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α- Amylase inhibitory activity of *Argyreia nervosa* (Burm. f .) Bojer a medicinal plant in southern western ghats

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ABSTRACT

A. nervosa plant have been historically ascribed an important role in the traditional ayurvedic systems of holistic health and herbal medicine of the east. It is also called elephant creeper. Almost all the parts of the plant like the roots, leaves and the seeds are used in ayurveda for its medicinal purposes. The aim of this study was to screen and determine significant α amylase inhibition of leaves of *A. nervosa* .This study was conducted to Alpha amylase inhibitory activity of different concentrations of ethanol extracts of *A. nervosa* leaves were assessed. The extract showed an IC_{50} value of 143.5 µg/ml. Acarbose was used as a reference standard. The Inhibiting of α amylase enzyme can regulate postprandial hyperglycemia and reduce the risk of developing diabetes.

Keywords: α- Amylase inhibition

INTRODUCTION

A. nervosa is a very attracting climbing plant with showy flowers and heart shaped silver backed leaves. Many pharmacognostical and preliminary phytochemical studies have present in *A.nervosa* plant.(Agarwal, & Rastogi, (1974). *A.nervosa* is best medicinal plant its commen name elephant creeper and related to *convolulaceae* family. Which has many therapeutic properties such as anti-inflammatory, analgesic and wound healing activities(Jeet *et al*., 2012). (Kamal *et al.*, 2012). (singhal *et al.*, 2011). α amylase is very important pancreatic digestive enzyme it offers an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch break down. The present investigation was initiated to screen the ethanol extracts of *A.nervosa* leaves exhibited much α amylase inhibitory activities. (Ponnusamy, *et al*., 2011).

Amylase belongs to the family of glycoside hydrolase enzymes that break down starch into glucose molecules by acting on α-1,4-glycosidic bonds. The α-amylases (EC 3.2.1.1) cleave at random locations on the starch chain, ultimately yielding maltotriose and maltose, glucose and "limit dextrin" from amylose and amylopectin. In mammals, α-amylase is a major digestive enzyme. Increased enzyme levels in humans are associated with salivary trauma, mumps due to inflammation of the salivary glands, pancreatitis and renal failure.

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Fig (1) *Argyreia nervosa* (Burm. f .) Bojer

Material and methods

Sample collection and extract preparation

The leaves of *A.nervosa* (Figure 1) was collected from Lalbag Botanical Garden Bengaluru, India and authenticated (TBGT 91091) in JNTBGRI Thiruvananthapuram Palode, Kerala, India. The collected leaves were shade dried and make in to fine powder and used for the preparation of ethanol extracts. α amylase solution was purchased from Sigma, USA. Sodium phosphate buffer, DMSO, starch, DNS reagent, sodium, potassium tartrate, NaOH was purchased from Merk, USA.

Procedure

The α- amylase inhibitory activity was assessed by the method described by with suitable modification. Briefly, (500, 250, 100, 50 and 10 µg/ml) of the eucalyptus test sample (AN- Ethanol) was remixed with 200 µl of α- amylase solution (1.0 U/ml in phosphate buffer pH 6.9), and incubated at 25^{o}C for 30 min. After pre-incubation, 400 µl of 0.25 % starch solution in the phosphate buffer (pH 6.9) was added to each tube to start the reaction. The reaction was carried out at 37^{o}c for 5 min and terminated by the addition of 1.0 ml of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then kept over a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted by making up the volume to 10 ml of distilled water and absorbent (A) was measured at 540 NM. Control incubations representing 100% enzyme activity were conducted in a similar way by replacing extracts with buffers. For blank incubation (to allow for absorbance produced by the extracts), enzyme solution was replaced by buffers solution and absorbance recorded. The α- amylase inhibitory activity was expressed as percent inhibition and was calculated as follows.

$$\% \text{ Inhibition} = \frac{\text{A control} - (\text{A test} - \text{A background})}{\text{Control}} \times 100$$

Where $A_{control}$, A_{test}, $A_{background}$ represented the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without the enzyme, respectively.

