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In vitro anti-diabetic activity of seed extracts of *Cassia auriculata* and *Cassia angustifolia*

Shravan Kumar Nanumala^{1*}, Tulasi P.² and Errabelli Sujitha³

¹Dept. of Pharmacology, Joginpally B. R. Pharmacy College, Hyderabad, Telangana, India

²Dept. of Pharmacology, Sarojininaidu Vanitha Pharmacy Mahavidyalaya, Hyderabad, Telangana, India

³Dept. of Pharmacology, Jyothishmathi Institute of Pharmaceutical Science, Karimnagar, Hyderabad, Telangana, India

ABSTRACT

To investigate the anti-diabetic potential of seed extracts of *Cassia auriculata* and *Cassia angustifolia* in-vitro. The current study evaluated the Anti diabetic activity of *Cassia auriculata* and *Cassia angustifolia* via in-vitro inhibition of Wheat α -amylase and Yeast α -glucosidase using ethanol seed extracts. Preliminary phytochemical analysis was performed with ethanol seed extracts of *Cassia auriculata* and *Cassia angustifolia*. The α -amylase inhibitory potentials of the extracts were investigated by reacting different concentrations of the extracts with α -amylase and starch solution while α -glucosidase inhibition was determined by pre-incubating α -glucosidase with different concentrations of the extracts followed by the addition of P-nitro, phenyl, glucopyranoside. The ethanolic extract of *Cassia auriculata* and *Cassia angustifolia* exhibited appreciable alpha amylase inhibitory activity with an IC_{50} values $149.6 \pm 0.21 \mu\text{g/ml}$ and $228.8 \pm 1.25 \mu\text{g/ml}$ respectively when compared with acarbose (IC_{50} values $102.76 \pm 0.65 \mu\text{g/ml}$). and glucosidase inhibitory activity with IC_{50} values $134.9 \pm 0.54 \mu\text{g/ml}$ and $170.53 \pm 0.59 \mu\text{g/ml}$ respectively. The results of phytochemical analysis of both extract revealed the presence of flavonoids, polyphenols, triterpenoids and steroids. The observed inhibitions of α -amylase and α -glucosidase suggest that the seed extracts of *Cassia auriculata* and *Cassia angustifolia* may be useful in the management of diabetes mellitus. The results of this work clearly indicate the potential of these extracts to manage hyperglycemia.

Key words: Ethanolic, Alpha amylase, Alpha glucosidase, Acarbose, *Cassia auriculata* and *Cassia angustifolia*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, its insulin action and both [1;2]. It includes a group of metabolic diseases characterized by hyperglycemia. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy [3], neuropathy [4], nephropathy [5], cardiovascular complications[6] and ulceration [7]. Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia [8]. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alphasglucosidase [9]. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch to glucose. Alpha amylase and Glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes [10]. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, abdominal

bloating etc [11]. Therefore natural alpha amylase and glycosidase inhibitors from the dietary plants can be used as an effective therapy for treating postprandial hyperglycemia with minimal side effects. *Cassia angustifolia* (family: Caesalpinaceae) is commonly known as Indian Senna. It is used in the treatment of spleen enlargements, tumors, typhoid, jaundice, rheumatism, gout, skin disorders and bronchitis [12]. *Cassia auriculata* Linn (Family: Caesalpinaceae) commonly known as Tanners Senna, is distributed throughout of India. The plant is used in the traditional system of medicine for urinary disorders, female antifertility, leprosy, worm infestation, diarrhea; bark is used in skin disorder and as astringent; leaves as anthelmintic; seeds for eye troubles, diabetes [13]. The present *in vitro* study was carried out for antidiabetic activity by investigating the inhibitory potentials of the ethanolic seed extract of *Cassia auriculata* and *Cassia angustifolia* on alpha amylase and alpha glucosidase, the key enzymes responsible for hydrolysis of carbohydrates.

MATERIALS AND METHODS

Plant Material

The seeds of *Cassia angustifolia* and *Cassia auriculata* were obtained from ranga reddy local surrounding, Hyderabad, India and were botanically authenticated by the botanists (Department of Botany of the Osmania University). The seeds of *Cassia angustifolia* and *Cassia auriculata* were shade dried for 15 days and Powdered.

