Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (3):267-272
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Assessment of *in vitro* anti-diabetic activity of *Ficus glomerata*

Merina Paul Das*, P. Vennila Devi and Y. Yasmine

Department of Industrial Biotechnology, Bharath University, Chennai, India

---

ABSTRACT

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Diabetes mellitus is a metabolic disorder which results from defects in insulin secretion. Among the various methods of Diabetes management, herbal products are best alternative drug with zero side-effects. The aim of our work was to assess the phytochemicals qualitatively and to evaluate the anti-diabetic activity of Ficus glomerata gum using different polar and non-polar solvent extract. The in vitro alpha-amylase and alpha-glucosidase inhibitory studies were performed using different concentration of extract and compared with a standard drug. There was a dose dependent increase in percentage inhibitory activity against these intestinal enzymes by all the extracts. Our findings revealed that among the all extracts, aqueous extract showed efficient anti-diabetic activity. This may be due to the presence of maximum phytochemicals in aqueous gum extract which is analyzed by preliminary phytochemical screening. At the concentration of 1000 µg/ml the gum showed appreciable alpha amylase and alpha glucosidase inhibitory activity of 82.76 % and 80.09 % respectively than the commercial anti-diabetic drug.

Keywords: Diabetes mellitus, Anti-diabetic, Phytochemicals, *Ficus glomerata*, α-amylase, α-glucosidase.

INTRODUCTION

Diabetes mellitus (DM) is characterized by group of chronic metabolic syndrome in which the deficiency or insensitivity of insulin causes glucose to accumulate in the blood, leading to various complications [1]. The intestinal enzymes like α-amylase and α-glucosidase are found to be very important in carbohydrate digestion and glucose absorption [2]. The suppression of the activity of such digestive enzymes would delay the degradation of starch and oligo saccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation [3], eliminates the symptoms of diabetes. Increase in blood glucose damages many of the body's systems, in particular, the blood vessels and nerves. The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes and hyperglycemia due insufficient insulin utilization is called Type-2 diabetes [4]. Out of these two types, Type -2 diabetes is a major problem of today and it account for nearly 95% of total diabetic population of about 246 million [5]. The number of diabetes mellitus cases has been still increasing worldwide. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030 [6].

The commonly practiced treatment of diabetes includes oral antidiabetic drugs, insulin injection and management through diet and physical exercise. Oral hypoglycemic agents have been reported as highly effective for glycemic control, but the use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying their attendant side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic

injury, acute hepatitis, abdominal fullness and diarrhea [7, 8]. Management of diabetes without any side effect is still a challenge to the medical community. Thus there is continuous searching for alternative drugs which is essential to overcome diabetic problems [9]. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored [10]. Antihyperglycemic activities of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/or to stimulate insulin secretion [11]. The mechanism by which the plants exerted action may be due to its action on carbohydrate binding regions of α- glucosidase enzyme, α- amylase, endoglucanases that catalyse hydrolysis of the internal α-1, 4 glucosidic linkages in starch and other related polysaccharides have also been targets for the Suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Since α-amylases play an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion [12].

Ficus glomerata is a species of plant in the Moraceae family. Popularly known as the Cluster Fig Tree or Goolar (Gular), this is native to Australia, South-east Asia and the Indian Subcontinent. It is unusual in that its figs grow on or close to the tree trunk. In India, the tree and its fruit are called Gular in the north and Atti in the south. The fruits are a favourite staple of the common Indian macaque. Medicinally it has been various pharmacological activities astringent, antidiabetic, refrigerant, antiasthmatic, anti-inflammatory, hepatoprotective, antioxidant, antiulcer, anti-pyretic, antidiuretic, Antihyperglycemic, antidiarrhoel [13]. In this study, research has been carried out to assess the *F. glomerata* gum extracts as a potential antidiabetic agent at *in vitro* condition by investigating the effect of extracts on inhibition activity of alpha amylase and alpha glucosidase enzyme. To better understand the biological activities of *F. glomerata*, we determined the phytochemicals present in the various extracts of fig gum.

MATERIALS AND METHODS

Collection and pretreatment of plant material

Fig gum resin was collected from the incised trunk of *Ficus glomerata* in the forest area of Tamil Nadu, India. The fig gum was dried to completely remove the moisture and then hydrated in distilled water for one day with intermittent stirring; extraneous materials were removed by straining through a muslin cloth. The gum was precipitated from solution using absolute acetone. The precipitate was separated and dried on water bath at 50 °C. A fine powder of dried gum was obtained using a blender and stored in air-tight container.

