Research Article

Amylase and Lipase Inhibitory Activity of *Spondias pinnata* Leaf Extract

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ABSTRACT

Spondias is genus's of flowering plants in the cashew family, Anarcardeaceae[1]. They are native to the neotropics. They are deciduous or semi evergreen trees. The leaves are spirally arranged, pinnate or simple. It is the tree with rich tradition in the ancient health system of Ayurveda and North-East people for the management of rheumatism.

INTRODUCTION

The fruit spondias pinnata is aromatic, astringent, refrigerant used to give tone and treatment of rheumatic articular and muscular pain. [2]Leaves of this plant are aromatic, acidic and astringent and find use in dysentery. Ethanolic extract of pulp of *S. pinnata* has been reported to show antimicrobial activity [3]. Antioxidant effects of methnolic extract of *S.pinnata* bark has been studied by Hazra et al. [4]. A crude extract of *S. pinnata* has been reported to show antibacterial activity [5]. Gupta et al. [6] reported antimicrobial activity of resin of *Spondias pinnata.*

Absorption of glucose can be delayed by reducing the rate of digestion of starch. Inhibition of the mammalian alpha amylase enzyme in the intestine would delay the degradation of starch and oligosaccharides to monosaccharide's before they can be absorbed. This would decrease the absorption of glucose and consequently reduce postprandial blood glucose level[7]. Therefore, screening of alpha-amylase inhibitors in medicinal plants has received much attention.

Lipase is an enzyme that actively breaks down dietary fats into simpler, absorbable molecules. The main groups of dietary lipids are a class called triacylglycerols. "Lipase hydrolyzes the ester bonds in triglycerides, to form fatty acids and glycerol. Fats require special digestive action before absorption because the end products must be carried in a water medium (blood and lymph) in which fats are not soluble. . Recently, newer approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase (PL) as this is the major source of excess calories. Natural products provide a vast pool of PL inhibitors that can possibly be developed into clinical products.

EXPERIMENTAL SECTION

Collection, Authentification and Extraction of Material

Fresh air leaves were collected in the month of June 2014 from the medicinal garden of our Institute and was authetified. Extraction done in Soxhlet apparatus using 10 gm of air dried drug in 100 ml aqueous alcoholic solution.

Methodology of Amylase Inhibitory activity [11]

Alpha amylase hydrolyses alpha 1, 4-linkages of starch molecules in a random manner. The reducing sugars (mainly maltose) produced by the action of alpha amylase react with dinitrosalicylic acid and reduce it to a brown/orange –red coloured product, nitroaminosalicylic acid. The starch hydrolyzed product concentration under a specified level of alpha-amylase enzyme, with and without inhibitor is used to express the alpha amylase inhibitory activity.

PROCEDURE

METHOD

Preparation of maltose calibration curve; Pipette aliquots of 0.1 to 1.0 ml of maltose (100-1000µg) solution into test tubes and make up the volume to 1ml with suitable addition of distilled water. To each tube add 2ml of dinitrosalicylic acid reagent. Cover tubes with marbles. Keep the tubes in water bath for 10 minutes. Cool the tubes and add 10 ml of distilled water to each test tube. The orange red colour formed is measured at 540nm against a reagent blank.

Determination of α – Amylase inhibitory activity

Preincubate the entire reagents for 15 minutes at 37^0 C in a water bath. Pipette 0.5 ml of 1% starch solution, add it to 0.25 ml of phosphate buffer (0.2M, p H 7) and 0.25 ml of α amylase enzyme solution. Similarly a second set of test tubes (blank) by using phosphate buffer in place of enzyme solution. Prepare a third set of test tubes containing 0.5 ml of starch solution,2ml of dinitro salicylic acid reagent.0.25 ml of α-amylase enzyme solution; this set is called the zero time control. Incubate all the tubes at 37^0C for three minutes. At the end of the incubation add 2 ml of dinitro salicylic acid reagent to first and second set of tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes. After cooling under cold water, add 10 ml of distilled water mix thoroughly and take absorbance at 540nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve. One unit of enzyme activity is defined as that amount which liberate1µmol of reducing sugars (calculated as maltose) /min from soluble starch at 37^0C ,p H 7, and under the specified experimental condition.

