

**$\alpha$ - AMYLASE INHIBITORY ASSAY OF DIFFERENT FOLIAR EXTRACTS OF ANTHOCEPHALUS CADAMBA (ROXB.) BY USING DNSA METHODS**

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**ABSTRACT**

*Anthocephalus cadamba* is a medium size plant belongs to Rubiaceace family and found in all over in India. Many studies suggest herbal remedy by cadamba to treat diabetes or overcome blood glucose level. Diabetes is a metabolic disorder syndrome. A therapeutic approach for treating diabetes is to decrease the carbohydrates hydrolyzing enzyme such as  $\alpha$ -amylase.  $\alpha$ -amylase is a major digestive enzyme, which hydrolyze the 1,4-  $\alpha$ -linked glycosidic bond of polysaccharides and converts it into disaccharides and trisaccharides. Three different solvents such as petroleum ether, ethanol and distilled water were used for the extraction using soxhlet method and obtained extract were used

for the  $\alpha$ -amylase inhibition assay.  $\alpha$ -amylase inhibition assay was performed by using DNSA i.e 3,5- dinitro salicylic acid. The absorbance was recorded at 540nm by using UV-spectroscopy (PerkinElmer). Result of this study revealed that leaves extract showed  $\alpha$ -amylase inhibition in a dose dependent manner. Three different concentrations i.e 2.5, 5, 10 mg/ml of each extract was used for the present study. The extracts showed maximum inhibition at a concentration of 10 mg/ml which is increased with increasing concentration. The prepared extract showed significant inhibition of  $\alpha$ - amylase at all the selected concentration i.e 2.5, 5, 10 mg/ml with the increase in concentration.

**KEYWORDS:**  $\alpha$ -amylase, Polysaccharides, *Anthocephalus cadamba*, DNSA Method.

## INTRODUCTION

Diabetes is a metabolic disorder syndrome, resulting in abnormal high blood sugar levels, generally occurs due to combination of hereditary and environmental causes.<sup>[1]</sup> It is chronic disease and spreading worldwide. An observation indicate that there were 171 million people in world with diabetes in year 2000 and may be assumed to increase up to 336 million by 2030.<sup>[2,3]</sup>

A therapeutic approach for treating diabetes is to decrease the function of carbohydrates hydrolyzing enzyme such as  $\alpha$ -amylase and  $\alpha$ -glucosidase.<sup>[4]</sup> Both of these enzymes are important for the digestion of carbohydrates.  $\alpha$ -Amylase involves in the breakdown of long chain of carbohydrates.<sup>[5]</sup>

Amylase is a digestive enzyme that aids in the breakdown of carbohydrates by breakings the bonds between sugar molecules in polysaccharides through hydrolysis reaction (i.e chemical breakdown of a molecule by adding water to produce smaller molecule). Amylase is a major digestive enzyme present in the saliva (pH-6.7 to 7) of human and some other mammals. Pancreas and salivary gland make amylase to hydrolyse dietary starch into disaccharides and trisaccharides. It can be present in the animal, plants and bacteria.  $\alpha$ -amylase,  $\beta$ -amylase and  $\gamma$ -amylase are the three main types of amylase. Only  $\alpha$ -amylase is found in the animal tissue.<sup>[6]</sup> It is a protein enzyme that hydrolyses  $\alpha$  bond of large,  $\alpha$ - linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. *Anthocephalus cadamba* (Roxb) is one of the important medicinal plants found in all parts of India. It is used in treatment of various disease like- fever, anemia, diabetes, dysentery, upset stomach and wound healing. It is also known as wild cinchona in English.<sup>[7]</sup> Many studies suggest herbal remedy by cadamba to treat diabetes or overcome blood glucose level. Leave and bark extract of cadamba contains flavonoids and phenols which have antidiabetic effect.<sup>[7]</sup>

## MATERIALS AND METHODS

**Solvent Extraction of *Anthocephalus cadamba* leaves:** Fresh and disease free leaves of *Anthocephalus cadamba* were collected from the botanical garden and used for solvent extraction. Collected leaves were washed with distilled water and shed dried at room temperature. The shed dried leaves were grinded and stored in air-tight container for the further use of extraction with solvents. Three different solvents such as petroleum ether, ethanol and distilled water were used for the extraction using soxhlet method.