Extraction of plant material

For extraction, two polar solvents such as aqueous, methanol and two non-polar solvents, heptane, benzaldehyde were used. About 100 g of gum powder was mixed with 300 ml of each solvent. The extraction was carried out by continuous percolation method using Soxhlet apparatus for 36 h accompanying with occasional shaking and stirring. The extract was underwent a coarse filtration by muslin cloth followed by a filtration through Whatmann filter paper. Each extract was concentrated by distilling off the solvent and evaporated to dryness under vacuum. The crude extracts were used for the phytochemical analysis. Different concentration (50 - 1000 µg/ml) of plant extract was used for anti-diabetic analysis extracts by dissolving dried extract in 1 % carboxy methyl cellulose (CMC).

Qualitative phytochemical screening

Phytochemical screening was done to investigate the plant material in terms of its active constituents. In order to establish the profiles of the extracts for their nature of chemical composition and identification of various phytoconstituents, different qualitative chemical tests were performed [14, 15].

Evaluation of *in vitro* anti-diabetic activity

Inhibition of alpha amylase enzyme assay

A total of 500 µl of test samples and standard drug (50 - 1000 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/ml) solution and were incubated at 25 °C for 10 min. After these, 500 µl of a 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic (DNS) acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Acarbose was used as a standard drug for assay. The control samples represent 100 % enzyme activity

and were prepared without any plant extract [16]. Each test was performed three times and the mean absorption was used to calculate the percentage α- amylase inhibition.

% Inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{\text{A540 Control} - \text{A540 Sample}}{\text{A540 Control}} \times 100$$

Inhibition of alpha glucosidase enzyme assay

The α-glucosidase inhibitory effect of *F. glomerata* was determined according to the Standard method using different concentration (50 - 1000 µg/ml) of gum extracts [17]. 10 µl of α-glucosidase enzyme solution and varying concentrations of the extract is incubated together for 10 min, at 37 °C, and the volume was made up to 210 µL with maleate buffer, pH 6.0. The enzyme reaction is started by adding 200 µl of 2 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution and further incubated at 37 °C for 30 min. Enzymatic reaction was stopped by adding 2 ml 0.2 M sodium carbonate solution. After the addition of 1.0 ml of 0.1 M disodium hydrogenphosphate solution, the absorption of liberated p-nitrophenol is read at 400 nm. Each test was performed three times and the mean absorption was used to calculate the percentage α-glucosidase inhibition. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing the plant extracts prepared with different solvents.

% Inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{\text{A400 Control} - \text{A400 Sample}}{\text{A400 Control}} \times 100$$

Statistical Analysis

All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences. Values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Diabetes is a major degenerative disease in the world today [18], affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [19]. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body [20]. In this context, in this study, *Ficus glomerata* gum extracts were used for the assessment anti-diabetic activities.

Table 1 Qualitative phytochemical analysis of *Ficus glomerata* gum extract

Phytoconstituents	Aqueous	Methanol	Heptane	Benzaldehyde
Terpenoids	+	-	-	+
Phenols	+	+	+	+
Flavonoids	-	-	-	-
Coumarins	+	+	+	+
Tannins	+	-	+	+
Amino acids	+	-	+	-
Alkaloids	+	+	+	+
Cardiac glycosides	-	-	-	-
Saponins	+	-	-	-
Carbonyls	+	+	-	+

(+) Presence of Phytoconstituents, (-) Absence of Phytoconstituents

Qualitative analysis of phytoconstituents

Table 1 shows the preliminary phytochemical screening of *F. glomerata* using different polar and non-polar solvent extracts, which revealed the presence of various phytoconstituents, such as, tannins, terpenoids, phenols, coumarins, alkaloids, and carbonyls. Usually phytochemicals have been known to possess medicinal properties and hence widely used in Indian systems of traditional medicine [21]. Alkaloids, tannins, flavanoids have anti-diabetic and

anti-inflammatory activity [22, 23]. Thus these secondary metabolites could be may be in combination and/or separately responsible for the anti-diabetic activity of this plant.

