**Original Article**

# Assessment of phytochemical components and antioxidant activity of Rheum turkestanicum Janisch

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## Abstract

***Background & Aims:*** *Plants contain high antioxidant activities due to their redox and the chemical* properties are affluent in secondary metabolites such as phenols, flavonoids, and other components. Rheum turkestanicum Janisch is a plant from polygonaceae that is widely used for diabetes. At this project that is a part of national thesis, relative levels of antioxidant activity, total phenols, total flavonoid, total anthocyanin, soluble and non-soluble sugar content of Rheum turkestanicum were measured.

***Materials & Methods*****:** The shoots of Rheum turkestanicum were collected and verified from Dargaz region in north-east of Iran and then they were dried at room temperature.

The aerial portion of the plant was powdered by grinding, and five grams of the herbal powder were mixed with 300 mL of deionized water and after 24 h, the resulting mixture was filtered using Whatman No. 1 filter paper. Determination of total phenol, total flavonoid, anthocyanin, soluble sugars and antioxidant properties of aqueous extract was performed by standard Folin chicaletto, aluminum chloride colorimeter, Wagner, phenolic sulfuric acid, DPPH methods using a spectrophotometer.

***Results*****:** The results of this project showed that the amount of total phenolic and flavonoid acids in Rheum turkestanicum extract was high at 123.8 and 116 mg/g dry weight, respectively. DPPH scavenging activity was observed to be 6.42 mg/g dry weight of ascorbic acid.

The results of this project showed that DPPH scavenging activity was observed to be 6.42 mg/g ascorbic acid dry weight. Total phenolic acid and total flavonoid content of the investigated Rheum turkestanicum were higher in comparison to other components.

***Conclusion*****:** This fact indicates that phenolic acids and flavonoids play a major role in the antioxidant and anti-diabetic properties of Rheum turkestanicum. The results also indicate that Rheum turkestanicum can be used as an important source of antioxidants in the food and pharmaceutical industries due to its high levels of secondary metabolites such as phenols and flavonoids.

***Keywords*****:** Rheum turkestanicum, antioxidant activity, phenols, flavonoids

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## Introduction

Due to the changes in environmental conditions, a variety of free radicals are emerging while plants have to cope with them in order to survive. Reactive oxygen species (ROS) including $OH^{\bullet}$, $OH^{-}$, and $O_2^{\bullet}$, are highly reactive and toxic and they are produced generally in cells through metabolism (1-3). They generate severe oxidative damage to tissues, membranes, enzymes, inflammation, and etc. (4-6). Much attention has been paid to secondary metabolites and natural antioxidant agents since they are capable of scavenging free radicals (7-10). Free radicals are involved in the progress of several disarrays such as cancer, degeneration of neuron cells, and inflammation (11-13), and investigations are conducted on antioxidants to prevent and treat these particular infections. The existence of antioxidants such as phenols, flavonoids, anthocyanins and reducing sugars in plants can furnish the safety procedure in contrast to numeral infections; for example, the consumption of natural antioxidants completely related to limited of disease and humanity from progressive disorders (14-16). Thus, medicinal plants are enquired into antioxidants materials and nutrition preservatives are massively growing (17-20). *Rheum turkestanicum* (commonly known as Eshghan) is an important medicinal plant that grows widely in central Asia particularly in the north-east of Iran (21). *Rheum turkestanicum* from Polygonaceae is a significant medicinal plant of Khorasan province that is utilized for jaundice by local inhabitants in the north-east of Iran (22) and the shoots extracts have a crucial role in antibacterial activity (23, 24). *Rheum turkestanicum* has been used in traditional medication and, it has been reported to contain cytotoxicity effect on human breast cancer cell line (MCF-7) (25). The purpose of this project was to verify the content of bioactive molecules as well as the antioxidant properties of the shoot parts of *Rheum turkestanicum* (Fig. 1).


