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Research Article

**EVALUATION OF  $\alpha$ - AMYLASE INHIBITION ASSAY IN  
VARIOUS FOLIAR EXTRACT OF SARACA ASOCA (ROXB.)****Abhishek Kumar<sup>1\*</sup>, Basant Kumar<sup>2</sup> and Dr. Garima Bartariya<sup>3</sup>**<sup>1</sup>Department of Biotechnology, ITM University Gwalior, Madhya Pradesh, India  
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Email- drgarimabartariya@gmail.com, Whatsapp- 9977188900**Abstract:**

Diabetes is a metabolic illness, caused by the deficiency in production of insulin or ineffectiveness of the insulin. According to the report of WHO on diabetes indicate that in 2014, 8.5% of adults aged 18 years and more than 18 years had diabetes and in 2012 approximately 105 million death were caused by diabetes. A theoretical approach to reduce the risk of diabetes is reduction in the function of carbohydrates hydrolyzing enzymes such as  $\alpha$ -amylase.  $\alpha$ - amylase enzyme is major digestive enzyme and involves in the breakdown of long chain of carbohydrates. It is a protein enzyme that hydrolyses  $\alpha$  bond of large,  $\alpha$ - linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. In this study foliar extracts of *Saraca asoca* were used. For preparation of foliar extract, three different solvents such as petroleum ether, ethanol and distilled water were used. *Saraca asoca* oftenly used in all parts of India as medicine in the treatment of various diseases. Results of this study showed significant inhibition of alpha amylase at all the selected concentration. Distilled water extract showed inhibition of 55.84%, 44.86% and 38.55% respectively, in case of ethanol the reduction was 54.15%, 46.42% and 43.39% respectively whereas in petroleum ether it was 52.85%, 46.42%, 31.95% respectively.

**Keywords:** Diabetes, Foliar extract, DNSA method, *Saraca asoca*,  $\alpha$ - amylase.**Corresponding author:****Abhishek Kumar,**

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**INTRODUCTION:**

Diabetes is a metabolic disorder syndrome, caused by the deficiency in production of insulin or ineffectiveness of the insulin produced by pancreas. The deficiency of insulin results in high concentration of glucose in blood which may damage many cells and tissues of the body [1]. According to the report of WHO on diabetes indicate that in 2014, 8.5% of adults aged 18 years and more than 18 years had diabetes and in 2012 approximately 105 million death were caused by diabetes [2]. Based on insulin production, diabetes is mainly classified into two categories i.e Type 1 (insulin dependent diabetes mellitus) and Type 2 (non insulin dependent diabetes mellitus). Type 1 occurs when  $\beta$  cell of pancreas do not produce insulin or produce it in less amount, and Type- 2 occurs when body resists the effects of insulin or less amount of insulin is produced [3, 4].

An approach to overcome diabetes is to drops the function of carbohydrates hydrolyzing enzyme such as  $\alpha$ -amylase and  $\alpha$ - glucosidase [5]. These enzymes are major digestive enzymes and also important for the digestion of carbohydrates.  $\alpha$ -amylase involves in the breakdown of long chain of carbohydrates through a reaction named as hydrolysis [6]. It is produced by pancreas and salivary gland and present only in animal tissue [7]. In 1831, Eriedrich Leuchs (1800-1837) describe the hydrolysis of starch by saliva, due to the presence of an enzyme in saliva, "ptylin" an amylase [8, 9]. In 1833, French chemists Anselme Payen and Jean-Francois Persoz isolated an amylase complex from germinating barley and named it "diastase [10, 11]. It is a protein enzyme that hydrolyses  $\alpha$  bond of large,  $\alpha$ - linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. Alpha amylase is also known as  $\alpha$ -1,4-glycano hydrolase. It consists of 496 amino acids, one calcium ions, one chloride ions and 170 water molecules. The  $Ca^{++}$  ion bounds to an, asparagine, argine, asparte, histadine, and three water molecules where as the  $Cl^-$  ion bound to an argine, asparagine and one water molecules [12].

*Saraca asoca* is common widespread tree of India. It is small evergreen plant of 7-10 m high. The leaves of this plant are 15-20 cm long with 6-12 leaflets, peripinnates, obolong and lanceolate leaves which are arranged opposite to each other. It is reported that leaves of this plant contains alkaloids, steroids, flavanoids, tannins, saponins, terpenoids, polyphenolics, glycosides and many carbohydrates [13, 14, 15, 16, 17]. *Saraca asoca* contains many medicinal compound and oftenly used in all parts of India as medicine in the treatment of uterine fibroids, leucorrhoea, piles, dysentery, wound healing along with diabetes [18].