Preparation of extract and quantification of α- amylase inhibitor activity

Take 1 gm of sample and extract with 75 ml of distilled water and 75 ml of ethanol for 2 hrs, at 40^0C. Centrifuge the suspension at 5000rpm.Collect the supernatant. Take 0.25 ml and incubate with0.25 ml of enzyme solution for 15 minutes at 37^0C. Incubate all the reagents also at 37^0C for three minutes. At the end of the incubation add 2 ml of dinitro salicylic acid reagent to first, second and sample tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes. After cooling under cold water, add 10 ml of distilled water mix thoroughly and take absorbance at 540nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve. One unit of enzyme activity is defined as that amount which liberate1µmol of reducing specified experimental condition.

LIPASE INHIBITORY ACTIVITY OF THE PLANT EXTRACT

METHOD

Determination of Enzyme Inhibition The potency of crude inhibitor extract to inhibit pancreatic lipase, pancreatic amylase, intestinal glucosidases (maltase,sucrase)was assayed. Pancreatic lipase inhibition was determined by turbidimetric method 20.1 mL of the sample was added to 100 mL of ethanol and shaken vigorously. 1mLof this suspension was added to 9 mL of 0.05MTris-HCl buffer, pH 8.0 containing 0.025 M of sodium deoxycholate. This emulsion was used as substrate. Reaction mixture containing enzyme and inhibitor (in requisite amount) was incubated at room temperature for 10 min. Reaction was started by addition of 1 ml of substrate.

Incubation lasted for 10 min at 37°C. The decrease in turbidity was measured at 660 nm. Inhibitors present in the reaction prevented the decrease of turbidity of the mixture. And compare the decrease in turbidity before and after the addition of substrate.

RESULTS AND DISCUSSION

The maltose calibration curve was plotted, from the graph the concentration at which the sample absorbance value intercepts are taken as the appropriate sample concentration. The percentage α-amylase inhibition of *s.pinnata leaf extract was found to be 57.85%.*

LIPASE INHIBITION BEFORE AND AFTER THE ADDITION OF SUBSTRATE

The turbidity produced is measured at 610 nm using UV Spectrophotometer And the lipase inhibition was found to be 31.04 %.

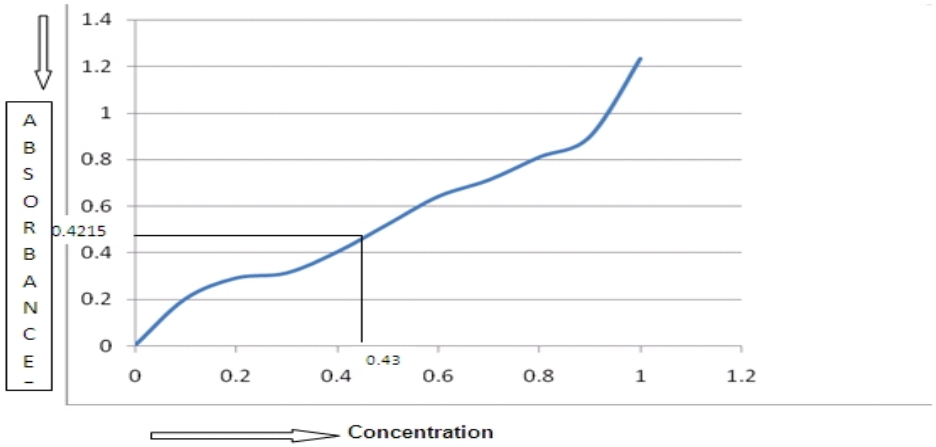
Table 1: Maltose calibration curve

Concentration	Absorbance
0	0
0.1	0.2013
0.2	0.2904
0.3	0.3121
0.4	0.4025
0.5	0.5215
0.6	0.6414
0.7	0.7125
0.8	0.8108
0.9	0.8988
1	1.2346
Extract	0.4215

Table 2: Lipase inhibitory activity

Sample	Before the addition of substrate (%) Inhibition is	After the addition of substrate (%) Inhibition is
Spondias Pinnata leaf extract	21.48	52.52

MALTOSE CALIBRATION CURVE



CONCLUSION

The literature shows a variety of plant species used as antidiabetics in various traditional systems of medicines including species in the family, *Rutaceae*. But few examples of assays with substances inhibiting enzymes that can promote the therapy of diabetes and obsesity. The observed results for compounds of *Spondias pinnata* demonstrate their potential for developing drugs of natural origin for the prevention or treatment of metabolic syndrome in which the inhibition of digestive enzymes is desirable. These compounds differentiate from those currently used in therapy due to their ability to inhibit more than one enzyme complex. And this compound can be used in the treatment of some chronic disorders.

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