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In Vitro Evaluation of Alpha-amylase Inhibition, Antioxidant Potential and Phytochemical Analysis of *Acacia Modesta*

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Abstract: Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, which affects insulin secretion. Among several approaches to treat diabetes, controlling postprandial hyperglycemia is an effective way to manage diabetes type II. In this regard, medicinal plants are considered the best source of new chemical entities with fewer side effects. This study aims to evaluate the alpha-amylase inhibitory potential of *Acacia modesta* ethanol and aqueous extracts in correlation with its antioxidant potential and develop an anti-diabatic drug against the alpha-amylase enzyme that can control the post-prandial hyperglycemia. We used the starch-iodine color change method for α-amylase, a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay for determining the antioxidant potential and determined different phytochemicals, including phenols, flavonoids, and tannins by spectrophotometry. The results indicated that the gum of *A. modesta* possesses potent α-amylase inhibitory potential with IC_{50} values of 100.4 ± 0.04 µg/mL in ethanol and 91.8 ± 0.05 µg/mL in the aqueous extract, which was approximately 3-folds higher compared to standard acarbose (286.8 ± 0.04 µM). The antioxidant activity was also higher in gum compared to any other part. As for phytochemical analysis, this plant possesses higher amounts of tannins and phenols, while flavonoids are in lesser amounts. The gum of *A. modesta* shows a dual mode of action, i.e., antioxidant and amylase inhibitor, which makes it a better candidate for diabetes management. The novelty is that, to our knowledge, this study is the first to report the anti-diabetic and antioxidant properties of *A. modesta* using pancreatic amylase as a drug target from Khyber Pakhtunkhwa, Pakistan.

Keywords: *Acacia modesta*, alpha-amylase, flavonoids, tannins, antioxidant.

金合歡的α澱粉酶抑制、抗氧化潛力和植物化學分析的體外評價

摘要：糖尿病是一種以高血糖為特徵的代謝性疾病，影響胰島素分泌。在治療糖尿病的幾種方法中，控制餐後高血糖是控制II型糖尿病的有效方法。在這方面，藥用植物被認為是副作用較少的新化學實體的最佳來源。本研究旨在評估金合歡乙醇和水提取物的α-澱粉酶抑制潛力與其抗氧化潛力的相關性，並開發一種針對可控制餐後高血糖的α-澱粉酶的抗糖尿病藥物。我們使用α-澱粉酶的澱粉-碘顏色變化法，一種1,1-二苯基-2-苦基肼自由基清除試驗來測定抗氧化潛力，並通過分光光度法測定不同的植物化學物質，包

括酚類、類黃酮和單寧.結果表明，金合歡的樹膠具有強大的α-澱粉酶抑制潛力，乙醇中的IC_{50}值為100.4 ± 0.04微克/毫升，水提取物中的IC_{50}值為91.8 ±0.05微克/毫升，與標準相比高約3倍阿卡波糖(286.8 ± 0.04微摩爾)。與任何其他部分相比，口香糖的抗氧化活性也更高。至於植物化學分析，這種植物含有較多的單寧和酚類物質，而類黃酮的含量較少。金合歡的口香糖顯示出雙重作用模式，即抗氧化劑和澱粉酶抑製劑，這使其成為糖尿病管理的更好候選者。新穎之處在於，據我們所知，這項研究首次報導了金合歡的抗糖尿病和抗氧化特性，使用來自巴基斯坦開伯爾普赫圖赫瓦的胰澱粉酶作為藥物靶點。

