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Anti-diabetic potential of fruit extracts of *Flacourtia indica* (Burm. F.) Merr-An *in-vitro* study

Sudhina Makuttan[1], Jennifer Fernandes[2]* and Ambily P G[2]

[1]Department of Pharmaceutical Chemistry, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Deralakatte 575018, Mangaluru, Karnataka, India

[2]Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Paneer, Deralakatte 575018, Mangaluru, Karnataka, India

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Diabetes mellitus is one of the biggest global health problems requiring preventive and new therapeutic interventions. There is a need for safe, reliable, and cost-effective anti-diabetic drugs, and investigating medicinal plants for new anti-diabetic medications is an exciting research field. Thus, the present study examined the *in-vitro* anti-diabetic activity of ethanolic and aqueous extracts of *Flacourtia indica* (Burm. F.) Merr (Flacourtiaceae) fruits by different enzyme inhibition assay methods. Alpha-amylase and Alpha-glucosidase are the principal enzymes present in the human body which helps in the digestion of carbohydrates. Inhibition of these enzymes slows down the absorption of glucose and lowers the sugar level in the blood. Both extracts showed potent inhibitory activity against these enzymes in a dose-dependent manner. The highest percentage of inhibition is exhibited by ethanolic extract at a concentration of 100 µg/mL with an IC_{50} value of 84.02. The results were compared with the standard drug Acarbose, a competitive inhibitor of both enzymes. The ethanolic extract was subjected to preliminary phytochemical analysis to find out different chemical constituents. It revealed the presence of reducing sugars, flavonoids, phenolic compounds, terpenoids, fatty acids, and steroids. Therefore, the current study proved that both ethanolic and aqueous extracts of *F. indica* fruits possess bioactive constituents that could be responsible for the anti-diabetic activity.

Keywords: Acarbose, Alpha-amylase, Alpha-glucosidase, Anti-diabetic, *Flacourtia indica*.

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Introduction

Medicinal plants play an important role in the development of modern herbal medicines. The bioactive constituents present in the medicinal plants are used as anti-diabetic, chemotherapeutic, anti-inflammatory, and anti-arthritic agents, whereas modern medicines fail to provide a satisfactory cure. Medicinal plants have been used as dietary supplements and for treatment purposes without proper knowledge about their medicinal values. Traditional medicine makes extensive use of secondary metabolites found in plants, which could lead to the discovery of new medications[1]. WHO developed its first comprehensive traditional medicine strategy in 2002 to support efforts to promote the use of traditional medicine and complementary alternative medicine that is inexpensive, effective, and safe for the treatment of various ailments.

Diabetes mellitus is a complex multifactorial condition defined by hyperglycemia (very high blood glucose levels) and glucose intolerance, which can be caused by a lack of insulin or an ineffective insulin action to improve glucose uptake. Over recent years the population suffering from diabetes mellitus has been increasing dramatically[2]. It is also expected that by 2030, India, China, and the United States will have the highest number of diabetic patients[3]. Diabetic patients experience oxidative stress, which causes lipid peroxidation and tissue damage, such as retinopathy, nephropathy, and coronary heart disease[4]. The control of blood glucose levels is a critical strategy as many anti-diabetic therapies with conventional drugs are frequently not a single-dose programme, as most drugs require frequent injections, sometimes for the rest of the diabetic patient's life. However, many of these conventional medicines are ineffective and have significant negative side effects. One therapeutic way to prevent postprandial hyperglycaemia is to retard the digestion and absorption of carbohydrates in the gastrointestinal tract through the

*Correspondent author
Email: fernandesj@nitte.edu.in

inhibition of enzymes such as alpha-amylase and alpha glucosoidase[5]. Alpha-amylase and alpha-glucosidase are the major enzymes in the human body which catalyse the hydrolysis of the 1,4 glycoside bond of complex carbohydrates into simple sugars[6]. Inhibition of these enzymes is found to be effective in the treatment of diabetes. Nowadays many synthetic oral hypoglycaemic agents that have an inhibitory effect on alpha-amylase and alpha-glucosidase are developed for the treatment of diabetes mellitus, however, due to their undesirable side effects; it is required to search for herbal medicines obtained from plants for the cureness of diabetes mellitus[6]. Most herbal plants contain phytocompounds like alkaloids, flavonoids, tannins, glycosides, fatty acids, terpenoids etc., that possess multipotential activities for the treatment of various diseases.

