Inhibition of Carbohydrate Hydrolyzing Enzyme Activities by *Flacourtia inermis* Fruit Extracts

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***Abstract*----**Fruits of *Flacourtia inermis* Roxb. (Family Flacourtiaceae), an underutilized fruit crop in Sri Lanka was tested for carbohydrate enzyme inhibition activities during this study. Fruit extracts were tested for α-glucosidase and *α*-amylase enzyme inhibition activities in in-vitro conditions using standard protocols and ethyl acetate extract exhibited considerable activities against those enzymes. The total phenolic content was also determined and recorded as 1.28 g of gallic acid equivalents per 100 g of fresh fruits. According to the findings of this study, *F. inermis* fruits have a potential to be used as a controlling agent of blood glucose level.

Keywords*---Flacourtia inermis*, Inhibition activities, α-glucosidase, α –amylase

I. Introduction

FLACOURTIA inermis is a small, evergreen tree which is native to Malaysia and can be found in South Asian and African regions as well. *F. inermis* is common in Sri Lanka, especially in the wet zone up to 600 m. Tree branches are spiny and leaves have scalloped or serrated margins. Fruits are edible, red, pulpy berries with an acidic taste and contain 4-8 hard seeds. This tree is grown in home gardens and has an ornamental value because of its beautiful foliage.

F. inermis has not been studied for their biochemical properties. However, there are a few previous reports about their antioxidant activity [1], anti-fungal activity against human opportunistic pathogens [2] and anti-protozoal activities [3].

During the current study fruit extracts of *F. inermis* were subjected to glycosidase enzyme inhibition bioassays in order to assess the value of these fruits and fruit extracts as a nutritional supplement for diabetes mellitus type II patients.

Controlling the release of glucose from dietary carbohydrates is one way of reducing blood glucose level and treating diabetes mellitus type II. This can be achieved by inhibiting or reducing the activities of glycosidase enzymes in the digestive organs such as α-amylase which catalyses the hydrolysis of starch into maltose and glucose.[4]

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There are synthetic glycosidase enzyme inhibitors such as acarbose (*O*-4,6-dideoxy-4-[[(*1S,4R,5S,6S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl] amino] α-D-glucopyranosyl-(1→ 4)-*O*-α-D-glucopyranosyl-(1 →4)-D-glucose) to control postprandial hyperglycemia.

However, as there are several side effects reported on these synthetic enzyme inhibitors, finding a natural remedy to control carbohydrate digestion was the main objective of this study.

II. Procedure

Extraction

Fresh fruits of *F. inermis*, (1.5 kg), collected from the Central Province of Sri Lanka, were blended, the fruit pulp separated from the wine red fruit juice and extracted sequentially with ethyl acetate (EtOAc) and methanol (MeOH), while the fruit pulp residue was partitioned sequentially with EtOAc and n-butanol (n-BuOH). TLC indicated that the EtOAc extracts from the fruit pulp and fruit juice were identical and were combined.

α-Glucosidase Enzyme Inhibition Bioassay [5]

A 2 units/ml solution of α-glucosidase (G5003, from *Saccharomyces cerevisiae*, 100U/mg of protein) and 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution were prepared in 20 mM phosphate buffer (pH 6.0). Plant extracts were dissolved in distilled water with 1% dimethylsuphoxide (500 ppm -5000 ppm). The reaction was carried out at 25°C using 10 µl enzyme, 10 µl plant extract, and 50 µl substrate for 7 minutes. Color was measured at 415 nm after the addition of 80 µl 0.4 M glycine, pH 10.4, to stop the reaction.

A reaction control was performed using 10 µl enzyme, 50 µl substrate and 10 µl of aqueous 1% DMSO instead of plant extract. This control was incubated for 7 minutes at 25°C and color formation was measured as before following the addition of 80 µl 0.4 M glycine solution, pH 10.4. Experiment was replicated six times.

$$Percent\ inhibition = \left(\frac{A-B}{A}\right) \times 100$$

Where; A- Absorbance of reaction control at 415 nm, B- Absorbance of test sample at 415 nm

The IC_{50} value was calculated by plotting % inhibition of enzyme activity against sample extract concentration.

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Acarbose tablets (1 ppm – 100 ppm) (ACARB 25 – Orchid Healthcare, Mumbai, India) were used as the positive control.

