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Research Article

Evaluation of α-glucosidase inhibition and antioxidant potential of *Acacia modesta* root bark

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ABSTRACT

The α-glucosidase inhibition and antioxidant potential of dichloromethane and methanolic extracts of *Acacia modesta* root bark were evaluated to provide basis for isolation and structure elucidation of bioactive compounds. In α-glucosidase inhibition assay, dichloromethane and methanolic extracts exhibited inhibitory activity of 98.9 % and 99.9 % with IC_{50} of 6.8 µg/ml and 1.2 µg/ml respectively. The results were compared with standard, acarbose, which showed 59.1 % inhibition with IC_{50} of 540 µg/ml. While antioxidant assay results indicated that both dichloromethane and methanolicextractswere active with percentage radical scavenging activities of 76 % and 88 % respectively. IC_{50} values were also calculated which were 283.33 µg/mlfor dichloromethane extract and 236.49 µg/ml for methanolic extract. Ascorbic acid was used as standard. These results confirm that *Acacia modesta* root bark extracts possess α-glucosidase inhibitors and antioxidants, thereby providing worthy justification for isolation of novel bioactive compounds.

Keywords: *Acacia modesta*, α-glucosidase inhibition, antioxidant, dichloromethane, methanolic

INTRODUCTION:

Since antiquity to date, plants have been providing medicines to humans for the treatment of various ailments [1, 2].In the beginning, these plant derived drugs were available in crude form such as tinctures, poultices, teas, powders and other home remedies [3].According to World Health Organization, nearly 80 % of the world's population rely on plant products for their basic health care needs [4]. Until today, people are interested in herbal remedies for such infections in which existing medications are less effective.

Acacia modesta belongs to family Fabaceae (subfamily Mimosaceae). Commonly, it is known as phulai and locally called palosa. It is a medium or small size tree that grows in dry areas of Pakistan, India and Afghanistan. In Pakistan, it is found in Balochistan, Khyber Pakhtunkhwa and Punjab [5]. Different parts of the plant such as bark, leaves, roots, flowers and gum are utilized for various medicinal purposes. The plant is used to treat dysentery, wounds, leprosy, trachoma and venereal diseases [6-9]. Branches of *Acaciamodesta* are commonly used as miswak (tooth brush). Traditionally, ash of its wood was applied in severe pain. While a solution of gum, called Zhublesharbat, was taken as health tonic. Different parts of the plant were also used for back pain, sex and cough [10-14].

In view of traditional importance of the plant, current study was undertaken to investigate the α-glucosidase inhibition and antioxidant activity of the root bark extract of *Acacia modesta*.

MATERIALS AND METHODS

Chemicals:

All used solvents, chemicals and materials were of analytical grade. While preparation of solutions and reagents were carried out according to the procedure mentioned in United States Pharmacopoeia and British Pharmacopoeia.

Collection of plant material:

The plant root was collected from Chak No. 120/13 AL, Chichawatni forest, district Sahiwal in the month of April 2015. It was identified as *Acacia modesta* Wall (Family: Fabaceae) by Dr. Zafar Ullah Zafar, taxonomist of Bahauddin Zakariya University, Multan, Pakistan.

Extraction:

The plant material was dried under a shade for 30 days. After drying, it was grinded to powder by an electric blender. For extraction, 350 g of powdered drug was taken and soaked in dichloromethane for

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a whole day, thrice, with random shaking. Every time the mixture was filtered and the filtrate was evaporated till dryness, using rotary evaporator. A crude dichloromethane extract (8.9 g) was acquired in a separate bottle and labelled as AMRBD. Similarly the marc was macerated with methanol and the above procedure was repeated thrice to obtain crude methanolic extract (27.46 g). This was also collected in a separate sample bottle designated with code AMRBM.

Phytochemical analysis:

According to previous standard methods, preliminary phytochemical tests were performed on powdered root bark[15].

In vitroα-glucosidase inhibition assay:

Inhibition of α-glucosidase by dichloromethane and methanolic extracts were assayed by previously reported standard method [16].Sample extract was prepared in 70 % DMSO and 20 µl of that sample was added to 96-well microplate containing 135 µl of 0.05 M phosphate buffer (pH 6.8). Then 20 µl of α-glucosidase was transferred to the wells and incubated at 37^{o}C for 15 minutes. Subsequently pre-read was taken on spectra max. After pre-reading, 25 µl of 0.7 mMsubstrate (*p*-nitrophenyl-α-D-glucopyranoside) was poured and again incubated at 37^{o}C for 15 minutes. Thenreadings were recorded at 400 nm by microplate reader and matched with the control having only buffer solution. EZ-Fit Enzyme Kinetics program was used to calculate the IC_{50} values.

