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In-vitro alpha amylase inhibitory activity (Antidiabetic) of star fruit: *Averrhoa carambola* L.

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Abstract
Indian medicinal plants used in the Ayurvedic traditional system to treat diabetes are a valuable source of novel anti-diabetic agents. *Averrhoa carambola* L. belonging to the family Oxalidaceae, traditionally known as "kamrakh" and commonly known as star fruit because of its peculiar shape. Fruits and leaves are used widely in Ayurveda preparations for diabetic and general debility. The aim of this work was to evaluate the toxicity and α-amylase inhibitory activities of the crude fruit extracts of *A. carambola* in petroleum ether, chloroform, ethyl acetate, acetone and methanol. *In vitro* α-amylase studies result reveled the significant inhibition of α -amylase activity in dose depend manner. Among all the extracts methanolic fruit extract showed maximum of 72% of Inhibition of α- Amylase which was similar to that of acarbose (standard) with 76% of Inhibition. Further *in vivo* study is necessary to confirm the antidibetic activity of star fruit.

Keywords: Star fruit, *in-vitro*, alpha amylase and antidiabetic

Introduction
Diabetes describes a group of metabolic diseases which cause high blood sugar levels. According to the World Health Organization, around 1.5 million people worldwide died due to diabetes in 2019. It is estimated that 463 million people are living with diabetes all over the world. By 2045, projections show this number rising to some 700 million diabetics globally [1]. India has an estimated 77 million people with diabetes, which makes it the second most affected in the world, after China [2]. India's population as calculated in October 2018 was about 17.5% of the global total [3]. In India, type 1 diabetes is rarer than in western countries. Only about one-third of type 2 diabetics in India are overweight or obese [4]. A 2004 study suggests that the prevalence of type 2 diabetes in Indians may be due to environmental and lifestyle changes resulting from industrialization and migration to urban environment from rural.

Type-1 Diabetes mellitus: Type-1 DM can occur due to the improper production of insulin from the pancreas. It is also called as insulin dependent diabetes mellitus. If the patient is having type I diabetes mellitus, they are required to take insulin in every day to stay alive [6]. Studies have demonstrated that the Type 1 DM patients have a better quality of life in comparison to type 2 DM patients [7].

Type -2 Diabetes mellitus: Type 2 DM is a chronic metabolic disorder that can arise from defects in insulin as well as insulin action. Hence, this type of DM can be called as non-insulin dependent diabetes mellitus [8]. Type 2 DM, obesity, and dyslipidemia are considered as independent risk factors for CHD and cerebrovascular disease [9]. Type-2 diabetes mellitus occurs mainly due to lack of physical activity, stress, poor diet and obesity [10].

Gestational Diabetes mellitus: Gestational diabetes mellitus is like type-2diabetes mellitus. This type of diabetes mellitus usually has grown up in some women when they are pregnant. Unfortunately, this condition also results in significant increase in the incidence of macrosomia delivery [11]. The syndrome of gestational diabetes mellitus includes skeletal muscle abnormalities and congenital heart, in some case GD may also cause placental dysfunction, then damages the foetus [12].

Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes [13]. Generally, plants are rich sources of constituents with antidiabetic, antihyperlipedaemic and antioxidant properties such as flavonoids, gallotannins, amino acids and other related polyphenols [14]. Furthermore, medicinal plants having antihyperglycaemic activities are being more desired, owing to their lesser side-effects and low cost [15].

Averrhoa carambola L. is cultivated extensively in India [16] for its edible fruits [17 18]. The leaves and fruits *of A. carambola* have been used in folk medicine as an appetite stimulant, a diuretic, an antidiarrheal, and a febrifugal agent, as well as in the treatment of eczemas [19]. Also, decoction of the leaves has been used in diabetes treatment [20]. Hypoglycemic [21-22], hypocholesterolemic [23], antimicrobial [24], antioxidant [25] and anti-inflamatory [26] effects.

2 Materials and Methods

2.1 Collection of plant material

The fruits of *Averrhoa carambola* L were collected from Rural Technology Park, Rajendra Nagar, Hyderabad district of Telangana state India during the month of August / September in the year 2018. The plant was authenticated by the Department of Botany, Osmania University (O.U), and the specimen was submitted to the Herbarium, Hyderabadens, Department of Botany, O.U, Hyderabad (Accession no. OU-0148).