Flacourtia indica (Burm. F.) Merr (syn. *Flacourtia ramontchi*), commonly known as Batako plum or Indian plum belongs to the family Flacourtiaceae. It is one of the essential nutraceutical plants widely distributed in Bangladesh and India from Punjab east to Bihar, Deccan and Southern Peninsula[7]. It is a small bushy shrub or tree that grows at a height of 900 m. Flowers are greenish-yellow, unisexual or occasionally bisexual. The flowering period is from December to April. The fruits are fleshy and globular with a diameter of 8-10 mm. The unripe fruits are green in colour, when ripe it becomes reddish. The ripening period of fruit is from March to July[8]. Fruits can be eaten raw and used to make preservatives, jams, and jellies. This plant has been reported as an important herb in Ayurveda where infusions of plant parts are used medicinally to cure various diseases. The juice of fresh leaves is used to treat fever. Dried leaves are used for bronchitis, asthma, and catarrh of the bladder. Decoction of the roots is taken for treatment of urinary calculi[9].

Based on Palani *et al.*[10], ethanolic extract of *Flacourtia* can prevent heart damage in rats caused by DOX-induced myocardial infraction, and this action is likely mediated by the plant's antioxidant activity. *F. indica* leaves were found to possess free radical scavenging and anti-oxidant activity[11]. *F. indica* histopathological tests revealed a relative degree of reversal of Methotrexate-induced necrosis, with considerably improved levels of marker enzymes for liver function and oxidative stress[12]. Lalsarea *et al.*[13] reported that methanolic extracts of *F. indica* possess anti-inflammatory and broad-spectrum antimicrobial activity. The barks are strong antibacterial agents and can be used for the treatment of Diarrhoea[14]. The animals treated with *F. indica* demonstrated a significant increase in pre-convulsion time when exposed to histamine, suggesting that its anti-asthmatic effect could be attributable to its bronchodilator and cell stabilising properties[15]. The anti-diabetic effects of ethanolic extract of leaves of *F. indica* were evaluated by Singh *et al.*[16], with significant alteration in parameters like fasting blood glucose, serum cholesterol and triglycerides, liver glycogen, glycosylated haemoglobin, and body weight on STZ-induced diabetic rats. The plant also lowers lipid levels in hyperlipidemic animals and this might be because of the presence of a substantial amount of flavonoids in the active extract of plants[17]. The polyphenolic compounds present in the root extract of the plant proved to have significant diuretic activity[18]. Although studies of various plant parts have demonstrated anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, antimalarial, anti-diabetic, anti-asthmatic and antibacterial activity, the potential of fruit extract is yet to be resolved, therefore this study aims to evaluate the anti-diabetic activity of *F.indica* fruit extracts.

Materials and Methods

Collection of plant material

F. indica fruits were collected from the local area of Mangalore city, Karnataka from March to August 2020. The authentication of the plant was done by Dr Raju Krishna Chalannavar, Chairman of the Department of Applied Botany, (voucher specimen Number 19PC007R) at Mangalore University. Fruits were washed and cleaned with deionized water and dried under shade for 2 weeks. The shade dried *F. indica* fruits were powdered mechanically and were stored under an airtight container.

Preparation of extracts

Ethanolic extract

About 250 g of finely powdered powder was subjected to extraction by cold maceration procedure for 7 days using ethanol as solvent. After the completion of the extraction process, the solvent was distilled out by a simple distillation method. The recovered solvent was used for further extraction procedure. The finely obtained ethanolic extract was filtered through Whatman filter paper, concentrated under a vacuum, and stored in a desiccator for further experiment.

Aqueous extract

About 200 g of powder kept under cold maceration process using distilled water as the solvent for 24 h.

The finely recovered semisolid residue is concentrated and kept under the desiccator.

Preliminary phytochemical screening

The preliminary phytochemical analysis of residue obtained was done to determine the presence or absence of various classes of chemical constituents i.e. primary and secondary metabolites such as alkaloids, reducing sugars, flavonoids, phenols, terpenoids and steroids[19].

