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Antioxidant, α-amylase inhibitory and antiglycation activity of *Berberis royleana* roots

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ABSTRACT

In the current research work, the plant roots were investigated for their antioxidant, α-amylase inhibitory and antiglycation activities. The results obtained revealed that the maximum antioxidant activity 1.984% was exhibited by an aqueous fraction at a concentration of 1mg/ml. The maximum α-amylase inhibition was exhibited by ethyl acetate 1.864% and n-hexane fraction 1.483% at a concentration of 1mg/ml while the chloroform and aqueous fraction exhibits minimum inhibition 0.075% and 0.073% at 0.613mg/ml concentration. The chloroform fraction exhibited maximum antiglycation potential of 12% at 0.5µg/ml while the minimum potential was 5.5% exhibited by ethyl acetate fraction at a concentration of 1µg/ml. The results obtained revealed the medicinal importance of the plant and will help the researchers to exploit the phytochemicals for antioxidant, α-amylase inhibitory and antiglycation activities.

Keywords: *Berberis royleana*, *antioxidant, α-amylase, antiglycation.*

1. INTRODUCTION

The genus *Berberis* belongs to the family *Berberidaceae* is comprised of approximately 500 species distributed in Siberia, Nepal, Afghanistan, China, India, Europe, North and South America [1]. The *Berberis* species being comprised of alkaloids are very important from the pharmacological point of view. Some species of this genus are comprised of important constituent and are used in herbal medication systems such as in Unani, Ayurvedic, Eastern and modern system of medicines [2-3]. It has been shown by various studies that the aqueous and ethanolic extracts of roots of *B. aristata* has greater antioxidant potential and are helpful in decreasing oxidative stress [4]. In addition, antioxidant status in CCl4- induced liver injury is notably improved by aqueous and methanolic extracts of the aerial parts of *B. Aristata* [5]. The fruits of *B. lycium* have also been studied in order to check its antioxidant properties, with respect to DPPH radical scavenging potential of its photochemical which included compounds 4, 4 dimethyl hexadeca 3-ol, berberine, β-sitosterol, 3-[4-(6- methyl butyl) phenyl] propan-1-ol and butyl -3-hydroxypropyl phthalate [6].

The root bark extracts of *B. lycium* were synthesized and screened in various solvents including water, aqueous methanolic, methanolic, chloroform and n-hexane for their anti-diabetic activities in alloxanized rabbits. Amongst the above-mentioned extracts, it was seen that water extract (500 mg/kg) showed maximum hypoglycemic activity when administered orally, for almost 6 h. Blood glucose levels were also reduced for 4 h with the help of similar doses of aqueous methanol, methanol, and n-hexane extract. The chloroform extract did not show any significant anti-diabetic activity [7-8]. In normal and alloxanized rats, ethanolic and aqueous extracts of the roots of the plant were administered and approximately 20 mg/kg dose of glibenclamide was used as a control drug. Water extract was further checked and compared in combination with insulin. The results reveal that after 3 to 5 h of administration, 50 and 100 mg/kg doses decreased hyperglycemia level. Oral glucose tolerance tests expressed that serum's glucose decreased in a dose-dependent manner by plant extracts [8]. The mechanism followed in the hypoglycemic effects may involve insulin-like effects, possibly through increased peripheral glucose consumption [9]. Ethanolic root extract of *B.lycium* was checked and compared with that of pure berberine in order to examine the anti-diabetic activity of normal and alloxan-induced diabetic rats using similar doses (50 mg/kg) of each. Both treatments reduced blood glucose levels significantly and demonstrated significant effects on glycosylated hemoglobin, glucose tolerance, serum lipid profiles and animal body weights. *B. brevissima* and *B. parkeriana* are also having anti-diabetic activities [10]. *B. royleana* is rare species amongst the members of *Berberis (Berberidaceae)*. This specie is still imperfectly known and the flower is not specified. It differs from other species of the genus by its smaller leaves, narrow fruits, inflorescence and pedicels [11].