Evaluation of *in vitro* α-amylase inhibitory activity

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide [24]. Fig.1 revealed that fig gum extract showed a significant inhibition of α-amylase enzyme. Five different concentrations were tested with four various solvents, among them aqueous extract showed good inhibitory effect at all the tested concentrations (50, 100, 200, 400, 800 and 1000 µg/ml**).** The inhibitory activity increased in dose-dependent manner. At minimum concentration of 100 µg/ml of *F. glomerata* aqueous extracts of gum showed a percentage inhibition 48.9 % and a maximum inhibitory effect 82.76 % at concentration of 1000. The aqueous extract of *F. glomerata* gum exhibited higher α-amylase inhibition as compared with the standard drug, Acarbose (77.1 % at 1000 µg/ml). The numerous polyphenolic compounds, triterpenoids and other chemical compounds present in the plant may account for the observed antidiabetic effect of the gum.

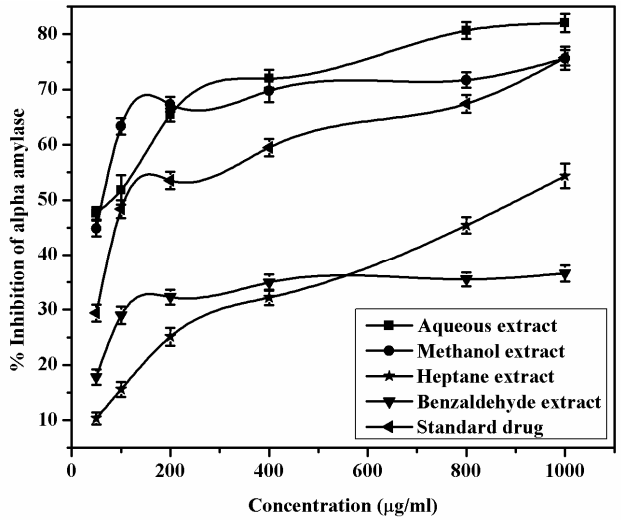

Figure 1 *In vitro* alpha amylase inhibition of gum extract of *Ficus glomerata*

Evaluation of *in vitro* α-glucosidase inhibitory activity

Percent of alpha glucosidase inhibition of the two polar and non-polar extracts was plotted as a function of concentration in comparison with Acarbose as shown in Fig.2. The data presented here indicate that aqueous extract of *F. glomerata* possesses significant *in vitro* inhibitory action on α- glucosidase enzyme by all solvent extracts. The results indicate that aqueous gum extract exhibited well anti alpha glucosidase activity, methanol and heptanes extracts showed appreciable inhibition activity (71.78 %, 68.76 % respectively) and benzaldehyde extract (57.1 %) showed the least inhibitory activity. The percentage inhibition at 100-1000 µg/ml concentrations of all the extracts showed a concentration dependent increase in percentage inhibition. Thus the highest concentration of 1000 µg/ml tested showed maximum α-glucosidase inhibition of nearly 80.09 % by aqueous gum extract. This potent inhibition activity may be explained by the ability of the phytoconstituents because phenolic compounds do inhibit α-glucosidase enzyme [25].

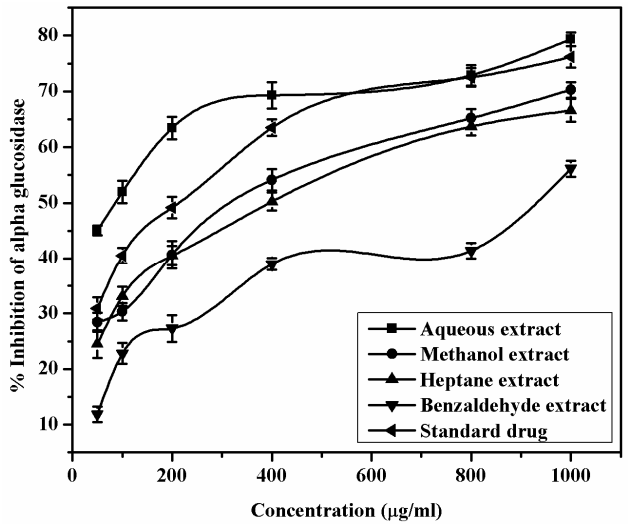

Figure 2 *In vitro* alpha glucosidase inhibition of gum extract of *Ficus glomerata*

CONCLUSION

The hypoglycemic effect of plants has been paid more attention because of increasing incidence of diabetes and predominance of plant derived product in the therapy. The medicinal properties of these plants could be attributed to the presence of one or more of the detected plant natural products. The present study showed that aqueous extract of *Ficus glomerata* gum possessed significant anti-diabetic activity which suggests the presence of biologically active phyto components. This made proper attempt to isolate the active principles from *F. glomerata* gum which might help in the findings of new lead compounds in the fields of anti-diabetic drug research after extensive investigation on bioactivity, mechanism of action, pharmacotherapeutics, and toxicity and after proper standardization and clinical trials.

