

Antioxidation and Antiglycation Properties of *Bunium Bulbocastanum* Fruits Various Fractions and its Possible Role in Reducing Diabetes Complication and Ageing

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Abstract

Over the centuries medicinal plants have benefited mankind against different diseases. Advanced Glycation End-products (AGE's) formation due to non-enzymatic glycation and oxidative stress has been demonstrated in the pathogenesis of diabetic complications and aging processes. In this study we investigated the antiglycation and antioxidation potential of different fraction *Bunium bulbocastanum* fruits. Our results indicated that the chloroform fraction of *B. bulbocastanum* fruits had more antiglycation ability with a Minimum inhibitory concentration (MIC₅₀) of 132.88 µg/ml as compared to the MIC₅₀ of 189.92, 199.08, 202.50 and 261.41 µg/ml of aqueous, crude methanolic, ethyl acetate and *n*-hexane fractions, respectively. While the results of antioxidation assay showed that Ethyl acetate has more antioxidation potential than the standard (ascorbic acid). Ethyl acetate, aqueous, crude methanolic, *n*-hexane and chloroform fractions had 31.24, 26.07, 20.67, 15.51 and 5.72% antioxidation potential at 0.5 mg/ml respectively.

Keywords: AGE's; Antiglycation; Antioxidation; *Bunium bulbocastanum*

Introduction

Generation of free radicals inside human body takes place from both endogenous and exogenous sources [1]. Endogenous include oxidative enzymes and respiratory chain while exogenous include smoking and other air pollutants [1]. These sources create overproduction of free radicals which ultimately potentiate development of various diseases because of oxidative stress [1-3]. Some of the destructive effects of oxidative stress include damaged proteins, nucleic acid and lipids which lead to conditions like diabetes, Alzheimer's, cancer, cardiovascular diseases [1-3].

This oxidative stress is also an instrumental factor in glycation process [4]. Glycation is basically a reaction of amino groups in amino acid of proteins and carbonyl group in sugar resulting in formation of Amadori products [5-7]. These intermediate Amadori products then through a series of reactions; including oxidation reactions, ultimately forms advanced glycation end products (AGE's) [5-7]. These AGE's are very dangerous to our health and cause most of diabetic mellitus complications and result in speeding up of ageing process [7-10]. From pathogenesis point of view AGE's accumulation is hazardous to our body cells and results in acceleration of aging and diabetic complications including retinopathy, cataract, neuropathy and atherosclerosis [11]. Hence any source that has both antioxidation and antiglycation capabilities can be very helpful against such disease conditions.

B. bulbocastanum is found primarily in northern India, Kashmir, Afghanistan, Tajikistan and Persia [12]. *B. bulbocastanum* has been used for edible purposes for years. Its seeds can be used either in raw or cooked form to enhance food flavors or to improve its taste [12]. Medicinally it is also used as an astringent [12]. Keeping in view the novelty of *B. bulbocastanum*, the current study was aimed to screen the fruits of *B. bulbocastanum* for possible antioxidation and antiglycation potential.

Materials and Methods

Plant material

Fruits of *B. bulbocastanum* plants were collected from Kashmir

and kindly identified by Prof. Dr. Abdur- Rasheed, Plant taxonomist, Department of Botany, University of Peshawar, Khyber Pukhtunkhwa, Pakistan.

Extraction

The *B. bulbocastanum* fruits were kept in shade for drying and were then chopped and grounded to powder. The powdered materials were soaked in methanol (twice) for 15 days at room temperature. Each time the filtrate was filtered and the filtrates were combined and concentrated to crude methanolic extracts using rotatory evaporator at 40°C.

Fractionation

The crude methanolic extract of *B. bulbocastanum* fruits (137 g) was suspended in distilled water (350 ml). It was further partitioned with *n*-hexane (3×500 ml), chloroform (3×500 ml) and ethyl acetate (3×500 ml), respectively, to yield the *n*- hexane (44 g), chloroform (29 g), ethyl acetate (15 g) and aqueous (36 g) fractions. 20 g of the crude methanolic extract of was left for biological/pharmacological activities. All the fractions will only contain particular compounds based on the solubility of these compounds from the crude extract. For instance, the *n*- hexane fraction will contain only those compounds which are non-polar and soluble in non-polar solvents and so on.

Materials

The materials used for *in vitro* antiglycation assay were: Bovine Serum Albumin (BSA), D-glucose, Sodium dihydrogen phosphite (Na₂HPO₄), Potassium dihydrogen phosphite (KH₂PO₄), Sodium

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Chloride (NaCl), Potassium Chloride (KCl), Aminoguanidine (Merck, Pakistan), Retinoid (Merck, Pakistan) and Tri-Chloro Acetic Acid (TCA) (Sigma, Pakistan). While alkaline PBS (137 nM NaCl, 8.1 nM Na₂HPO₄, 2.68 mM KCl, 1.47 mM KH₂PO₄) was prepared and its pH adjusted to 7.4 with 0.25 N NaOH.