B. royleana is a deciduous plant with height up to 3 m. The roots are thick and broaden easily. The stem has red-brown color and has spines. Leaves are usually 7-15 mm long, 6-12 mm broad. The ripe fruit is ovoid with red color and about 1 cm in length. The fruits developed in clusters form are bitter to taste. Berries are somewhat black, pruinose grey, oblong, 8 mm long and 3.5 mm broad (immature) [11]. *B. royleana* roots have antibacterial, antifungal [12], and insecticidal activity against insect *Kelosobrocus meleticulatis* [12]. *B. lycium* have a significant ability to combat with the HCV virus and reduce/stop the growth of the virus [13]. The hypoglycemic activity of the extracts of *B. aristata* is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting

glucose transport across the cell membrane as revealed by simple in vitro model of yeast cells [14]. *Berberis aristata* DC have been studied for anti-inflammatory, antidiabetic, anti-granuloma activity [15,16]. *B. lycium* is rich in flavonoids, alkaloids, tannins, carbohydrates [17]. The extract of *Berberis orthobotrys* exhibits anti-tumor effects on different types of breast as well as bone cancer cells in vitro [18]. *Berberis vulgaris* and berberine, its main component, traditionally have been used for treatment of various disorders [19]. The ethanol extract of Moroccan *Berberis vulgaris* shows cytotoxic effect against the MCF-7 tumour cell line [20]. The methanolic extracts and aqueous solutions of the bark of the roots of *Berberis vulgaris* shows antioxidant activity and antidiabetic activity [21]. *B. vulgaris* showed positive effects on contrast media-induced nephrotoxicity [22]. Keeping in view the above mentioned pharmacological importance of *genus Berberis*, its species *B. royleana* was selected to investigate its antioxidant, α-amylase inhibitory and antiglycation activities to further explore its hidden medicinal potential.

2. EXPERIMENTAL SECTION

2.1. Plant material. *B. royleana* roots were collected from Trarkhal in July 2017. A voucher specimen number BRZ-31 of the plant sample was kept as a record in the Department of Botany, Abdul Wali Khan University, Pakistan.

2.2. Extraction and fractionation. The plant *B. royleana* roots (4 kg) were shade dried for two months. The dried roots were chopped, crushed and powdered. The powdered material (4kg) was soaked in methanol, with occasional stirring at room temperature for one week. The filtrate was concentrated through the use of rotary evaporator at 45 OC. The crude extract obtained (1 kg) of *B. royleana* was suspended in a minimum amount of water and fractionated with n-hexane thrice which afforded n-hexane fraction weighed (180g). The remaining water-soluble part was further fractionated with an adequate amount of chloroform which afforded chloroform fraction weighed (200g). The remaining aqueous soluble part was partitioned with ethyl acetate three times which resulted into ethyl acetate fraction weighed (350g) and aqueous fraction (190 g). These crude fractions were further tested for biological activities.

2.3. Antioxidant assay. The antioxidant potential was determined according to the thiocyanate method [23]. Each sample in 0.5ml methanol was combined with 5ml DMSO suspension (2.5mL, 0.02M, pH 7.0) and phosphate buffer (2 mL, 0. 2M, pH 7.0) in a test tube and located in dark at 37 OC to accelerate oxidation. The peroxide value was obtained by noting the absorbance at 750 nm with a spectrophotometer (Hitachi U-2000) after coloring with FeCl3 and thiocyanate at intervals during incubation.

2.4. α-Amylase inhibitory assay. Quantified amount of reducing sugar (maltose equivalent), which is liberated during the assay conditions was used for the estimation of α-amylase inhibition activity. The amount of maltose liberated during the reaction in units was used to represent the enzyme inhibition activity. A modified Dinitrosalicylic acid (DNS) procedure was used for the determination of maltose equivalent [24]. The experiment was initiated by pre-incubation of 1ml of each fraction of *B. royleana* for 30 min with α-amylase 1U/ml and then 1 ml (1% w/v) of starch solution was added. After that mixture was further incubated at 37°C for 10 min. At the end, 1 ml of DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) was added in the mixture and then stop the reaction. Meanwhile, contents were heated in a boiling water bath for 5 min. Two further tests were made, one blank test which was prepared without plant sample and the second which was prepared without enzyme amylase, being replaced by equivalent quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, at pH 6.9 and 20oC). The calculated absorbance was 540 nm. With the help of a standard graph, maltose equivalent was determined by the amount of reducing sugar which was released from starch during the course of the reaction. Acarbose acts as a positive control. To obtain the final concentration of 0.0625, 0.5, 0.25, 0.125 and 1mg/ml, the different crude fractions of *B. royleana* roots were diluted in buffer. The antidiabetic activity was determined by the inhibition of α-amylase enzyme which was expressed as a percent inhibition and calculated by the following formula.