Preparation of Plant Extract

The dried seed powder of individual plant was extracted separately using ethanol as a solvent in Maceration method for 72 hr at room temperature. The extracts were filtered while the residue was further extracted under the same condition and extracts was concentrated by simple evaporation under reduced pressure.

Preliminary phytochemical screening

A portion residue from extract was subjected for phytochemical analysis in order to see the presence of phytoconstituents [14].

Extraction of Wheat alpha amylase

Malted wheat flour (500g) was added to 1000ml of 0.2% calcium acetate solution at room temperature with continuously stirred for 2 hours on a stirrer then suspension was centrifuged at 40C at 10000g for 10minutes. The resultant clear brown supernatant was stored at 2°C to 3°C prior to heat treatment. Since beta amylase interferes with the enzymatic determination of alpha amylase it was inactivated by heating the extract at 70°C for 15 minutes. Alpha amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to 6.6 with cold 4% ammonium hydroxide. Heat treatment was carried out at 85°C to 90°C and other at 72°C to 74°C using a water bath with continuous stirring. The extract was then cooled to 2°C to 3°C until use [15].

Determination of Wheat alpha amylase inhibitor activity

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in concentration range 10-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula [16].

$$\text{Inhibition (\%)} = \frac{\text{Abs}(\text{control}) - \text{Abs}(\text{extract})}{\text{Abs}(\text{control})} * 100$$

The IC₅₀ values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

Determination of Yeast alpha glucosidase inhibitor activity

The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts were used in the concentration ranging from 20-100 µg/ml. Different concentrations of plant extracts were mixed with 320 µl of 100 mM phosphate buffer

pH 6.8 at 30 °C for 5 minutes. 3ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any plant extracts [17].

The % inhibition was calculated according to the formula

$$\text{Inhibition (\%)} = \frac{\text{Abs (control)} - \text{Abs (extract)}}{\text{Abs (control)}} * 100$$

Statistical Analysis

All results are expressed as mean \pm standard error. The data was Analyzed using one ways of analysis of variance (ANOVA). The statistical significance of the different of the means was evaluated by Dennett's test.

RESULTS

Preliminary phytochemical screening revealed the presence of steroids, flavonoids, polyphenols, tannins and triterpenoids in *Cassia auriculata* and sterols, triterpenoids, flavonoids, naphthalene glycosides in *Cassia angustifolia*. The inhibitory potential of ethanolic extracts of *Cassia auriculata* and *Cassia angustifolia* on wheat alpha amylase and yeast alpha glucosidase was investigated in this study and the results are shown in Table 1 and 2. In the alpha amylase inhibition assay, Acarbose (at concentration 100 $\mu\text{g/ml}$) showed 50.35% inhibitory effect on alpha amylase activity with IC_{50} values $102.76 \pm 0.65 \mu\text{g/ml}$ at $P < 0.01$. The ethanolic extract of *C. auriculata* and *C. angustifolia* (at concentration 100 $\mu\text{g/ml}$) exhibited 38.94% and 29.56% on alpha amylase inhibitory effect with IC_{50} values $149.6 \pm 0.21 \mu\text{g/ml}$ at $P < 0.01$ and $228.8 \pm 1.25 \mu\text{g/ml}$ at $P < 0.05$ respectively (Table: 1). Both plant extract showed appreciable on alpha amylase inhibition effect when compared with acarbose. In the alpha glucosidase inhibition assay, the ethanolic extract of *C. auriculata* and *C. angustifolia* (at concentration 100 $\mu\text{g/ml}$) exhibited 36.2% and 30.45% on alpha glucosidase inhibitory effect with IC_{50} values $134.9 \pm 0.54 \mu\text{g/ml}$ at $P < 0.01$ and $170.53 \pm 0.59 \mu\text{g/ml}$ at $P < 0.05$ respectively (Table: 2). Both plant extract showed inhibitory effect on alpha glucosidase activity.

