Preliminary phytochemical and *in vitro* anti-diabetic activity of *Ficus racemosa (L.)* stems bark extract

[a]Rajendra Chary Vijayagiri, [a]Estari Mamidala
[a]Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal, Andhra Pradesh, India.

Abstract

Ficus racemosa Linn. (Family Moraceae) is traditionally used in the treatment of metabolic disorders and skin problems. The present study is aim to identify the phytochemical components in *Ficus racemosa* plant extract and to test the anti-diabetic activity by *in vitro* study. Phytochemical analysis was performed by various qualitative methods. In vitro anti-diabetic activity was done by measuring the α-amylase and α-glucosidase enzyme inhibitory activity. The extraction of *Ficus racemosa* stem bark was carried out using sequential extracts of solvents with varying polarity; hexane, chloroform, ethyl acetate, acetone and methanol respectively. The qualitative phytochemical analysis shows the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, phenols, flavonoids, quinones, steroids, amino acids and terpenoids in various extracts. The chloroform extract shows highest α-amylase and α-glucosidase enzyme inhibitory activity when compared to other solvent extracts. The results obtained in the present investigation indicated *Ficus racemosa* stem bark as a rich source of herbal medicines and having anti-diabetic compounds.

KEYWORDS: *Ficus racemosa (L)*, phytochemicals, TLC, plant extracts, R_f (retention factor).

Introduction

Diabetes mellitus is an endocrine and metabolic disorder characterized by chronic hyperglycemia, dyslipidemia, and protein metabolism that result from defects in both regulations of insulin secretion and/or insulin action (Bloc et al, 2006). There has been a dramatic increase in the number of diabetic patients worldwide because of changes in life-style and diet. The major complications associated with diabetes include retinopathy, neuropathy, nephropathy, atherosclerotic coronary artery disease and peripheral atherosclerotic vascular disease. Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis (Kaczmer, 1998). However, the challenge is to optimize glycaemic control with minimum number of medication while taking into consideration the cost of the therapy, adverse effect profiles, ease of administration, and the urgency for blood sugar normalization. Insulin and Insulin sensitizers, as well as enzyme inhibitors such as α-glucosidase and α-amylase inhibitors contribute as an important therapeutic option in the treatment of diabetes (Akhtar and Al, 1984).

Many traditional medicinal plants are good sources of unique phytochemical compounds such as polyphenols and flavonoids. Recent studies have shown that some medicinal

plants containing high total polyphenolic compounds and flavonoids yield can be linked to intestinal α -glucosidase and pancreatic α -amylase inhibitory activities in vitro (Koh et al, 2010). Thus, efforts have been directed at investigating intestinal α-glucosidase and pancreatic α–amylase inhibitors from medicinal plants that are largely free of major undesirable side effects.

Ficus racemosa linn is commonly known as Cluster fig and Udumbara (sanskrit) belongs to the family Moraceae, is an evergreen, moderate sized, deciduous tree and used as herbal medicine from ancient times. Its leaves are dark green, ovate and having traditional medicinal importance (Abu Hasant. 2011). This is native to Australia, South East Asia and the Indian subcontinent. It is unusual in this plant that its figs grow on or close to the tree trunk (Joseph. 2010). *F. racemosa* is widely distributed genus in North East India and abundant in Warangal district, Andhra Pradesh having used by Warangal peoples to reduce diarrhea and stop bleeding along with some other ethno medicinal practices (Rajendra Chary Vijayagiri. 2012; Vinatha Naini and Estari Mamidala, 2013). So, it was the need of time to explore some of the species of this genus specially *Ficus racemosa* which is available in southern part of Andhra Pradesh, India, for better upgradation of knowledge regarding the phytochemicals and its biological activity of this genus. Therefore, the aim of this work is to carry out a phytochemical screening of stem bark extracts of *Ficus racemosa* and *in vitro* antidiabetic activity testing.

Materials and Methods:

Plant material

Based on ethno botanical investigation and in our previous study (Rajendra chary Vijayagiri. 2012), the *Ficus racemosa* plant was selected for study. *Ficus racemosa* stem bark was collected from rural areas of Venkatapuram forest, Warangal dist., Andhra Pradesh, India. The taxonomic identities of these plants were confirmed by Department of Botany, Kakatiya University, Warangal, India. The collected fresh stem bark of plant free from diseases was bought to laboratory in sterile polyethylene bags and washed thoroughly 2-3 times with running tap water and then shade dried for three weeks, subsequently ground into fine powder using mechanical grinder and motor driven grinding mill. The powder was used for extraction of crude extracts.

