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In vitro antidiabetic activity of Important Plants of Caesalpiniaceae Family

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ABSTRACT
About 150 genera and 2,200 species make up the Caesalpiniaceae, which are found primarily in tropical and subtropical regions. Antidiabetic, antiulcer, anticancer, antibacterial, anti-inflammatory, and antirheumatic activity are just some of the pharmacological qualities described for these species, and they have been shown to be effective in ethnomedicinal use. The purpose of this research was to determine the in vitro inhibitory activity of various Caesalpiniaceae plant extracts against pig pancreatic amylase. Ethanol was used to extract the plants. For each extract, we used a rotary evaporator to evaporate the liquid at a low temperature and pressure. Each extract was prepared with dimethyl sulfoxide (DMSO) and then tested for its ability to inhibit the activity of -amylase using starch azure at doses of 20, 60, 120, 200, and 300 µg/mL. With the aid of a spectrophotometer, we were able to measure the absorbance at 597 nm. The IC50values and percentage of α-amylase inhibition was determined using this procedure for each extract. Ethanol extracts of Cassia bonducella, Cassia alata, Caesalpinia coriaria, Brownea coccinea, Cassia javanica, Cassia siamea, Amherstia nobilis, and Bauhinia acuminata showed anti-diabetic action. Findings indicated that plant extract inhibited alpha amylase activity in a dose-dependent fashion. When compared to other Caesalpiniaceae plant extracts, those from Cassia bonducella found most effective. With an IC50 value of 52.23 ± 1.01µg/mL, it showed much higher α-amylase inhibitory action than acarbose (IC50value 14.17 ± 0.34µg/mL). It can be concluded from the study that Caesalpiniaceae possess antidiabetic activity. Further, tests on animals and clinical study are required to make this drug available in the market.
Key words: Alpha amylase, diabetes, hyperglycemia, enzymatic activity, herbal medicine.

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INTRODUCTION
Hyperglycemia caused by insulin secretion abnormalities characterises the metabolic diseases known as diabetes [7]. Diabetes is caused by both dietary choices and hereditary factors. Evidence suggests that as rural India becomes more urbanised, the prevalence of diabetes there has increased by 100%. [5, 12]. Predictions put the number of Indians living with diabetes at 57 million by the year 2025, up from 19 million in 1995. The National Urban Diabetic Survey indicated that the prevalence of diabetes was high in urban areas. Type 2 diabetes accounts for 80% of the cases in these populations [14, 17, 11]. Clinical trials on multiple animal species have demonstrated that eating less can mitigate the onset of diabetes and cardiovascular disease.
Although there has been some progress in treating Type 2 diabetes, prevention is still preferable. To cure Type 2 diabetes, reducing postprandial hyperglycemia is one potential treatment target [8]. Diabetes can now be treated using cutting-edge pharmaceuticals including biguanides, sulfonylureas, and thiazolidinediones. Nonetheless, their applications are not without negative consequences [19, 4].
Diabetes can also be treated with a variety of alternative treatments, most of which are herbal drugs. Herbal medicines have several benefits, including their efficiency, safety, and general appeal. The therapeutic plants and natural items work by blocking the enzymes responsible for breaking down carbohydrates, like pancreatic amylase, which slows down the body's ability to absorb glucose. Delaying glucose absorption and dampening the postprandial plasma glucose rise, inhibition of this enzyme delays carbohydrate digestion and protracts overall carbohydrate digestion time. Several native medicinal herbs show promising results in blocking the action of the α-amylase enzyme [10].

The genus Caesalpinia (Caesalpiniaceae) is very large, and the majority of plant have not been evaluated for their potential pharmacological effect [2]. Caesalpinia species have been shown to contain compounds from a wide variety of chemical families. *Caesalpinia sappan* L., a member of the family Caesalpiniaceae, is a small to medium-sized tree that can reach heights of 4-10 metres and is native to India and Malaysia. It is thorny and shrubby [6]. The plant has been credited with a wide range of pharmacological actions, including those that are anti-diabetic, anti-inflammatory, antioxidant, anti-fungal, anthelmintic, cytotoxic, hepatoprotective, wound-healing, analgesic, anticonvulsant, insecticidal, and antiplasmodial. C. bonduc has been use in the treatment of inflammation and impaired blood circulation in addition to its more well-known uses as an anthelmintic and antimalarial [13, 15, 16, 20]. In the medical and scientific literature, the genus Caesalpinia is praised for its vast range of chemical contents and therapeutic importance. Hypoglycemia may be caused by the phyto-constituents' tannins, flavonoids, triterpenoids, and phenolic compounds, which, according to traditional pharmacological models, have anti-diabetic properties [18]. Caesalpinia (*Cassia bonducella, Cassia coriaria, Cassia alata, Brownea coccinea, Cassia siamea, Cassia javanica, Amherstia nobilis,* and *Bauhinia acuminata*) extracts were tested for their ability to suppress pig pancreatic amylase activity in vitro.

