



An *In Vitro* Study on the inhibitory Activities of *Eugenia jambolana* Seeds Against Carbohydrate Hydrolyzing Enzymes

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ABSTRACT

Herbal medicines have been used since prehistoric times by different cultures worldwide for treatment of diabetes. In the present investigation, the effect of untreated and heat-treated aqueous extracts from *Eugenia jambolana* Lam (Myrtaceae) seeds on carbohydrate hydrolyzing enzymes, namely, porcine pancreatic α -amylase, rat intestinal α -glucosidase, and sucrase, have been studied using *in vitro* model systems. Untreated *E. jambolana* extract (EJU) significantly inhibited ($p \leq 0.01$) α -amylase, α -glucosidase, and sucrase activities in a dose-dependent manner, with the exception of sucrase, where the increased sample concentration did not increase the sucrase inhibitory activity. Heat treatment of the sample resulted in a significant increase ($p \leq 0.01$) in the α -amylase inhibitory activity of the sample, while a marginal increase in the α -glucosidase and sucrase inhibitory activities were observed, however, they did not reach statistical significance. EJU showed IC₅₀ values of 3.4%, and 68 and 56 $\mu\text{g mL}^{-1}$ for α -amylase, α -glucosidase, and sucrase, respectively, while the IC₅₀ values for heat-treated *E. jambolana* extract (EJH) were 2.4%, and 66 and 54 $\mu\text{g mL}^{-1}$, respectively. Further, a significant correlation ($p \leq 0.01$; $r = 0.833$) was observed between α -amylase, α -glucosidase, and sucrase inhibitory activities of both EJU and EJH. These findings emphasize that inhibition of carbohydrate hydrolyzing enzymes is one of the mechanisms through which *E. jambolana* exerts its hypoglycemic effect *in vivo*.

Key words: A-amylase, α -glucosidase, sucrase

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.^[1] Control of postprandial hyperglycemia is critical in the early treatment of diabetes mellitus,^[2] as it could induce nonenzymatic glycosylation of various proteins, resulting in the development of chronic complications such as, micro- and macrovascular diseases,^[3] and it has also been proposed as an independent risk factor for cardiovascular

diseases.^[4] Postprandial hyperglycemia can be controlled by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes such as α -amylase, α -glucosidase, and sucrase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong the overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption, and consequently blunting postprandial hyperglycemia.^[5] Examples of such inhibitors that are in clinical use are acarbose, miglitol, and voglibose.^[6]

Eugenia jambolana seeds are being used since historic

times for the cure of various diseases and disorders including diabetes,^[7] metrorrhagia,^[8] diarrhea, and dysentery, enlargement of spleen, and as a diuretic in scanty or suppressed urine.^[9] Scientific studies have established various pharmacological effects of different parts of *E. jabolana*, with particular reference to the antidiabetic effect of the seeds. Although a number of studies have established the antidiabetic potential of *E. jabolana* seeds in experimental diabetes,^[10-15] the effect of aqueous extracts on carbohydrate hydrolyzing enzymes, namely, α -amylase, α -glucosidase, and sucrase are not reported. Hence, the present study has evaluated the effect of untreated and heat-treated aqueous extracts of *E. jabolana* seeds on the activity of carbohydrate hydrolyzing enzymes *in vitro*.

MATERIALS AND METHODS

Chemicals and reagents

α -amylase (23 u/mg solid) was purchased from Sigma Aldrich, India. p-nitrophenyl- α -D-glucopyranoside and 3,5-dinitrosalysilic acid were purchased from Sisco Research Laboratory, India. Glucose oxidase peroxidase assay kit was purchased from Agappe Diagnostics, India. All the chemicals and reagents used in the study were of extra pure analytical grade.

Collection of plant material

Eugenia jabolana seed powder was purchased from a local Ayurvedic dispensary. It was dried at 50°C for 1 hour and passed through a 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use.

Heat treatment

The seed powder was subjected to heat treatment in a vacuum oven at 100°C for 60 minutes, cooled in a desiccator and used for the preparation of the heat-treated extract (EJH).