Antioxidant assay:

Antioxidant activity of the extracts of *Acacia modesta*root bark were evaluated by DPPH assay which involved the use of 2,2-Diphenyl-1-picrylhydrazyl free radical[17].DMSO was mixed with extract to form sample solution. While methanol was used to form DPPH solution. In 96-well microplate, 5 µl of sample solution was added and pre-reading was noted at 515 nm. Then 95 µl of 300 µM DPPH solution was decanted to each well and incubated at 37^{o}C for 30 minutes. To record the final absorbance, microplate reader was employed. Extract mixture with methanol was taken as blank. Whilst ascorbic acid,prepared in the same concentration as sample, was used for comparison. Percentage antioxidant activity (% AA) was calculated by following formula:

$\% AA = 100 - [\{(Abs_{sample} - Abs_{blank}) \times 100\} / Abs_{control}]$

Where Abs = Absorbance

RESULTS

Phytochemical analysis:

During preliminary screening of root bark of *Acacia modesta*, it revealed that saponins, flavonoids, alkaloids, cardiac glycosides and tannins were present while anthraquinones were absent.

In vitroα-glucosidase inhibition studies:

The in vitro α-glucosidase inhibitory activity of the plant extracts is summarized in **Table 1**. At 500 µg/ml concentration, dichloromethane and methanolic extracts exhibited inhibitory activity of 98.9 % and 99.9 % with IC_{50} of 6.8 µg/ml and 1.2 µg/ml respectively. The results were compared to standard, acarbose, which showed59.1 % inhibition with IC_{50} of 540 µg/ml.

Table 1: α-glucosidase inhibition results of dichloromethane and methanolic extracts of *Acacia modesta* root bark

Sample code	Conc. (µg/ml)	Percentage inhibition	IC_{50} ± SEM (µg/ml)
AMRBD	500	98.9	6.8 ± 0.15
AMRBM	500	99.9	1.2 ± 0.50
Acarbose	640	59.1	540 ± 1.73

Antioxidant assay:

Antioxidant potential of root bark extract of *Acaciamodesta* was assessed by DPPH assay model. The results indicated that dichloromethane extract had IC_{50} of 283.33 µg/ml and percentage radical scavenging activity (%RSA) of 76 % at concentration of 500 µg/ml. Whilst at the same concentration, methanolic extract was 88 % active with IC_{50} of 236.49 µg/ml. These results in comparison to standard ascorbic acid are described in **Table 2**.

Table 2: Antioxidant activity results of dichloromethane and methanol extracts of *Acacia modesta* root bark

Sample code	Conc. (µg/ml)	% RSA	IC_{50} ± SEM (µg/ml)
AMRBD	500	76	283.33 ± 1
AMRBM	500	88	236.49 ± 0.99
Ascorbic acid	500	96	7.06 ± 1.2

DISCUSSION

Diabetes mellitus is among the world's greatest health hazards. It has affected almost 171 million people and many of them are suffering from type II diabetes mellitus [18]. This higher risk of type II diabetes is a serious health concern and accounts for 9 % of deaths worldwide. During last decade, although an improvement in drug treatment of type II diabetes has been observed but drug resistance is still a problem that has to be dealt. One approach is to explore new therapeutically active agents, especially α-glucosidase inhibitors, which inhibit the production of glucose from carbohydrates and impede the postprandial increase in blood glucose level[19]. Natural products are a vital source of such inhibitors thereby motivating to search medicinally important plants for biologically active compounds. The results of the present study specify that both extracts of *Acacia modesta* root bark showed α-glucosidase inhibition. The extracts essentially contain such bioactive constituents which are hindering the enzyme activity. These expected compounds could be flavonoids as literature reports described them as inhibitor of α-glucosidase[20-22]. It has also been reported that flavonoids have antioxidant potential[23, 24]. This may be the reason that dichloromethane and methanolic extracts of root bark exhibited radical scavenging activity in DPPH assay. In the body, antioxidants avert free radicals from oxidizing proteins, nucleic acids and lipids. Similarly, they also maintain the level of free radicals in our systems which is valuable because high level may cause disorders like multiple sclerosis and carcinomas [25]. Hence it is seen that plants have been investigatedfor natural antioxidants [26, 27].

CONCLUSION

In light of above findings, we conclude that the plant root bark should be considered for isolation of lead compounds having antidiabetic and antioxidant activities.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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