**MATERIALS AND METHODS:**

The experiments were conducted in June- July 2017 in ITM University, Gwalior, Madhya Pradesh, India. Disease free leaves of *Saraca asoca* were collected from the botanical garden, washed with distilled water, shed dried and used for solvent extraction. Extraction was done by using three different solvents i.e petroleum ether, ethanol and distilled water. Study was conducted by using DNSA method.

 **$\alpha$ - Amylase assay by DNSA Method:**

Alpha- amylase assay was performed by following methodology of Juvekar et al. 2014 and Gayathri and Jeyanthi 2013 [19, 20]. One hundred twenty micro-liter ( $\mu$ l) 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). 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  $p^H$  6.9) and kept at room temperature for 3 minutes. After 3 minutes 600 $\mu$ l of the mixture was taken out into separate test tube and mixed with 300 $\mu$ l of DNSA color reagent (1g 3,5- dinitrosalicyclic acid, 30g sodium potassium tartrate and 20 $\mu$ l of sodium hydroxide to get final volume of 100 ml in distilled water). Test tubes were kept at 85-90  $^{\circ}C$  into the water bath for 15 minutes. After that 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-visible Sepectroscopy (PerkinElmer). The control was prepared by replacing 120 $\mu$ l of solvent in place of plant extract. The inhibition % was calculated by using formula-

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

**RESULTS AND DISCUSSION:** **$\alpha$ - Amylase inhibition assay:**

$\alpha$ - amylase is a carbohydrate hydrolyzing enzyme reseponsible for Type (II) diabetes. In present study foliar extract of *Saraca asoca* has been used to evaluate its ability to inhibit  $\alpha$ -amylase activity by using standard method of Jevekar et al. 2014 and Gayathri and Jeyanti 2013 [19, 20]. Results of this study revealed that leaves extract of *Saraca asoca* indicate  $\alpha$ -amylase inhibition in a dose dependent manner. Three different concentrations i.e 2.5, 5, 10 mg/ml of each extract were used for the present study. The extracts showed maximum inhibition at a concentration of 2.5 mg/ml and which is decreased with increasing concentration i.e 5 and 10 mg/ml. The prepared extract possess significant inhibition of  $\alpha$ - amylase at all the selected

concentration i.e 2.5, 5, 10 mg/ml with the decrease in concentration.

Aqueous extract of leaves of *Saraca asoca* also indicated significant inhibition at all the concentrations. At a dose of 2.5 mg/ml, 5 mg/ml, 10 mg/ml distilled water extract showed inhibition of 55.84%, 44.86% and 38.55% respectively. Study of Gayathri and Jeyanthi 2013 [20] on bark of *Saraca indica* also reported that aqueous extract of bark of this plant exhibited 82% inhibition of  $\alpha$ -amylase.

Ethanol foliar extract of *Saraca asoca* showed significant inhibition at all the concentration.

Different concentration used for the assay were 2.5, 5, 10 mg/ml which exhibited significant inhibition of  $\alpha$ -amylase i.e 54.15%, 46.42% and 43.39% respectively. Result of a study on ethanolic extract of bark of *Saraca asoca* also reported that the bark extracts inhibit 95% of  $\alpha$ -amylase action [20].

Petroleum ether extract of leaves of *Saraca asoca* showed significant inhibition at all the concentration. To evaluate the inhibition activity of *Saraca asoca* leaves extract, different concentrations of extracts were used i.e 2.5, 5, 10 mg/ml which exhibited significant inhibition of  $\alpha$ -amylase i.e 52.85%, 46.42%, 31.95% respectively.

**Table 1:  $\alpha$ - Amylase Inhibition Assay in Distilled Water Foliar Extract of *Saraca asoca***

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	0.428 $\pm$ SD 0.260
Test 1 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.189 $\pm$ SD 0.090
Test 2 (5mg/ml)	120	480	1.2	600	300	2.7	0.236 $\pm$ SD 0.185
Test 3 (10mg/ml)	120	480	1.2	600	300	2.7	0.263 $\pm$ SD 0.143