Table 1: The percent inhibition of wheat alpha amylase by ethanolic extract of cassia auriculata and cassia angustifolia at varying concentrations

S.No	Concentration ($\mu\text{g/ml}$)	% Inhibition by <i>C.auriculata</i>	IC_{50} value of <i>C.auriculata</i>	%Inhibition by <i>C.angustifolia</i>	IC_{50} value of <i>C.angustifolia</i>	% Inhibition by Acarbose	IC_{50} value (Acarbose)
1	10	16.79	$149.6 \pm 0.21^{**}$	15.86	$228.8 \pm 1.25^*$	18.12	$102.76 \pm 0.65^{**}$
2	20	19.52		17.58		22.25	
3	40	23.48		21.12		28.85	
4	60	26.79		23.63		34.25	
5	80	33.45		27.75		41.17	
6	100	38.94		29.56		50.35	

Table 2: The percent inhibition of Yeast alpha glucosidase by ethanolic extract of cassia auriculata and cassia angustifolia at varying concentrations at varying concentrations

S.No	Concentration ($\mu\text{g/ml}$)	% Inhibition by <i>C.auriculata</i>	IC_{50} value (<i>C.auriculata</i>)	%Inhibition by <i>C.angustifolia</i>	IC_{50} value (<i>C.angustifolia</i>)
1	10	8.61	$134.9 \pm 0.54^{**}$	6.45	$170.53 \pm 0.59^*$
2	20	14.8		14.25	
3	40	22.8		19.85	
4	60	29.4		23.23	
5	80	34.5		28.47	
6	100	36.2		30.45	

Figure 1: % inhibition of wheat alpha amylase enzyme by ethanolic extract of cassia auriculata and cassia angustifolia at varying concentrations and reference alpha amylase inhibitor, Acarbose

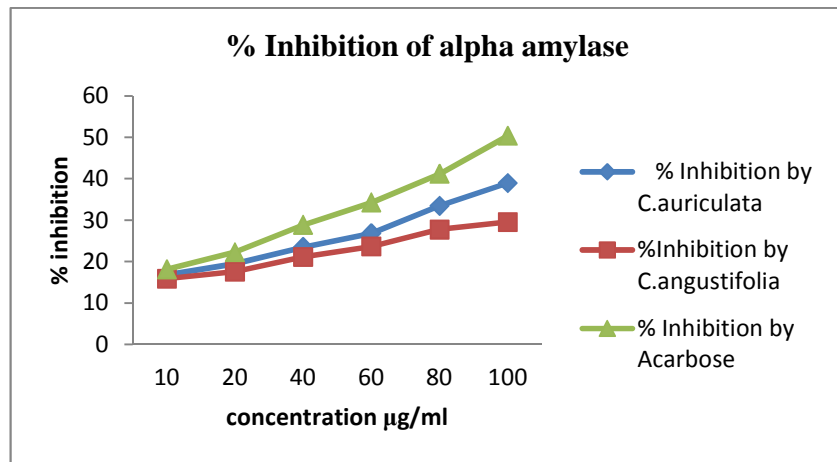


Figure 2 : IC 50 Values on inhibition of wheat alpha amylase enzyme by ethanolic extract of cassia auriculata and cassia angustifolia at varying concentrations and reference alpha amylase inhibitor, Acarbose