关键词：金合歡、α-澱粉酶、類黃酮、單寧酸、抗氧化劑。

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia that affects insulin secretion. Defects or alterations in the secretion or action of insulin disturb carbohydrate, lipid, and protein metabolism [1]. This shocking disease is prevalent in all parts of the world. According to the current data, the occurrence of diabetes mellitus type 2 is on the rise from 143 million people to 300 million people by 2025 [2]. Among various therapeutic approaches to cure diabetes, lowering postprandial hyperglycemia is essential [3]. This approach prevents glucose absorption by inhibiting carbohydrate-hydrolyzing enzymes, such as alpha-glucosidase and alpha-amylase [4]. The α-amylase is one of the major secretory products of the pancreas and salivary glands, playing a role in the digestion of starch and glycogen, and can be found in microorganisms, plants, and higher organisms [5]. Alpha-amylase enzyme catalyzes the initial step in the hydrolysis of starch to a mixture of oligosaccharides consisting of maltose, malt-triose, and branched oligosaccharides of 6-8 glucose units that contain both α-1,4 and α-1,6 linkages. They are further degraded to glucose by α-glycosidase. Therefore, it can apply as an effective diabetic drug target. By inhibiting amylase, the breakdown of carbohydrates into glucose can be controlled and, thus, in patients with diabetes, it can lower the hyperglycemic index that will ultimately stop the production of Advanced Glycated End products (AGEs), which is the main reason for all complications generated because of diabetes [6]. Oral hypoglycemic agents/drugs may be effective for glycemic control, but they come with side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness, and diarrhea. Over time, these medicines lose their efficacy for controlling the disease and finally fail to alter the course of diabetic complications [7].

Medicinal plants are essential in preventing diseases with fewer side effects due to their bioactive constituents, such as flavonoids, phenol, and tannin [8]. Recent evidence suggests that oxidative stress may contribute to the pathogenesis of Diabetes mellitus type 2 by increasing insulin resistance or impairing insulin secretion. The physiological burden of free radicals causes an imbalance between oxidants and antioxidants in the body [9]. This imbalance leads to oxidative stress, suggested as the root cause of aging and various human diseases, including atherosclerosis, stroke, diabetes, cancer, and neurodegenerative diseases such as Alzheimer's and Parkinsonism, and vice versa is the role of antioxidants [10].

Acacia modesta (Palosa tree) is native to Asian countries such as Pakistan, Afghanistan, and India. In Pakistan, it is found below 1200 m altitude in the foothill ranges of the Himalayas, Salt Range, Sulaiman Hills, Baluchistan, and the Kirthar Range. It is also found in the plain areas close to these mountains [11]. *Acacia modesta* (AM) is a thorny, moderate-size tree having a length of 3-9 m and a diameter of up to 2 m. Its leaves are compound, 1.2-5 cm long, growing in bunches, and appear between March to May, depending on the geographic location. The pods are 5-7.5 cm long and mature between May to November [12]. It is an intolerant, drought-resistant tree that survives on various soils, including dry and shallow soils. It showed some frost resistance. At present, no disease or insect problems have been identified [13]. The gum of AM applies as a tonic and to cure dysentery, and branches apply as toothbrushes, locally called Miswak [14]. AM is widely used in the local system as a medicine for treating various diseases such as diabetes and muscular problems, to ease backache for women after delivery, and chronic stomach complaints from time immemorial [15]. Keeping the abovementioned facts, the present study answered the question if any parts of AM possess the alpha-amylase inhibitory potential and antioxidant properties to cure diabetes. Crude ethanol and aqueous extracts were prepared for different parts of AM, including root, stem bark, seed, gum, and flower. IC_{50} values were calculated for both

alpha-amylase inhibition and antioxidant activity. Furthermore, important phytochemicals, including phenols and polyphenols, were also determined in this study.

2. Materials and Methods

Fig. 1 shows the methodology schematic workflow.

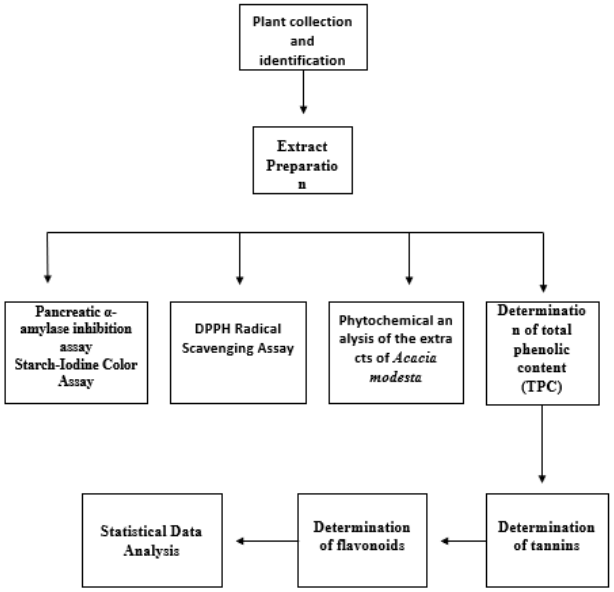


Fig. 1 Schematic workflow of methodology

2.1. Plant Collection and Identification

Root, stem, seeds, gum, and flower parts of AM were collected during December to May from district Nowshera (34°01′ 53 ″N 71°35′E), Khyber Paktunkhwa, Pakistan. A taxonomist in the Department of Botany, Abdul Wali Khan, University Mardan, Pakistan, identified the plant parts. Collected plant parts were air dried for three weeks under the shade and ground into fine powder.

