

Biocomponents and Antioxidant Activity of *Ribes khorasanicum*

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Abstract

Introduction: Currently, there is great interest in phytochemicals as bioactive components. The roles of fruits, vegetables, and red pigments in preventing diseases have been partially attributed to the antioxidant features of their constituent polyphenols, flavonoids, anthocyanins, and so on.

Methods: Biochemistry parameters including the relative levels of antioxidant activity, total phenolic content, total flavonoid, total anthocyanin, and soluble and insoluble sugar contents of *Ribes khorasanicum* were measured.

Results: The total phenolic and flavonoid content of the fruits were calculated as 33.6 mg/g dry weight and 23.36 mg/g dry weight, respectively. Anthocyanin was measured as 62.9 mg/g dry weight, indicating anthocyanin as the predominant antioxidant component in this plant. The content of soluble sugar was 5.65 mg/g dry weight, while the amount of insoluble sugar was 4.60 mg/g dry weight. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was observed to be 6.42 mg/g dry weight.

Conclusion: Anthocyanin was the predominant antioxidant component in the investigated plant. This particular plant has the potential to serve as an important source of anthocyanin which is useful in medicinal and food industries.

Keywords: *Ribes khorasanicum*, Anthocyanin, Antioxidant activity

Introduction

Reactive oxygen species (ROS), including superoxide, hydroxyl, and nitric oxide radicals, may damage the living cells in biological systems and lead to the oxidative stress in cells.¹⁻⁴ Generally, the antioxidant molecules can scavenge free radicals and as a result, keep the balance between the oxidation and anti-oxidation pathways. Nevertheless, exposure to pollution or ecological toxins can cause the generation of extreme ROS and upset the balance between oxidant and antioxidant systems, resulting in certain enduring and progressive infections.^{5,6} An increase in the consumption of exogenous antioxidants

would end up in ameliorating the damage induced by oxidative stress via reducing the oxidative reaction, acting in removal of free radicals.⁷ The antioxidant molecules are found in high volumes in medicinal plants, fruits, crops, and aromatic plants.⁸⁻¹¹ The natural antioxidants with plant resources mostly consist of phenols, flavonoids, anthocyanins, vitamins (E and C), and reducing sugars.¹² Natural antioxidant molecules generally exert a wide range of biological effects such as anti-inflammatory, antibacterial, antiviral, and anticancer effects.¹³⁻¹⁷ *Ribes khorasanicum* from Grossulariaceae family is an important medicinal plant which grows in

north of Khorasan province, Iran, and its fruits are useful in medicine.¹⁸ This study intended to determine the bio-components of *R. khorasanicum* fruit and its antioxidant properties.

Materials and Methods

Plant Materials and Chemicals

All the used chemicals were pure (<99%). Solvents were purchased from Merck (Germany) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was acquired from Sigma Chemical Company (Sigma-Aldrich, USA). Plants were collected from Dargaz region, Iran.

Extraction and Fractionation Procedure

The fruit parts of plants (3.0 g) were dried and transformed into powder. They were then macerated in double distilled water for a duration of 24 hours. After being filtered, the crude extract was preserved in refrigerator (Figure 1).

DPPH Radical Scavenging Test

The antioxidant capacity of the extracts was estimated by the DPPH test and compared with ascorbic acid as the positive control.¹⁹ The DPPH has a purple deep color and its maximum absorption is at 515 nm.²⁰ The reducing capacity could be analyzed through evaluating the decrease in its absorbance. In brief, 23 mg/mL solution of DPPH was added to ethanol, and its absorbance was measured at 517 nm. DPPH is a purple-colored, stable free radical which is changed to yellow diphenylpicrylhydrazine after oxidation. All the samples were evaluated in triplicate. The prevention capacity of active radicals was acquired against standard curve obtained from ascorbic acid.

Folin–Ciocalteu Reagent Assay

Folin–Ciocalteu Reagent (FCR) test is a general and simple procedure for determination of phenol contents of fruits and medicinal plants.²¹ These particular blue pigments have maximum absorption within the range of 700–760 nm, which depends on the qualitative and/or quantitative composition of phenolic mixtures. In brief, 50 µL of extract was mixed with 450 µL of deionized water, 250 µL of Folin–Ciocalteu chemical, and 1.2 mL of sodium bicarbonate (20%, w/v). Afterward, it was put aside at 25°C for 20 minutes, and then centrifuged at 4000



Figure 1. *Ribes khorasanicum* (A) and its Aqueous Extract (B).

rpm for 10 minutes. The absorbance was measured at 730 nm. Aqueous solutions of gallic acid concentrations were utilized for calibration.²²

Anthocyanin Assay

Anthocyanin assay was carried out via the standard procedure.²³ Anthocyanin was determined in 0.3% HCl in methanol at room temperature utilizing the extinction coefficient:

$$E_w = 33\,000 \text{ [cm}^2\text{/mol]}.$$

Estimation of Total Flavonoid Content

The aluminum chloride colorimetric technique²⁴ was applied to estimate the total flavonoid content. Gallic acid was utilized to produce the calibration curve. One hundred mg of fruit parts was rubbed in distilled water. The diluted solutions (0.5 mL) were then 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 incubation for 30 minutes at 25°C, the absorbance of the reaction mixture was measured at 415 nm.