**Fig.(1).** The photograph of Rheum turkestanicum (A) and its shoots (B).

## Materials and Methods

***Materials:***

All of the prepared chemical materials were of analar grade and solvents and DPPH radical have been purchased from Sigma (USA). The shoots of *R. turkestanicum* were collected from Zarrin-Kuh Protected Area, Razavi Khorassan Province (NE Iran). A voucher specimen was identified and deposited (No.21433) in Dargaz Payame Noor University Herbarium. The *Rheum turkestanicum* shoots were cleansed and dried at ambient temperature, while more shoots part were grounded to powder and stored for future research. The experiments in this work were done using double distilled water.

***Extraction procedure:***

The shoots part of *Rheum turkestanicum* (3.0 g) were put to be dried and powdered; then they were extracted with maceration in distilled water for 24 hours. After being filtered, the crude extract was preserved in the refrigerator (Fig. 2).

**Fig (2).**The aqueous extract of Rheum turkestanicum

***Antioxidant activity assay:***

The antioxidant capacity of the extract was estimated and compared with ascorbic acid as a positive control through the use of a DPPH. Briefly, 23mg/ml solution of DPPH was arranged in ethanol, while its absorbance was measured to be at 517 nm. DPPH is a purple colored, constant free radical and when the antioxidants are added, its color turns from purple to yellow. All the samples were evaluated in triplets. Active-radical's prevention capacity was acquired through the standards of ascorbic acid.

***Total phenolic content:***

Total phenols were ascertained by the use of Folin–Ciocalteau reagents (26). 100 mg shoots of *Rheum turkestanicum* were rubbed with methanol and placed in the dark for 48 hours. 50 µL of this solution was mixed with 450 µL of deionized water, 250 µL of Folin–Ciocalteu chemical, and 1.2 mL of sodium bicarbonate (20%, w/v). Then it was set aside to standpoint at 25 ºC for 20 min, afterwards it was centrifuged for 10 minutes. The absorbance was measured at 735 nm. Aqueous solutions of gallic acid concentrations were utilized for calibration.

***Anthocyanin assay:***

Anthocyanin assay was operated via standard technique (27). Anthocyanin was confirmed in 0.3% HCI in methanol at room temperature by applying the extinction coefficient:

ew = 33,000 [$cm^2$/mol]

***Estimation of flavonoid content:***

The aluminum chloride colorimetric procedure (28) was used to estimate the total flavonoid content. Gallic acid was used to make the calibration curve. 100 mg of aerial parts were rubbed in distilled water. The dilute solutions (0.5 mL) were individually mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1.0 M potassium acetate, and 2.8 mL of distilled water. After being incubated at 25 ºC for thirty minutes, the absorbance of the reaction solution was measured at 415 nm using a spectrophotometer.

***Saccharides content:***

Saccharides (soluble sugars and starch) were measured through the utilization of the phenol sulfuric acid method (29).100 mg dried shoots of plant were obtained with 80% ethanol and after applying $Ba(OH)_2$ and $ZnSO_4$ to discard the pigments from extracts and adding 5% phenol and sulfuric acid, the absorbance of extracts was recorded at 485 nm.

## Results

The DPPH radical is widely utilized in estimating the removal of free radical properties since the reaction is quite simple and easy. DPPH scavenging activity was observed to be 6.42 mg/g ascorbic acid dry weight. The total phenolic content of the shoots that was quantified from the calibration curve was 123.8 mg/g dry weight and the total flavonoid content was 116.7 mg/g dry weight (Fig. 2). Phenolic compounds have proved to contain redox activity that enables phenols to take action as antioxidants (30, 31). As their free radical eliminate activities that are smoothened by their hydroxyl groups, the total phenols content might be employed as a source for a fast selection of antioxidant action. Flavonoids, involving flavones, and flavanols, are among the plant secondary metabolites, and their antioxidant properties rely on the existence of free OH groups. Flavonoids in plants have antioxidant activities *in vitro* and doing *in vivo* as well (32, 33).

