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Der Pharmacia Lettre, 2015, 7 (4):26-30
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ISSN 0975-5071
USA CODEN: DPLEB4

Phenolic profile, antioxidant, and antibacterial effects of ethanol and aqueous extracts of *Rheum ribes* L. roots

Khalida K. Abdulla[1], Ekhlass M. Taha[2] and Saleh M. Rahim[1]*

[1]*Department of Biology, Faculty of Education for Pure Sciences, University of Tikrit, Salah Al Deen, Iraq*
[2]*Department of Chemistry, Faculty of Science for women, University of Baghdad, Baghdad, Iraq)*

ABSTRACT

Rheum ribes is a traditional medicinal plant collected from mountainous areas in northern Iraq and widely used to treat various ailments. The antioxidant potential of the ethanol and aqueous root extracts of this plant were examined using 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay. The antibacterial activity of the extracts on selected Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli, Proteus mirabilis, and Pseudomonas aeruginosa) bacterial species was also evaluated. Ten individual phenolics compounds (emodin, aloe-emodin, physcion, chrysophanol, rhein, chlorogenic acid, gallic acid, tannic acid, kaempferol, and rutin) were also determined by HPLC. The contents of phenolic compounds were significantly higher in ethanol than aqueous root extracts. Both root extracts exhibited antioxidant and antibacterial activities. The highest antioxidant activity (IC_{50}) was found with ethanol extract (4.73 ± 0.21 μg/mL), which was closer to that of vitamin C (0.046 ± 0.05 μg/mL) than that of aqueous extract (25.62 ± 0.85 μg/mL). The ethanol extract of roots exhibited the highest antibacterial effect against all bacterial species assayed compared with standard drugs. The high content of phenolic compounds in R. ribes root extracts were found to be responsible for the high antioxidant and antibacterial activities against the tested microorganisms. Thus, R. ribes root extracts can be used as a therapeutic agent against various ailments, which confirms the use of this plant in traditional medicine.

Keywords: Phytochemicals, antibacterial, antioxidant, *Rheum ribes*, DPPH, HPLC

INTRODUCTION

Plants comprise a vast resource of potential drug targets and other active drug molecules. Thus, medicinal plants serve as the basis for traditional medicinal systems and natural products, which provide remarkable leads for new drug development. The World Health Organization estimated that 80% of global population resort to traditional medicine for primary health care needs. The majority of this therapy involves the use of plant extracts and their active components, which are non-nutritive compounds synthesized from plants that have disease protective properties [1–3]**.** Antibacterial active components isolated from higher plants are some of the important alternative approaches to contain antibiotic resistance and manage disease. The increasing application of drugs has resulted in the resistance of pathogenic microorganisms to existing antimicrobial compounds. Hence, exploring and designing alternative drugs from natural products is necessary to combat microbial infections. Plant-based drugs, which probably evolved as a chemical defense against predation or infection, is perceived to have less or no side effect compared with synthetic antibiotics [4,5]. Phenolic compounds from plants are known to be good natural antioxidants because of their hydroxyl groups. These components protect cells against the damaging effects of free radicals or reactive oxygen species such as singlet oxygen, superoxide, peroxide radicals, hydroxyl radicals, and peroxynitrite [6,7]. Natural flavonoids are known for their significant scavenging properties on oxygen radicals *in vivo* and *in vitro.* In addition, these compounds have membrane-stabilizing properties and also affect some processes of intermediary metabolism. Numerous studies have shown the importance of the antiradical activity of flavonoids [6,8]. Hence, these compounds have been extensively investigated and have been described to demonstrate