***In-vitro* anti-diabetic activity**

Alpha-amylase inhibitory activity

The inhibitory activity of alpha-amylase was performed by the chromogenic DNSA method[1]. The alpha-amylase activity was determined by the reducing group arising from the hydrolysis of starch by alpha-amylase. The assay mixture consists of 200 µL of 0.02M sodium phosphate buffer (pH 6.9), 20 µL of enzyme solution (0.5 µg/mL in phosphate buffer) and fruit extract of varying concentrations (10, 20, 40, 60, 80, 100 µg/mL). The above mixture was kept for incubation at 25°C for 10 min. To the above solution add 200 µL of 1% starch solution and further incubate for 10 min at 25°C. The reaction was stopped by adding 400 µL of di-nitrosalicylic acid reagent. A colour shift occurs when 3,5-dinitrosalicylic acid is reduced to nitro-aminosalicylic acid, which is followed photometrically by a change in absorbance at 540 nm. The same procedure was repeated for control where the fruit extract was replaced by phosphate buffer. Both control and test extracts were placed in the boiling water bath for 10 min and were cooled. After cooling, added 15 mL distilled water and the absorbance was measured at 540 nm. Acarbose at different concentration (10, 20, 40, 60, 80, 100 µg/mL) was taken as standard. The percentage inhibition is calculated by the formula

$$\% \text{ Inhibition } = \frac{\text{Abs Control } - \text{ Abs Extract}}{\text{Abs Control}} \times 100$$

Where Abs Control and Abs Extract are absorbance values of control and extract respectively.

Alpha-glucosidase inhibitory activity

The inhibition of -glucosidase enzymes was performed using Kim *et al.*, approach with minor modifications[20]. When the enzyme -glucosidase is incubated with the substrate p-nitrophenyl—D-glucopyranoside (p-NPG), it hydrolyzes the substrate top-nitrophenol and D-glucose, respectively. In a 96 well plate, 20 µL of fruit extracts of different concentrations (10, 20, 30, 40, 50 µg/mL), 50 µL of phosphate buffer (pH 6.8), 10 µL of alpha-glucosidase enzyme solution (1 U/mL) were added. The plates were kept for incubation at 37°C for 15 minutes. Thereafter add 20 µL of p-nitrophenyl-α-D-glucopyranoside solution (5 mM) and again incubate 20 minutes at 37°C. After incubation 50 µL of sodium carbonate (0.1 M) solution is added to stop the reaction. The absorbance is measured at 405 nm. Acarbose at concentration of (10, 20, 40, 60, 80,100 µg/mL) taken as standard. The percentage inhibition of fruit extract is calculated by

$$\% \text{ Inhibition } = \frac{\text{Abs Control } - \text{ Abs Extract}}{\text{Abs Control}} \times 100$$

Where Abs Control and Abs Extract are Absorbance values of Control and Extract respectively.

Statistical analysis

The results were expressed as mean±SEM, statistical analysis was performed by one-way ANOVA followed by Dunnet Multiple comparison tests using graph pad software, *P* values <0.05 were considered as significant.

Results

Phytochemical analysis

The various preliminary phytochemical tests reveal the presence of reducing sugars, flavonoids, phenolic compounds, saponins, glycosides, steroids, and terpenoids in solvents like ethanol and aqueous extract (Table 1).

***In-vitro* anti-diabetic activity**

Alpha-amylase inhibitory assay

Alpha-amylase is one of the important enzymes present in the intestine which helps in the degradation of starch and oligosaccharides into monosaccharides

Table 1 — Phytochemical screening of ethanolic and aqueous extract of *Flacourtia indica* fruit

S. No.	Phytochemical	Ethanolic extract	Aqueous extract
1	Alkaloids	-	-
2	Reducing sugars	+	+
3	Flavonoids	+	+
4	Phenols	+	+
5	Saponins	+	+
6	Glycosides	+	+
7	Steroids	+	+
8	Terpenoids	+	+

before absorption. Inhibition of these enzymes retards glucose absorption and thereby reduces the blood glucose level.

