Biomedical & Pharmacology Journal, December 2018. Vol. **11**(4), p. 2043-2050

In vitro and *In vivo* Evaluation of Potential Anti Diabetic Efficacy On *Cassia Auriculata* Flowers

G. Nagarajaperumal, V.E. Idachristi, M. Karpakavalli and S. Mohan

Karpagam College of Pharmacy, Coimbatore, Tamil Nadu, India.

http://dx.doi.org/10.13005/bpj/1581

(Received: 30 July 2018; accepted: 30 October 2018)

Diabetes mellitus (DM) is most significant health problem in various developed and developing countries due to alteration of various clinical and pathological factors. Current work was intended for examine *in vitro* and *in vivo* anti diabetic competence of *Cassia Auriculata* flowers and its phytochemical analysis. The inhibitory effects on carbohydrate digestive enzyme á- amylase interaction with various extracts of *Cassia Auriculata* were contrast with acarbose. Hypoglycemic activity was executed with standard as a glibenclamide. Study indicated ethanolic extract showed higher action on á- amylase inhibition with assessment of IC_{50} value; 43.6%. Based on the above results of *in vitro* studies were used to selected ethanolic extract of *Cassia Auriculata* flower used for further study. Results of animal studies indicated that the ethanolic extract of *C. Auriculata* has shown dose dependent action (200 mg/ kg (1.20±0.91"!) and 400 mg / kg (4 ± 0.01"!) when compared to control and standard drug treated groups. Our study confirmed to ethanolic extract work through the á- amylase inhibition mechanism. Our view bioactive constituents confirm anti diabetic capacity and afford methodical source for validation of *Cassia Auriculata* flowers in ayurvedic formulations on diminution of DM prevalence.

Keywords: *Cassia Auriculata,* Acarbose, Glibenclamide, Alfa Amylase and Hypoglycemic.

Free radicals were extremely hasty chemical species frequently produced in the human system by usual organic reactions on various exogenous systems[1]. A number of radicals were involved in the process of biological functions. Consequently, their effects on the organism are plaid by protection system that includes various enzymes which are excoriated oxidative stress and dent cells. Amplified oxidative stress directed diabetes mellitus and complications. Accordingly, endorsement on curative natural agents requires methodical exploration evaluation of effectiveness with variety of *Ex vivo* systems, besides properties involving whole animal preparation. [2]

Identified plant has with splendid alluring brilliant golden yellow blossoms yellow dispersed in dry parts of India and Asia. A dissimilar fraction of plant has been reported on the survey articulate practice of plant against constipation, skin disorder and various other disorders. In addition, flora used to prepared various formulations counteractive effect on DM.[3] Dissimilar extracts ready by use flowers expressed an assortment of actions.[4 and 5] Traditional medicine flower part of *Cassia Auriculata* incited investigated in features, avert oxidation efficiency, anti diabetic action, plummeting clout, shifting ferrous ions, anti-peroxidation and ROS amend capabilities

were *in vitro* conditions. Survey report of *C. Auriculata* has been illustrated assorted limitation were determined whole animal preparation but no proper scientific validation. In current research required to be finding as a plant based new entity for the treatment against diabetes mellitus and also perform complete scientific validation of above plant.[6 and7]

MATERIALS AND METHODS

Identification and authentication

Cassia Auriculata blossoms were collected at Coimbatore District. Plant matters recognized and genuinely checkered at Botanical survey of India, Coimbatore No: BSI / SRC / 5 / 23 / 2012-13 Tech / 496.

Preparation of extract

The blossoms were isolated, washed altogether with water and shade dried for 6 days. 1000 grams of powdered blossoms was subjected to extraction with methanol, ethanol, chloroform, petroleum ether, ethyl acetate (2000ml) in a round bottom flask at room temperature for 15days[8]. After fifteen days decanted and press the mark up to collect the fluidized product which were resolute utilizing rotating vacuum evaporator under lessened weight for collected extract 17.8%.

Phytochemical Test

Screening and identification of phytochemical constituents observational study was done in standard methodology.

Mayer's reagent

Required quantity of mercuric chloride + 60 ml of refine water + 5.0 g of potassium iodide were mix 20 ml of refine water water. Both solutions were mixed and volume was raised to 100 ml with distilled water[9].