REFERENCES

[1] Akanksha, A.K. Srivastava, R. Maurya R, *Indian J. Exp. Biol.*, **2010**, 48(3), 294.
[2] M.P. Das, Y. Yasmine, P. Vennila Devi, *Int. J. Pharma Bio. Sci.*, **2015**, 6(3), 711.
[3] S.N. Davis, D.K. Granner; Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas, In: L.L. Brunton, J.S. Lazo, K.L. Parker (ed.), Goodman and Gilman's: The pharmacological basis of therapeutics, 11th ed. McGraw-Hill Medical Publication Division, New York, **2001,** 1706.
[4]W. Marshal, S.K. Bangret, *Clinical Chemistry, Elsevier Ltd*, **2004**, 191.
[5]J.M. Mycek, S. Harvey, P.C. Chape, Insulin and oral hypoglycemic drugs in Lippincott's illustrated Reviews, Pharmacology 2nd (ed) Lipincott Williams and Wilkins, USA, **2000**.
[6]S. Wild, G. Roglic, A. Green, R. Sicree, H. King H, *Diabetes care*, **2004**, 27, 1047.
[7]S.L. Vishwakarma, S. Rakesh, M. Rajani, R.K. Goyal, *Indian J. Exp. Biol.*, **2010**, 48, 26.
[8]S. El-Kaissi, S. Sherbeeni, *Curr. Diabetes Rev.*, **2011**, 7(6), 392.
[9]Syamsudin, *Int. J. Phytomed.*, **2010**, 2, 430.
[10]S. Kumar, R. Malhotra, D. Kumar, *Indian J. Pharm. Sci.*, **2010,** 72(4), 531.
[11]A.M. Gray, Y.H.A. Abdel-Wahab, P.R. Flatt, *J. Nutr.*, **2000**, 130, 15.
[12]V.K. Prabhakar, A. Jaidka, R. Singh, *Int. J. Sci. Res. Pub.*, **2013**, 3(8), 1.
[13]G. Jagtap Supriya, S. Shelar Rohan, M. Munot Neha, R. Ghante Minal, D. Sawant Sanjay, *Int. Res. J. Pharm.*, **2012**, 3(5), 281.
[14]P.A. Mary Helen, Prinitha, S. Jaya Sree, S.M. Madoen Abisha, A. Jacob, *Res. J. Pharm. Biol. Sci.*, **2012**, 3(3), 49.
[15]S.G. Patil, B.K. Honrao, *J. Econ. Taxon. Bot.*, **2000**, 24(3), 688.
[16]N.R. Thalapaneni, K.A. Chidambaram, T. Ellappan, M.L. Sabapati, S.C. Mandal, *J. Complement Integr. Med.*, **2008**, 5(1), 1.

[17]R.T. Dewi, Y.M. Iskandar, M. Hanafi, L.B.S. Kardono, M. Angelina, I.D. Dewijanti, S.D. Banjarnahor, *Pak. J. Biol. Sci.*, **2007**, 10(8), 3131.
[18]S.O. Ogbonnia, J.I. Odimegwu, V.N. Enwuru, *Afr. J. Biotechnol*., **2008**, 7(15), 2535.
[19]D.O. Edem, *Eur. J. Sci. Res*., **2009**, 33(4), 669.
[20]M. Sham Shihabudeen, H. Hansi Priscilla, D. Kavitha Thirumurugan, *Int. J. Pharma Sci. Res*., **2010**, 1(10), 430.
[21]A.S. Upadhye, A.A. Rajopadhye, *J. Sci. Ind. Res*., **2010**, 69, 700.
[22]K.T. Augusti, S. Cherian, *Indian J. Exp. Biol.*, **2008**, 33, 608.
[23]P. Paanakh, V.D. Ravichandra, *Int. J. Pharm. Pharm. Sci.*, **2011**, 3(2), 131.
[24]D. Gupta, Chandrashekar, L. Richard, Yogendra, N. Gupta, *Der Pharmacia Lettre*, **2012**, 4(2), 614.
[25]K. Tadera, Y. Minami, K. Takamatsu, T. Matsuoka, *J. Nutri. Sci. Vitaminol*., **2006**, 52, 149.