Preparation of extracts

Aqueous extracts (Untreated and heat-treated) were prepared by extracting the untreated and heat-treated seed powders with distilled water (1:8 w/v) on a mechanical shaker, for 24 hours, at room temperature.

Assay of α -amylase inhibitory activity

The effect of *E. jabolana* on α -amylase activity was studied

using an enzyme-starch system.^[16] *E. jabolana* seed powder (1 – 5%) was mixed by stirring with 25 mL of 4% potato starch in a beaker; 100 mg of α -amylase was added to the starch solution, stirred vigorously, and incubated at 37°C for 60 minutes. After the incubation period 0.1 M NaOH was added, to terminate enzyme activity. The mixture was centrifuged (3000 xg; 15 minutes) and the glucose content in the supernatant was determined.

Assay of α -glucosidase inhibitory activity

A crude enzyme solution of rat intestinal α -glucosidase and sucrase, prepared according to the method of Dahlqvist,^[17] was used to assay the α -glucosidase and sucrase inhibitory activities, according to the method of Honda and Hara.^[18] Ten milliliters of enzyme solution and varying concentrations of the aqueous extract (100 – 500 μ g) were incubated together for 10 minutes, at 37°C, and the volume was made up to 210 μ L with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 μ L of 2 mM p-nitrophenyl- α -D-glucopyranoside solution and further incubated at 37°C for 30 minutes. The reaction was terminated by treating the mixture in a boiling water bath for five minutes. After the addition of 1.0 ml of 0.1 M disodium hydrogenphosphate solution, the absorption of liberated p-nitrophenol was read at 400 nm.

Assay of sucrase inhibitory activity

The effect of *E. jabolana* on sucrase activity was assayed according to the method of Honda and Hara.^[18] The enzyme solution (10 μ L) and varying concentrations of the aqueous extract (100 – 500 μ g) were incubated together for 10 minutes at 37°C, and the volume was made up to 200 μ L with maleate buffer (pH 6.0). The enzyme reaction was started by adding 100 μ L sucrose solution (60 mM). After 30 minutes, the reaction was terminated by adding 200 μ L of 3,5- dinitrosalysilic acid reagent and treating the mixture in a boiling water bath for five minutes. The absorbance of the solution was read at 540 nm.

The percent inhibitory activities were calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, *Abs control* is the absorbance of the control reaction (containing all reagents except the test sample), and the *Abs sample* is the absorbance of the test sample. An untreated enzyme solution was used as the control. All the experiments were carried out in triplicate.

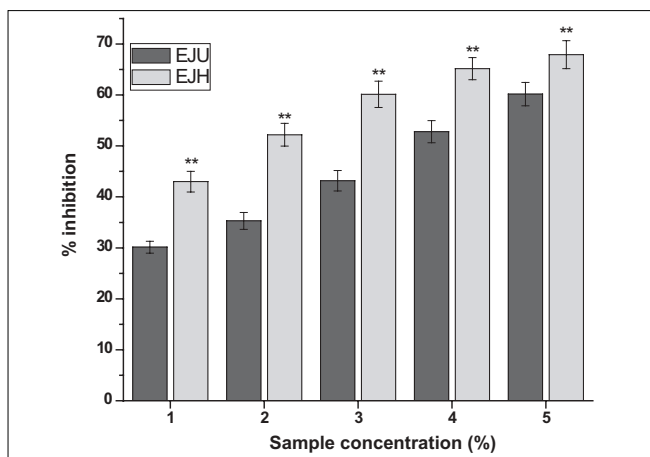


Figure 1: Effect of *Eugenia jambolana* aqueous extracts on α -amylase activity. *EJU: untreated *Eugenia jambolana* extract, EJH: heat-treated *Eugenia jambolana* extract

