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Preliminary Phytochemical Screening and *In-Vitro* Alpha Amylase Inhibitory Activity of Aqueous Methanolic Extract of *Acacia catechu*

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Abstract

This study investigated the phytochemicals and therapeutic effects of *Acacia catechu* aqueous methanolic extract by *in-vitro* alpha-amylase inhibitory activity. Hardwood of *Acacia catechu* was extracted with aqueous methanol using soxhlet extraction method and tested for presence of phytochemical constituents and α-amylase inhibitory activity. The findings showed that the extract had a presence of 12 phytochemicals except flavonoids, anthocyanin and betacyanin. The extract exhibited inhibitory effect on α-amylase (14.1342±0.6611 µg/mL). The findings suggest that hardwood of *Acacia catechu* may be used as a potential source for the development of new oral hypoglycemic agent.

Keywords: *Acacia catechu*, Phytochemicals, α-Amylase inhibition, oral hypoglycemic agent.

1 Introduction

Phytochemicals occur naturally in the leaves, vegetables, fruits, roots and barks of plants [1]. Phytochemicals exhibit defense mechanism and exploited for curing various human diseases. Phytochemicals are biologically active compounds either called as primary and secondary metabolites. Proteins, common sugars and chlorophyll are called as primary constituents and terpenes, nitrogen-containing secondary metabolites such as phenolic compounds and alkaloids are called as secondary metabolites [2,3]. Phytochemicals exhibit vital pharmacological activities like anti-inflammatory, inhibition of cholesterol synthesis, anti-viral, anti-malarial, anti-bacterial activities, anti-cancer and anti-diabetetic activities [4]. Diabetes mellitus is a chronic metabolic disorder caused by inherited or acquired deficiency in insulin secretion and by decreased sensitivity of the organ to secrete insulin. Such a deficiency results in increased glucose level in blood which in turn can damage several functions, including blood vessels and nerves [5]. One of the remedial approaches is to reduce the postprandial hyperglycemia by slow down the absorption of glucose by inhibition of carbohydrate hydrolyzing enzymes, such as alpha-amylase and alpha glucosidase [6]. From this point of view, many efforts have been made to look for more effective and safe inhibitors of alpha-amylase from natural materials to develop a physiological system to treat diabetes [7]. Many traditional plants have been reported in India for diabetes, but only a small number of these have received scientific and medical evaluation to assess their efficacy.

Acacia catechu belonging to the family Leguminosae is used by many traditional healers in most of the herbal preparations [8]. It is considered as an astringent and used in the Indian sub-continent in Ayurvedic medicine [9]. Folk medicinal practitioners use it for alleviation of pain and for the treatment of gastrointestinal disorders like dysentery, diarrhea, colitis, and ulcers, as well as hemorrhoids and skin eruptions [10]. *Acacia catechu* possesses antioxidants, antimicrobial activities, antipyretic, hepatoprotective, immunomodulatory and anthelmintic activity [11-14]. Considering the above facts in view, this study was aimed to evaluate the phytochemicals and therapeutic effects of *Acacia catechu* aqueous methanolic extract by *in-vitro* alpha-amylase inhibitory activity.

2 Experimental

2.1. Chemicals and reagents

All the chemicals and solvent used were analytical grade and were obtained from Hi-media, India.

2.2. Collection of plant material

The hardwoods of *Acacia catechu* were collected from Yelagiri Hills in Tirupattur District of Tamil Nadu. The collected hardwoods were brought to the laboratory and shade-dried. Shade dried hardwoods were powdered using a blender mixer.

2.3. Preparation of extract

Powdered *Acacia catechu* hardwoods (25 g) were extracted with 200 mL of solvent mixture containing 20 mL distilled water and 180 mL methanol. The sample was

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extracted at ambient temperature for 48 hours. The mixture was filtered and the solvent was evaporated under reduced pressure. The extract was transferred into a conical flask and stored at 4°C.

