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EVALUATION OF α-AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF *AEGLE MARMELO'S* BY *IN-VITRO* METHOD

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ABSTRACT

An α-amylase inhibitor acts as an anti-nutrient that obstructs the digestion of starch and absorbtion of glucose. Acarbose is a complex oligosaccharides that delays the digestion of carbohydrates, thereby resulting in a smaller raise in blood glucose concentration following food intake. Acarbose, inhibit the action of pancreatic amylase in breaking down starch, thereby achieving this effect. Our finding reveals that *Aegle marmelos* extracts (CAM & PEAM) efficiently inhibits α-amylase suggesting that *Aegle marmelos* extract Is a starch blocker. α-glucosidase inhibitors retard the digestion of carbohydrates to simpler carbohydrates and slows down the absorbtion of the later in the small intestine. Thereby, preventing high glucose concentration in the blood after a meal. The antidiabetic action of *Aegle marmelos* extracts is attributed to the intestinal α-glucosidase inhibitory activity. In conclusion it may be stated that, there occurs selective decrease in the hyper glycemic state after the administration of the *Aegle marmelos* extract.

Keywords: α-amylase, Acarbose, α-glucosidase, and *Aegle marmelos.*

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INTRODUCTION

The first written records on the medicinal uses of plants appeared in about 2600 BC. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. Bael (Aegle Marmelos (Linn), family Rutacae, is also known as Bale fruit tree (Fig-1), is a moderate sized, slender, aromatic tree, 6.0 -7.5 m in height, and 90 to 120 cm in girth, with a some what fluted bole of 3.0-4.5 meter growing wild throughout the deciduous forests of India, ascending to an altitude of 1200 meter in the western Himalayas and also occurring in Andaman island. This is generally considered as sacred tree by the Hindus, as its leaves are offered to Lord Shiva during worship. Aqueous extract of *Aegle marmelos* leaves, was evaluated for hypoglycemic and antioxidant effect by Upadhya, by using alloxon induced diabetes in male albino rats and proposed AML may be useful in the long-term management of diabetes. In the present study we aimed to evaluate the

in vitro α-amylase and α-glucosidase inhibitory activity of *Aegle marmelo's.*

Fig-1 *Aegle marmelos*

MATERIALS AND METHODS

Collection and authentication of plant

The fresh bark of *Aegle marmelos* were collected from the Guntur district, Andhrapradesh. They were identified and authenticated in Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India and a specimen was preserved.

Preparation of the extract

Fresh stem bark of *Aegle marmelos* were collected and air dried in shade under the room temperature. The dried stem bark material was powdered mechanically and sieved through No.20 mesh sieve. The fine powder was kept separately in an airtight container until the time of use. Around 100 g of finely powdered bark material was evenly packed in a soxhlet apparatus and the extraction was done with chloroform and Pet ether for 48 hours. The solvent was then evaporated under reduced pressure. The percentage (%) yield of the extract was calculated. The extracts were named CAM and PEAM respectively (2).

Inhibition of α-amylase using *in vitro* method

Starch solution (1 ml) was mixed with increasing concentration (50, 100, 200, 400, and 800 µg/ml) of the sample CAM and PEAM (0.1ml) and to this 1ml of enzyme solution was added and left to react with starch solution at 25° C for 3 minutes. After this 1ml of colorimetric reagent was added and the contents were heated for 10 to 15 minutes on a boiling water bath. The final volume (13 ml) was made up with distilled water and the absorbance was measured at 540 nm spectrophotometrically (1).

Determination of percentage inhibition

Percentage of inhibition was calculated by using the following equation

$$\text{Percentage inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

Inhibition of α-glucosidase by using *in vitro* method

α-glucosidase activity was determined by incubating a solution (0.1 ml) of enzyme preparation with tris buffer, pH 8 (0.1ml) containing increasing concentration (5, 10, 20, 40, 80 and 100 µg/ml) of the (0.1 ml) sample CAM and PEAM at 37°C for 60 min. The reaction mixture was heated for 2 min in a boiling water bath to stop the reaction. The amount of liberated glucose measured by glucose oxidase method. The absorbance was measured at 540 nm (3).

Determination of percentage inhibition

Percentage inhibition = Enzyme activity in the absence of inhibitor - Enzyme activity in the presence of inhibitor/ Enzyme activity in the absence of inhibitor x 100 (4, 5)

Standard plot for glucose

Glucose concentration 100 µg/ml was used as working solution to plot standard graph (Fig-2).

RESULTS AND DISCUSSION

In vitro α-amylase inhibitory activity

Aegle marmelos (CAM and PEAM) at varying concentrations (100, 200, 400, 800, 1600 µg/ml) were analysed for α-amylase inhibitory action. There was a dose dependent increase in the percentage inhibition for all the concentration tested. The results were listed in the table-1 (6, 7).