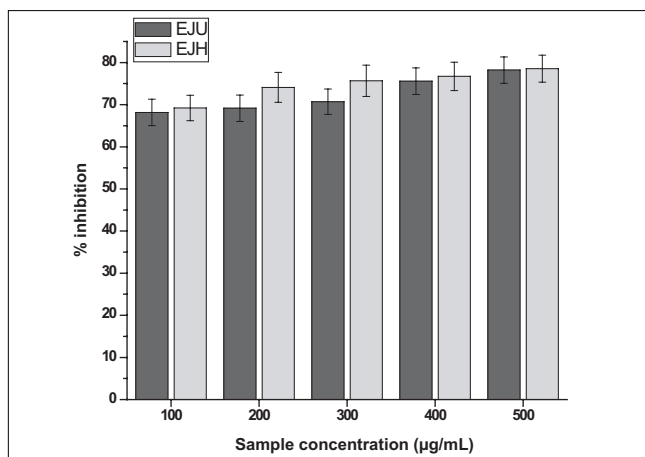


Figure 2: Effect of *Eugenia jambolana* aqueous extracts on α -glucosidase activity. *EJU: untreated *Eugenia jambolana* extract, EJH: heat-treated *Eugenia jambolana* extract

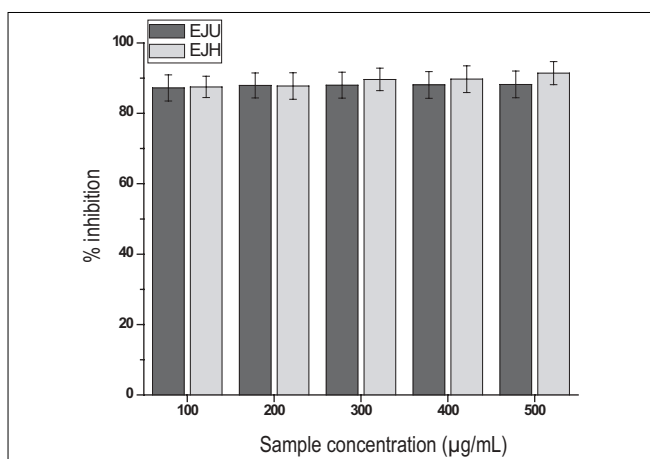


Figure 3: Effect of *Eugenia jambolana* aqueous extracts on sucrase activity. *EJU: untreated *Eugenia jambolana* extract, EJH: heat-treated *Eugenia jambolana* extract

Statistical analysis

The data was analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences, using SPSS 14.0 computer software. The values were considered significant when $p \leq 0.05$. IC_{50} values were calculated by Boltzmann's dose response analysis using Origin 6.1 software.

RESULTS

Effect of *Eugenia jambolana* on α -amylase activity

The α -amylase inhibitory activity of *E. jambolana* was studied using an α -amylase-starch model system and the results are presented in Figure 1. Both untreated and heat-treated extracts exhibited significant inhibition of

α -amylase activity in a dose-dependent manner and the inhibitory activities ranged between 30-60% and 42-67% for EJU and EJH, respectively. The α -amylase inhibitory activity of EJH was significantly higher ($p \leq 0.01$) when compared with that of EJU, and as a result the IC_{50} value of EJH was lower than that of EJU.

Effect of *Eugenia jambolana* on α -glucosidase activity

The α -glucosidase inhibitory activities of EJU and EJH are presented in Figure 2. Both EJU and EJH showed dose-dependent inhibition of α -glucosidase. The inhibitory activities ranged between 68-78% and 69-78% respectively, for untreated and heat-treated *E. jambolana*. Although the IC_{50} value of EJH was comparably lower ($66 \mu\text{g mL}^{-1}$) than EJU ($68 \mu\text{g mL}^{-1}$), no statistical differences were observed.

Effect of *Eugenia jambolana* on sucrase activity

The effect of EJU and EJH on sucrase activity is shown in Figure 3. The sucrase inhibitory activity ranged between 87-88% and 88-91% for EJU and EJH, respectively. Here the dose-dependent inhibition of the enzyme sucrase ceased to exist in both the untreated and heat-treated *E. jambolana* extracts, and their inhibitory effects did not differ statistically between any of the doses tested. Consequently the IC_{50} values also did not differ for EJU and EJH [Table 1].

A significant correlation ($p \leq 0.01$; $r = 0.833$) was observed between α -amylase, α -glucosidase, and sucrase inhibitory activities of both untreated and heat-treated *E. jambolana* extracts, and the enzyme inhibitory activities of *E. jambolana* extracts were directly proportional to the sample concentration, with the exception of sucrase.