2.2. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade.

2.3. Extract Preparation

The powdered plant materials, i.e., roots, stems, seeds, gum, and flowers, were exhaustively extracted with distilled water (1:10) and ethanol (1:10) using the reflux method. All samples were then filtered and centrifuged with the residue collected in the second round of extraction. This process has been repeated three times with extract storage at -20°C for further analysis.

2.4. Pancreatic α-Amylase Inhibition Assay and Starch-Iodine Color Assay

The screening of AM extracts (as α-amylase inhibitors) was conducted according to [15] with slight modifications based on the starch-iodine test. The total reaction volume was 120µl that contained sodium phosphate buffer (0.02M) having pH 6.9 (containing 6 mM sodium chloride), pancreatic α-amylase (PA) solution (1.5 ml; 3 units/ml), and plant extracts at a concentration of 0.1-1.5 µg/ml (w/v). The reaction mixture was incubated at 37°C for 10 min, followed by adding soluble starch (1%, w/v) and again at 37°C for 15 min. Adding 1 M HCl (60 µl) stopped the enzymatic reaction, with further adding a 300 µl of iodine reagent (5 mM, I2 and 5 mM KI). The color changed with reading the absorbance at 620 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included. The known amylase inhibitor, acarbose, was used as a positive control in a concentration range of 6.5-32.8 µg/ml. A dark-blue color indicates starch; a yellow – the absence of starch, while a brownish color – partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded, thus giving a dark-blue indication.

2.5. DPPH Radical Scavenging Assay

The antioxidant activity of plant extracts against stable 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was assayed following a standard method with slight modifications [16]. Briefly, 2 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of plant extract in a concentration range of 62.5-1000 µg/ml. The equivalent positive control used L-Ascorbic acid (1-100 µg/ml) as standard; a negative one - mixing 1 ml ethanol and 2 ml DPPH solution, and the same for the aqueous extract. The reaction mixture was allowed to incubate for 5 min at room temperature in the dark, and decolorization quantified the scavenging activity of each plant extract at 517 nm using a UV-Vis spectrophotometer (Hitachi U-2900, Tokyo, Japan). The reaction was in triplicate with the percentage of free radical scavenging activity of each extract expressed as a percent inhibition from the given formula.

$$\% \text{ Inhibition of DPPH radical} = Ac\text{-}As/Ac \times 100$$

where Ac is the absorbance of the control As is the absorbance of the sample.

2.6. *Acacia Modesta* Extract Phytochemical Analysis

The aqueous and ethanol extracts of AM were evaluated for the quantitative determination of various classes of active chemical constituents.

2.7. Determination of the Total Phenolic Content (TPC)

The total phenolic content (TPC) of the crude extracts of seeds, flowers, gum, stem bark and root bark were determined using the Folin-Ciocalteu reagent method with slight modifications [17]. Briefly, 1-mL extract (sample) was mixed with 1 ml of Folin-Ciocalteu reagent incubated at room temperature for 3 min. After incubation of 3 min, 1 1ml of saturated sodium carbonate solution (20%) was added to the mixture and the volume was adjusted to 10ml with distilled water. The reaction mixture was kept in the dark for 90 min, and absorbance was measured at 725 nm using UV-Visible spectrophotometer (Hitachi U-2900, Tokyo, Japan). A standard curve was generated using different concentrations of phenol (50-500mg) as standard and a blank was also prepared using the same method [17].

2.8. Determination of Tannins

Tannins were extracted by dissolving 50-µl ethanol and an aqueous extract with 950 µl of distilled water. Then, the reaction mixture was prepared thoroughly with 0.5 ml of folin-phenol reagent and 2.5 ml of 20% sodium carbonate solution with incubation of the reaction mixture at room temperature for 40 min. The absorbance measurement at 725 nm used a UV-Vis spectrophotometer (Hitachi U-2900, Tokyo, Japan). Absolute ethanol and distilled water were used as blanks for ethanol and H_2O extracts, respectively. A standard curve used tannic acid as a standard for its generation.