2.2 Extraction

Dried fruits were powdered in a mechanical grinder, about 25 grams of the powder was packed in a thimble of filter paper prepared manually. The thimble was then inserted into the Soxhlet apparatus and extraction was done by using 250 ml round bottom flask. The fruit powder was extracted successively with Methanol at 65° C and finally stored at 40°C for use [27].

2.3 Alpha-amylase inhibition assay

2.3.1 Chemicals

Potato starch, trichloroacetic acid, Folin-Ciocalteau reagents were purchased from SD Fine Pvt. Ltd., Mumbai, 3,5-dinitrosalicylic acid, Tris buffer, linoleic acid, ammonium molybdate, were purchased from Hi-Media Pvt. Ltd., Mumbai, α-amylase, α-glucosidase enzymes, xanthineoxidase, quercetin, hypoxanthine, pyrocatechol were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Agappe diagnostic Pvt. Ltd., Bangalore, Acarbose was obtained from Bicon Pvt. Ltd., Patancheruvu, Hyderabad, ferrozine, (2'2-azobis (2-amidinopropane) dihydrochloride), butylatedhydroxy toluene from Loba Cheme. All other chemicals used in the study were obtained commercially and were of analytical grade.

2.3.2 Instrument used

UV-visible Spectrometer (Systronic double beam- UV-2201).

2.3.3 Preparation of extract

Fruit extractions used in invitro studies (petroleum ether, chloroform, ethyl acetate acetone and methanol).

2.3.4 Experimental procedure for α-amylase inhibition assay

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 di nitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle [28].

2.3.5 Calculation of 50% inhibitory concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract.

Percentage inhibition (I%) was calculated by

$$I\,\% = \frac{(Ac-As)}{Ac} \times 100$$

Where

Ac = Absorbance of the control,

As = Absorbance of the sample

3. Results

Invitro antidiabetic studies result revels that 125 µg of all extracts i.e., petroleum ether, chloroform, ethyl acetate, acetone and methanol fruit extracts showed the significant alpha-amylase Inhibition when compared with other concentration. *In vitro* α-amylase studies result revels that increased concentration of all the extract has been shown significant inhibition of α -amylase activity hence its dose depend manner. Among all the extract methanolic fruit extract shown the 72% of Inhibition of α- Amylase which similar that of acarbose (standard) 76% of Inhibition of α- Amylase. The data were expressed as the mean ± standard deviation (Table - 1 & Fig 1-8)

Table 1: α-Amylase Inhibition of various extracts of *Averrhoa carambola* fruit

Conc (µg/ml)	Petroleum ether (%inhibition)	Chloroform (%inhibition)	Ethyl acetate (%inhibition)	Acetone (%inhibition)	Methanol (% inhibition)	Acarbose (Positive control) (% inhibition)
0	0	0	0	0	0	0
25	36	32	42	45	34	32
50	39	38	48	49	40	49
75	48	47	55	56	53	60
100	59	58	63	64	66	70
125	60	64	66	68	72	76
Mean ±SD	40.33 ±22.096	39.83 ±22.87	45.66 ±24.10	47 ±24.60	44.16 ±26.08	47.83 ±28.18