$$\% \text{ reaction} = (\text{maltose}) \text{ test} / (\text{maltose}) \text{ control} \times 100$$

$$\% \text{ inhibition} = 100\% \text{ reaction}$$

2.5. Antiglycation assay. 60 µl of each fraction was prepared by mixing with DMSO and the mixture of the sample (20 µl BSA + 20 µl of glucose anhydrous and test sample 20 µl). The glycated control contained 20 µl glucose, 20µl sodium phosphate buffer and 20 µl BSA, while the blank control contained 20 µl BSA and 40 µl sodium phosphate buffer. The test samples were placed in incubator using 96 well plates for approximately 7 days at 37°C and then removed from incubator and cooled out at room temperature. After incubation 60 µl of 100% TCA was putted into each well followed by centrifugation (15000 rpm) for 4 min at 4°C. After centrifugation and agitation performed at 14000 rpm for 4 min, the supernatant was removed which was contained on glucose, inhibitor, interfering substance and AGE-BSA pellet that was dissolved in PBS. In this bioassay of AGEs Spectrofluorimeter RF-1500 (Shimadzu, Japan) was used to monitor the assessment of fluorescence spectrum (ex. 370 nm) and change in fluorescence intensity (ex. 370 to 440 nm). The standard inhibitor used in this activity was Rutin. With the help of spectrofluorimeter, the fluorescence intensity at 370 nm excitations and emission at 440 nm was compared with each other. Percentage inhibition was calculated using the following formula.

$$\text{Inhibition (\%)} = (100) - [\text{OD (test)} / \text{OD (blank)}] \times 100$$

3. RESULTS SECTION

3.1. Antioxidant activity. The effects of different crude extract fractions of *B. royleana* roots on the per-oxidation of DMSO are shown in **(Figure 1)**. The oxidative activity of DMSO was inhibited by the crude extract fractions of *B. royleana* roots compare with the control assay. Among the extract fractions, the maximum antioxidant activity was examined in an aqueous fraction which was 1.984% while the minimum antioxidant activity was observed in chloroform fraction which was 0.998% at a concentration of 1mg/ml. This reveals the presence of poly-phenols which are the most plentiful group of compounds in the crude extract fractions of *B. royleana* roots and seem to be responsible for the antioxidant potential.

3.2. α-Amylase inhibitory activity. In this bioassay various concentrations viz., 1, 0.5, 0.25, 0.125 and 0.0625 mg/ml of the crude fractions of *B. royleana* roots were tested for the inhibition of α-amylase activity **(Table 1)**. The results obtained revealed that ethyl acetate and n-hexane fraction shows maximum inhibition which is 1.864 and 1.483 respectively with 1mg/ml concentration while chloroform and aqueous fraction exhibits minimum inhibition of 0.075 and 0.073 respectively with 0.0625mg/ml concentrations. From the results, it can be deduced that *B. royleana* root has greater potential for the reduction of digestion-rate and carbohydrates absorption and thereby play an important role for the efficient management of diabetes.

3.3. Antiglycation activity. In this bioassay the crude fractions of *B. royleana* roots were tested for antiglycation activity based on serial dilution method with concentration ranges from 1µg/ml to 0.0078125 µg/ml **(Figure 2)**. The results obtained revealed that n-hexane fraction showed maximum anti-glycation activity 11.5% while the ethyl acetate fraction showed minimum anti-glycation activity 5.5% at a concentration of 1µg/ml. The chloroform and ethyl acetate fraction exhibit 12% and 7.5% anti-glycation activity at the concentration of 0.5µg/ml while at concentration of 0.25µg/ml the chloroform and ethyl acetate exhibit 9.5% and 8% anti-glycation activity. Similarly, at concentration 0.125µg/ml maximum anti-glycation activity was observed in ethyl acetate fraction which found to be 11% while minimum anti-glycation activity was 7% given by aqueous fraction. On the other hand, at a concentration of 0.0625µg/ml the maximum anti-glycation activity was revealed by ethyl acetate and chloroform fraction which was 9.5% while minimum anti-glycation activity was 6.5% showed by n-hexane. At concentration 0.03125µg/ml maximum anti-glycation activity was shown by ethyl acetate fraction which was 10.5% while minimum anti-glycation activity was 7% shown by an aqueous fraction. In addition to it at a concentration of 0.015625µg/ml maximum anti-glycation potential was shown by ethyl acetate fraction which was observed to be 10% while minimum anti-glycation activity was 6% observed by n-hexane. Next, to the concentration of 0.0078125µg/ml maximum anti-glycation activity was shown by ethyl acetate fraction which was 9.5% while minimum anti-glycation activity was 6.5% shown by n-hexane. Overall maximum anti-glycation activity was exhibited by chloroform fraction which was 12% at 0.5µg/ml while minimum anti-glycation activity was 5.5% exhibited by ethyl acetate fraction at a concentration of 1µg/ml.