2.9. Determination of Flavonoids

Total flavonoid contents were determined by a spectrophotometric method [17]. The flavonoid extract (250 µl) was mixed with 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. The reaction mixture was allowed to incubate for 5 min at room temperature in the dark. After 5 min, 150 µl of 10% $AlCl_3 \cdot H_2O$ was added with further incubating the reaction mixture for 6 min at room temperature. After 6 min, 500 µl of 1M NaOH and 275 µl of distilled water were added to the mixture. The solution was mixed thoroughly with absorbance measurement at 415 nm using a UV-Vis spectrophotometer (Hitachi U-2900, Tokyo, Japan). A standard curve used Quercetin with varying concentrations (50-250 µg) for its generation.

2.10. Statistical Data Analysis and Determination of the 50% Inhibitory Concentration for the Plant Samples

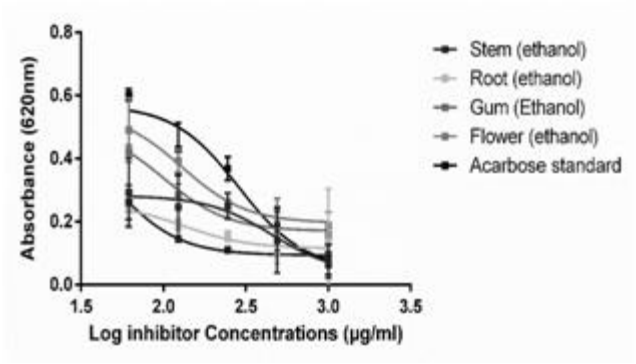
Statistical analysis used Prism 6.01 software (GraphPad, La Jolla, CA, USA). All experiments were performed in 3 different sets, with each set-in triplicate. The data are expressed as mean ± SEM (standard error of the mean). The IC_{50} values for alpha-amylase inhibition and DPPH scavenging activity were calculated from plots of log inhibitor concentration versus absorbance in Prism 6.01 software (GraphPad, La Jolla, CA, USA) using non-linear regression analysis.

3. Results and Discussion

Drug discovery and development is a time-consuming and very expensive process in today's context. The minimum cost of new drug discovery is about 1 billion USD in about 12 years [18]. Medicinal chemistry, along with combinatorial chemistry, which succeeds in developing several chemical libraries, initially started the success stories of new drug discovery [19]. In terms of overall success, this approach is limited by various side effects [20]. The only source for new drugs with fewer side effects became the natural source [8]. Semi-synthetic analogs and natural sources comprise about 80% of the drug substance [19]. From 1981 to 2010, out of 1184 new chemical entities, about 60% were derived from natural sources [21]. Several herbal treatments are available against diabetes in the local market that involves formulations to control postprandial hyperglycemia, but these need scientific validation. The acacia family is a family that is famous for its anti-diabetic potential [22]. In literature, most works relate to the *Nilotica* species commonly called the Babul tree [23]. Although fewer data are available on *Modesta* species, [24] published the usage of different parts of *AM* in various clinical manifestations, including anti-inflammatory, anti-carcinogenic, and analgesic properties. However, now no study is available on the antidiabetic potential of any part of AM regarding alpha-amylase inhibition. This study evaluated the anti-diabetic properties of different parts of *AM* (commonly called Palosa tree in the native language). Crude ethanol and aqueous extracts, prepared for various parts included stem bark, roots, seed, gum, and flower. Each part used a concentration of 60-1000 µg/mL with percentage inhibitions calculations. Absorbance was plotted versus log inhibitor concentration to generate a dose-response curve that gave a 50% occupancy value.

In the first series of experiments, the alpha-amylase inhibitory potential of the abovementioned plant parts was evaluated in ethanol and aqueous extracts, as shown in Fig. 1 a, b. The data indicated that the alpha-amylase inhibitory potential for each part increased with the increase in concentration. However, at higher concentrations, the increase reached a constant value. The comparison of percentage inhibitions demonstrated that the root part showed a percentage inhibition ranging from 83-90%, the stem part showed 78-94%, the seed part showed 81-94%, gum 69-88% and flower 64-86% for pancreatic amylase activity. In addition to ethanol extract, the alpha-amylase inhibitory potential has been evaluated in aqueous extracts (Fig. 1b). The data indicated the increase in percentage inhibition with the increase in concentration. The inhibition range