### $\alpha$ - Amylase assay by DNSA Method

Alpha- amylase assay was performed by following methodology of Juvekar et al. 2014 and Gayathri et al. 2013.<sup>[8,9]</sup> One twenty micro-liter of plant extract was mixed with 480 $\mu$ l of distilled water and 1.2ml of starch solution (1g starch in 0.02M sodium phosphate buffer containing 0.0067 M of sodium chloride in 100ml) was added. The reaction was initiated by adding 600 $\mu$ l of enzyme solution (1mg of  $\alpha$ - amylase in 10ml of 0.02M of sodium phosphate at PH 6.9) were added into the mixture and kept at room temperature for 3 minutes. After 3 minutes 600 $\mu$ l of the mixture was transferred into separate test tube which contains 300 $\mu$ l of DNSA colour reagent (1g 3,5- dinitrosalicylic acid, 30g sodium potassium traterate and 20 $\mu$ l of sodium hydroxide to final volume of 100 ml in distilled water), test tube were kept into the water bath for 15 minutes at 85-90 <sup>0</sup>C . After water bath sample was allowed to cool down at room temperature and 2.7 ml of distilled water was added into each test tube. The absorbance was recorded at 540nm by using UV- spectroscopy (PerkinElmer). The control was prepared by using 120 $\mu$ l of solvent in place of plant extract. The inhibition % was calculated by using formula.

$$\text{Inhibition \%} = \frac{(\text{Control}_{540} - \text{Sample}_{540}) \times 100}{\text{Control}_{540}}$$

### RESULTS AND DISCUSSION

$\alpha$ - Amylase is a carbohydrates hydrolyzing enzyme responsible for Type (II) diabetes. In present study foliar extract of *Anthocephalus cadamba* has been used to evaluate its ability to inhibit  $\alpha$ -amylase activity by using standard method of Juvekar et al. 2014, Gayathri and Jeyanti et al. 2013.<sup>[8,9]</sup> Result of this study revealed that leaves extract showed  $\alpha$ -amylase inhibition in a dose dependent manner. Three different concentrations i.e 2.5, 5, 10 mg/ml of each extract was used for the present study. The extracts showed maximum inhibition at a concentration of 10 mg/ml which is increased with increasing concentration. The prepared extract showed significant inhibition of  $\alpha$ - amylase at all the selected concentration i.e 2.5, 5, 10 mg/ml with the increase in concentration.

Petroleum ether extract of leaves of *Anthocephalus cadamba* showed significant inhibition at all the concentration. To evaluate the inhibition activity, different concentration of extract were used i.e 2.5, 5, 10 mg/ml which exhibited significant inhibition of  $\alpha$ -amylase i.e 2.37%, 5.21% and 16.62% respectively.

Ethanollic extract of leaves of *Anthocephalus cadamba* showed significant inhibition at all the concentrations. Different concentration for the assay were used i.e 2.5, 5, 10 mg/ml which exhibited significant inhibition of  $\alpha$ -amylase i.e 7.73%, 22.86% and 28.11% respectively. Result of a study on ethanollic extract of bark of *Saraca asoca* also reported that the bark extracts inhibit 95% of  $\alpha$ -amylase action.<sup>[9]</sup>

Aqueous extract of leaves of *Anthocephalus cadamba* also indicated significant inhibition at all the concentration. At the dose of 2.5 mg/ml, 5 mg/ml and 10 mg/ml, distilled water extract showed inhibition of 33.87%, 51.63% and 65.42% respectively. Study of Gayathri and Jeyanthi et al. 2013<sup>[9]</sup> conducted on bark of *Saraca indica* also reported that aqueous extract of bark of *Saraca indica* exhibited 82% inhibition of  $\alpha$ - amylase.

**Table. 1:  $\alpha$ - Amylase Inhibition Assay in Petroleum Ether Foliar Extract of *Anthocephalus cadamba*.**

Sample (concentration)	Volume ( $\mu$ l)	Distilled water ( $\mu$ l)	Starch (ml)	Enzyme solution( $\mu$ l)	DNSA ( $\mu$ l)	Distilled water (ml)	Absorbance at 540nm $\pm$ SD
Control (sample)	120	480	1.2	600	300	2.7	0.842 SD $\pm$ 0.63
Test 1 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.822 SD $\pm$ 0.071
Test 2 (5mg/ml)	120	480	1.2	600	300	2.7	0.798 SD $\pm$ 0.430
Test 3 (10mg/ml)	120	480	1.2	600	300	2.7	0.702 SD $\pm$ 0.210

$\mu$ l= micro litre, ml= milli litre, SD=Standard deviation.