numerous biological activities such as antioxidant, anti-inflammatory, cytotoxic, antitumor, and antiviral activities [9,10]. *Rheum ribes* belongs to the family Polygonaceae, and the genus *Rheum* consists of approximately 60 perennial species distributed worldwide [11]. *R. ribes* roots have been used as antihelmintic and expectorant as well as in the treatment of treat diabetes, hypertension, obesity, ulcer, and diarrhea [12]. Chemical studies on the roots of *R. ribes* collected from Erzincan in Turkey have been conducted. Chrysophanol, physcion, rhein, aloeemodin, physcion-8-O-glucoside, aloeemodin-8-O-glucoside, sennoside A, and rhaponticin have been isolated [12]. These compounds represent several bioactivities ranging from anti-inflammatory, antioxidant, and antimicrobial, among others [13]. In Iraq, *R. ribes* is found in the mountainous regions at an elevation of more than 1500 m north of the country. In this country, the plant is called Rewaze. Its fresh stalk is peeled and consumed during the spring season from April to July. *R. ribes* roots has been prescribed by herbalists to treat various ailments. The antibacterial susceptibility of *R. ribes* root ethanol and aqueous extracts against four bacterial strains was investigated in the current study. The phenolics content and antioxidant activity of these extracts were also evaluated using high performance liquid chromatography (HPLC) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging.

MATERIALS AND METHODS

Plant Material

The roots of *R. ribes* were obtained from the local market in northern Iraq. The specimen was identified and authenticated by Prof. Khalil I. Al-Shemmary, Plant taxonomist, Biology Department, Faculty of Science, Tikrit University, Tikrit, Iraq. The certified specimen was deposited at the herbarium of the Faculty of Science, Tikrit University.

Instruments and Chemicals

Most of the chemicals used were laboratory-grade compounds purchased from Merck. DPPH was obtained from Sigma Chemical. Solvents for HPLC were obtained from Merck (HPLC grade). All other reagents, such as solvents, DMSO, and acetic acid, were of analytical grade and available commercially.

Preparation of the extracts:

The rhizomes and roots of plant were thoroughly cleaned to remove soil and other debris that may contaminate the sample. The dried powdered samples (100 g) were subjected to continuous extraction using absolute ethanol and distilled water for 24 h under constant stirring at regular intervals at laboratory temperature. The extracts were filtered through Whatman No.1 filter paper and concentrated at 40 °C in a rotary evaporator. The solid samples of ethanol and aqueous extracts obtained weighed 7 (yield = 7%) and 15 g (yield = 15 %), respectively. The crude ethanol extract was kept in an air-tight container and stored in a refrigerator at 4 °C until use.

Antibacterial activity

Antimicrobial activity was tested by agar diffusion method. Mueller Hinton agar was inoculated with different selected bacterial strains using a streak plate method. Wells were made on the agar surface using a 6 mm cork borer, and 200 µL ethanol and aqueous extracts at concentrations of 25, 50, and 75 mg/mL were poured into the wells using a sterile micropipette [14]. After incubation at 37 °C for 24 h, the antimicrobial efficiencies of the extracts were quantitatively assessed on the basis of the inhibition zone [15]. Antibacterial activity was tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. The bacterial strains were obtained from the Laboratory of Bacteriology of the Tikrit Teaching Hospital in Tikrit, Iraq. The results were compared with cefotaxime and gentamycin.

Antioxidant activity

DPPH scavenging method

The antioxidant activities of the ethanol and aqueous extracts from the rhizome and root of *R. ribes* were assessed on the basis of the radical scavenging effect of stable DPPH [10]. DPPH methanol solution (1 mL, 0.2 mM) was added to 4 mL extracts with various concentrations in different test tubes. The mixture was shaken vigorously and left to stand at laboratory temperature. After 30 min, the absorbance of the solution was measured at 517 nm, and antioxidant activity was calculated using the following equation:

Scavenging capacity % = 100 – [(Ab of sample – Ab of blank) – 100/Ab of control] × 100.