Analysis of Saccharides

Saccharide content (soluble sugars and starch) was calculated by applying the phenol sulfuric acid method²⁵ and the absorbance was recorded at 485 nm.

Results

The total phenolic content of the fruit parts of *R. khorasanicum* was calculated from the calibration curve as 33.6 mg/g dry weight and the total flavonoid content was measured as 23.36 mg/g dry weight (Figure 2). Anthocyanin content was determined as 62.9 mg/g dry weight. The content of soluble sugar was 5.65 mg/g dry

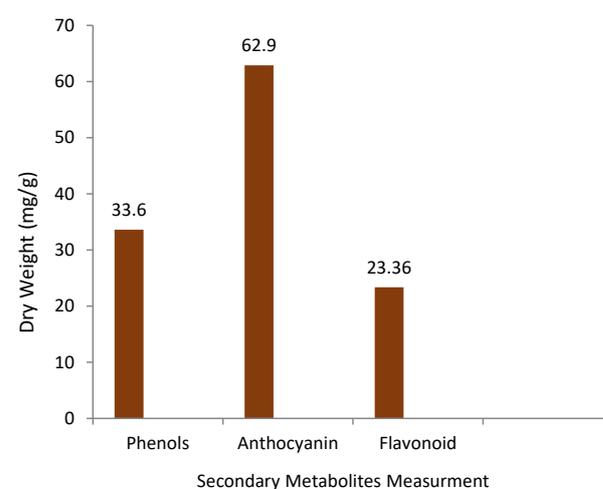


Figure 2. The Amount of Some of the Reducing Agents in *Ribes khorasanicum*.

Note: All of the data are in mg/g dry weight.

weight, while the amount of insoluble sugar was 4.60 mg/g dry weight. DPPH radical scavenging activity was observed to be 6.42 mg/g dry weight.

Discussion

Plants are prone to different kinds of ecological stresses like salinity, water deficit, excess temperature, and radiation, throughout their life cycle. Depending on the severity of stress, these particular environmental factors can limit their growth and productivity to varying degrees and lead to the production of ROS such as OH[•], O₂^{•-}, and H₂O₂. These species extensively damage the cells by peroxidation of lipids, proteins, and DNA. These ROS are removed by antioxidant molecules.

Phenolic compounds are the secondary metabolites²⁶ and play a vital role against pathogens.²⁷ Phenols have health benefits that are derived from ingesting high levels of fruits and vegetables.²⁸ The useful effects obtained from phenols have been attributed to their antioxidant properties.^{29,30} Flavonoids are the largest group of plant phenols.³¹ In this study, anthocyanin was the dominant constituent among other components of *R. khorasanicum*. Anthocyanin plays a critical role as a reducing agent. More than 600 different anthocyanins and their substituents have been reported in recent years.³² They play a vital role in the color quality of plants. Due to their color properties, extracts which have huge amount of anthocyanins are gradually more utilized in food industries as natural alternatives to artificial dyes.³³ Anthocyanins have proved to be non-toxic and water soluble, attracting excessive attention for their application as natural water resolvable pigments.³⁴ They possess antioxidant properties that are effective in preventing cancer and cardiovascular diseases.³⁵

In this study, the content of soluble sugar was more prominent than the amount of insoluble sugar in *R. khorasanicum*. Till now, the protective effects of soluble sugars against oxidative stress have mainly been assigned to the signaling effects resulting in elevation of various ROS scavengers^{36,37}; however despite this fact, it has been recently proposed that soluble sugars may themselves act as ROS scavengers, specifically at higher concentrations.³⁸ The role of sugars is not limited to only nourishing growth and developing; they also play a significant and regulatory role as signaling molecules.^{39,40} Soluble sugars are usually assumed to be associated with oxidative stress; on the other hand, endogenous sugars can create reducing power for glutathione (GSH) assisting in H₂O₂ reduction.⁴¹

This study is one of the first reports on the antioxidant activity and on bioactive components of *R. khorasanicum*, thus phytochemical analyses must be done to point out the active phenolic and flavonoid components. Plants that are rich in phenols, flavonoids, and anthocyanins contain antioxidant properties because of their redox properties and chemical structures. The DPPH radical is widely utilized in assessing free radical scavenging activity.

Superoxide is known as a reactive oxygen species that has the power to damage cells and DNA, resulting in various diseases.⁴² The high phenolic and flavonoid contents seem to be accountable for the bioactivity of these basic extracts. Flavonoids are very effective scavengers among the most oxidizing molecules.²⁷ Flavonoids can scavenge reactive species and up-regulate and promote antioxidant defenses.⁴³ Likewise, phenols have conferred oxidative stress tolerance in plants and have many benefits for health.

Conclusion

Our results indicated that *R. khorasanicum* can be considered as a potential source of anthocyanin, which could be applied as a natural antioxidant and preservative in food and medical industries. Phytochemical analysis is needed to isolate the compounds displaying a wide range of pharmacological properties in *R. khorasanicum*.

Ethical Approval

This research was ethically approved by Urmia University (code of ethics: 2A-438).

Competing Interests

The authors declare that they have no competing interests.

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