2.4. Phytochemical screening

Phytochemical constituents of *Acacia catechu* extract were assessed using standard methods [15, 16].

Test for Tannins: About 1 mL of the extract was added to 1 mL 5% ferric chloride. The formation of dark blue or greenish-black indicates the presence of tannins.

Test for Saponins: About 1 mL of the extract was added to 1 mL distilled water and shaken in a graduated cylinder for 15 minutes; the lengthwise formation of a 1 cm layer of foam indicates the presence of saponins.

Test for Quinones: About 1 mL of the extract was added to 1 mL concentrated sulphuric acid. The formation of red color indicates the presence of quinones.

Test for Flavonoids: About 1 mL of the extract was added to 1 mL 2N sodium hydroxide. The formation of yellow color indicates the presence of flavonoids.

Test for Alkaloids: About 1 mL of the extract was added to 2 mL concentrated HCl and Mayer's reagent. The presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides: About 1 mL of the extract was added to 3 mL chloroform and 10% ammonia. The formation of pink colour indicates the presence of glycosides.

Test for Cardiac Glycosides: About 1 mL of the extract was added to 2 mL glacial acetic acid and few drops of 5% ferric chloride. This was under layered with 1 mL of concentrated sulphuric acid. The formation of the brown ring at interface indicates the presence of cardiac glycosides.

Test for Terpenoids: About 1 mL of the extract was added to 2 mL chloroform along with concentrated sulphuric acid. The formation of red-brown color at the interface indicates the presence of terpenoids.

Test for Phenols: About 1 mL of the extract was added to 2 mL distilled water followed by a few drops of 10% ferric chloride. The formation of blue or green color indicates the presence of phenols.

Test for Steroids: About 1 mL of the extract was added to 2 mL chloroform and 1 mL sulphuric acid. The formation of the reddish-brown ring at interface indicates the presence of steroids.

Test for Coumarins: About 1 mL of the extract was added to 1 mL 10% sodium hydroxide. The formation of yellow color indicates the presence of coumarins.

Test for Anthocyanin and Betacyanin: About 1 mL of the extract was added to 1 mL 2N sodium hydroxide and heated for 5 min at 100°C. Formation of bluish-green color indicates the presence of anthocyanin and the formation of yellow color indicates the presence of betacyanin.

Test for Fixed oil: About 1 mL of the extract was added to 1 mL 1% copper sulfate and then added 10% sodium hydroxide. The formation of a blue color indicates the presence of fixed oil.

Test for Reducing sugar: About 1 mL of the extract was added to the 1 mL Fehling A and B solutions and kept in boiling water bath for 5 minutes. The formation of Reddish-brown color indicates the presence of reducing sugar.

Test for Proteins and Amino acids: About 1 mL of the extract was added to 2 mL ninhydrin and kept in boiling water bath for 5 minutes. The formation of bluish violet color indicates the presence of proteins.

2.5. *In-Vitro* α-Amylase inhibitory assay

This inhibition assay of alpha-amylase was determined using a modified procedure of McCue and Shetty [17]. About 250 µL of extract (1.25 to 10 µg/mL) were placed in a test tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) were added to each tubes. The content of the tubes was pre-incubated at 25°C for 10 minutes in 250 µL of buffer solution, after which 250 µL of 1% starch solution was added.

The reaction mixtures were incubated at 25°C for 10 minutes. Then 500 µL of dinitrosalicylic acid (DNS) reagent was added and kept in boiling water for 10 minutes and cooled to room temperature. The reaction mixture was then diluted with 5 mL of distilled water and the absorbance was measured at 540 nm in a spectrophotometer. A control was prepared using the same method except that the extract was replaced with distilled water. The α-amylase inhibitory activity was calculated as follow;

$$\text{Percentage of Inhibition (\%)} = \frac{\text{Ac} - \text{Ae}}{\text{Ac}} \text{ x } 100$$

Where, Ac and Ae are the absorbance of the control and extract. The concentration of extract resulting in 50% inhibition of enzyme activity (IC_{50}) was determined graphically using Microsoft Excel.