α-Amylase Inhibition Bioassay [4]

The dinitrosalicylic acid (DNSA) color reagent was prepared using 20 ml of 96 mM 3,5-dinitrosalicylic acid in de ionized water, 5.31 M sodium potassium tartrate in 2 M NaOH (8 ml) and deionized water (12 ml). α-Amylase (Porcine pancreas, Type VI-B, 23 U /mg of protein) enzyme was dissolved in phosphate buffer (20 mM, pH 6.9, 6.7 mM NaCl). Plant extracts were dissolved in 1% DMSO in deionized water (500 ppm -5000 ppm). A 100 µl of α-Amylase (8U /ml) was mixed with plant extract and incubated at 25°C for 30 minutes. A 100 µl of this mixture was mixed with starch (0.5% w/v) solution (100 µl), incubated at 25°C for 3 min and DNSA reagent (100 µl) was added, incubated at 85 °C water bath for 15 mins, allowed to cool and then diluted with distilled water (900 µl). Negative controls were conducted in an equal manner replacing plant extracts with 1% DMSO (100 µl) in de ionized water. For blanks, the color reagent was added prior to the addition of starch solution to denature the enzyme, kept in 85°C water bath for 15 minutes and then diluted with distilled water (900 µl) as before. Absorbance was measured at 540 nm and percent inhibition was plotted against concentration to calculate IC_{50}. Acarbose (1 ppm – 100 ppm) was used as the positive control. Experiment was replicated six times.

$$Percent\ inhibition = \left(\frac{A - B}{A}\right) \times 100$$

Where; A- Absorbance of reaction control at 540 nm, B- Absorbance of test sample at 540 nm

Total Polyphenol Content [6]

A concentration series of anhydrous gallic acid (GA) ranging from 1 mg/ mL– 100 mg/mL was used as the standard. A 500 µL of each GA solution was mixed with 500 µL of distilled water and 2.5 mL of 10 –fold diluted Folin-Ciocalteu reagent. A 4.0 mL of 7.5% (w/v) Na_2CO_3 solution was added After 5 min, and allowed to stand at room temperature for 60 min. Developed blue color was measured at 765 nm against distilled water. Absorbance was plotted against mass of GA in each solution and a calibration curve was constructed.

A 10 g of fresh fruits were extracted with 20 mL of 70% aqueous methanol for five successive times and evaporated to obtain dry extract (yield- 1.40 g). A 1 mg/mL solution of this plant extract was prepared using distilled water. A 500 µL of this solution was mixed with 500 µL of distilled water and 2.5 mL of 10 –fold diluted Folin-Ciocalteu reagent. A 4.00 mL of 7.5% (w/v) Na_2CO_3 solution was added After 5 min, allowed standing at room temperature for 60 min and developed blue color was measured at 765 nm. Mass of polyphenols in this extract was read from the calibration curve.

III. Results And Discussion

In the absence of an inhibitor, starch is broken down to maltose by the α-amylase enzyme. Maltose is a reducing sugar and reacts with 3, 5-dinitrosalicylic acid (DNSA) in alkaline medium, converting it to 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm. If the α-amylase enzyme is inhibited, it will reduce the amount of maltose produced and this will be indicated by a reduction of absorption at 540 nm.

The substrate used in α-glucosidase bioassay was *p*-nitrophenyl-α-D-glucopyranoside. α-Glucosidase enzyme hydrolyses the glycosidic bond in this substrate and releases *p*-nitrophenol with the development of a yellow colour in the reaction mixture. The intensity of the yellow colour was estimated by measuring the absorbance at 415 nm. In the presence of an inhibitor which of the α-Glucosidase enzyme, release of *p*-nitrophenol will be inhibited and reduction of absorbance at 415 nm can be observed.

The EtOAc, MeOH and n-BuOH extracts of *F. inermis* were tested using these procedures and the IC50 values were given in **table 1**.

TABLE I
.IC_{50} VALUES OF *F. INERMIS* EXTRACTS AND ACARBOSE (POSITIVE CONTROL) AGAINST *A-GLUCOSIDASE AND A-AMYLASE INHIBITION*

Extract	**IC_{50} value (ppm)**	
	a-glucosidase inhibition	***a-amylase inhibition***
EtOAc	549.13± 3.47	1022.91 ± 10.55
MeOH	710.69 ± 1.08	1948.39 ± 11.81
n-BuOH	661.86 ± 4.33	1186.19 ± 13.15
Acarbose	13.83 ± 1.27	19.85 ± 0.98

These values are significant for a crude extract when compared with the IC_{50} values of clinical drugs such as α-amylase and α-glucosidase inhibitor Acarbose (19.85 ppm, 13.83 ppm) under the identical conditions. According to Nickavar, Abolhasani and Izadpanah (2008), it is found that polyphenols and flavonoids are among the natural active antidiabetic agents. Polyphenols show various important biochemical properties including carbohydrate hydrolyzing enzyme inhibition such as of α-amylase and α-glucosidase enzymes. Polyphenols have the ability to partially or completely inhibit the activities of digestive enzymes as they can bind with proteins. The inhibitory activities of plant phytochemicals, including polyphenols, against carbohydrate hydrolyzing enzymes can be used effectively in lowering of postprandial hyperglycemia in the management of diabetes. Total polyphenol content of *F. inermis* fresh fruits was determined using Folin-Ciocalteu method and it was 1.28 g per 100 g of fruits as gallic acid equivalents.

The results of this study indicate that *F. inermis* fruits are rich in polyphenols and have the potential to be used in controlling blood glucose levels and can be used to identify lead compounds in anti diabetic drug discovery. However, further studies with animal models are needed to confirm these results.

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