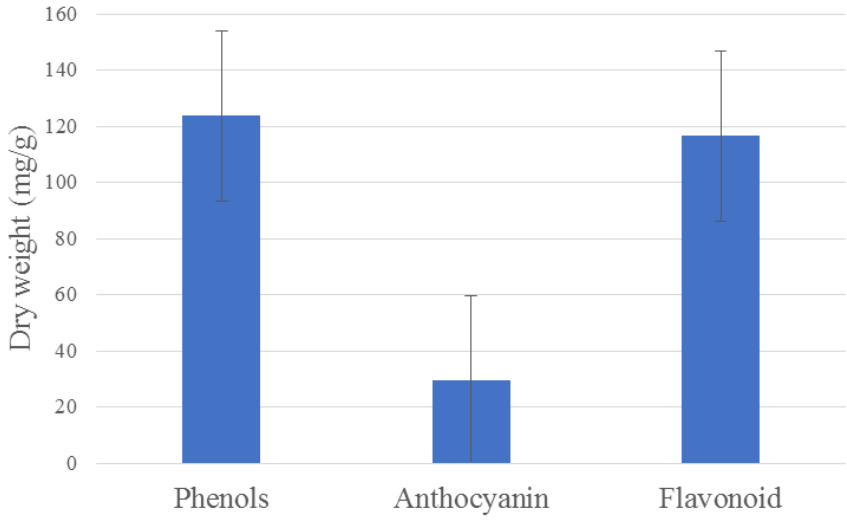


**Fig (3).**The content of some important reducing agents (All of the data are in mg/g dry weight).

The content of soluble sugar was observed to be 4.90 mg/g dry weight and the content of insoluble sugar was 3.85 mg/g dry weight. Soluble sugars that are usually interpreted as monosaccharides and disaccharides have a main effect in the assembly and function of all alive cells. Their source seems to be closely connected with prebiotic and early biotic evolution (34). Soluble sugars appear to presume a crucial effect with attribute to reactive oxygen species. The amount of soluble sugars might be included in, or associated with metabolism ways that produce ROS and pathways that can contribute to ROS removing. Consequently, soluble sugars could be anticipated to balance the defense system in contrast to different ROS-producing stresses. Besides, the probability that singlet oxygen may take action as an apoptogenic sign in plant cells (35, 36). Oxidative stress may play an important role in $HgCl_2$-induced hepatorenal injury and *R. turkestanicum* extract may be useful and help protect the kidney and liver against $HgCl_2$-induced oxidative damage(37). The extract of *R. turkestanicum* has a protective effect against cisplatin-induced nephrotoxicity by reducing oxidative stress in kidney tissue (38).

Anthocyanins are well known as the major and most vital cluster of water resolvable pigments in nature (39) and they may be the cause of various biological activities such as preventing or depressing the danger of cardiovascular ailment, diabetes, and cancer. They are responsible for the colors of many fruits and vegetables (40). Anthocyanins possess the potential of scavenging hydroxyl radicals via the restriction of $OH^{\bullet}$ production by chelating iron (41). Since this is the first report on the antioxidant activity and bioactive component of *Rheum turkestanicum*, the thorough phytochemical examination is required to recognize the active phenols and flavonoids components. Phenols, flavonoids, and anthocyanin in plants contain antioxidant activities. The DPPH radical is widely employed in assessing free radical scavenging activity since the reaction is easily performed (42). The secondary metabolites are responsible for the bioactivity of these basic extracts. Flavonoids are useful scavengers among the oxidizing molecules and numerous free radicals are involved in various infections (43). Flavonoids have the power to moderate ROS formation and chelated trace elements that are included in free radical formation while scavenging reactive species as well as up-regulating and protecting antioxidant defenses (44). Ghorbani et al. reported that R.turkestanicum inhibited the development of nephropathy, liver injury, and myocardial destruction in diabetes by inhibiting oxidative stress-mediated lipid peroxidation and this is done by the flavonoids in the extract (45). In another study, high antioxidant activity for R.turkestanicum root is reported (24). Equally, phenols confer the oxidative stresses forbearance on plants. Crude extracts of fruits, vegetables, crops, and other plant materials that are rich in the phenolic compounds are progressively utilized in food trade because of antioxidative properties and many health profits.

## Conclusions

As it is the first report of a study on the bioactive components of *Rheum turkestanicum*, it is indicated by our results that it is a possible source of antioxidant agents and could be utilized as a natural antioxidant and protective in food and medical industry. Additional biochemical assays are needed to isolate the components of the plant which has displayed a wide range of pharmacological properties.

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## Conflict of interests

The authors declare no conflict of interest

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