Preparation of plant extracts

Around 2 kg of stem bark of *Ficus racemosa* was powdered by using of electric grinder to obtain coarse powdered which is best suited for extraction. The powder was weighed and plant powder material was extracted successively with maceration method. In this method using solvents ranging from non polar to polar i.e., hexane, chloroform, ethyl acetate, acetone and methanol for 24 hrs. The extract was concentrated under vacuum using rotary vacuum evaporator. The obtained extracts were stored at 4^{o}C till to use for phytochemical investigation.

Preliminary phytochemical analysis

Preliminary phytochemical screening of the extracts for alkaloids, carbohydrates, glycosides, saponins, tannins, phenols, flavonoids, quinones, steroids, amino acids and

terpenoids using standard phytochemical screening methods (Harbone. 2009, Siddiqui. 1997 and Earnsworth. 1974).

Inhibition of α–amylase:
Starch solution (0.5% w/v) was prepared in Tris HCl buffer with 6.7 mM sodium chloride (pH 6.9) in boiling water for 5 min and preincubated at 37°C for 5 min. The extracts of *F. racemosa* were dissolved in DMSO to obtain concentration of 10, 20, 40, 60, 80 and 100 µg/ml. Then 0.2 ml of plant extract was added to the tubes containing starch solution. A total of 0.1 ml pancreatic amylase solution (PPA-Sigma Aldrich) prepared in Tris HCl buffer (2 units/ml) was added to the tube containing plant extract and starch solution. The reaction was carried out at 37°C for10 min. The reaction was stopped by adding 0.5 ml 50% acetic acid. The reaction mixture was centrifuged at 3000 rpm for 5 min 4°C. The absorbance of supernatant was measured at 595 nm (Conforti et al, 2005).

Percentage inhibition = Control-Test/Control × 100

Inhibition of α – glucosidase
Enzyme solution prepared in Tris buffer (pH 8) was added to the tubes containing increasing concentration of extracts of *F. racemosa* (10, 20, 40, 60, 80 and 100 µg/ml) at 37°C for 60 min. Then the reaction mixture was heated for 2 min in boiling water to stop reaction. The absorbance was measured at 540 nm. Percentage inhibition was calculated by using the following equation (Hansawasdi et al, 2000 and Vogel, 2002).

Percentage inhibition = Control-Test/Control × 100

Results:

Yield of *Ficus racemosa* stem bark extracts
The yield of sequential extracts (g) is shown in Table 1. The 500 grams of the plant material used for extraction with each solvent and the amount of the hexane extract obtained from the extraction was (7.3) (1.46 % w/w yield), chloroform extract was 6.6 g (1.32 % w/w yield), ethyl acetate extract 5.3 g (1.06 % w/w yield), acetone extract 2.9 g (0.58 % w/w yield) and methanol extract was 47.6 g (9.52 % w/w yield).

Phytochemical screening of *Ficus racemosa* stems bark extracts
Phytochemical screening of the sequential extract of *Ficus racemosa* stem bark revealed the presence of various bioactive components of which phenolics, saponins, steroids, alkaloids, flavonoids, terpenoids, tannin, and cardiac glycosides are the most prominent components and the result of phytochemical test is presented in Table 2. Among these phytochemical tests, methanol extract was found to contain maximum saponin content along with plant phenolics such as alkaloids, glycosides and tannins.

Inhibition of α–amylase
All solvent extracts of *F. racemosa* as a test drug and Acarbose as reference standard were analysed for α-amylase inhibitory activity at concentration of 10, 20, 40,

60, 80 and100 µg/ml. A dose dependent, gradual rise in inhibition of α-amylase was observed for test and standard drugs as shown in Figure 1. The chloroform extract of *F. racemosa* shows highest α-amylase inhibitory activity when compared remaining solvent extracts. The IC_{50} value for chloroform extract and and Standard drug were found to be 64.5 µg/ml and 51.8 µg/ml, respectively. The chloroform extract shows 81% α–amylase inhibitory activity where standard drug acarbose shows 84% at 100 µg/ml concentration.

Inhibition of α–glucosidase:

A gradual rise in inhibitory activity of α-glucosidase was observed for all solvent extracts of *F. racemosa* (Test Drug) and acarbose (Standard reference) as shown in Figure 2. The chloroform and acetone extracts shows highest α-glucosidase inhibitory activity when compared to methanol, ethyl acetate and hexane extracts. The IC_{50} value for test drug chloroform extract and standard drug were found to be 71.2 µg/ml and 52.9 µg/ml, respectively. The chloroform extract shows 66% α-glucosidase inhibitory activity compared to acarbose (68%).

