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EVALUATION OF α-GLUCOSIDASE, UREASE INHIBITION AND ANTIOXIDANT POTENTIAL OF *ACACIA JACQUEMONTII* AND *RHAMNUS PERSICA*.

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Abstract:

The aim of this study was to investigate antioxidant, urease and α-glucosidase inhibition activities of the plants Acacia jacquemontii and Rhamnus persica. Dichloromethane and methanol extracts of the plants were evaluated for described activities. In α-glucosidase inhibition assay, dichloromethane and methanolic extracts of Acacia jacquemontii exhibited inhibitory activity of 97.9 % and 98.9 % with IC_{50} of 4.8 µg/ml and 1.2 µg/ml respectively while that of Rhamnus persica showed 68.8 % and 24.5% with IC_{50} values of 29.3 and 614.5 µg/ml respectively. The results were compared with standard, Acarbose, which showed 70.1 % inhibition with IC_{50} of 520 µg/ml. DPPH inhibition assay indicated that both dichloromethane and methanolic extracts of Acacia jacquemontii were active with percentage inhibition of 86.8% and 94.11 % respectively with IC_{50} values of 24.51 µg/ml and 9.51 µg/ml. The dichloromethane and methanolic extract of Rhamnus persica exhibited the percentage inhibition of 75.9 and 94.9 respectively with IC_{50} values 30.95 µg/ml and 34.77 µg/ml. Ascorbic acid was used as standard. Both the plants showed non significant activity in urease inhibition assay. These results confirm that both plant extracts possess significant α-glucosidase inhibitors and antioxidant potential, thereby providing worthy justification for isolation of novel bioactive compounds. Further study is needed to screen out antioxidants and potent enzyme inhibitors.

Keywords: ***Acacia jacquemontii, Rhamnus persica, Antioxidant, α-Glucosidase inhibition, Urease inhibition***

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INTRODUCTION:

The genus *Acacia* (Fabaceae) is comprised of almost 1380 species. A majority of these are native to Australia and rest belongs to tropical and subtropical parts of the world [1]. *Acacia jacquemontii* Benth. (Fabaceae), known as Banolii (Hindi) and Chhoti kikrii (Urdu) is native to "Thar desert" of Indo-Pak subcontinent. It is an erect shrub with multiple branches coming from below ground [2]. Traditionally, the decoction of the plant bark is used to combat fever, muscular pain and cough [3].Various species of genus *Acacia* have been reported with variety of pharmacological potential. Different compounds and extracts of *A. nilotica* have been reported to possess potent antioxidant activity. Results from in vitro assay of kaempferol from methanol extract showed strong dose dependent activity in the concentration range of 1–50µg/ml for DPPH assay and 1–100µg/ml for deoxyribose degradation assay [4]. α-D- glucosidase inhibitory activity of the polysaccharide isolated from Acacia *tortilis* was tested in both in vitro as well as in vivo models. The results revealed significant inhibitory activity against α –D- glucosidase [5]. Rhamnaceae, the Buck thorn family contains 50 genera and about 900 species of flowering plants distributed globally but mostly in tropical and subtropical areas. Represented in Pakistan by 6 genera and 21 species. *Rhamnus* fairly a large genus of 160 species, cosmopolitan in distribution. Represented in Pakistan by 6 species only. *Rhamnus persica*, belongs to the genus *Rhamnus* and family Rhamnaceae, is widely distributed in Pakistan and Iran. In Pakistan it is distributed in the area of Baluchistan and surroundings [6]. Several species of *Rhamnus* are used in different parts of the world by the peoples for treatment of various ailments. The bark and fruit of *Rhamnus* species have been used for centuries in folk and official medicine as purgatives and for blood detoxication [7] an infusion prepared from the fruits of *Rhamnus cathartica* is used in Bulgarian folk medicine as an antiseptic for wounds. In folk medicine in Bosnia and Herzegovina the bark of *Rhamnus fallax* is used to treat skin diseases [8]. Biological assays have validated the traditional uses of various *Rhamnus* species. Various species of genus *Rhamnus* exhibited various biological activities including antimicrobial, antioxidant, antiproliferative, antimutagenic and antigenotoxic activities. Flavonoids isolated from the leaves of *Rhamnus alaternus* L. showed antioxidant and free radical-scavenging properties [9]. Ethanol and methanol extracts of aerial parts *Rhamnus prinoides* showed antimicrobial activities [10]. Methanol extracts of flower and leaves of *R. kurdica* showed significant antioxidant activity [11]. Strong antioxidant capacity was exhibited by methanol extracts of leaf and bark of *Rhamnus intermedia* [12].

