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Evaluation of *in vitro* anti-diabetic activity of *Elaeocarpus serratus* fruit

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Abstract

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Recent decades have experienced a sharp increase in the incidence and prevalence of Diabetes mellitus. Pancreatic α-amylase and α-glucosidase inhibitors offer an effective strategy to lower levels of post prandial hyperglycemia via control of starch breakdown. The objective of the present work was to evaluate the ethanolic extract of fruit of *Elaeocarpus serratus* by using the *in vitro* anti-diabetic activity. The results suggested that the maximum α-amylase inhibitory activity and moderate α-glucosidase inhibitory activity. Acarbose was used as a standard drug.

1. INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves[1]. For its therapy, along with the synthetic drugs, many agents of the plant origin are also in use particularly for the treatment of non-insulin dependent diabetes mellitus. According to world ethno botanical information reports, almost 800 plants may possess anti-diabetic potential[2]. Medicinal plants with anti-diabetic potentials decrease the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, such as pancreatic amylase[3,4]. In the past decade, research has been focused on scientific evaluation of traditional drugs of plant origin and screening of more effective and safe hypoglycemic agents has continued to be an important area.

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity[5]. *Elaeocarpus* is a genus of tropical and subtropical evergreen trees and shrubs belonging to family Elaeocarpaceae. Studies indicate that various species contain chemical constituent such as triterpenes, tannins, indolizidine alkaloids, flavonoids, and ellagic acid derivatives[6-7]. Several species have been known to possess anti-inflammatory[8], antimicrobial[9], analgesic[10], antioxidant activity[11] and antihypertensive[12] activities. *Elaeocarpus serratus* leaves are used in the treatment of rheumatism[13], diuretic, cardiovascular stimulant and as antidote to poison, antimicrobial activity[14] while the fruits are locally prescribed for the treatment of diarrhea and dysentery. The fruit juice is given for stimulating secretions from taste buds thus increasing appetite in patients[15]. Leaves contain Myricitrin, Myricetin, Mearnsitrin and Ellagic acid. Fruits contain tannin and large amount of plant acids[16-17]. In continuation of the above studies, the present work is aimed to evaluate the anti-diabetic activity of ethanolic fruit extract using *in vitro* assay system.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The fruit of *Elaeocarpus serratus* L. were collected from Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), India and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech. 454 has been deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode

(T.N), India. Coarse powder from the shade dried fruit of *E. serratus* (500g) were exhaustively extracted using soxhlet apparatus with absolute ethanol (78.5°C). The extract were dried (free of solvent) using a vacuum evaporator. The extract thus obtained was stored in refrigerator and used for the experiment.

2.2 Chemicals

The chemicals used in the present work were acquired from E. Merck (INDIA) Limited, Hi-media, Pvt. Ltd, India and Sigma-Aldrich, India.

2.2.1 In Vitro α-amylase Inhibition Assay

The α-amylase inhibitory activity was determined according to the method described by[18]. 20-100µg/ml of plant extract and 1ml enzyme solution were mixed in a tube and incubated at 25°C for 30 minutes. To 1ml of this mixture was added 1ml of 0.5% starch solution and the tube was incubated at 25°C for 3 minutes. Then, 1ml of the color reagent Dinitro Salicylic acid (DNSA) was added and the closed tube was placed in a water bath at 85°C. After 15minutes, the reaction mixture was removed from the water bath and cooled. Thereafter, it was diluted with 9ml distilled water and the absorbance value determined at 540nm. Acarbose was used as positive control**.** The percentage of α-amylase inhibition was assessed using the formula-:

$$\textbf{Percentage of α-amylase inhibition} = \frac{A_{540}\ \text{control} - A_{540}\ \text{test}}{A_{540}\ \text{control}} \textbf{ X 100}$$

2.2.2 In Vitro α-glucosidase Inhibition Assay

The assay was carried out following the standard protocol[19]. α-glucosidase inhibitory activity was determined by premixing α-glucosidase (0.07units) with 20-100µg /ml of plant extract. Then 3mM p-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37°C for 30minutes and the reaction was terminated by addition of 2ml of sodium carbonate. The α-glucosidase activity was determined by measuring the p-nitrophenyl release from p-nitrophenyl glucopyranoside at 400nm. Acarbose was used as positive control**.** The percentage of α-amylase inhibition was assessed using the formula:

Percentage of α - glucosidase inhibition =1- B/A×100

A - Absorbance of control; B - Absorbance of sample containing extracts.

3. RESULTS

The results showed that ethanolic extract of fruit of *Elaeocarpus serratus* exhibited different degree of α-amylase and α-glucosidase inhibitory activities in *in vitro* assays using starch and p-nitrophenyl glucopyranoside as substrate, respectively. The percentage inhibition at 20, 40, 60, 80 and 100µg/ml concentration of plant extract showed a dose-dependent reduction in percentage inhibition. Maximum inhibition of 89.54±2.99% was shown by 100µg/ml concentration of the fruit extract. The standard drug acarbose showed 94.65± 0.82% at 100µg/ml (Figure 1). The plant extract displayed moderate α-glucosidase inhibitory activity (6.68±0.59% to 25.93±0.34%). The standard acarbose (at concentrations 20-100µg/ml) showed α-glucosidase inhibitory activity from 43.12±0.59% to 75.80±1.01%. According to the experimental results, it was certainly confirmed that the plant extract exhibited weak α-glucosidase enzyme inhibition when compared to α-amylase enzyme inhibition (Figure 2).