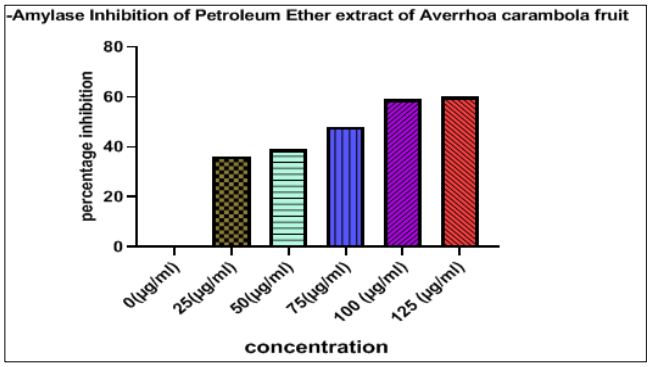

Fig 1: α-amylase inhibition of Petroleum ether fruit extract of *A. carambola*

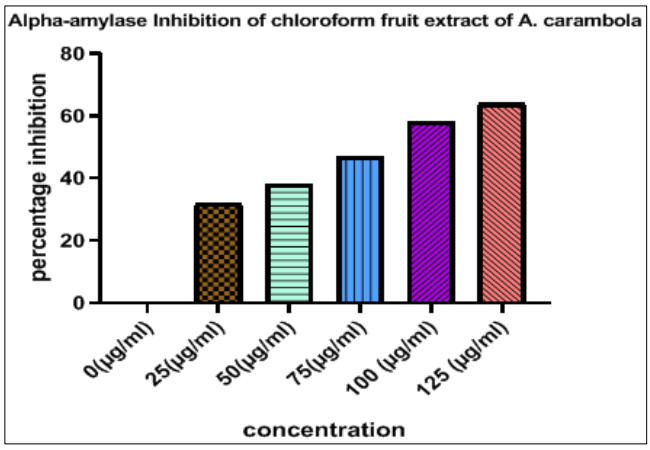

Fig 2: α-amylase inhibition of chloroform fruit extract of *A. carambola*

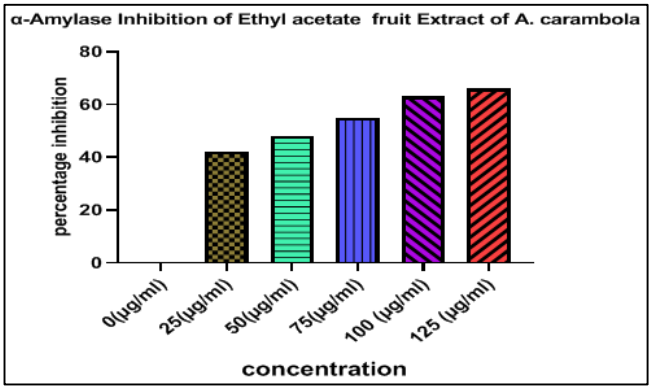

Fig 3: α-amylase inhibition of ethyl acetate fruit extract of *A. carambola*

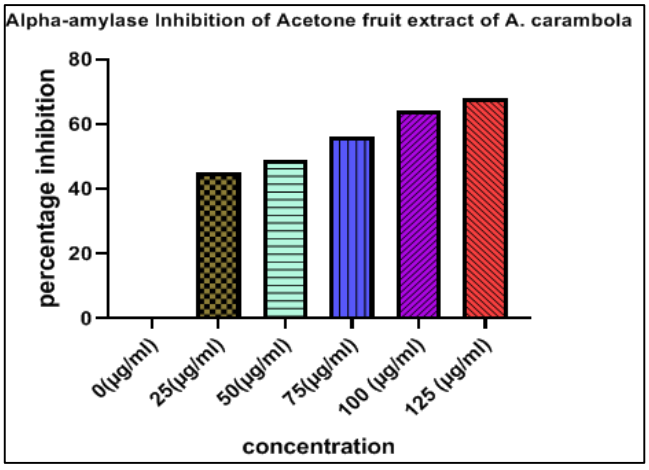

Fig 4: α-amylase Inhibition of Acetone fruit extract of *A. carambola*

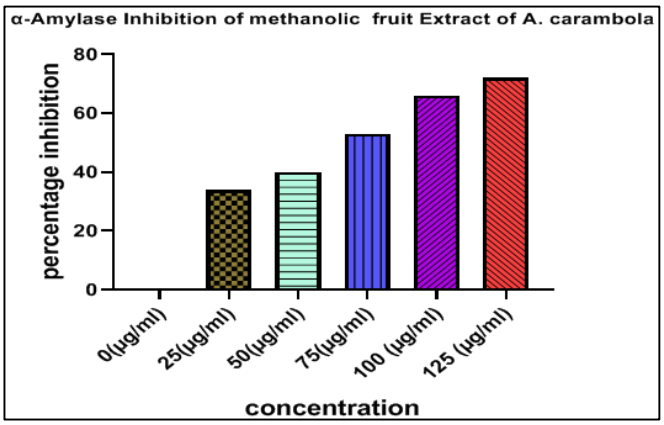

Fig 5: α-Amylase Inhibition of methanolic fruit Extract of *A. carambola*

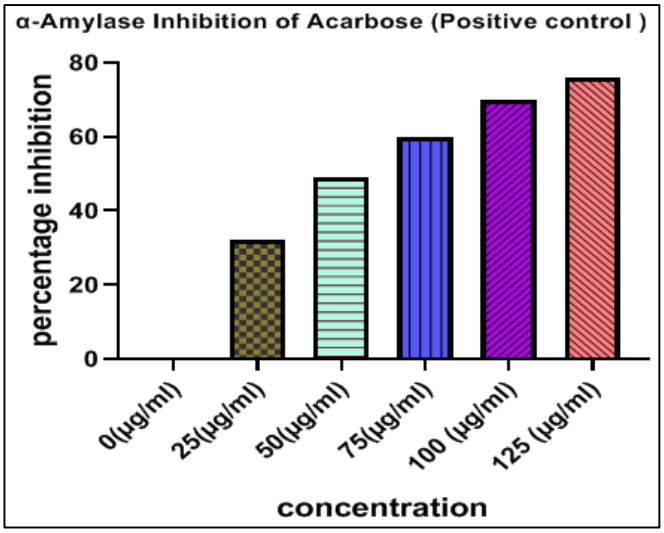

Fig 6: α--amylase Inhibition of positive control

Table 2: Percentage of inhibition

Extract	% of Inhibition
Petroleum ether Extract	60%
Chloroform extract	64%
Ethyl acetate extract	66%
Acetone	68%
Methanol	72%
Acarbose(positive)	76%

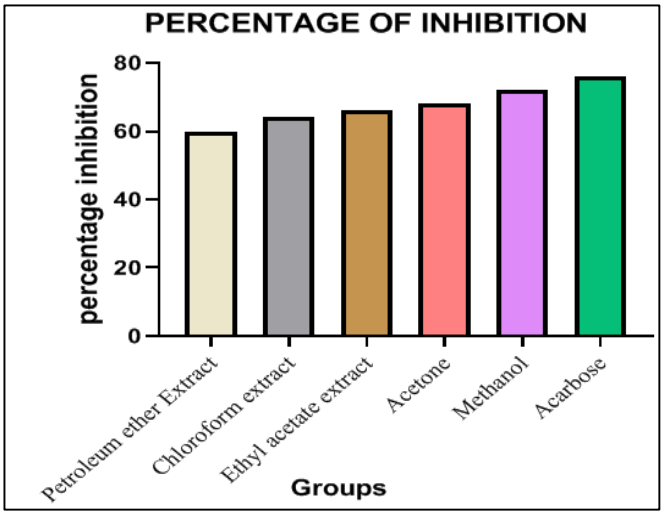

Fig 7: α--amylase percentage Inhibition of various extracts along with positive (Acarbose)

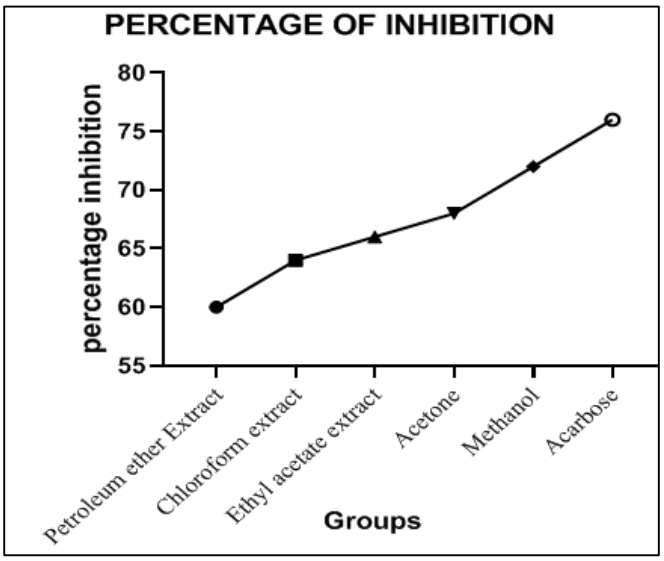

Fig 8: Graphical representation of alpha-amylase percentage Inhibition along with positive (Acarbose)

4. Conclusion

The current study was to evaluate the amylase inhibitory potential of the fruits of *A. carambola.* The crude fruit extracts of *A. carambola* exhibit remarkable α-amylase inhibitory activity of petroleum ether, chloroform, ethyl acetate, acetone and methanolic extracts. The extent of inhibition by the different extracts was compared to the Acarbose (positive). The methanolic extract of the fruits was found to possess maximum potent amylase inhibitor activity and similar to positive control. The results of the work therefore clearly indicate the potential of these extracts to manage hyperglycemia. To understand the inhibitory mechanisms more clearly, further *in vivo* study is required. Furthermore, this study has opened opportunities for future research in searching for novel effective drugs for diabetics that possess anti-diabetic activity.

5. Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

6. Acknowledgement

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