In the current study, different concentrations (10-100 µg/mL) of ethanolic and aqueous extract of *F. Indica* fruits were investigated for alpha-amylase inhibitory activity and were compared with the standard drug Acarbose. The maximum percentage of inhibition (62.96±1.82%) was obtained by ethanolic extract at a concentration of 100 µg/mL followed by aqueous extract (55.07±1.46%). The standard drug acarbose showed alpha-amylase inhibition of 13.37-67.37% on varying concentrations from 10-100 µg/mL. The IC_{50} value of ethanolic extract was found to be 84.02 which is comparable with Acarbose having an IC_{50} value of 72.83 (Table 2). The IC_{50} value is the concentration of each extract or standard drug needed to inhibit 50% of the enzyme in the reaction. A lower IC_{50} value indicates more potency and therapeutic efficacy. The IC_{50} value of chloroform extract is nearly comparable to that of Acarbose, making it an excellent-amylase inhibitor. The comparison of inhibitory activity between standard drugs and fruit extracts is graphically shown in Fig. 1.

Alpha-glucosidase inhibitory assay

Alpha-glucosidase is the enzyme located in the intestinal lumen and brush border membrane of the intestine which helps in the digestion of polysaccharides and disaccharides before their absorption. Inhibition of the alpha-glucosidase enzyme is one of the ideal treatments for the management of type 2 diabetes.

In this study, the inhibitory activity of the alpha-glucosidase enzyme against the ethanolic and aqueous extract of *F. Indica* fruit was examined. Among the two extracts, the highly potent inhibitory activity (72.44±0.70%) is shown by ethanolic extract at a concentration of 100 µg/mL (Table 2). The standard drug Acarbose shows the maximum inhibition (83.61±1.46%) at 100 µg/mL. The IC_{50} value of ethanolic extract (70.47) is again close to standard drug acarbose 62.49 indicating its potent nature. A comparison of alpha-glucosidase inhibitory activity

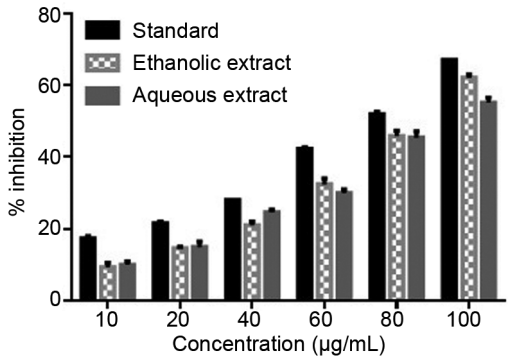

Fig. 1 — Correlation between fruit extracts concentration and percentage of α-amylase inhibition.

Table 2 — Effect of aqueous and ethanolic *Flacourtia indica* fruit extracts on alpha-amylase and alpha-glucosidase

Tested material	Concentration (µg/mL)	% Inhibition of alpha- amylase ±SEM	IC_{50} value	% Inhibition of alpha glucosidase ±SEM	IC_{50} value
Acarbose	10	17.37±0.75	72.83	14.61±1.48	62.49
	20	21.65±0.37		19.43±1.11	
	40	28.20±0.85		36.30±1.13	
	60	42.30±0.42		44.17±0.52	
	80	51.99±0.62		58.15±0.98	
	100	67.37±0.79		83.61±1.46	
EEFIF	10	9.35±1.21	84.02	9.79±1.06	70.47
	20	14.61±0.50		16.86±1.60	
	40	21.44±1.94		25.06±1.66	
	60	32.45±1.62		36.22±1.67	
	80	42.80±1.56		60.32±1.88	
	100	62.96±1.82		72.44±0.70	
AEFIF	10	10.39±1.24	89.36	13.08±1.52	83.43
	20	14.93±1.58		20.83±0.45	
	40	24.61±0.72		26.69±2.26	
	60	30.82±1.56		32.94±1.26	
	80	45.45±1.78		44.27±1.29	
	100	55.07±1.46		61.13±1.46	

Each value represents the mean±SEM, experimental groups were compared with control P <0.05, considered extremely significant; Ethanolic extract *of Flacourtia indica* fruit (EEFIF), Aqueous extract of *Flacourtia indica* fruit (AEFIF).

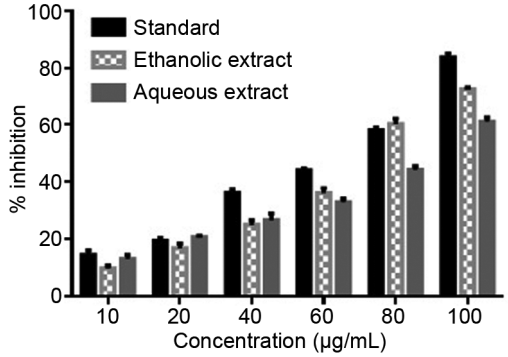

Fig. 2 — Correlation between fruit extracts concentration and percentage of α-glucosidase inhibition.

between standard drugs and fruit extracts is represented in Fig. 2.