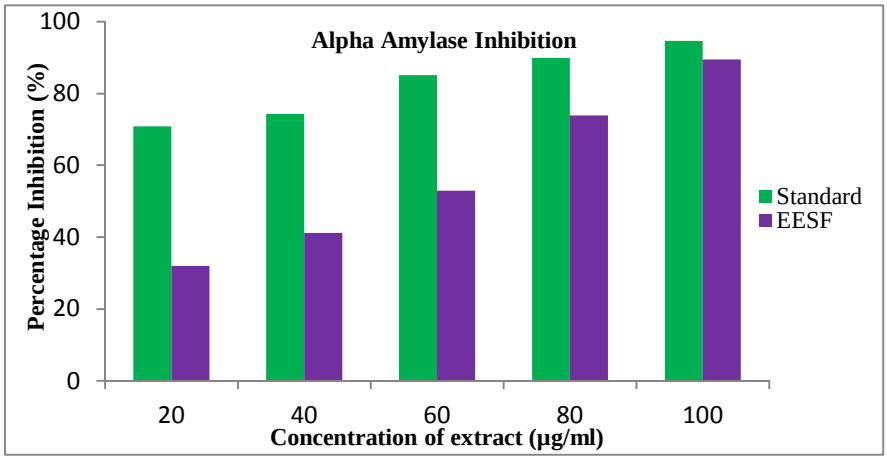

Figure 1: Effect of ethanolic extract of fruit of *E. serratus* on α-amylase inhibition

Values are mean of three independent analyses of the extract ± standard deviation (n=3)
Standard - Acarbose (20-100µg/ml), EESF - Ethanol extract of E. serratus fruit (20-100µg/ml)

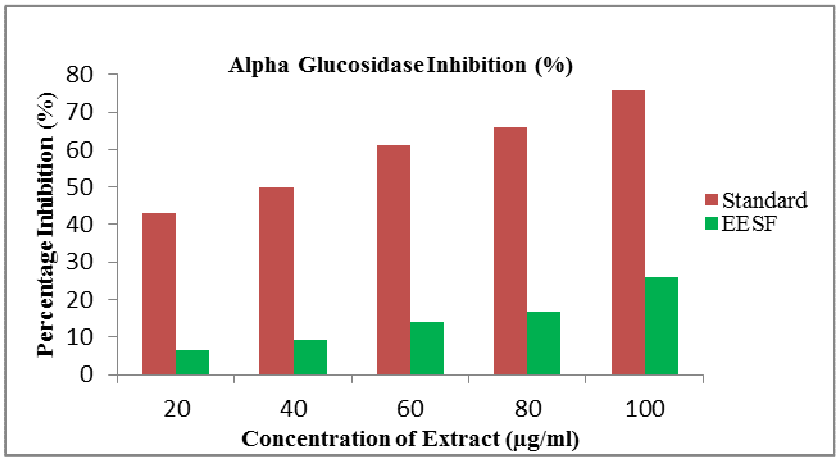

Figure 2: Effect of ethanolic extract of fruit of *E. serratus* on α-glucosidase inhibition

Values are mean of three independent analyses of the extract ± standard deviation (n=3)
Standard - Acarbose (20-100µg/ml), EESF - Ethanol extract of E. serratus fruit (20-100 µg/ml)

4. DISCUSSION

Diabetes is a debilitating disease affecting millions of people worldwide. Since the disease has no known modern allopathic cure, it requires lifelong health. One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including α-amylase and α-glucosidase present in the brush borders of intestine. A major goal in the treatment of diabetes mellitus is to maintain near normal blood glucose levels in both the fasting and postprandial state[20]. Furthermore, some inhibitors of α-amylase and α-glucosidase such as phaseolamin, acarbose and vogiblose are currently used to suppress postprandial glucose levels in diabetic patients[21]. Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful anti-diabetic drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available *in vitro* assays[22, 23].

The present investigation indicated that the plant extract exhibited weak α-glucosidase enzyme inhibition when compared to α-amylase. In earlier studies, the hypoglycemic potential of *Carpesium abrotanoides* was evaluated by the α-amylase and α-glucosidase inhibition assay. The optimal concentration of *C. abrotanoides* required for the 50% inhibition (IC_{50}) against α-glucosidase was 44.22µg/ml. Acarbose was used as positive control with IC_{50} value of 2.5µg/ml [24]. According to[25] the acetone extract of both fruits (68.9%) and leaves (89.6%) of *Terminalia bellirica* showed strong inhibitory activity against α-amylase whereas the aqueous extract of fruit (6.89%) and leaf (7.5%) was found to exhibit highest α-glucosidase activity. Similar trend was noted by[26,27] who showed that the methanolic extracts of *Amaranthus cruentus* and *Moringa oleifera* showed promising levels of α-amylase (10-45%) and α-glucosidase (13-80%) inhibition activities. According to[28] the methanolic extract of leaves of *Psidium guava* efficiently inhibited both α-amylase (89.4%) and α-glucosidase (96.3%) enzymes *in vitro* in a dose-dependent manner. Our experiment was in accordance with the results reported by[29-31].

5. CONCLUSION

We, for the first time, have demonstrated the ability of ethanolic fruit extract of *Elaeocarpus serratus* to inhibit glucose diffusion using and *in vitro* model of glucose absorption. Further studies are required to elucidate whether *in vitro* effects represented therapeutic potential by limiting postprandial glucose absorption and for improving glycemic control in type 2 diabetic subjects.

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