was 67-89% for stem bark, 80-93% for the seed part, 48-94% for the gum part, and 65-91% for the flower part is a clear indication of the following: first, the inhibitory potential increased with the increase in concentration. Second, the seed part possesses the highest inhibitory potential compared to all other parts, which became more prominent when considering the IC_{50} values calculated from the dose-response curve generated by the graph pad prism using non-linear regression analysis (Fig. 1a, b). Table 1 shows the IC_{50} values. For the gum part, the IC_{50} value was 100.4±/0.04 µg/mL in ethanol extract and 91.8±0.05 µg/mL in the aqueous extract, which was the lowest concentration compared to root, stem bark, and flower that gave an IC_{50} value of 133.2± 0.07 µg/mL, 458.2 ± 0.08 µg/mL and 129.1±0.01 µg/mL respectively in ethanol extract and 118 ± 0.01 µg/mL, 175.2 ± 0.03 µg/mL and 227.9 ± 0.02 µg/mL respectively in aqueous extracts. When compared with standard acarbose (286.8 ± 0.04 µM), the IC_{50} value of the gum part was approximately 3-folds higher in both ethanol and aqueous extract. In comparison, both ethanol and aqueous extracts are almost equally effective in inhibiting pancreatic amylase, indicating the therapeutic importance of AM gum concerning postprandial control of hyperglycemia.



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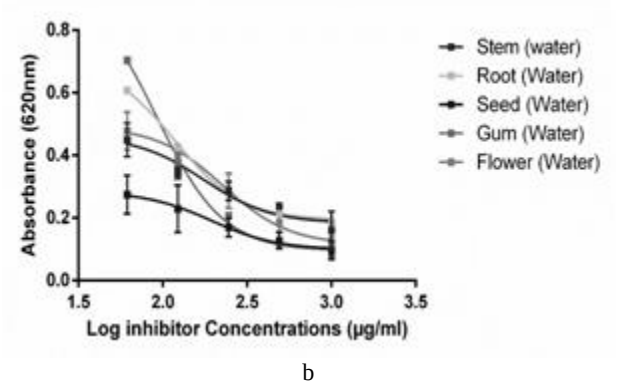

b

Fig. 1 Inhibition of Alpha-amylase activity by *Acacia modesta* extracts (Developed by the authors)

Fig. 1a is a graphical representation of the inhibition activity of ethanol extract for different parts (root, stem, seed, gum, and flower) of AM. The concentration (60-1000 µg/mL) is shown on a logarithmic scale on the abscissa, and absorbance for alpha-amylase activity is on the ordinate to show a composite graph for all parts used in all experiments. Solid lines represent hyperbolic dose-response curves generated using non-linear regression analysis in the graph pad prism. The curves showed that the inhibition increased with the increase in concentration till the maxima reached.

Fig. 1b is a graphical representation of the inhibition activity of aqueous extracts of different parts (root, stem, seed, gum, and flower) of AM. Again, the concentrations (60-1000 µg/mL) of all parts are shown on a logarithmic scale on the abscissa, and percentage inhibition of alpha-amylase activity is on the ordinate to show a composite graph of all experiments. Solid lines represent hyperbolic dose-response curves generated in the graph pad prism.

In another series of experiments, the antioxidant potential for each part of AM for evaluation used a DPPH radical scavenging assay. At 25°C, DPPH is a stable free radical and accepts a proton to become a stable diamagnetic molecule, and the purple of the DPPH solution changed to yellowish color. At 517 nm, the DPPH solution shows maximum absorbance. The decrease in absorbance values demonstrates the reduction capability of the DPPH radical caused by antioxidants. The reaction between antioxidant molecules and free radicals results in radical scavenging by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity. Figs. 2a and 2b show the results of the DPPH scavenging activity. We calculated percentage inhibitions using the absorbance value. The data indicated that the percentage inhibition increased with an increase in the concentration of antioxidants in both ethanol and aqueous extract. The absorbance was also plotted against the log inhibitor concentration to generate a dose-response curve. We calculated the IC_{50} values using nonlinear regression analysis in the graph pad prism. After comparing the IC_{50} values, the gum part was more active compared to all other parts (Table 1).