Methanol (1 mL) and plant extract solutions (4 mL) were used as blank, while DPPH solution plus ethanol/distilled water was used as a negative control. Ascorbicacid was taken as standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

HPLC analyses of phenolic contents of *R. ribes* ethanol and aqueous root extracts

Ten phenolic components of *R. ribes* ethanol and aqueous root extracts were quantitatively analyzed using HPLC. Each extracted sample (100 mg) was dissolved into 5 mL ethanol–water (80:20 v/v) in glass tubes. The suspensions were ultrasonicated (Branson sonifier, USA) at 60% duty cycles for 25 min at 25 °C, followed by centrifugation at 7500 rpm for 15 min. The clear supernatant of each extract was subjected to charcoal treatment to remove pigments prior to evaporation in a vacuum (Buchi Rotavapor Re Type, Switzerland). Dried samples were re-suspended in 1.0 mL HPLC-grade methanol by vortexing, and the mixture was passed through 2.5 µm disposable filter and stored at 4 °C for further analyses. The samples (20 µL) were injected into an HPLC system according to the optimum conditions: phenomenex C-18 column, 3 µm particle size (50 × 2.0 mm I.D); mobile phase, linear gradient of solvent A (0.1% phosphoric acid in deionized water) and solvent B was acetonitrile, with gradient program from 0% B to 100% B for 10 min; flow rate, 102 mL/min; detection wavelength, 280 nm; and column temperature, 25 °C. The optimum condition of column used with gallic acid and tannic acid was a Zorbax clipse XDB-C18 (2.0 × 50 mm) column. Gradient elution was performed with 0.1% acetic acid (A), methanol (B) using a linear gradient program from 0% B to 100% B for 8 min at a constant flow rate of 1 mL/min. Detection wavelength was 370 nm. HPLC was conducted using a Shimadzu 10 AV- LC equipped with binary delivery pump model LC-10 A Shimadzu. The eluted peaks were monitored by UV-Vis 10AV-SPD specrophotometer (Shimadzu, Kyoto, Japan). The peaks found in the root extracts of *R. emodi* samples were identified by comparison with the mixture of standard materials, and each standard was 25 µg/mL [16].

RESULTS AND DISCUSSION

Emodin, aloe-emodin, physcion, chrysophanol, rhein, chlorogenic acid, rutin, gallic acid, tannic acid, and kaempferol found in *R. ribes* ethanol and aqueous root extracts were analyzed by phytochemical HPLC. The first five phenolics are appointed as the standard compounds, and their total amount is viewed as the quality control standard of *R. ribes* [17]. The individual phenolic content were found to be higher in ethanol extract compared with those in the aqueous extract (Table 1). Phenolic content was ranked in ethanol extract in descending order as follows: chrysophanol > emodin > physcion > aloe-emodin > chlorogenic acid > rhein > gallic acid > rutin > kaempferol > tannic acid. By contrast, the decreasing phenolic contents in the aqueous extracts were ranked as follows: emodin > physcion > chrysophanol > aloe-emodin > rutin > rhein > gallic acid > kaempferol > tannic acid > chlorogenic acid. These phytochemicals have been shown to possess medicinal activity and physiological activity, which confirms the traditional medicinal uses of this plant.

The antioxidant activity of *R. ribes* extracts was measured using DPPH radical scavenging activity. In the DPPH assay, the antioxidants reduced the stable DPPH radical (purple) to the non-radical form DPPH-H (yellow). The DPPH scavenging activities of antioxidants are attributed to their hydrogen-donating abilities [18]. A significant decrease *($p < 0.05$)* in the concentration of DPPH radicals was exhibited by all extracts and standards. Vitamin C was used as the reference compound. The scavenging effect of the samples on the DPPH radical decreased in the following order: ascorbic acid > ethanol extract > aqueous extract. The IC_{50} values for vitamin C, ethanol extract, and aqueous extract were 0.046 ± 0.05, 4.73 ± 0.21, and 25.62 ± 0.85 µg/mL, respectively (Table 2). The antioxidant activity was due to the presence of various secondary metabolites, such as phenols, flavonoids, and other secondary metabolite compounds, in *R. ribes* root extracts, which were higher in ethanol extract as shown in Table (1).Positive correlations between total phenolic content and antioxidant capacity have been reported [19]. Moreover, several reports have revealed that the contribution of phenolic compounds to antioxidant activity was much greater than those of vitamin C and carotenoids [20].