Acarbose was used as the standard drug for the determination of α-amylase inhibitory activity. The concentration of acarbose varied from 50 to 1000 µg/ml. Acarbose at concentration 50 µg exhibited a percentage inhibition of 10.88 and for 1000 µg/ml it

was found to be 95.66. A graded increase in percentage of inhibition was observed for the increases in the concentration of acarbose. The IC_{50} value of acarbose was found to be 290 μg/ml. All determinations were done in triplicate and the mean values were determined. An increase in IC_{50} value was observed for *Aegle marmelos* (CAM and PEAM) when compared with the standard drug acarbose (290 μg/ml)

***In vitro* and α-glucoside inhibitory activity.**

The *Aegle marmelos* (CAM and PEAM) showed a significant inhibitory action of α-glucosidase enzyme, which is shown in table-2. There was a proportionate increase in the percentage of α-glucosidase inhibition in all the concentrations of *Aegle marmelos* (CAM and PEAM) (100, 200, 400, 800, and 1600 μg/ml). Acarbose was used as a reference standard for the evaluation of α-glucosidase inhibitory action.

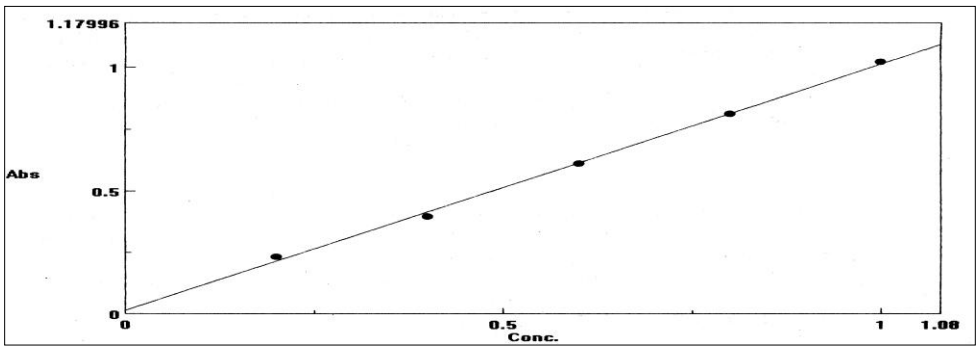
The concentration of acarbose employed in the test varied from 0.1 μg/ml to 2 μg/ml. The dose 0.1 μg/ml produced an inhibitory percentage of 19.45 and the same at 2 μg/ml produced 90.58 inhibition. A dose dependent increase in the percentage of inhibition could be observed for all the concentrations tested. The IC_{50} value of acarbose against α-glucosidase was found to be 0.42 μg/ml. All determinations were done in triplicate and the mean values were determined (8, 9, 10).

Table-1 *In vitro* α-amylase inhibitory activity

concentration	**Percentage In]]]]]hibition(CAM)**	**Percentage inhibition(PEAM)**	**Standard Acarbose**	**IC50value**
100	6.76 ± 0.81	10.88 ± 1.24	16.88 ± 0.14	CAM 220 μg/ml
200	21.14 ± 0.97	24.44 ± 1.46	32.44 ± 1.16	
400	40.13 ± 0.93	43.59 ± 1.01	63.59 ± 1.31	PEAM 166 μg/ml
800	60.97 ± 1.01	68.51 ± 1.21	72.51 ± 0.21	
1600	75.12 ± 1.91	85.07 ± 0.97	95.23 ± 1.47	Acarbose 290 μg/ml

Table-2 *In vitro* and α-glucoside inhibitory activity.

concentration	**Percentage inhibition(CAM)**	**Percentage inhibition(PEAM)**	**Standard Acarbose**	**IC50value**
100	10.75 ± 1.21	19.45 ± 0.89	26.88 ± 0.14	CAM 402 μg/ml
200	26.89 ± 1.46	39.12 ± 0.93	42.44 ± 1.16	
400	36.70 ± 1.31	47.07 ± 0.86	63.59 ± 1.31	PEAM 222 μg/ml
800	46.20 ± 1.27	55.43 ± 1.07	72.51 ± 0.21	
1600	56.43 ± 0.97	70.08 ± 1.23	95.23 ± 1.47	Acarbose 0.44 μg/ml



Slope value = 0.0145

Fig-2 Standard plot for glucose

CONCLUSION

The *in vitro* studies have shown an inhibitory effect on α-amylase and α-glucosidase enzymes which are involved in postprandial hyperglycemia. The present study suggest that *Aegle marmelos* can be successfully utilized for the management of diabetes due to their hypoglycemic action. Further studies on the nature of functional group involved would enlighten the exact mechanism and thus help to rationalize their use in the treatment of diabetes more effectively.

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