The materials used for Nitric Oxide (NO) free radical scavenging assay were: Sodium Nitroprusside (Na₂[Fe(CN)₅NO]·2H₂O) (SNP), Sulphanilic acid (SA) (0.33% in 20% Acetic acid), [N-(1-Naphthyl) Ethylene diamine Dihydrochloride] (NED) (0.1% in H₂O), Phosphate buffer (10 mM, pH=7.4), UV double beam spectrophotometer, micropipettes, test samples (crude methanolic extract), Methanol (as blank) and Ascorbic Acid (Vitamin-C) as positive control.

In vitro glycation assay

The method of Matsuura et al. [13] was followed with little modifications. Stock solutions of the plant extracts were prepared by dissolving 3 mg in 1ml of alkaline PBS. From the stock solution 10, 50, 90, and 130 µL solutions were taken using micropipette and were mixed with a solution containing 400 µg BSA and 200 mM glucose. These reaction mixtures were kept in a water bath at 55°C for 48 hours. BSA and glucose without any inhibitor was used as control. After the incubation time the reaction mixture was transferred into separate Eppendorf tubes and 10 µL of 100% w/v TCA was added and centrifuged at 14500 rpm at 4°C for 4 minutes. Supernatant then discarded and the pellet was re-dissolved in 400 µL alkaline PBS.

Using fully automated UV double beam spectrophotometer, the degree of absorbance for both the control and the test reaction mixtures were taken at 350 nm. Percent inhibition was calculated using the following formula:

$$\text{Percent inhibition} = [1 - (A_s - A_o) / (A_b - A_o)] \times 100$$

Where A_s is absorbance of test samples, A_b is absorbance of reaction mixture without plant extract and A_o is absorbance of blank control.

Nitric Oxide (NO) free radical scavenging assay

To perform NO free radical scavenging assay the method of Ebrahimzadeh et al. [14] was followed. Stock solutions of test samples were prepared by dissolving 3mg of the test samples in 1 ml of methanol. Different dilutions i.e. 0.5, 0.25 and 0.125 mg/ml of test sample were made from the stock solution and 1ml of each dilution was introduced into separate test tubes along with 1ml of SNP to make the reaction mixture. This mixture was then incubated for 90 minutes at 27°C. After incubation 0.5ml of the reaction mixture was added to 1ml of SA and incubated at 27°C for 5 minutes. Add 1ml of NED to it and again incubate for 30 minutes at 27°C.

Results were obtained by taking absorbance at 546 nm. Methanol and Vitamin C were used as blank and positive control respectively. Percentage antioxidation potential was calculated by the following formula:

$$\text{Percent inhibition} = [A_o - A_t / A_o] \times 100$$

Where A_o is absorbance of control; reaction mixture without extract, A_t absorbance of test samples.

Results

Antiglycation assay

The UV double beam spectrophotometric analyses of the reaction and test mixtures of *B. bulbocastanum* fruits are shown in the Figure 1. It can be seen from the figure that as the concentration of the *B.*

bulbocastanum fruits extract increases from 10 µg/mL to 390 µg/mL the absorbance decrease indicating an increase in antiglycation ability of that fraction. However the relatively slow decrease from 30 µg/mL onwards is attributed to the effect of interfering color agents present in *B. bulbocastanum* fruits fractions.

The minimum inhibitory concentrations (MIC₅₀) of aminoguanidine and *B. bulbocastanum* fruit fractions were also calculated. We found that the MIC₅₀ of aminoguanidine, crude methanolic, *n*-hexane, chloroform, ethyl acetate and aqueous extracts were 70.14, 199.08, 261.41, 132.88, 202.50, 189.92 µg/ml, respectively. These values can also be inferred from Figure 1. Figure 2 shows the percent inhibition of Millard reaction by aminoguanidine and *B. bulbocastanum* fruit fractions verses the amount of test sample used i.e. Sample concentration. Hence from the MIC₅₀ and both Figures 1 and 2 it is confirmed that the chloroform fraction had most appreciable antiglycation ability with MIC₅₀ of 132.88 µg/ml while the *n*-hexane had least antiglycation potential with MIC₅₀ of 261.41 µg/ml.