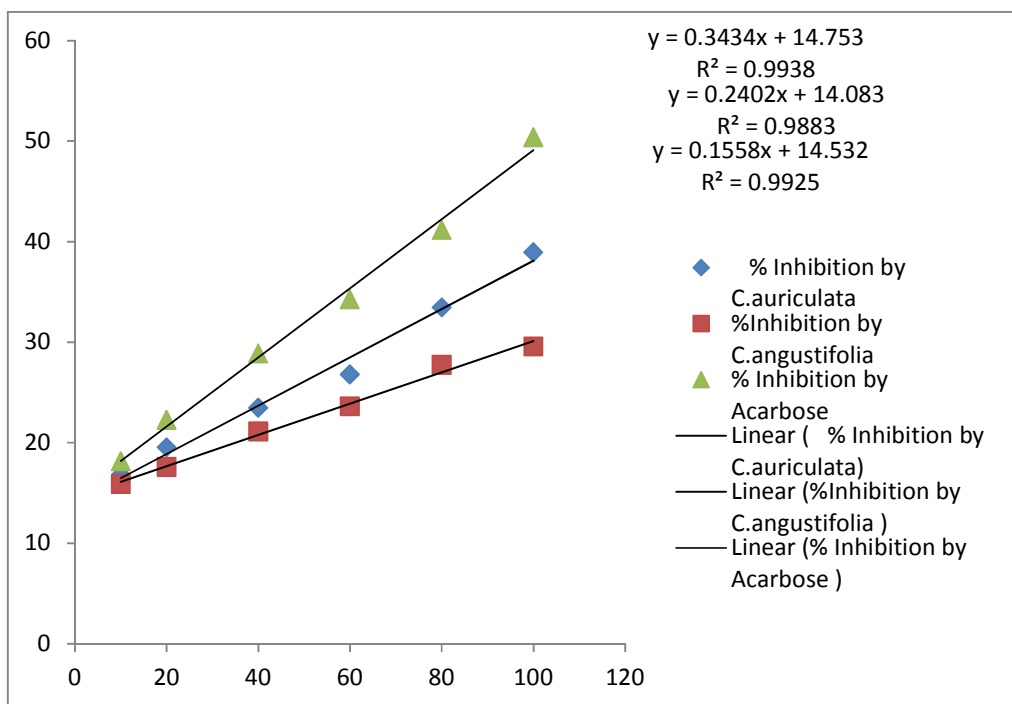


Figure 3: % inhibition of yeast alpha glucosidase enzyme by ethanolic extract of cassia auriculata and cassia angustifolia

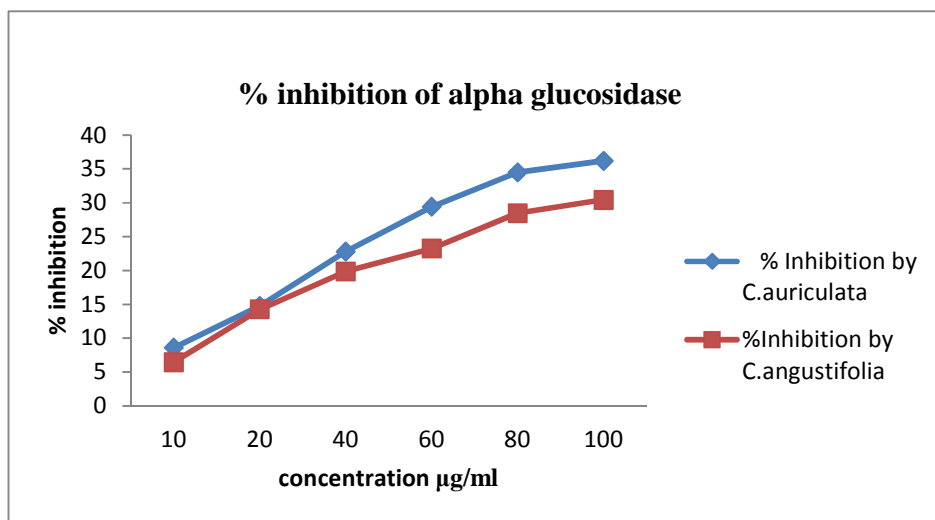
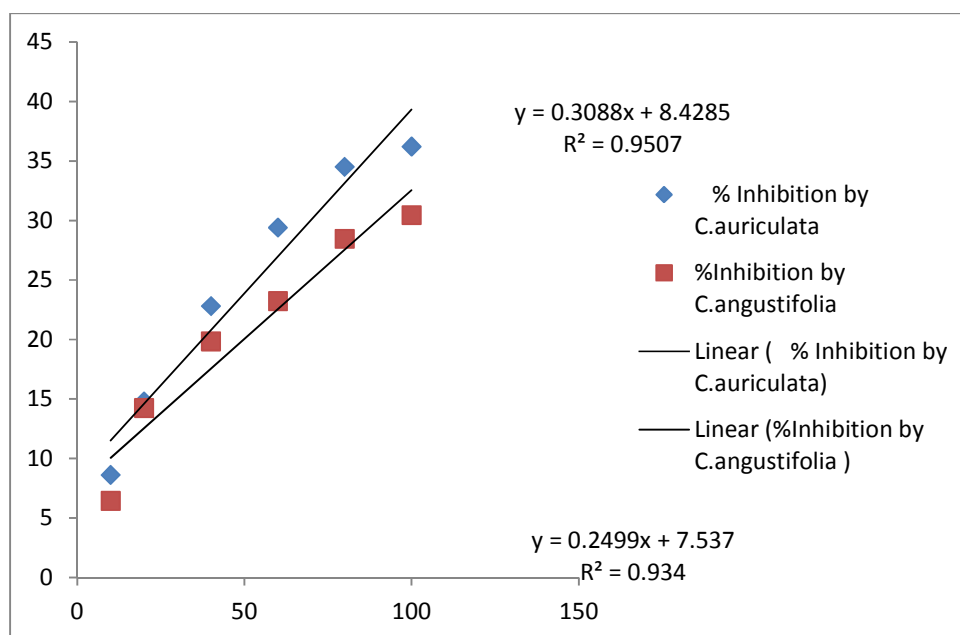