MATERIAL AND METHOD:

Plant Collection and extraction

The plant *Acacia jacquemontii* was collected from the surroundings of Muzaffargarh power plant, District Muzaffargarh while Aerial parts of *Rhamnus persica* from Fort Manro.The plants were identified as *Acacia jacquemontii* and *Rhamnus persica* by Prof. Dr.Altaf Dasti, taxonomist at The Institute of pure and applied Biology of Bahauddin Zakariya University Multan and voucher Specimen was deposited in the herbarium of the department for future reference. The root bark of *Acacia jacquemontii* was separated from the underground parts of the collected plant, chopped into small pieces and dried at room temperature for 15 days. Similarly the aerial parts of *Rhamnus persica* were shade dried. The dried material was powdered and 400 g each of the powder was macerated in dichloromthane and methanol respectively and filtered; the filtrate were concentrated under vacuum using a rotary evaporator to obtain both extracts. The extracts were assigned the codes for plant *Acacia jacquemontii* AJRBD, AJRBM and for *Rhamnus persica* RPAPD and RPAPM respectively.

Phytochemical analysis:

Standard preliminary phytochemical screening tests were used for detection of various secondary metabolites in the extract. Briefly, Borntrager's test was used to detect anthraquinones, $FeCl_3$ test for tannins, Dragendorff's test for alkaloids, Keller-Kiliani test for cardioactive glycosides, froth test for saponins and alkaline reagent test for confirmation of presence of flavonoids [13].

Antioxidant assay:

Antioxidant activity of the extracts was evaluated by DPPH assay which involved the use of 2, 2-Diphenyl-1-picrylhydrazyl free radical with slight modification [14]. The concentration of DPPH 0.1 mM Total assay volume was 100µl containing 10 µl of the test solution and 90 µl of DPPH solution in a 96 well plate. The contents were mixed and incubated at 37°C for 30 minutes. Synergy HT Biotech USA microplate reader was used to determine the diminution in absorbance at 517nm.Standard antioxidant used was ascorbic acid. All experiments were carried out in triplicate. Serial dilutions were made for IC_{50} values which were computed by using Amherst USA software Ez-fit5 Perrella Scientific Inc. The decrease in absorbance indicated increased radical scavenging activity which was determined by the following formula.

Inhibition (%) = (Absorbance of control – Abs. of test solution) × 100 / Abs. of control
Where
Absorbance of control = Total radical activity without inhibitor.
Absorbance of Test = Activity in the presence of test compound

***In vitro* α-glucosidase inhibition assay:**
Inhibition of α-glucosidase by dichloromethane and methanol extracts were assayed by previously reported standard method [15]. 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 37oC for 15 minutes. Subsequently pre-read was taken on spectra max. After pre-reading, 25 µl of 0.7 mM substrate (*p*-nitrophenyl-α-D-glucopyranoside) was poured and again incubated at 37oC for 15 minutes. Then readings 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.

***In Vitro* urease inhibition assay:**
Anti urease assay was performed according to (Weatherbum 1967 and Xio et al 2007) with slight modification [16]. First 5 ul of test compound, 25ul (0.25mg/ml) of enzyme were incubated at 37^0C for 15 minutes .Then 55ml of substance(urea) was added and re incubated at the same condition. After incubation absorbance was measured at 630nm.This was taken as per read. Then 45ml of phenol and 70 ml of alkali reagent was added into the mixture and incubated for 50 minutes. After incubation absorbance was measured at 630nm and taken as after read. Methanol was taken as control. For IC_{50} serial dilution was done, results were measured by this formula.

% Inhibition= 100− (Absorbance of test compound/ Absorbance of control) * 100

RESULTS:

Phytochemical analysis:
Presence of various secondary metabolites was carried out by standard methods. The result is given below in table 1.

In vitro α-glucosidase inhibition studies:
The in vitro α-glucosidase inhibitory activity of the plant extracts is summarized in Table 2. At 500 µg/ml concentration, dichloromethane and methanolic extracts of *Acacia jacquemontii* exhibited inhibitory activity of 97.9% and 98.9 % with IC50 of 4.8 µg/ml and 1.2 µg/ml respectively. The dichloromethane and methanol extracts of *Rhamnus persica* showed inhibitory activity of 68.8 and 24.5 with IC_{50} values of 29.3 µg/ml and 614.5 µg/ml respectively The results were compared to standard, acarbose, which showed 59.1 % inhibition with IC_{50} of 540 µg/ml.