NO free radical scavenging Assay

The antioxidation potential of *B. bulbocastanum* fruits fractions at different concentrations was compared with ascorbic acid (Vitamin-C) as standard. Results obtained from NO scavenging assay are shown in Table 1. It can be seen from the table that among the different fractions of *B. bulbocastanum* ethyl acetate fraction had more potential of antioxidation and even exceeds the antioxidation potential (31.24%)

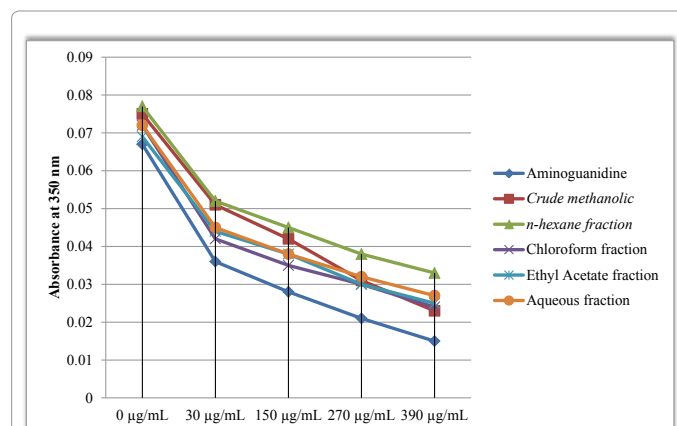


Figure 1: Antiglycation properties of *B. bulbocastanum* fruits fractions at different concentrations.

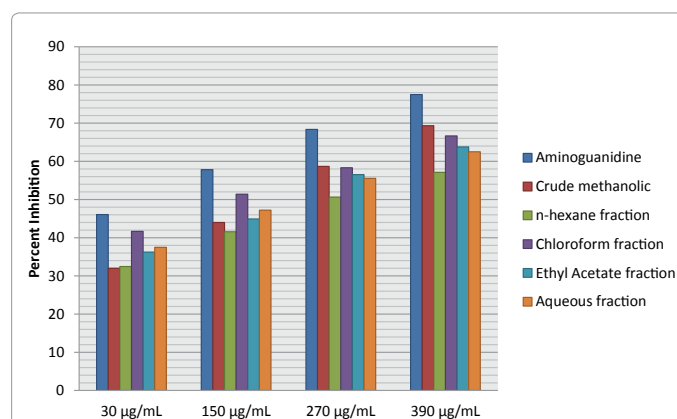


Figure 2: Percentage inhibition of *B. bulbocastanum* fruits fractions at different concentrations.

Test Sample	Percent antioxidation activity		
	0.5mg/ml	0.25mg/ml	0.125mg/ml
Ascorbic Acid	26.50	26.28	24.34
Crude methanolic extract	20.67	17.38	11.84
<i>n</i> -hexane fraction	15.51	14.92	8.32
Chloroform fraction	5.72	5.24	4.58
Ethyl acetate fraction	31.24	24.89	21.66
Aqueous fraction	26.07	16.06	12.98

Table 1: Percent antioxidation activity of *B. bulbocastanum* fruits fractions at 0.5, 0.25 and 0.125 mg/ml concentration.

of the standard (26.50) at 0.5 mg/ml concentration. Aqueous fraction also showed significant antioxidation potential of 26.07% at 0.5 mg/ml concentration. However the rest of the three fractions had lower antioxidation activity from the standard at all the concentration.

Discussion

As far as we had investigated there have been no antiglycation and antioxidation studies on *B. bulbocastanum* fruits. This study is the first one that reports that its fractions do have antiglycation and antioxidation potentials. Our findings for the antiglycation *in vitro* assay showed that among the test samples chloroform fraction was the most effective antiglycation agent with MIC₅₀ of 132.88 µg/ml followed. We also found that the percent inhibition of glycation is concentration dependent.

Our result for the NO free radical scavenging assay shows that as the concentration of test samples increases the percent antioxidation potential increases. Therefore indicating concentration dependence of antioxidation ability in both the standard and test samples. In our study we found that all fractions of *B. bulbocastanum* had antioxidation potential. The percent antioxidation inhibition of 31.24 and 26.07% at 0.5 mg/ml for ethyl acetate fraction and aqueous fraction presents an encouraging finding. However in particular the ethyl acetate fraction antioxidation ability of 31.24% as compared to the standard (Ascorbic acid) value of 26.50% represents its possible therapeutic use as antioxidant.

Chloroform fraction of *B. bulbocastanum* fruits could be used as antiglycation agent in therapeutics. While the ethyl acetate and aqueous fraction of *B. bulbocastanum* fruits can be used as therapeutic agents against harmful oxidation reactions. Therefore we conclude

that further studies on *B. bulbocastanum* fruits extracts could lead to therapeutics for reducing diabetic complications and process of ageing.

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