Table 1 IC_{50} values for alpha-amylase inhibition and free radical scavenging by different parts of *Acacia modesta* (Developed by the authors)

Acacia modesta	**Alpha-amylase inhibition IC_{50} (µg/mL)± SE**	**DPPH Radical Scavenging IC_{50} (µg/mL) ± SE**
Ethanol extract		
Root	133.2 ± 0.07	188.2 ± 0.80
Stem	458.2 ± 0.08	251.4 ± 0.04
Gum	100.4 ± 0.04	98.37 ± 0.03
Flower	129.1 ± 0.01	186.1 ± 0.01
Water extract		
Root	118 ± 0.01	61.51 ± 0.01
Stem	175.2 ± 0.03	108.4 ± 0.01
Seed	194.9 ± 0.03	211.8 ± 0.02

Continuation of Table 1		
Gum	91.8 ± 0.05	57.16 ± 0.01
Flower	227.9 ± 0.02	203.1 ± 0.02
Acarbose ascorbic acid	286.8 ± 0.04	38 ± 1.15

The IC_{50} values were 98.37 ± 0.03µg/mL and 57.16 ± 0.01 µg/mL in ethanol and aqueous extract, respectively, which were higher compared to the root (61.51 ± 0.01 µg/mL), stem (108.4 ± 0.01 µg/mL), seed (211.8 ± 0.02 µg/mL) and flower (203.1 ± 0.02 µg/mL). Although compared with positive control ascorbic acid (IC_{50}, 38 ± 1.15 µg/mL), the gum part was less active; however, the duality in the mode of action still makes it a better candidate as an anti-diabetic agent. In the final series of experiments, the classes of phytochemicals, including phenols, flavonoids, and tannins were quantified in both ethanol and aqueous extract to see if the alpha-amylase inhibitory and antioxidant potential of AM is due to the presence of phenolic or polyphenolic compounds (Table 2).

Table 2 Photoactive compound determination in ethanol and aqueous extract of different parts of *Acacia modesta* (Developed by the authors)

Acacia modesta	**Phenols, µg ± SD**	**Flavonoids µg ± SD**	**Tannins µg ± SD**
Ethanol extract			
Root	543 ± 6.01	398 ± 11.9	808 ± 1.34
Stem	737 ± 1.42	476 ± 15.2	687 ± 3.19
Seed	194 ± 1.87	302 ± 42.3	504 ± 1.94
Gum	110 ± 15.8	---	361 ± 0.74
Flower	633 ± 4.31	214 ± 18.9	212 ± 2.61
Water extract			
Root	453 ± 1.61	35.3 ± 1.45	923 ± 4.14
Stem	624 ± 10.8	19.6 ± 1.92	738 ± 80.7
Seed	270 ± 20.7	247 ± 1.68	395 ± 5.02
Gum	---	41.2 ± 4.28	456 ± 16.6
Flower	572 ± 11.5	487 ± 4.05	403 ± 48.3

(---) Negligible amounts

From the data, phenols, and tannins were in higher amounts in all studied parts of AM, compared to flavonoids in ethanol extract. In the case of the gum part, phenols and tannins are in lesser amounts compared to any other parts. Moreover, flavonoids were in negligible amounts. For the same classes, quantified in aqueous extract, we found consistent results that, again, tannins and phenols were in much more amounts compared to flavonoids. Data indicated the high therapeutic potential of AM gum extract regarding alpha-amylase inhibition as well as antioxidant potential, and this high activity might be due to the presence of higher contents of tannins.

Considering the literature, [24] reported the medical importance of the leaves of AM regarding their antioxidant activity, enzyme inhibition activity, and cytotoxicity potential. It also reported the presence of various phytochemicals in AM, including phenols and flavonoids; however, these activities do not correlate to anti-diabetic potential, which is the main focus of our study. Moreover, regarding the enzyme activity, their study focused on the acetylcholine esterase enzyme. Our study is consistent with this one in showing various phytochemicals in AM and enzyme inhibitory potential, which was amylase. Our results agree with that of [21], which has reported the anti-inflammatory potential of pod extracts from *Acacia nilotica*. The study of anti-inflammatory activity for methanol extract and tannin fraction revealed that the tannin fraction gave much more activity compared to the methanol extract. Our results also suggest the same for *AM* that the higher activity of seed extract might be due to the higher amounts of tannins in it. [26] determined the tannin concentration in the aqueous extract of various *Acacia* species belonging to Sudan and determined that the leaves and fruit contained much more amount of tannins compared to bark. Moreover, among all species under study, *A. nilotica* is the richest source of tannins; however, that study lacked data on AM species because this species is uncommon in Sudan. Interestingly, our results also revealed a higher amount of tannins in AM.