Dragendorff's reagent

First preparation: 1.7 grams of basic bismuth nitrate and 20 g of tartaric acid added 80 ml of refine water in a 100 ml standard flask. Second preparation: 16 grams of potassium iodide + 40 ml of refine water. First + Second mixed equal ratio[9].

Test for alkaloids

About 0.6 g of plant extract + 8 milliliter of 1% Hydrochloric acid temperate and filtered. Required quantity of filtrate mixed both reagents such as Mayer's and Dragendorff's

Test for steroids

0.7g gram of plant extract was diverse with two milliliter of acetic anhydride chase by two milliliter of sulphuric acid[9].

Test for terpenoids

Required quantity of plant extract was adding two milliliter of chloroform in a test tube and added three milliliter of concentrated sulphuric acid.[8]

Test for flavonoids

Substance treated alcohol, a couple of magnesium turnings and few drops of concentrated HCL were added and bubbled for five minutes.[9]

Test for tannins

0.5 gram of sample boiled in twenty milliliter of refine water in test tube and filtered.[9]

Test for Phytosterol

Sample was liquefying in Two milliliter of acetic anhydride, animated for sweltering, cooled and one milliliter of concentrated sulfuric acid was added.[9]

1. Foam Test: 5 milliliter test solution taken single test tube was disturbed well for 5 mins.
2. Olive oil test: - Additional a couple of olive oil to required quantity in test tube congaing sample.[9]

Test for glycosides

Keller -Killiani test: Additional required quantity of glacial acetic acid + few drops of 5 % ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.[9]

***In vitro* á- Amylase Inhibition activity [10-11]**

Five hundred micro liter of samples in a test tube and additional to Five hundred micro liter of 0.20 mM buffer of phosphate containing á-amylase solution and incubate at 25°C for 10 minutes. Five hundred micro liter of one percentage starch solution in 0.02 M sodium phosphate buffer additional each tube. Reaction combination was incubate at 25°C for 10 minutes and mix with 3, 5 dinitro salicylic acid colour reagent which incubate boiling water bath for five minutes and cooled to room temperature then make up 10 ml refine water which absorbance estimated at 540 nm.

Proportion inhibition in each examine was intended formulae

Inhibition (%) = [(Control- Test)/ Control] X 100

Experimental animals

Mice for acute toxicity study

The Adult female *S*wiss mice weighing between (20-30 grams) were used to calculate LD_{50}. Housed animals in clean cages and kept up under standard states of light (12 hours amid elective day/night cycles), relative humidity (60-70%) and temperature (26 ± 1 °C). Experimental groups of mice was treated orally with aqueous ethanolic extract of *Cassia Auriculata* leaves at dose of 2000 mg/kg and observed for 15 days to register possible mortality.[12-15]

Preliminary *In vivo* activity

The Adult rats weight were measured range between 200-230 grams and used to perform the hypoglycemic action and Institutional Animal Ethical Committee (1164/ac/08/CPCSEA). [16-21]

Group 1: Control group.

Group 2: Glibenclamide 200µg/kg.

Group 3: *C. Auriculata* flower extract 200mg/kg.

Group 4: *C. Auriculata* flower extract 400mg/kg.

Animals were fasted for 48 hours and weigh the animals. Divided animals above mentioned format. Collected first drop of blood by tail nipping method and glucose level monitored by using Glucometer, which considered as a 0 hour reading. All the extracts and drug administer orally by using oral feeding needle. After the administration glucose level were constantly measured different time intervals ½ and 1 hours.

Statistical Analysis

Results were articulated as mean± SD assesses by one way analysis of variance pursued by Dunnett's technique of several assessment.

Table 1. Phyochemical Analysis

Constituents	Deduction
Alkaloids	+
Steroids	+
Terpenoids	+
Flavonoids	+
Tannins	+
Phytosterol	+
Saponin	+
Glycosides	+

+ = Presence

Table 2. α-Amylase Inhibition of Petroleum Ether extract of *C. Auriculata* leaves

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	28
50	33.5
75	42.3
100	53.1
125	56.1

RESULTS AND DISCUSSION

Appearance and percentage yield of Extract

AEECA (Aqueous Ethanolic Extract of *Cassia Auriculata*) were a semisolid brownish color extract and the percentage yield was found to be 17.8%.