Table 1 Results of phytochemical screening of both plants *Acacia jacquemontii* and *Rhamnus persica.*

Secondary metabolites	*Acacia jacquemontii*	*Rhamnus persica*
Alkaloids	Absent	Absent
Anthraquinones	Absent	Present
Tannins	Present	Present
Cardioactive glycosides	Present	Absent
Saponins	Present	Present
Flavonoids	Present	Present

Table 2: In vitro α-glucosidase inhibitin assay results of *Acacia jacquemontii* and *Rhamnus persica.*

Sample code	Conc.(µg/ml)	% Inhibition	IC_{50} ± SEM (µg/ml)
AJRBD	500	97.9	4.8 ± 0.14
AJRBM	500	98.9	1.2 ± 0.55
RPAPD	500	68.8	29.3 ± 0.45
RPAPM	500	24.5	614.5 ± 0.31
Acarbose	640	70.1	520 ± 1.73

Table 3: DPPH inhibition assay results of *Acacia jacquemontii* and *Rhamnus persica*

Sample code	% Inhibition(500 µg/ml)	IC_{50} (µg/ml)
AJRBD	86.8	24.51
AJRBM	94.11	9.51
RPAPD	75.9	30.95
RPAPM	94.9	34.7
Ascorbic acid	92	22

Table 4 Results of Urease inhibition assay of *Acacia jacquemontii* and *Rhamnus persica*.

Sample codes	% Inhibition(500 µg/ml)	IC_{50} (µg/ml)
AJRBD	4.1	Inactive
AJRBM	0.56	Inactive
RPAPD	35.1	Inactive
RPAPM	26	Inactive
Thiourea	99	19

Antioxidant assay:
Antioxidant potential of both plants *Acacia jacquemontii* and *Rhamnus persica* was assessed by DPPH assay model. The results indicated that dichloromethane extract of *Acacia jacquemontii* had IC_{50} of 24.51 µg/ml and 86.8 % of inhibition at concentration of 500 µg/ml. While at the same concentration, methanol extract was 94.11 % active with IC_{50} 9.51 of µg/ml.

The dichloromethane extract of *Rhamnus persica* showed 75.9 percent inhibition at IC_{50} value of 30.95 µg/ml while the methanol extract exhibited 94.9% inhibition at IC_{50} value of 34.7 µg/ml. These results in comparison to standard ascorbic acid are described in Table 3.

Urease inhibition assay:
Anti-urease assay was performed for both plants *Acacia jacquemontii* and *Rhamnus persica*. The results were compared with standard drug thiourea at concentration of 500mg/ml. Both plants showed non-significant activity in assay. The results are shown in table given below.

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 [17]. 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 [18]. 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 jacquemontii* 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 inhibitors of α-glucosidase [19, 20]. The development of the alpha-glucosidase inhibitor provides a new approach in the management of diabetes. By competitive and reversible inhibition of intestinal alphaglucosidases, alpha-glucosidase delays carbohydrate digestion, prolongs the overall carbohydrate digestion time, and thus reduces the rate of glucose absorption. After oral administration of alpha-glucosidase, the postprandial rise in blood glucose is dosedependently decreased, and glucose-induced insulin secretion is attenuated. Due to diminished postprandial hyperglycemia and hyperinsulinemia by alpha-glucosidase inhibitor, triglyceride uptake into adipose tissue, hepatic lipogenesis, and triglyceride content are reduced. Therefore, alpha-glucosidase inhibitor treatment not

only flattens postprandial glycemia, due to the primary and secondary pharmacodynamic effects, but also ameliorates the metabolic state in general [21]

Free radicals are known to play a pivotal role in the onset and exacerbation of several pathologies. By counteracting these free radicals, antioxidants help in preserving good health. Indeed, phytochemicals have received much interest owing to their molecular structure which consists of hydroxyl groups on aromatic rings and this has been associated with their functionality as oxidant scavengers [22].

It has also been reported that flavonoids have antioxidant potential [23, 24]. This may be the reason that dichloromethane and methanol extracts of both plants 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 are valuable because high level may cause disorders like multiple sclerosis and carcinomas [25].

CONCLUSION:

In light of above findings, we conclude that both the plants showed significant antioxidant potential and α-glucosidase inhibition activity. The dichloromethane and methanol extracts of *Acacia jacquemontii* exhibited significant α- glucosidase inhibition activity. The presence of flavonoids and saponins in plant extracts may possibly account for these activities. The isolation of biologically active compounds responsible for the observed effects is under way in our laboratory.

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