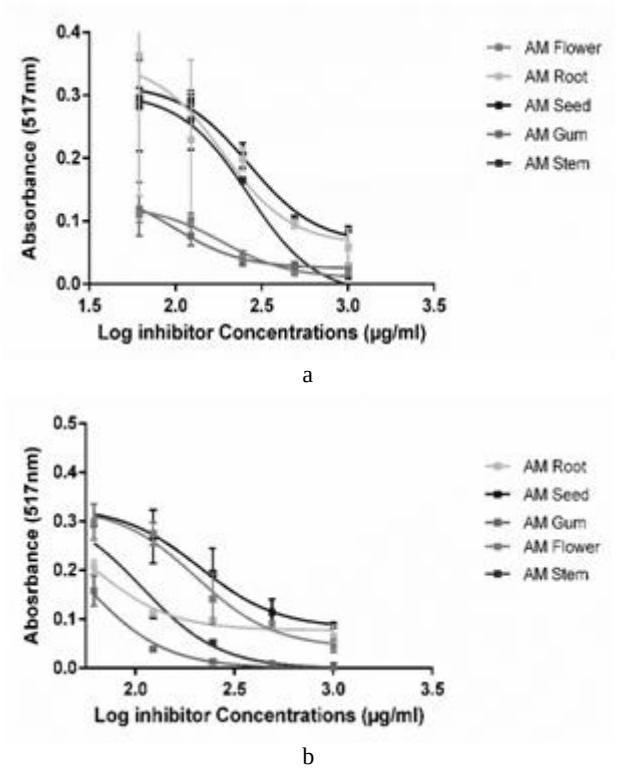

Fig. 2 DPPH radical scavenging activity of *Acacia modesta* extract (Developed by the authors)

Fig. 2 graphically represents the DPPH radical scavenging activity of ethanol (a) and aqueous (b) extract. The concentration (60-1000 µg/mL) of root, stem, seed, gum, and flower parts are shown on a logarithmic scale on the abscissa, and DPPH radical scavenging activity is on the ordinate to show a composite graph of all parts and experiments. Solid lines represent hyperbolic dose-response curves generated using non-linear regression analysis in the graph pad prism. The trend of curves showed that inhibition increased with the increase in the

concentration of inhibitors till attaining the maximum.

Several studies on different medicinal plants reported the correlation of alpha-amylase activity with that antioxidant activity. [27] revealed the possible use of antioxidant and alpha-amylase inhibitory potential for therapeutic applications of diseases emphasizing diabetes. Their results showed that aqueous and ethanol extracts of Pteris vittata L possess potent anti-hyperglycemic potential due to polyphenolic compounds possessing antioxidant and alpha-amylase inhibitory properties. Our results are consistent with this study by showing the same for ethanol and aqueous extract of AM. [28] also reported the correlation between alpha-amylase and the antioxidant potential of various Australian and Indian medicinal plants. Additionally, [5] studied the alpha-amylase inhibitory potential of 126 extracts of 17 different Indian medicinal plants, suggesting that alpha-amylase is a potential drug target for managing type 2 diabetes [5]. Our data also agree with all these studies by introducing AM, which is usually used as a fodder crop, as a natural source for the type 2 diabetes management by using its alpha-amylase inhibitory potential in correlation with its antioxidant potential.

4. Conclusion

The following conclusions can be drawn from our study. *First*, Gum of AM possesses high alpha-amylase inhibitory potential and thus can be used as a natural source to target pancreatic amylase in patients with diabetes by preventing the hydrolysis of polysaccharides to oligosaccharides. *Second*, the dual-mode action of AM, i.e., antioxidant and amylase inhibitor make it a better source for diabetes management. The implication and novelty of the study is that, to our knowledge, this study is the first to report the antidiabetic and antioxidant properties of AM using pancreatic amylase as a drug target from Khyber Pakhtunkhwa, Pakistan using pancreatic amylase as a drug target. As for future directions, however, in future compound isolation and purification as well as in vivo assays are needed to be done.

Acknowledgments

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