



Phytochemical Screening Antioxidant and Alpha-glucosidase Inhibitory Activities of *Fagopyrum tataricum* L. Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author MW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript.

Authors MU and BAC supervised this research work. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The objective of this work was the screening of different phytoconstituents and invitro antioxidant potential and α -glucosidase inhibitory activity of aerial parts of *Fagopyrum tataricum*.

Methods: Preliminary phytochemical screening was performed by using standard protocols. The antioxidant activity was determined by using spectroscopic method against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and α -glucosidase inhibition assay.

Results: The plant extract also shows potent antioxidant and α -glucosidase inhibitory potential against the tested methods.

Conclusion: From the results it seen that this plant exhibits significant antioxidant and α -glucosidase inhibitory potential.

Keywords: *Fagopyrum tataricum* L.; phytoconstituents; antioxidant; DPPH; α -glucosidase inhibition.

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1. INTRODUCTION

The over production of free radical in the human body for a long period of time may cause oxidative damage, eventually leading to chronic neurodegenerative disorders, diabetes mellitus [DM] and cancer [1]. Some biochemical and physiological reactions in the body and external environment is the cause of production of free radicals i.e. hydroxyl radicals, superoxide, peroxides, and singlet oxygen, as a byproduct [2]. The oxidative shielding acts as a defense mechanism by either decreasing chemicals in the body or the cellular uptake of toxic chemicals or to kill the cell by apoptosis and thus avoid the spreading to the neighboring cells [3]. Free radicals are major factor that are responsible for causing many chronic and neurodegenerative diseases like inflammation, coronary heart disease, stroke, cancer and DM [4]. Many synthetic antioxidants have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants [5]. Plants are the good source of biologically active compounds known as phytochemicals [6]. Because of their benefits on health, antioxidants are considered an important phytoconstituents. Vegetables, fruits, condiments and herbs contain a wide variety of free radical scavenging molecules such as phenolic compounds, nitrogen compounds, vitamins, and terpenoids, which have high antioxidant activities [7,8].

Formation of free radicals in the body are also the cause of diabetes mellitus (DM) [9]. DM is the most common endocrine disorder that affects 200 million people of whole world population [10]. The prevalence of DM will increase up to 366 million in 2030 [11]. The prevalence of DM has been increased under developed and middle-income countries due to different factors such as lack of physical activity and obesity due to dietary changes [12]. The indication of DM is hyperglycemia which is mainly due to lack of insulin action, insufficient insulin secretion or both [13]. The common mechanism for the treatment of DM is to retarding the conversion of polysaccharides to glucose by inhibiting α -glucosidase (carbohydrates-hydrolyzing enzyme) for absorption in the blood stream [14]. For the therapy of type II DM some synthetic α -glucosidase inhibitors, such as miglitol, voglibose and acarbose are clinically used now a day [15]. The efficacy of modern synthetic hypoglycemic drugs start to decline with long term use; because these synthetic agent causes gastro

intestinal tract side effects and these drugs are very costly [16,17]. The major gastro intestinal side effects due to the use of these synthetic α -glucosidase inhibitors are diarrhea, abdominal discomfort, bloating and flatulence [18].

Free radicals play a significant role in development of DM. It is suggested that oxidative stress is the most common pathogenic cause to insulin resistance, β -cell dysfunction, impaired glucose tolerance and ultimately leading to type II DM [19]. Oxidative stress produces type II DM. In diabetic patients, the proposed mechanism that increases the oxidative stress consist of compromised antioxidant defenses, formation of advanced glycated end products, glucose autooxidation and a change in the glutathione redox status [20]. For relieving type II DM consumption of food which is rich in antioxidant is help full.

F. tataricum commonly known as a buckwheat is an herbaceous plant that belongs to the family polygonaceae [21]. The genus *Fagopyrum* consists of 16 species, a few of which have been newly discovered and only 4 species are found in Pakistan [22]. *F. tataricum* tea was shown to protect from leg oedema in patients with chronic venous insufficiency [23]. The use of *F. tataricum* herb is recommended for the prophylaxis and treatment of diabetic patients suffering from retinopathy [24]. *F. tataricum* flour is gluten free and is thus an important ingredient in diets or food products for people suffering from coeliac disease [25].

The evaluation of antioxidant and α -glucosidase inhibitory activities may be used for preliminary observations on pharmacological activities because natural compounds from plants that are considered to be safe have therapeutic effects and fewer health side effects than synthetic medicines [26]. The present study was designed to investigate the antioxidant and α -glucosidase inhibitory activities of aerial parts of *F. tataricum*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The plant was collected from the surrounding of Bahauddin Zakariya University, Multan in the month of August and was identified by Prof. Dr. Altaf Ahmad Dasti, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

2.2 Antioxidant Activity: DPPH (1, 1, Diphenyl, 2-Picrylhydrazyl) Method

In 96-well plates 10 μ l sample of *F. tataricum* was added after then 90 μ l of 100 μ M methanolic DPPH solution was added as well. The resultant 100 μ L solution was mixed and incubated at 37°C for 30 min. Quercetin was used as a standard antioxidant. The absorbance decrease was measured at 517 nm using a Synergy HT Bio Tek® USA micro plate reader. The DPPH inhibition (%) was calculated as following.

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 and A_1 are control and sample absorbance respectively. IC_{50} shows the level at which 50% of the radicals were scavenged.