Phyochemical Analysis

The phytochemical screening results revealed that the alkaloids were present due to turbidity formation. Changed from violet to blue was showed steroids. Reddish russet was formed and positive result for presence of terpenoid. Red color observed and present flavonoids. A colour change was observed in the test tube, which point out tannins present. A darker ring was development at the intersection and the turning of the upper layer to dim green which indicated the test for phytosterols present. Below two observations indicated presence of saponins due to formation of stable foam confirmed the test and formation emulsion. Formation of blue and red color. Above two color changes indicated presence of glycosides.

Acute toxicity

Plant a dose of 2000 mg/kg has rejection unfavorable consequences tested with mice up to

Table 3. α-Amylase Inhibition of Ethyl acetate Extract of *C. Auriculata* leaves

Concentration (µg/ml)	Percentage Inhibition(%)
0	0
25	35.1
50	40.2
75	48.3
100	56.2
125	61

15 days of observation. Not toxic signs were absent in the mice. There was no mortality observed and recorded weight loss normal. Based on the above observation fix the doses 200 and 400 mg/kg for anti diabetic activity.

***In vitro* anti diabetic study**

In vitro results revealed that the Alfa amylase percentage of inhibition 33.5% for Petroleum Ether extract of *C. auriculata* Flower by the indication pet ether extract have lesser activity when compared to ethyl acetate extract (40.2%), ethanolic extract(43.6%)of *C. auriculata* leaves and Acarbose (45%). In our comparative results stated that the ethanolic extracts have more alfa amylase inhibition property when compared to other extracts but acarbose have constantly higher activity when compared to extracts. Based on the above results various extracts of *C. auriculata* Flower working mechanism expressed given below:

Squalor of starch and complex glucose to single glucose by Alfa -amylase and Alfa-glucosidase enzymes if suppressed by block glucose absorption. Ultimately, the eminent postprandial blood sugar controlled. Numerous drugs were used to control DM and induction of stress, many are establish to adverse drug reactions. Based on the higher alfa amylase activities of Ethanolic Extract of *C. Auriculata* have been used for *In Vivo* studies.

TLC study

Percentage Inhibition of Petrolium ether extract of *C. auriculata* leaves = 91 µg/ml

Percentage Inhibition of Ethyl acetate extract of *C. auriculata* leaves = 81 µg/ml

Percentage Inhibition of Ethanolic extract of *C. auriculata* leaves = 74 µg/ml

Percentage Inhibition of Acarbose (Positive control) = 62 µg/ml

Minimum Percentage Inhibition was found in ethanolic extract of *C. auriculata* leaves which resemblance to Percentage Inhibition of positive control, So Ethanolic extract of *C.*