$\mu$ l= micro liter ; ml= milli liter ; SD- standard deviation

**Table 2:  $\alpha$ - Amylase Inhibition Assay in Ethanol Foliar Extract of *Saraca asoca***

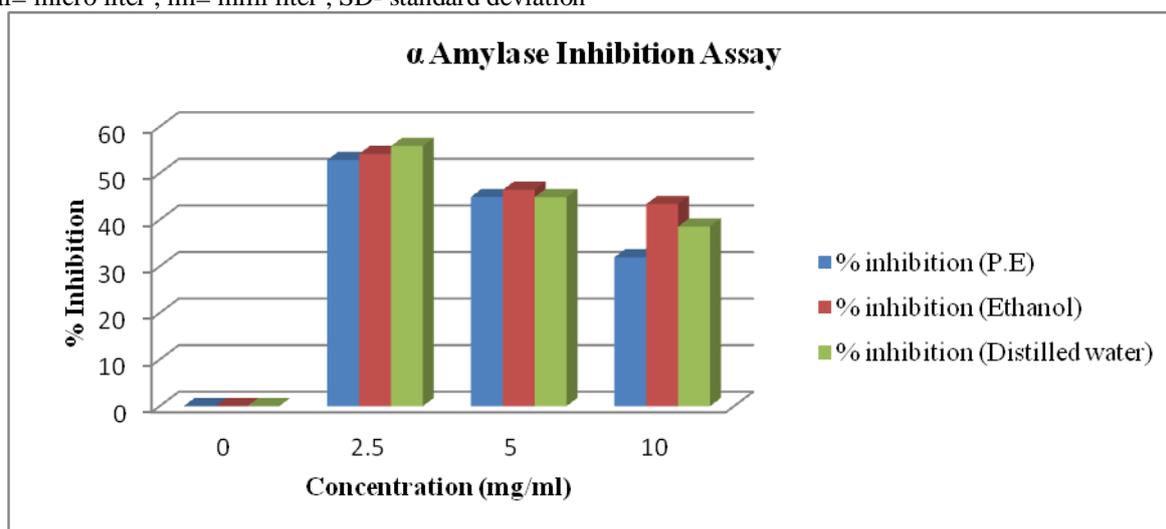
Sample (concentration)	Volume ( $\mu$ l)	Distilled water ( $\mu$ l)	Starch (ml)	Enzyme solution( $\mu$ l)	DNSA ( $\mu$ l)	Distilled water (ml)	Absorbance at 540nm $\pm$ SD
Control (sample)	120	480	1.2	600	300	2.7	0.530 $\pm$ SD 0.09
Test 1 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.243 $\pm$ SD 0.22
Test 2 (5mg/ml)	120	480	1.2	600	300	2.7	0.284 $\pm$ SD 0.256
Test 3 (10mg/ml)	120	480	1.2	600	300	2.7	0.300 $\pm$ SD 0.009

$\mu$ l= micro liter ; ml= milli liter ; SD- standard deviation

**Table 3:  $\alpha$ - Amylase Inhibition Assay in Petroleum Ether Foliar Extract of *Saraca asoca*.**

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	0.842 $\pm$ SD 0.63
Test 1 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.397 $\pm$ SD 0.02
Test 2 (5mg/ml)	120	480	1.2	600	300	2.7	0.464 $\pm$ SD 0.155
Test 3 (10mg/ml)	120	480	1.2	600	300	2.7	0.573 $\pm$ SD 0.115

$\mu$ l= micro liter ; ml= milli liter ; SD- standard deviation

**Fig.1: Comparison of  $\alpha$ - Amylase Enzyme Inhibition in various Solvent of Foliar Extracts of *Saraca asoca*.****CONCLUSION:**

It is reported that leaves of this plant contains alkaloids, steroids, flavanoids, tannins, saponins, terpenoids, polyphenolics, glycosides and many carbohydrates. Presence of various phytochemicals supports its medicinal importance in human health. In ancient time, leaves of ashoka were used to treat disease such as piles, uterine fibroids, diabetes and many others.  $\alpha$  Amylase is a protein enzyme that hydrolyses  $\alpha$  bond of large,  $\alpha$ - linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. Three different concentration i.e 2.5 mg/ml, 5 mg/ml and 10mg/ml of all the selected solvent such as petroleum ether, ethanol and distilled water decreased the  $\alpha$ -amylase activity. At a dose of 2.5 mg/ml, 5 mg/ml, 10 mg/ml, distilled water extract showed inhibition of 55.84%, 44.86% and 38.55% respectively whereas ethanol and petroleum ether revealed inhibition of 54.15%, 46.42% and 43.39% and 52.85%, 46.42%, 31.95% respectively. Hence *Saraca asoca* can be used in treatment of diabetic approach for future prospect.

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