in a concentration- and time-dependent manner[9].

We report herein the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters (**1**), β-sitosterol (**2**), phytyl fatty acid esters (**3**), triacylglycerols (**4**) and chlorophyllide a (**5**) from *Rheum ribes.* The structures of **1**-**5** are shown in Fig. 1.

MATERIALS AND METHODS

General Experimental Procedure

^1H (500 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26). Column chromatography was performed, with silica gel 60 (70-230

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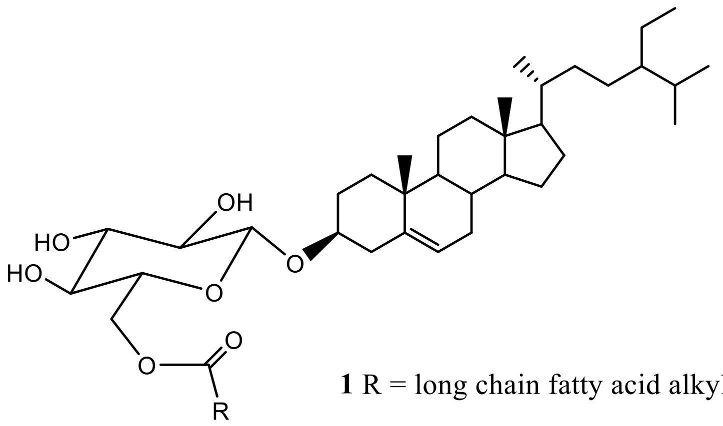

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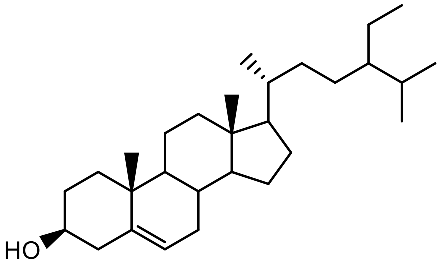

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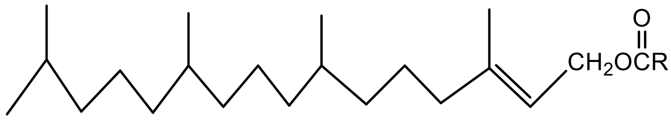

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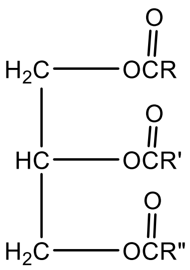

4 R, R', R" = long chain fatty acid alkyls

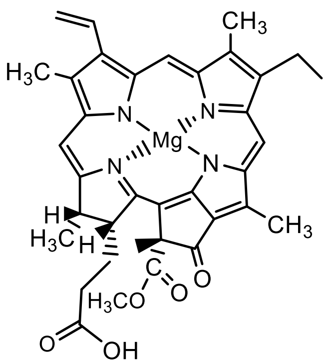

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Figure 1: Chemical structures of β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters (**1**), β-sitosterol (**2**), phytyl fatty acid esters (**3**), triacylglycerols (**4**) and chlorophyllide a (**5**) from *Rheum ribes.*

mesh). Thin layer chromatography, was performed with plastic backed plates coated with silica gel F254 and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Plant Material

Plant specimens were collected from the Zagros mountain areas of West Azerbaijan, Iran, with the kind assistance of Mr. Saeed Karami Ishghlo of Payame Noor University of Bukan. The identity of the samples was confirmed in comparison with voucher specimens in the Central Herbarium of Tehran University.

Isolation of the Chemical Constituents

The freeze-dried sample (53.9 g) was soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.4825 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to yield **3** (2 mg) after washing with petroleum ether. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to yield afford **4** (5 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford **2** (5 mg) after washing with petroleum ether. The 70% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford **1** (4 mg) after trituration with petroleum ether. The 80% to 90% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5, v/v) to afford **5** (3 mg) after washing with petroleum ether, followed by diethyl ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *Rheum ribes* L. afforded **1**-**5**. The NMR spectra of **1** are in accordance with data reported in the literature for β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters[10]; **2** for β-sitosterol[11], **3** for phytyl fatty acid esters[12], **4** for triacylglycerol[13], and **5** for chlorophyll a[14]. The fatty acids attached to the triacylglycerol were identified as linolenic acid, linoleic acid and oleic acid based on resonance intensities for the methyl triplet at δ 0.96 (t, J = 7.8 Hz), the double allylic methylenes at δ 2.78 and the olefinic protons at δ 5.34 (m) for the linolenic acid; methyl triplet at δ 0.86 (t, J = 6.6 Hz), the double allylic methylene at δ 2.80 and the olefinic protons at δ 5.34 (m) for the linoleic acid; and the ; methyl triplet at δ 0.86 (t, J = 6.6 Hz) and the olefinic protons at δ 5.34 (m) for the oleic acid[15].

Literature search revealed that **1-2** and **4-5** exhibited diverse biological activities. α-Sitosteryl-3α-glucopyranoside-6'-*O*-palmitate (**1**) was reported to exhibit cytotoxicity against Bowes (melanoma) and MCF7 (breast) cancer cell lines with IC_{50} values of 152 μM and 113 μM, respectively[16]. Furthermore, **1** exhibited cytotoxicity against human stomach adenocarcinoma (AGS) cell line with 60.28% growth inhibition[17]. In another study, **1** isolated from *Orostachys japonicus* was found to exhibit potent anti-complement activity (IC_{50} = 1.0 ± 0.1 μM) on the complement system expressed as total hemolytic activity[18].

β-Sitosterol (**2**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells[19]. It was shown to be effective for the treatment of benign prostatic hyperplasia[20]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench the radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis[21]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake[22]. It has also been reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells[23].

Triacylglycerols (**4**) from Tuna (1000 mg/kg) have been reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically implanted Lewis lung carcinoma[24]. Triacylglycerols exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes*[25]. Another study reported that triacylglycerols showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation[26]. Linoleic acid belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces the risk of colon and breast cancer[27] and lowers cardiovascular disease risk and inflammations[28]. Linolenic and linoleic acids inhibited parasites growth by 70% and 64% respectively, against *P. berghei* using the 4-day suppressive test. The two compounds, when used in combination, inhibited the parasites by 96% on day 4 of treatment[29].

Chlorophyll and its various derivatives are used in traditional medicine and for therapeutic purposes[30]. Natural chlorophyll and its derivatives have been studied for wound healing[31], anti-inflammatory properties[32], control of calcium oxalate crystals[33], utilization as effective agents in photodynamic cancer therapy[34-36], and chemopreventive effects in humans[37-38]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided[39].

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