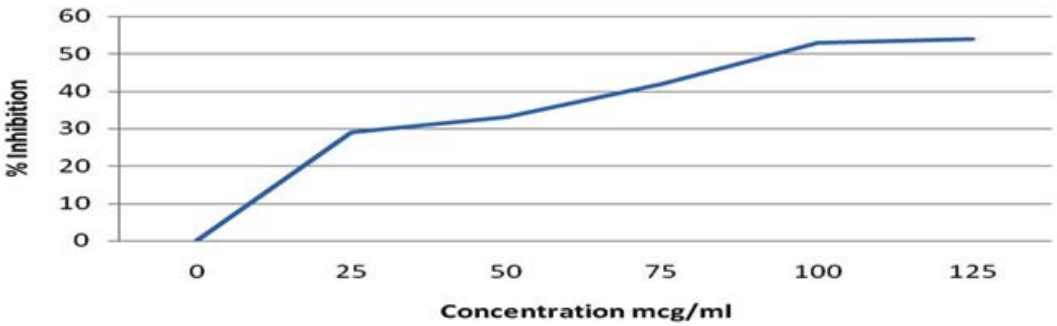

Fig. 1. α amylase inhibition of petroleum Ether extract of *C. Auriculata*

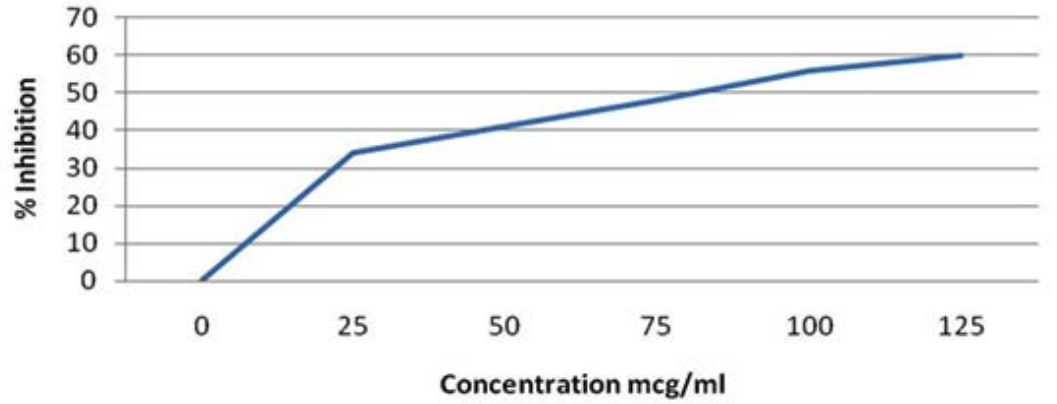

Fig. 2. α amylase inhibition of Ethyl acetate extract of *C. Auriculata*

auriculata contain active constituents of anti diabetic.

R_f Value range high-Polar substances present.

R_f value range low-Low polar substance present.

Solubility of compounds depend upon the polarity of solvents.

Obtained R_f values were confirmed by standard R_f values.

R_f values obtained for my extract ranges from 0.56 to 0.88, So my extract may contain compounds like flavanoids, glycosides and alkaloids. Quantity determination of total phenolics carried out with respect standard curve of gallic acid (r^2= 0.99) showed 115.8 mg of extract and also quercetin standard curve (r^2= 0.994) helps concentration of flavonoids observed114.2 mg of extract.

Hypoglycemic Test

In clinically oral hypoglymic drugs are evaluated by using above preclinical method which help to find the hypoglycemic property for the development of new chemical entity present in the ethanolic extract of *C. Auriculata*The hypoglycemic study demonstrated that the ethanolic extract of *C. Auriculata* flowers two dose levels of glucose level milder changes were

Table. 4. α-Amylase Inhibition of Ethanolic Extract of *C. Auriculata* leaves

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	33.6
50	43.6
75	51
100	55
125	62

Table 5. α-Amylase Inhibition of Acarbose (Positive control)

Concentration (µg/ml)	Percentage Inhibition(%)
0	0
25	25
50	45
75	53
100	56
125	65

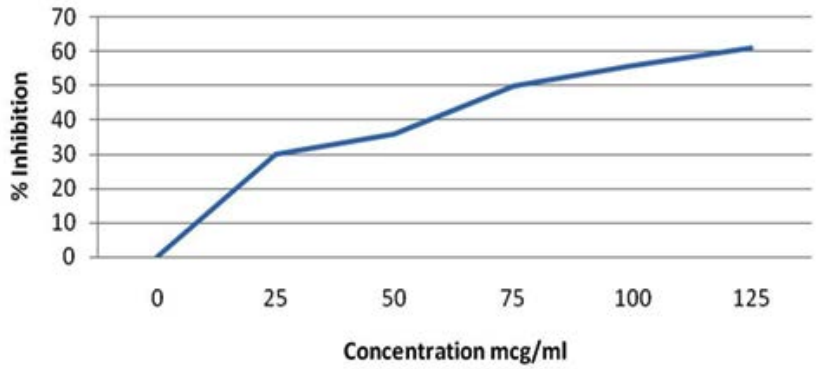

Fig. 3. α amylase inhibition of Ethanolic Extract of *C. Auriculata*

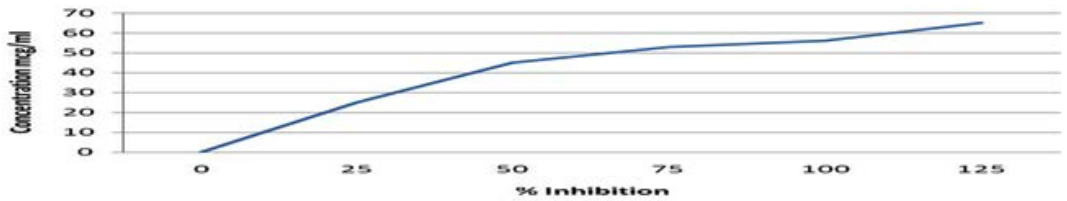

Fig. 4. α-Amylase Inhibition of Acarbose

Table 6. Results of Ethanolic extract of *C. Auriculata*

S. No.	Solvents	Concentration	R_f value
1.	Toluene + Ethyl acetate	7:3	0.61
2.	Toluene + Ethyl acetate + Glacial acetic acid	5:5:1	0.75
3.	Petrolium ether+Chloroform	7:3	0.55
4.	Ethyl acetate + Methanol	1:1	0.89
5.	Hexane+Dichloro methane	1:1	0.68
6.	Ethyl acetate + Methanol	3:1	0.60
7.	Dichloro methane +Hexane	3:1	0.73