MATERIAL AND METHODS

Chemicals and Culture Media

Sigma Aldrich, USA was procured for our supply of azure starch, porcine pancreatic amylase, and Tris-HCl. The chemicals chloroform, hexane, dimethyl sulfoxide (DMSO), ethanol, and acetic acid were purchased from Merck. The chemicals and solvents used were of the highest standard for analytical use.We have selected eight different plants (*Cassia bonducella, Caesalpinia coriaria, Cassia alata, Brownea coccinea, Cassia siamea, Cassia javanica, Amherstia nobilis,* and *Bauhinia acuminata*), all of which are native to the Mumbai, India and have leaves that are said to have healing capabilities in traditional literature (India).

Collection and extraction of plants

Plant components were gathered from JijimataUdyanof K.J.Somaiya College of Science and Commerce,Vidyavihar, Mumbai, India. After harvesting, the plants were washed twice or three times with regular tap water and once with sterile distilled water before being shade dried, crushed, and utilised in the extraction process. To create the extract, we mixed 50 gram of plant powder with 250 millilitres of 90% ethanol. First, the macerate was filtered through two layers of muslin fabric, and then it was centrifuged at 4000 rpm for 30 minutes. Mother extracts were obtained by filtering the supernatant through Whatman No. 1 filter paper; these extracts were then tested for their antibacterial and antioxidant potency [14].

In vitro α-Amylase Inhibitory Assay

The assay was performed with very minor adjustments to the conventional methodology (Wickramaratne et al., 2016). In 0.2 mL of a 0.5M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$, 2 mg of blue starch was suspended (substrate solution). Five minutes of boiling and five minutes of preincubation at 37°C were used to prepare the substrate solution in the test tubes. Caesalpinia ethanol extract was dissolved in DMSO to make 20, 60, 120, 200, and 300 μg/mL. 0.2 mL of plant extract was then added to the substrate solution. Porcine pancreatic amylase in Tris-HCl buffer was added to the plant extract and substrate solution. A 10-minute reaction at 37°C. The operation was stopped when each tube received 0.5 mL of 50% acetic acid. The reaction mixture was cooled to room temperature after 5 minutes at 3000 rpm and 4°C. Spectrophotometric absorbance of supernatant at 595 nm (Perkin Elmer Lambda 25 UV–VIS spectrophotometer). Different chloroform and hexane plant extracts were tested for beta-amylase inhibition using the same approach. Acarbose, a -amylase inhibitor, was the gold standard.

Statistical Analysis

All values were expressed mean ± SD. Graphpad prism 5 was used to do a comparative analysis and a linear regression.

RESULT

Acarbose showed 84% inhibitory effects on the α-amylase activity with an IC50 value 14.17 ± 0.34 μg/mL at a concentrations 300 μg/mL (Table 1). The ethanol extracts of *Cassia bonducella* exhibited 76.48% of α-amylase inhibitory activity with an IC50 values 52.23 ± 1.01 μg/mL. *Caesalpinia coriaria*extract exhibited 65.49% of α-amylase inhibitory activity with an IC50 values 123.35 ± 5.01 μg/mL.*Cassia alata* extract exhibited 73.29% of α-amylase inhibitory activity with an IC50 values 109.23 ± 4.44μg/mL.*Brownea coccinea* extract exhibited 62.48% of α-amylase inhibitory activity with an IC50 values 189.33 ± 6.34μg/mL.*Cassia siamea* extract exhibited 59.38% of α-amylase inhibitory activity with an IC50 values 199.43 ± 5.22μg/mL.*Cassia javanica* extract exhibited 71.49% of α-amylase inhibitory activity with an

IC50 values 118.34 ± 4.23µg/mL.*Amherstia nobilis* extract exhibited 70.44% of α-amylase inhibitory activity with an IC50 values 113.34 ± 7.12µg/mL. *Bauhinia acuminata* extract exhibited 68.39% of α-amylase inhibitory activity with an IC50 values 179.34 ± 4.65µg/mL (Figure 1).