Discussion

Diabetes mellitus is a life-threatening problem that is exponentially increasing in both developed and developing countries. Both men and women can have diabetes at any stage of life. Among the two types of diabetes (type 1 or insulin-dependent diabetes mellitus and type 2 or non-insulin-dependent diabetes mellitus), type 2 is the most common form and occurs in about 90-95% of all diabetic cases. In the early stages of diabetes, lowering post-prandial hyperglycaemia is a therapeutic strategy. This is achieved by inhibiting carbohydrate hydrolysing enzymes such as alpha-amylase and alpha-glucosidase in the digestive tract, which slows glucose absorption[21]. Acarbose is a complex oligosaccharide that synthetically inhibits alpha-glucosidase and alpha-amylase enzymes by reducing glucose absorption and thereby decreasing the blood glucose level. However, these synthetic medications cause side effects and fail to cure diabetic complications. Therefore, currently, medicinal plants are considered to be an important source of most potent hypoglycaemic properties as they produce fewer side effects and are less expensive compared to synthetic drugs. As a result, using a drug extracted from plants is a different way to treat diabetes[22].

The phytochemicals present in medicinal herbs are well known for their various pharmacological activities. Plant-derived phytoconstituents influenced metabolic glucose by inhibiting apoptosis, increasing translocated and expressed glucose transporters, decreasing gluconeogenesis, enhancing pancreatic beta-cell proliferation, and protecting pancreatic beta cells from oxidative stress and inflammation. These phytocompound mechanisms aided in the development of anti-diabetic drugs[23]. As per the literature survey, glycosides, terpenoids, and alkaloids can control the conversion of starch into sugar in the event of excess glucose production and act as alpha-amylase inhibitor[24]. Plant phenols serve as key antioxidants and scavengers of free radicals[25]. Rusasinghe *et al.*[26], reported that saponins possess hypocholesterolemic and anti-diabetic properties. Plant polyphenols and flavonoids are some of the naturally occurring anti-diabetic agents known to have an inhibitory effect on the inhibition of carbohydrate hydrolyzing enzymes by their ability to bind to proteins. In the present study, fruit extract shows the presence of these secondary metabolites which may be accountable for its anti-diabetic activity.

Regarding the *In vitro* anti-diabetic activity, both extracts of *F. Indica* fruits had an enormous inhibitory effect on alpha-amylase and alpha-glucosidase in a dose-dependent manner. Previous literature evidenced the phytoconstituents present in the extracts were responsible for the enzyme inhibition. Here the ethanolic extract reveals the highest percentage inhibition of 62.96±1.82% against alpha-amylase and 72.44±0.70% against alpha-glucosidase when compared to the reference standard drug Acarbose. In comparison to manufactured medications with multiple adverse effects such as gastrointestinal discomfort, bloating, flatulence, and diarrhoea, these active extracts could be employed as anti-diabetic agents. Natural alpha-amylase and alpha-glucosidase inhibitors from plant sources offer an attractive therapeutic approach to the treatment of postprandial hyperglycaemia by decreasing glucose release from starch and delaying carbohydrate absorption by inhibiting the activity of the carbohydrate hydrolyzing enzymes in the small intestine and may have the potential for use in the treatment of diabetes mellitus.

Conclusion

In our findings, we conclude that both ethanolic and aqueous extracts of *F. indica* possess anti-diabetic potential against alpha-amylase and alpha-glucosidase enzymes. However, ethanolic extract showed highest percentage of inhibition when compared to the aqueous extract. The phytoconstituents present in the extract were responsible for this anti-diabetic activity. Therefore, this study gives an idea that fruit extracts of the plant *F. indica* can be used as a lead compound for designing a potent anti-diabetic drug which can be used

for the treatment of hyperglycaemia. In future the current work can be extended to identify more chemical constituents through GC-MS analysis and NMR based techniques. Similarly, a functional food formulation could be produced with societal economic and nutritional benefits.

Conflicts of interest

The authors declare no conflicts of interest.

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