Table 7. Hypoglycemic Test

Treatment	Dosemg/kg	Blood Glucose Level (mg/dl)		
		0 min	0.5hr	1 hr
Control Carboxymethyl Cellulose	0.5 %	67.22±0.15	69.05±0.46	70.93±1.87
Positive Control(Glibenclamide)	0.2	68.93±0.54	53.75±1.06***	43.04±1.2***
Ethanolic Extract of *C. Auriculata*	200	68.45±0.76	67.65±1.67	67.95±0.95
Aqueous Ethanolic Extract of *C. Auriculata*	400	68.64±0.74	64.65±0.42*	65.66±0.58*

The blood glucose levels were expressed mean ± standard error and (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to each other groups

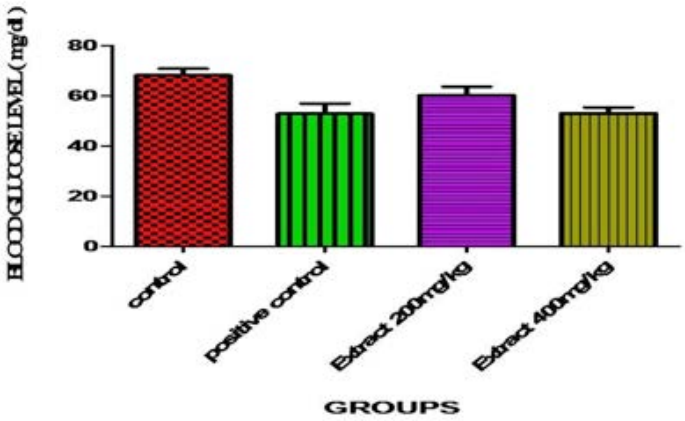

Fig. 5. Hypoglycemic Test – 30 Minutes

expressed in the form of reduction points of glucose level of Ethanolic Extracts of *C. Auriculata* 200 mg/ kg (1.20±0.91“!) and 400 mg / kg (4 ± 0.01“!) at 0.5 hrs when compared to standard drug. One hour later Ethanolic Extracts of *C. Auriculata* 200 mg / kg (0.35 ± 0.72“!), 400 mg / kg (1.1 ± 0.16“!) compared to Glibenclamide 200 µg / kg (10 ± 1.02“!). Based on the above results indicated that the ethanolic extract of C. Auriculata flower is working mechanism similar to acarbose..

CONCLUSION

The results of the present study provides scientific evidence for anti diabetic activity of flowers by the evaluation of various *in vitro* and *in vivo* models and hence supports the therapeutic usage of flowers in traditional medicines for treating DM and its associated complications. This work will be useful for diabetic research workers to

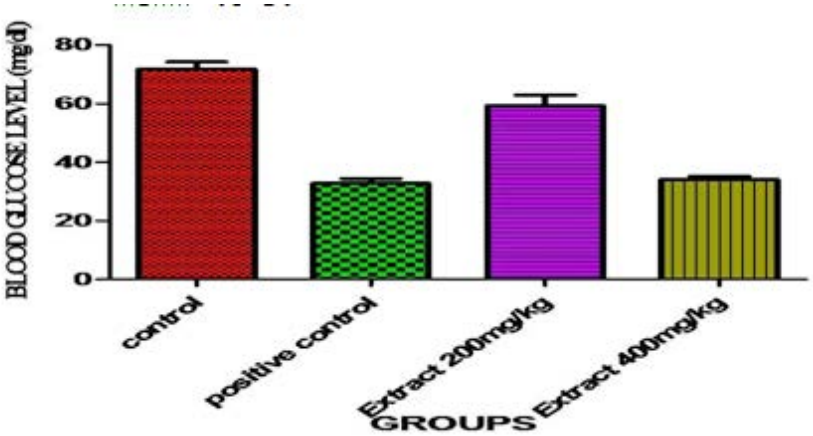

Fig. 6. Hypoglycemic Test – 1st hour

be found the new chemical entity for the treatment of DM and its associated diseases.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Vasanthakumar, Chairman, Karpagam Chairty Trust he valuable help rendered during the study.

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