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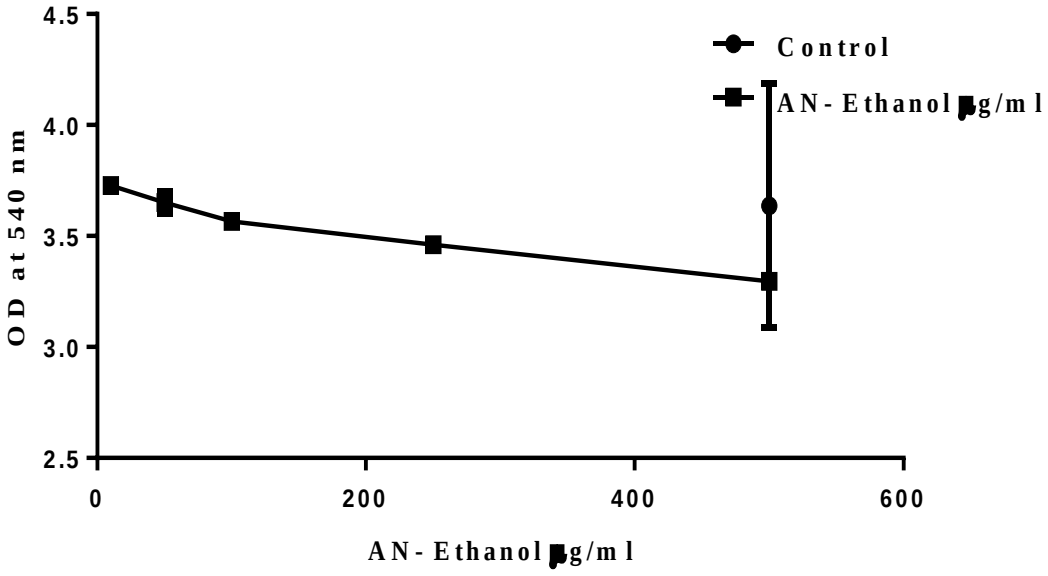
RESULTS

S. No	Tested sample concentration (µg/ml)	OD Value at 540 nm (in triplicates)			Mean OD value
1.	Control	3.985	3.102	3.920	3.669
2.	500 µg/ml	3.294	3.295	3.301	3.296
3.	250 µg/ml	3.451	3.446	3.487	3.461
4.	100 µg/ml	3.545	3.574	3.580	3.566
5.	50 µg/ml	3.616	3.629	3.706	3.650
6.	10 µg/ml	3.714	3.717	3.752	3.727
7.	Acarbose	1.680	1.652	1.884	1.738

OD Value at 540 nm



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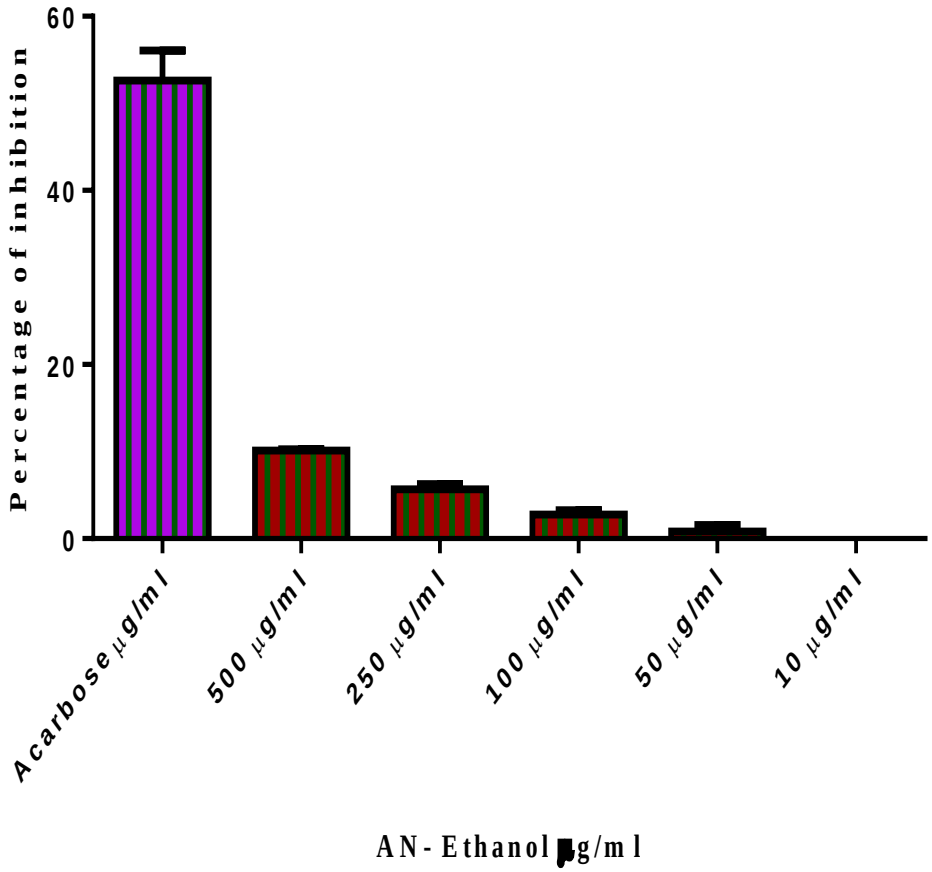
B. **Percentage of inhibition**