Table 1: Minimum inhibitory concentration of selected plants of family Caesalpiniaceae

S. No.	Drug/ Plant extracts	Concentration (µg/mL)					IC50 value (µg/mL)
		20 µg/mL	60 µg/mL	120 µg/mL	200 µg/mL	300 µg/mL	
1	Acarbose	60.34	69.42	76.33	81.32	84.32	14.17±0.34
2	*Cassia bonducella*	45.6	55.8	69.9	74.6	76.48	52.23±1.01
3	*Caesalpinia coriaria*	28.4	36.59	49.59	52.49	65.49	123.35±5.01
4	*Cassia alata*	29.49	40.33	55.28	61.44	73.29	109.23±4.44
5	*Brownea coccinea*	20.49	35.3	42.4	58.38	62.48	189.33±6.34
6	*Cassia siamea*	21.33	33.2	46.23	50.44	59.38	199.43±5.22
7	*Cassia javanica*	19.33	30.54	51.39	64.54	71.49	118.34±4.23
8	*Amherstia nobilis*	23.54	40.22	54.59	61.4	70.44	113.34±7.12
9	*Bauhinia acuminata*	18.84	32.43	43.5	58.93	68.39	179.34±4.65

Values are presented as mean ± SD (n=3).

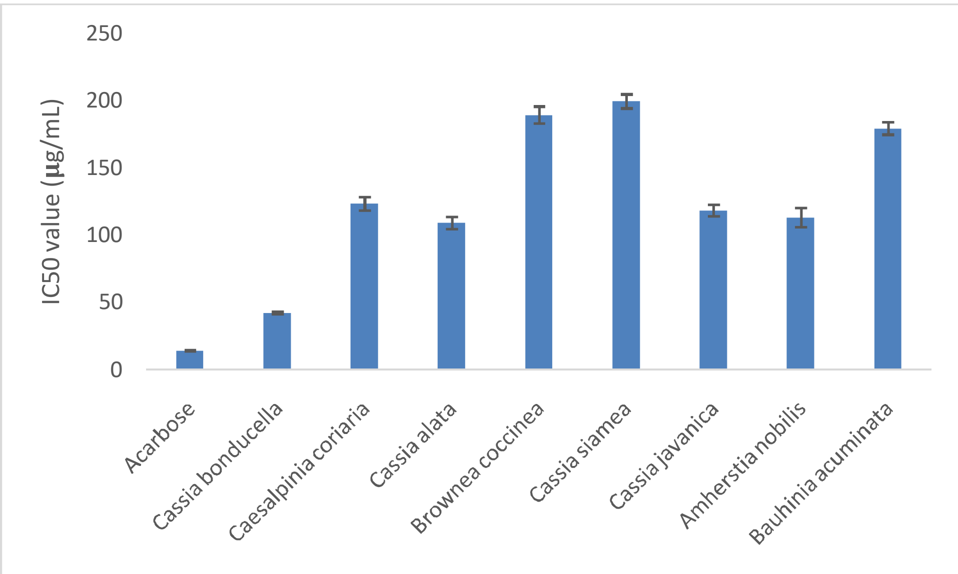

Figure 1 Percentage inhibition of α-amylase activity of ethanol extracts of Caesalpinia family

DISCUSSION

In Ayurveda, many different herbal extracts are utilised to treat diabetes. Many common pharmaceuticals today use herbal extracts either as an ingredient or complementary role. A variety of extracts from the caesalpinia family were tested for their ability to suppress amylase activity. These had led to a progressive reduction in blood glucose, maybe because of an increase in insulin [1]. The whole plant's antidiabetic effect in Type 2 diabetes was revealed in a different investigation. Five fractions of the *Caesalpinia bonducella* seed kernel were tested for their insulin secretagogue activity and their effects on a chronic type 2 diabetic rat by Chakrabarti et al. [3]. Blood sugar levels were observed to be lowered by using the extract. It can lower blood pressure, improve cholesterol and triglyceride levels, and reverse the effects of hyperlipidemia [9]. The half-inhibitory concentration (IC50) of α-amylase inhibition by ethanol extracts from the Caesalpinia family was investigated. The effects of plant extract on alpha amylase activity were found to be dose dependent. Among the Caesalpiniaceae plant extracts, *Cassia bonducella* was shown to be the most significant. It had significant α-amylase inhibitory activity, with IC50 values of 52.23 ±1.01 µg/mL, in contrast to acarbose's IC50value of 14.17 ±0.34 µg/mL.α-amylase inhibitors found in plants may one day be used as a treatment for diabetes (McCue et al., 2004). Compared to acarbose, all Caesalpinia plants in this investigation had much higher α-amylase inhibitory activities.

CONCLUSION

Plants *like Cassia bonducella, Caesalpinia coriaria, Cassia alata, Brownea coccinea, Cassia siamea, Cassia javanica, Amherstia nobilis,* and *Bauhinia acuminata* possess antidiabetic property and the current research validates this.Results demonstrated that several plant ethanol extracts had significant α-amylase inhibitory actions. This research lends credence to the ayurvedic belief that the Caesalpinia plant family may be helpful for diabetes treatment.

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