2.3 Alpha-glucosidase Inhibition Assay

Fagopyrum tataricum sample (10 μ L) was dissolved in 70 μ L of buffer phosphate (50 mM, pH 6.8) and 10 μ L of enzyme solution. The sample was mixed and pre-incubated at 37°C for 10 minutes and pre-read at 400 nm. Reaction was started by the addition of 10 μ L of substrate. Mixture were incubated at 37°C for 30 minutes. A control 0.5 μ M Acarbose were used after the incubation. Absorbance was measured at 400nm by using a 96 well plate reader.

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{(A_0)} \times 100$$

A_0 and A_1 are control and sample absorbance respectively.

2.4 Statically Analysis

Experiments were performed in triplicate. The results are expressed as mean \pm SE. The concentration response curve was obtained by

plotting inhibition percentages versus concentrations. IC_{50} value (half-maximal inhibitory concentration) of the sample was obtained graphically from an inhibition curve. Statistical calculations were carried out using IBM SPSS Statistics.

3. RESULTS

To evaluate this medicinal potential of *F. tataricum* extract was screened by using standards methods of in-vitro biological assays. These include, antioxidant and α -glucosidase inhibition assay. *F. tataricum* exhibited significant antioxidant activity. The % inhibition found to be 87.33 \pm 1.12 and its IC_{50} value was 159.51 \pm 1.29. Quercetin was used as a standard to check the antioxidant activity of *F. tataricum*. Methanol extract of *F. tataricum* also showed α -glucosidase inhibition activity. At concentration of 0.5 mg/mL % inhibition of α -glucosidase was 63.88 \pm 1.35 and its IC_{50} value was 391.55 \pm 1.44. Acarbose was used as a standard drug.

4. DISCUSSION

Free radicals are formed in our body due to different endogenous and exogenous factors which are responsible for different life threatening diseases like cardiovascular disease, cancer and DM. Antioxidants neutralize the free radicals that are responsible for cell damage. Natural antioxidants from plant sources are safer to health and have better potential than synthetic antioxidants [27]. Many plants have been reported to have free radical scavenging/antioxidants potential. At concentration of 100 μ g/mL methanol extract of *Ecbolium viride* (Forssk) have reducing power by DPPH 78.25 \pm 0.004 by using Quercetin as a standard [28].

Table 1. Antioxidant and alpha glucosidase inhibitory activities of methanol extract of aerial part of *F. tataricum*

Treatments	Antioxidants concentration (mg-mL ⁻¹)	Inhibition at 0.5 mg-mL ⁻¹ (%)	IC_{50} (μ g-mL ⁻¹)
<i>F. tataricum</i>	0.5	87.33 \pm 1.12	159.51 \pm 1.29*
Quercetin-control	0.5	91.50 \pm 0.91	16.96 \pm 0.14
<i>F. tataricum</i>	0.5	63.88 \pm 1.35	391.55 \pm 1.44*
Acarbose- control	0.5	92.23 \pm 0.14	38.25 \pm 0.12

One way anova test was applied and value of $p < 0.05$ was considered as significant and shown by*

Diabetes is one of the world's greatest health problems, affecting about 171 million people and most of these will be dominated by those suffering from type II DM [29]. One of the strategies for the treatment of DM is the inhibition of carbohydrates digesting enzyme such as α -glucosidase in the gastrointestinal glucose absorption thereby lowering postprandial glucose level [30]. α -glucosidase inhibitors delay the breakdown of carbohydrate in the small intestine and diminish the post-prandial blood glucose excursion [31]. Plant phenolics are natural α -glucosidase inhibitors because they inhibit intestinal carbohydrate digesting enzymes owing to their protein-binding capability [32].

Many plants have been reported to have antidiabetic activity and being used in ayurveda for the treatment of DM [33]. *Phoenix dactylifera* and *Capparis spinosa* plants extracts have shown α -glucosidase inhibition of 55.02 (%) and 53.88 (%) respectively when they was given at 25 mg-mL⁻¹ concentration [34]. While the % inhibition of *F. tataricum* extract was 63.88±1.35 and its IC₅₀ value 391.55±1.44. The results shows that *F. tataricum* extract is the one of most potent α -glucosidase inhibitor than the above mentioned plant extracts. So it causes delay in digestion, which would in turn decreases in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation.

Methanol extract of *F. tataricum* possess antioxidant and alpha glucosidase inhibitory activities. Plants which scavenge the free radicals and inhibit the α -glucosidase enzyme have antioxidant and anti-diabetic potential, which is directly related to the total amount of polyphenols and flavonols [35]. It is concluded that methanol extract of *F. tataricum* have scavenge the free radicals and inhibit the α -glucosidase enzyme, that might be due to polyphenols. Further studies on active constituents isolation along with in vivo animals study is needed to be investigated in detail to explore its pharmaceutical/neutraceutical use.

5. CONCLUSION

The results of this in-vitro study clearly indicated that methanolic extract of aerial part of *F. tataricum* have antioxidant and α -glucosidase inhibitory activity. These attributes when combined in one plant would be potentially useful to manage the DM and oxidative stress. The results suggested that phenolic compounds are the major contributor to the antioxidant and

α -glucosidase inhibitory capabilities of this plant. Consequently, this plant could be suggested as a potential natural source of antioxidant and antidiabetic compounds for the prevention or the treatment of diabetes and its complications. Further studies on active constituents isolation along with in vivo animals study is needed to be investigated in detail to explore its pharmaceutical/neutraceutical use.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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