The antimicrobial activity of *R. ribes* ethanol and aqueous extracts were tested using *E. coli*, *S. Aureus*, *P. aeruginosa*, and *P. mirabilis.* The results are shown in Table 3. The *R. ribes* ethanol and aqueous extracts showed significant zones of inhibition in a dose-dependent manner against all the tested microorganisms. The ethanol extract showed a higher zone of inhibition range (11.3 mm–24 mm) compared with the aqueous extract (9.3 mm–20 mm). These results indicate that the *R. ribes* ethanol and aqueous extracts exhibit a broad spectrum of activity. Gentamycin and cefotaxime exhibited zones of inhibition against all the test organisms. However, the inhibition zones of these standards were more or less remarkable only when compared with that of a low concentration (25 mg/mL) of the extracts and were nonremarkable compared with those of other extract concentrations. The antibacterial activity of *R. ribes* ethanol and aqueous extracts extract was mainly due to the presence of phytochemical compounds as shown above. The antibacterial effects of rhubarb are believed to have been caused by its inhibition of enzymes in the mitochondrial electron transport system [21]. Phenols are secondary metabolites broadly distributed in the plant kingdom with more than 8,000 phenolic structures currently known, ranging from simple molecules, such as phenolic acids, to highly polymerized substances, such as tannins. Positive correlations between total phenolics and antioxidant capacity have been reported [19]. These compounds result in various biological properties such as anti-bacterial, anti-inflammation, anti-aging, anti-carcinogen and cardiovascular

protection. Flavonoids are potent water-soluble free radical scavengers that are useful in preventing oxidative cell damage and have anti-cancer activity [22]. These chemicals positively affect human health and are known to exhibit biological activities such as antibacterial, antiviral, and anti-allergic activities [23].

Table (1) phenolics compounds content of *R. ribes roots* ethanol and aqueous extracts

Phenols and poly phenols	Aqueous extract µg/ml	Ethanol extract µg/ml
Aloe-emodin	323.5	1036
Emodin	1196	2020
Chrysophanol	636.79	2243
Physcion	1609	1096
Rhein	159.73	412.23
Chlorogenic acid	18.8	533
Gallic acid	131	284
Kaempferol	108.64	117.36
Tannic acid	40	100.4
Rutin	172.67	218.26

Table (2) Antioxidant activity of *R. ribes* ethanol and aqueous extracts on DPPH (1,1-diphenyl-2-picrylhydrazyl).

Extracts Sample	IC_{50} DPPH (µg/ml)
Aqueous extract	25.62
Ethanol extract	4.73
Vitamin C	0.046

Table (3) antibacterial activity of *R. ribes* ethanol and aqueous extracts and standard antibiotics

Organism	Extracts conc.(mg/ml),inhibition zones (mm),and standard antibiotics (µg/disc)							
	Ethanol extract			Aqueous extract			Gentamicin	Cefotaxime
	25	50	75	25	50	75	10	30
E. coli	11.3	18.6	20	9.3	10.3	16	13.3	12
S. aureus	18.6	22.6	24	12.3	15.6	20.3	6	16
P. aeruginosa	15.3	18.6	20	11.3	15.6	18	15.6	8
P. mirabilis	13.3	18.6	20	15.6	15.6	18	14.6	11

CONCLUSION

R. ribes possesses high medicinal values as shown by the activities of its crude extracts on various microorganisms. The presence of the phytochemicals justifies the traditional medicinal uses of this plant. The results from this study combined with those from previous studies on the roots of *R. ribes* may represent a reference to antioxidant and antibacterial effects of *R. ribes* with stable and biologically active components. Thus, a scientific foundation for the use of this plant in medicine can be established to improve the healthcare of local users.

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