3 Results and Discussion

Medicinal plants are used to treat many ailments and are considered to be less toxic than synthetic drugs [18]. Due to their pharmacological activities and economic advantages, scientific researchers have been increasing their interest in the traditional medicinal plants [19]. Table 1 shows the results of various phytoconstituents present in the aqueous methanolic extract of *Acacia catechu.* The extract showed the presence of alkaloids, saponins, quinones, tannins, glycosides, cardiac glycosides, terpenoids, phenols, steroids, coumarins, reducing sugar, fixed oil, proteins and amino acids. Three phytoconstituents namely flavonoids, anthocyanin and betacyanin were absent in the extracts.

Table 1. Preliminary Phytochemical screening of aqueos methanolic extract of *Acacia catechu*

Phytochemicals	Extract
Tannins	+
Saponins	+
Quinones	+
Terpenoids	+
Steroids	+
Flavonoids	–
Phenols	+
Alkaloids	+
Glycosides	+
Cardiac glycosides	+
Coumarins	+
Anthocyanin	–
Betacyanin	–
Reducing sugar	+
Proteins and Amino acids	+

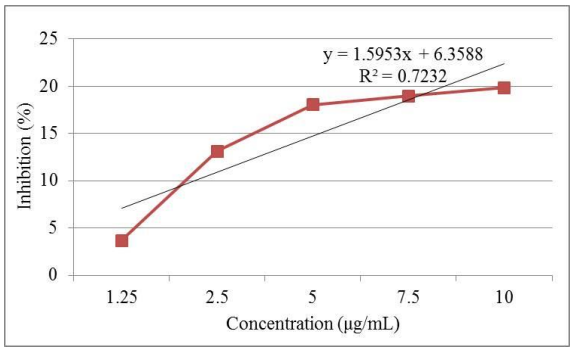
+ Presence; – Absence.

Alpha amylase is an enzyme responsible to catalyzes the hydrolysis of α-1,4-glycosidic linkages of starch, glycogen various oligosaccharides and disaccharides. Alpha amylase inhibitors are known as starch blockers, which prevent the absorption of starch from dietary sources and decreases postprandial glucose level and thus, inhibiting the breakdown of starch may have useful effects on insulin resistance and glycemic index control in diabetic people [20,21]. Table 2 and Figure 1 show the *in-vitro* antidiabetic activity of aqueous methanolic extract of *Acacia catechu.* There was a significant increase in the percentage of inhibitory activity noticed in a concentration dependent manner. The minimum concentration (1.25 µg) of the extract recorded 3.66% inhibitory activity and maximum concentration of 10 µg of the extract exhibited an inhibition percentage of 19.87 against alpha amylase enzyme. The IC_{50} value of the extract against alpha amylase enzyme was found to be 7.639 mg/mL.

Table 2. *In-vitro* antidiabetic activity of aqueous methanolic extract of *Acacia catechu.*

Extract concentration (µg/mL)	Percentage of inhibition	Regression equation	IC_{50} value
1.25	3.66	0.723	7.639
2.50	13.14		
5.00	18.04		
7.50	18.96		
10	19.87		

Fig. 1. *In-vitro* antidiabetic activity of aqueous methanolic extract of *Acacia catechu.*



4 Conclusions

Many people with type 2 diabetes mellitus need anti-diabetic drugs to manage their condition, but medications may be cause fewer side effects. Natural products have anti-diabetic properties which inhibit the carbohydrate hydrolytic enzymes and stimulate the pancreatic insulin secretion. The present study showed that hardwood of Acacia catechu contains many phytochemical constituents that may responsible for its inhibitory effects on alpha-amylase. Thus, the plant extract of hardwood of Acacia catechu may be more effective for type 2 diabetes mellitus through its alpha-amylase inhibition. Further assay and characterizations are necessary to test the extract for various pharmacological and therapeutic activities.

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