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Acarbose	54.21	54.97	48.65	52.61
2.	500 µg/ml	10.22	10.19	10.02	10.14
3.	250 µg/ml	5.94	6.07	4.96	5.66
4.	100 µg/ml	3.37	2.58	2.42	2.79
5.	50 µg/ml	1.44	1.09	0	0.84
6.	10 µg/ml	0	0	0	0



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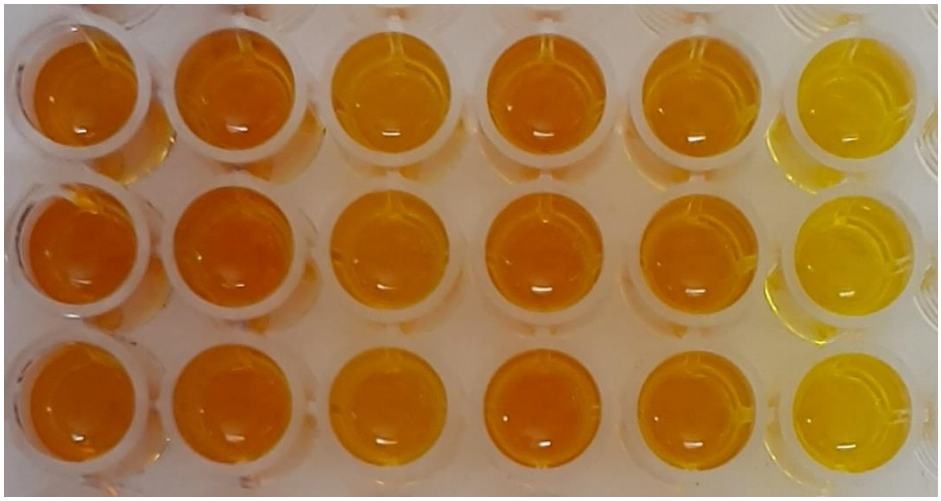
C. **IC_{50} Value of tested sample: 143.5 µg/ml**

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		2.157
HillSlope		-1.620
IC_{50}		143.5
Std. Error		
LogIC50		0.04061
HillSlope		0.2233
95% Confidence Intervals		
LogIC50		2.069 to 2.245
HillSlope		-2.102 to -1.137
IC50		117.3 to 175.6
Goodness of Fit		
Degrees of Freedom		13
R square		0.9413
Absolute Sum of Squares		1098
Sy.x		9.192
Number of points		
Analyzed	3	15



500 µg/ml 250 µg/ml 100 µg/ml 50 µg/ml 10 µg/ml STD Control

CONCLUSION

In the present study plant extracts showed α- amylase inhibition. enzymatic analysis for α-amylase inhibition used ethanol leaf extracts *A.nervosa* was successfully evaluated**.** The

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knowledge about the mechanism of inhibition by medicinal plant *A.nervosa* could give important use of the plant chemicals as drug targets.

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