Discussion

In the present study, we investigated F. racemosa stem bark extracts (n-hexane, chloroform, ethyl acetate, acetone and methanol) with anti-diabetic properties for intestinal α -glucosidase and pancreatic α -amylase inhibitory activities. There is an abundant medicinal plant throughout the world but only small amounts are investigated for its biological activity (Awadh Ali. 2001). Previous study of the phytochemical analysis of *Ficus racemosa* report as found that most of the biologically active phytochemicals were present in the ethanolic extract of *Ficus racemosa* bark (Poongothai, 2011). The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities (Ismail. 2011). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the stem bark of the plants studied (Ogu. 2012). Qualitative tests performed on the stem bark extracts of *Ficus racemosa* indicate the presence of alkaloids, carbohydrate, glycosides, saponins, tanins, phenolic compounds, proteins, flavonoids, terpenoids, quinines, steroids in different extracts.

The basic fundamental lying behind hyperglycemia involved overproduction and decreased utilization of glucose (Koh et al, 2010). Alloxan, destroys β cells of islet of langerhens of pancreas resulting in decrease in the insulin secretion and leads to decreased use of glucose by tissues (Conforti et al, 2005). α- amylase is main enzyme present in pancreas responsible for the digestion of starch and absorption of glucose. Its inhibitors such as acarbose inhibit the release of glucose in the blood and thereby achieving the anti-diabetic effect. Our finding revealed that the chloroform extract of *F. racemosa* efficiently inhibited the enzyme [Figure 1]. α-glucosidase is responsible for the digestion of carbohydrates to simpler carbohydrates and its absorption in small intestine (Abu hasant, 2011) Chloroform and ethyl acetate extracts of *F. racemosa* significantly inhibit the enzyme and thus attributed for anti-diabetic activity. Further purification, identification and characterization of the active compounds would be our priority in the future studies.

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Table-1: Yield of extracts

	Extract yield				
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Powder (g)	500	500	500	500	500
Yield (g)	7.3	6.6	5.3	2.9	47.6
Yield(w/w)(%)	1.46	1.32	1.06	0.58	9.52

Table-2: Qualitative phytochemical screening of *Ficus racemosa* stems bark

Phytochemicals	Test conducted	Extracts				
		Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids	Mayer's test	+	-	-	-	-
	Hager's test	+++	+++	+++	+++	+++
Phenols	Lead acetate test	+++	-	-	++	++
	Ferric chloride test	-	-	++	++	+++
Terpenoids	Test for terpenoids-1	+	+	+	+	+
	Test for terpenoids-2	+	+	+	+	+
Carbohydrates	Fehling's test	+++	+++	+++	+++	+++
	Benedict's test	+++	+++	+++	+++	+++
Starch	Iodine test	+++	+++	+++	+++	+++
Glycosides	Borntrager's test	+++	++	+++	+++	+++
	Brown ring test	+++	+++	+++	+++	+++
Flavonoids	Alkaline test	+++	+++	+	+	+
Quinones	Test for quinones	++	++	++	++	++
Tannins	Test for tannins	-	-	-	+	+
Saponins	Saponification test	++	-	+	+	+++
Steroids	Test for steroids	+	+	+	+	+
Proteins & amino acids	Millon's test	-	-	-	-	-
	Biuret test	-	-	-	-	-
	Nin hydrin test	-	-	-	-	-

Figure 1: α-amylase inhibitory activity

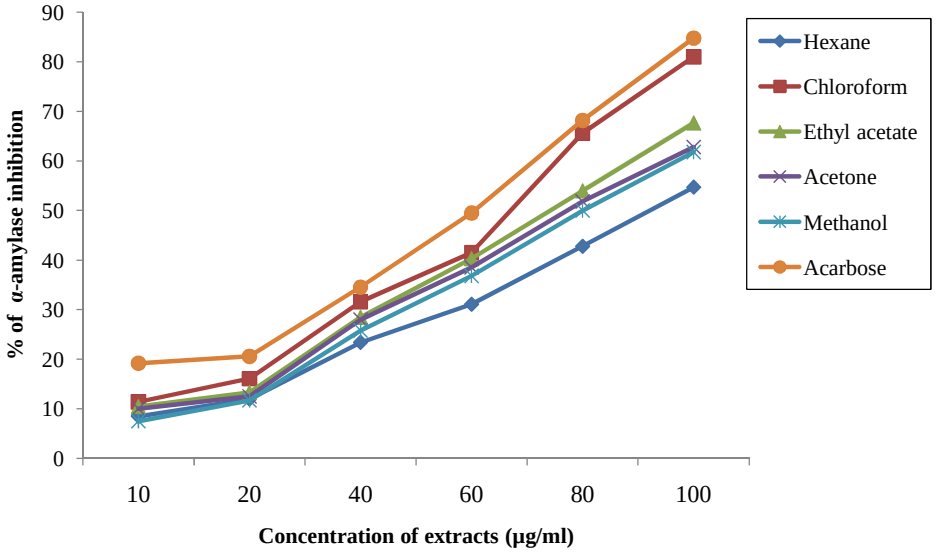

Figure-2. α-glucosidase inhibitory activity