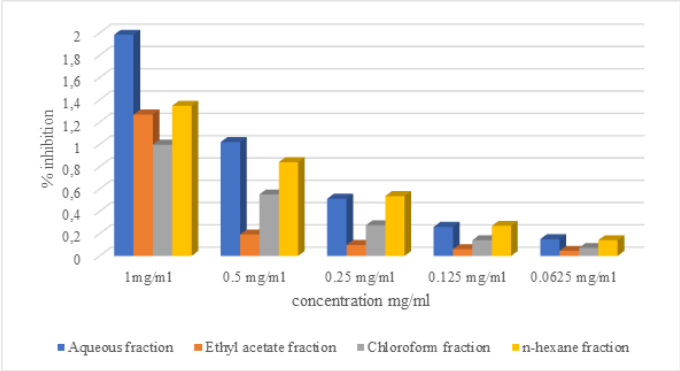

Figure 1. Antioxidant activity of crude fractions of *B. royleana* roots.

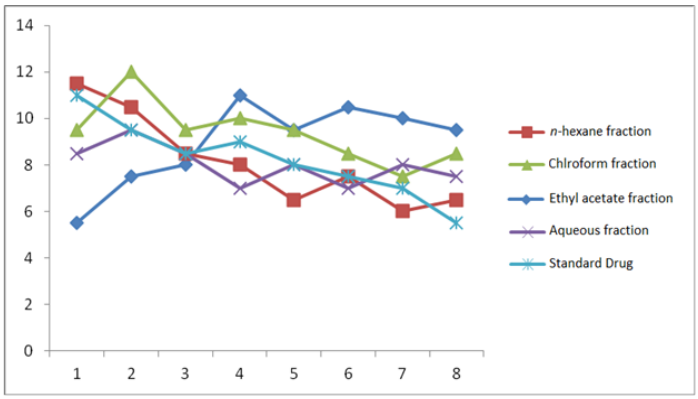

Figure 2. Antiglycation activity of the crude fractions of *B. royleana* roots.

Table 1. α-Amylase inhibitory activity of the crude fractions of *B. royleana* roots.

Sample	% Inhibition				
	1mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
***n*-hexane fraction**	**1.483**	**0.852**	**0.531**	**0.311**	**0.151**
Chloroform fraction	**0.931**	**0.452**	**0.257**	**0.152**	**0.075**
Ethyl acetate fraction	**1.864**	**1.052**	**0.831**	**0.591**	**0.252**
Aqueous fraction	**0.731**	**0.527**	**0.279**	**0.134**	**0.073**

4. CONCLUSIONS

In the current study, the medicinal plant *B. royleana* was investigated for antioxidant, α-amylase and antiglycation activity. The biological investigation of the crude extract fractions of the selected plant showed significant activity. The maximum antioxidant activity was observed in aqueous fraction which was 1.984% at a concentration of 1mg/ml. The ethyl acetate and n-hexane fraction showed maximum α-amylase inhibition which was 1.864% and 1.483% respectively at a concentration of 1mg/ml while the chloroform and aqueous fraction exhibits minimum inhibition 0.075% and 0.073% respectively at 0.613mg/ml

concentration. Similarly, the maximum antiglycation activity was exhibited by chloroform fraction which was 12% at 0.5µg/ml while minimum activity was 5.5% exhibited by ethyl acetate fraction at a concentration of 1µg/ml. The results obtained exhibit that this plant is very important from the medicinal point of view, and it needs further phytochemical exploitation to isolate phytochemical constituents having antioxidant, α-amylase inhibitory and anti-glycation activities.

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