Table 1: IC50 values for α -amylase, α -glucosidase, and sucrase activities

Enzyme	EJU	EJH
α -amylase	3.4 ^b \pm 0.73	2.4 ^a \pm 0.09
α -glucosidase	68.3 ^a \pm 1.4	65.7 ^a \pm 1.39
Sucrase	55.9 ^a \pm 1.75	53.9 ^a \pm 1.09

*EJU: untreated *Eugenia jambolana* extract, EJH: heat-treated *Eugenia jambolana* extract IC50 values for α -amylase activity are in %, while for α -glucosidase and sucrase they are in μ g mL⁻¹. **Mean values carrying different superscript letters a, b, c....., in columns, differ significantly ($p \leq 0.05$)

DISCUSSION

The present investigation reports the carbohydrate hydrolyzing enzyme inhibitory characteristics of *Eugenia jambolana* seed extracts. With a constant rise in the incidence of type II diabetes around the world it appears that more antidiabetic drugs with complementary mechanisms of action should be developed, in order to achieve durable glycemic control by inhibiting, in a reversible way, the hydrolysis of disaccharides and the ultimate steps of the digestion of dietary polysaccharides, to reduce the rise of postprandial blood glucose in diabetics.^[19]

Phytochemicals exhibit their hypoglycemic effect by several mechanisms, such as, inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β -cell regeneration, and enhancing the insulin releasing activity.^[20] In the present investigation, both untreated and heat-treated aqueous extracts of *E. jambolana* effectively inhibited porcine pancreatic α -amylase. It is a general opinion that medicinal plants inhibit α -amylase activity due to the presence of several possible factors / mechanisms, such as, fiber concentration, presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme, and direct adsorption of the enzyme on fibers, leading to decreased amylase activity.^[16] The α -amylase inhibitory activity of *E. jambolana* may due to the presence of flavonoid diglycosides, quercetin and myricetin,^[21] hydrolysable tannins, 1-0-galloyl castalagin and casuarinin,^[22] and a triterpene, oleanolic acid.^[23]

α -glucosidase is one among the number of glucosidases located in the brush-border surface membrane of intestinal cells, and is a key enzyme of carbohydrate digestion.^[24] α -glucosidase inhibitors block the actions of the enzyme in the small intestine, which is rate-limiting in the conversion of oligosaccharides and disaccharides to monosaccharides, necessary for gastrointestinal absorption. Postprandial glucose peaks may be attenuated by delayed glucose absorption. The inhibition of α -glucosidase by *E. jambolana* extracts can be attributed to the presence of tannins,

rutin, quercetin, and steroids,^[9] which are reported to act as strong antioxidants and anti-inflammatory agents, as reports indicate that phenolic-enriched extracts of *Solanum melongena*, with moderate free radical scavenging-linked antioxidant activity, have a high α -glucosidase inhibitory activity. Furthermore, since, heat treatment does not have any adverse effect on the α -glucosidase inhibitory activity of *E. jambolana*, it is inferred that it can also be used in the form of infusion, to derive its beneficial biological effects.

Rat intestinal sucrase occurs as a complex of sucrase and isomaltase, which converts sucrose into glucose.^[25] The inhibition of sucrase by *E. jambolana* extracts may also be due to the presence of flavonoids such as rutin, quercetin, and so on. The findings on α -glucosidase and sucrase inhibitory activities of *E. jambolana* are in good agreement with an earlier report, where an acetone extract the seed kernels exhibited a significant inhibition of α -glucosidase, sucrase, and maltase activities.^[26] The correlation observed between α -amylase, α -glucosidase, and sucrase inhibitory activities of both untreated and heat-treated extracts, represents a parallel and effective inhibition of these carbohydrate hydrolyzing enzymes in the digestive tract.

CONCLUSION

In conclusion, the results of the present study provide more insight into the mechanisms of the antidiabetic action of *Eugenia jambolana* seeds and also provide a scientific basis for its usage in the traditional systems of medicine, for the management of diabetes. The study also emphasizes that heat treatment does not have any adverse effect on its enzyme inhibitory effect. Furthermore, it is inferred that efforts need to be directed towards the development of nutraceutical products for their effective utilization as antidiabetic formulations.

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