**Table. 2:  $\alpha$ - Amylase Inhibition Assay in Ethanol Foliar Extract of *Anthocephalus cadamba*.**

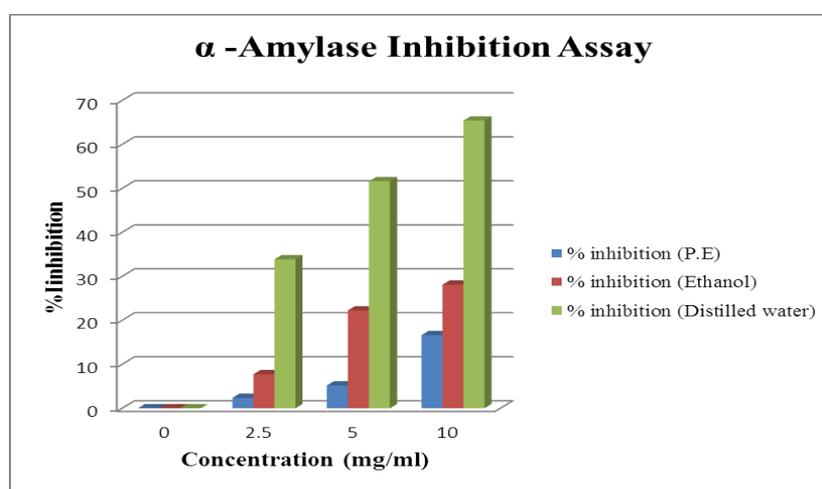
Sample (concentration)	Volume ( $\mu$ l)	Distilled water ( $\mu$ l)	Starch (ml)	Enzyme solution ( $\mu$ l)	DNSA ( $\mu$ l)	Distilled water (ml)	Absorbance at 540nm $\pm$ SD
Control (solvent)	120	480	1.2	600	300	2.7	0.530 SD $\pm$ 0.09
Test 1 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.489 SD $\pm$ 0.260
Test 2 (5mg/ml)	120	480	1.2	600	300	2.7	0.412 SD $\pm$ 0.50
Test 3 (10mg/ml)	120	480	1.2	600	300	2.7	0.381 SD $\pm$ 0.173

$\mu$ l= micro litre, ml= milli litre, SD= Standard deviation

**Table. 3:  $\alpha$ - Amylase Inhibition Assay in Distilled Water Foliar Extract of *Anthocephalus cadamba*.**

Sample (concentration)	Volume ( $\mu$ l)	Distilled water ( $\mu$ l)	Starch (ml)	Enzyme solution ( $\mu$ l)	DNSA ( $\mu$ l)	Distilled water (ml)	Absorbance at 540nm $\pm$ SD
Control (solvent)	NO	480	1.2	600	300	2.7	0.428 SD $\pm$ 0.15
Test 1 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.283 SD $\pm$ 0.060
Test 2 (5mg/ml)	120	480	1.2	600	300	2.7	0.207 SD $\pm$ 0.036
Test 3 (10mg/ml)	120	480	1.2	600	300	2.7	0.148 SD $\pm$ 0.03

$\mu$ l= micro litre, ml= milli litre, SD= Standard deviation.



**Figure. 1: Comparison of  $\alpha$ - amylase enzyme inhibition in various solvent of foliar extract of *Anthocephalus cadamba*.**

## CONCLUSION

*Anthocephalus cadamba* (Roxb) is used in treatment of various disease like- fever, anemia, diabetes, dysentery, upset stomach and wound healing. Diabetes is a metabolic disorder syndrome, resulting in abnormal high blood sugar levels, generally occurs due to combination of hereditary and environmental causes.<sup>[1]</sup> It is chronic disease and spreading world wise. A therapeutic approach for treating diabetes is to decrease the carbohydrates hydrolyzing enzyme such as  $\alpha$ -amylase and  $\alpha$ -glucosidase.<sup>[4]</sup> Pancreas and salivary gland make amylase to hydrolyse dietary starch into disaccharides and trisaccharides. It can be present in the animal, plants and bacteria.  $\alpha$ -amylase,  $\beta$ -amylase and  $\gamma$ -amylase are the three main types of amylase. Only  $\alpha$ -amylase is found in the animal tissue.<sup>[6]</sup> The experiments were carried out for in-vitro  $\alpha$ -amylase activity of powder extract of petroleum ether, ethanol and distilled

water of leaves of *Anthocephalus cadamba*. The absorbance was recorded at 540 nm by using uv-spectroscopy. The plant showed significant inhibition activity in petroleum ether, ethanol and distilled water. The extracts showed maximum inhibition at a concentration of 10 mg/ml which is increased with increasing concentration. The prepared extract showed significant inhibition of  $\alpha$ - amylase at all the selected concentration i.e 2.5, 5, 10 mg/ml with the increase in concentration. In conclusion, more research is required for developing a potential and valuable antidiabetic therapy by using  $\alpha$ - amylase inhibition.

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