Figure 4: IC 50 Values on inhibition of yeast alpha glucosidase enzyme by ethanolic extract of cassia auriculata and cassia angustifolia



DISCUSSION

Many herbal extracts are used in ayurveda for the treatment of diabetes and have been reported to have antidiabetic activity in the inhibition potential towards alpha amylase and glucosidase activity. In this study, an invitro inhibitory effect of *C. auriculata* and *C. angustifolia* on alpha amylase and alpha glucosidase activities was evaluated. Antidiabetic activity of *C. auriculata* has been reported on its flowers and whole plant part and on leaves of *C. angustifolia*. The various extracts of flowers and whole plant part of *C. auriculata* were successfully tested for diabetes in vivo in the doses range of 150 to 500 mg/kg. These finding had lowered the blood glucose level successfully, which may be due to increase the insulin level in blood [18]. Another previous study had reported for antidiabetic activity with leaves of *C. angustifolia*.

In our study, ethanolic extract of *C. auriculata* (at concentration 100 µg/ml) showed 38.94% and also *C. angustifolia* (at concentration 100 µg/ml) showed 29.56% on alpha amylase inhibitory effect. At the same time, results showed appreciable alpha amylase inhibition when compared with Acarbose. In the alpha glucosidase inhibition assay, the ethanolic extract of *C. auriculata* (at concentration 100 µg/ml) exhibited 36.2% and also *C. angustifolia* (at concentration 100 µg/ml) exhibited 30.45% on alpha glucosidase inhibitory effect. Present study also found active anti-diabetic *C. auriculata* extract contain of high phenolic compound and flavonoid (Rao et al. 2000). Several other classes of chemicals also have been found in *C. auriculata* that are triterpenoids and steroids anthracene derivatives and some dimeric procyanidins. In *C. angustifolia* extract contain alkaloids, flavonoids, glycosides, carbohydrates, proteins, tannins and triterpenoids in *cassia angustifolia* [20]. Our findings also revealed that the ethanolic extracts of the plant extract efficiently inhibited alpha glucosidase enzyme *in vitro*. There was a dose dependent increase in percentage inhibitory activity against alpha glucosidase by two plant extracts. According to results, *C. auriculata* extract showed more inhibitory potential compared with *C. angustifolia* extract. It may be due to the presence of phytoconstitute such as flavonoids, polyphenols, triterpenoids and steroids in responsible for antidiabetic activity in both extracts and proceed further to isolation of active principles responsible for antidiabetic activity. The plant based alpha amylase and glycosidase inhibitory potential offers a prospective therapeutic approach for the management diabetes.

CONCLUSION

In conclusion, more research is required for developing a potential and valuable phytochemical constitute for anti diabetic therapy using alpha amylase and alpha glucosidase inhibitors. Further studies will be carried out to isolation of active principles and elucidate the exact mechanism of action of seed extract of *C. auriculata* and *C.angustifolia* on diabetes and its antiperoxidative effect.

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