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ASSESSMENT OF *IN VITRO* ANTI-DIABETIC ACTIVITY OF *Ficus tinctoria* STEM EXTRACTS

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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. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α- amylase and α-glucosidase and α-amylase. Inhibition of amylase and glucosidase enzymes involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in management of blood glucose.The aim of the current study was to screen the water, hydroalcholic, methanol and ethyl acetate extracts of stem of *Ficus tinctoria* for its in vitro antidiabetic activity. The in vitro studies for alpha-amylase and alpha-glucosidase inhibitory action were performed using different concentrations of the extracts and the results were compared with that of a standard drug. Our findings revealed that among all the extracts, hydroalcholic and ethyl acetate extracts showed efficient anti-diabetic activity. Ethyl acetate extract of *Ficus tinctoria* stems has shown an IC_{50} value 229 µg/ml for alpha-amylase inhibitory activity. The same extract was found to have an IC_{50} value 79.60 µg/ml for alpha-glucosidase inhibitory activity. Hydro alchlolic stem extract of *Ficus tinctoria* has shown an IC_{50} value 96.49 µg/ml for alpha-glucosidase inhibitory activity and an IC_{50} value 340 µg/ml for alpha-amylase inhibitory activity.

Key words: *Ficus tinctoria*, ethyl acetate, Diabetes mellitus, anti-diabetic, hydroalcholic, methanolic, α-amylase, α-glucosidase.

INTRODUCTION

Ficus tinctoria is a climbing strangler, forming a tree with prop-roots and grows up to 25m [1]. It has alternately arranged leaves which are oval, glossy dark green above and pale green below with a rounded tip and base. Leaves [2-5] are often asymmetrical with stalks of thickness around 1.5 cm. Fruit is a fig, appearing in leaf axils, usually paired, round. It ripens through orange to red or purple. The fruits are the source of a red dye used in traditional fabric making in parts of Indonesia. *Ficus tinctoria* belongs to family *Moraceae.* Other names of the plant are Dye fig, Humped fig, Datir, kaliatthi, Itthi, gudumittemara, Umbar.

Diabetes mellitus (DM) is a chronic metabolic syndrome in which the deficiency or insensitivity of insulin causes glucose to accumulate in the blood, leading to various complications [6]. The intestinal enzymes like α-amylase and α-glucosidase are very important in carbo hydrate digestion and glucose absorption [7]. The suppression of the activity of such digestive enzymes would delay the breakdown of starch and oligo saccharides to glucose, and in turn causes a decrease in glucose absorption. Consequently elevation [8] of postprandial blood glucose level is controlled.

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 [9]. Out of these two types, Type -2 diabetes is a major problem of today and it accounts for nearly 95% of total

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diabetic population of about 246 million [10]. 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 [11]. To better understand the biological activities of *Ficus tinctoria* we determined the photochemicals present in the various extracts of leaves.

MATERIALS AND METHODS

Plant Collection, Drying and Pulverization

Stem of *Ficus tinctoria* were collected from Rampachodavara, Boduluru village, East Godavari district in the month of April. They were authenticated by Dr. K. N. Reddy, Dept. of taxonomy, Laila impex R&D Centre, Vijayawada. The plant materials were deposited in raw drug museum, Laila impex, R&D centre. The voucher no. of *Ficus tinctoria* stem was 3320. The plant material was air dried under shade at Dept. of taxonomy, Laila Impex R&D Centre and powdered mechanically to coarse or fine powder.

Extraction

The stems of plant material were extracted with water, methanol, ethyl acetate and hydro alcohol (60 % methanol in water) to extraction of *Ficus tinctoria.* Codes of extracts were named as below and in the further discussion the extracts were mentioned as their codes.

FTS 01- *Ficus tinctoria* stem water extract
FTS 02- *Ficus tinctoria* stem hydroalcoholic extract
FTS 03- *Ficus tinctoria* stem methanolic extract
FTS 06- *Ficus tinctoria* stem ethyl acetate extract

IN-VITRO ANTI DIABETIC ACTIVITY

Alpha- glucosidase inhibitory activity

The enzyme α- glycosidase inhibitory activity for *Ficus tinctoria* was determined by incubating solution (0.1 ml) of an enzyme preparation with 0.2 M Tris buffer, pH 8.0 (1.0 ml) containing different concentrations of methanolic and Hydro alcoholic extracts at 37ºC for 60 minutes by using Acarbose as working standard. The reaction mixture was heated for two minutes in boiling water bath to stop the reaction. The amount of liberated glucose was measured by glucose oxidation method. (Prashanth D., 2001) (Assay condition 37ºC±0.1ºC, pH-8.0; O.D at 540 nm)

Alpha-amylase inhibitory activity

A starch solution (0.1 % w/v) was obtained by stirring 0.1 g of potato starch in 100 ml of 16 mM sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylases in 100 ml of cold distilled water. The colorimetric reagent is prepared by mixing a sodium potassium tartarate solution (12 g of sodium potassium tartarate, tetra hydrate in 8 ml of NaOH 2M) and 3, 5-dinitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with α-amylase solution under alkaline conditions at 25 ^{0}C. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5-dintrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction (corresponding to the color change from orange-yellow to red) is detectable at 540 nm.

RESULTS

Ficus tinctoria stem extracts were evaluated for *in-vitro* anti-diabetic activity by α –Glucosidase inhibitory activity and α -amylase inhibitory activity. The extracts FTS 02 and FTS 06 were found to be potent compared to other extracts with IC_{50} of 96.49µg/ml and 79.60µg/ml in α –Glucosidase inhibitory activity. The standard used was Acarbose with IC_{50} 7.18ng/ml. The extracts FTS 02 and FTS 03 showed α-amylase inhibitory activity with IC_{50} of 340µg/ml and 300µg/ml. The Acarbose was used as standard with IC_{50} of 34.94µg/ml.

Table 1. Extraction of *Ficus tinctoria*

Extract	Crude drug	Quantity	Extract obtained	Solvent used
FTS 01	*Ficus tinctoria* stem	100 g	7.97 g	Water
FTS 02	*Ficus tinctoria* stem	100 g	9.36 g	60% Methanol
FTS 03	*Ficus tinctoria* stem	100 g	7 g	Methanol
FTS 06	*Ficus tinctoria* stem	500 g	12 g	Ethyl acetate

Table 2. α –Glucosidase inhibitory activity for extracts of *Ficus tinctoria*

S.No	Test compound	Dose (µg/ml)	Percent Inhibition	IC_{50} (µg/ml)
1	FTS 01	5	1.33	>150
		50	5.28	
		150	23.56	
2	FTS 02	5	4.60	96.49
		50	34.93	
		150	72.33	

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3	FTS 03	10	22.84	29.06
		25	43.40	
		50	80.62	
10	FTS 06	5	13.03	79.60
		50	42.25	
		150	79.67	
11	Standard (Acarbose)	2.5(ng/ml)	18.52	7.18 (ng/ml)
		5 (ng/ml)	46.67	
		10 (ng/ml)	74.63	

Table 3. α-Amylase inhibitory activity of *Ficus tinctoria*

S.No	Test compound	Dose (µg/ml)	Percent Inhibition	IC_{50} (µg/ml)
1	FTS 01	50	13.92	225
		100	22.61	
		200	31.22	
2	FTS 02	50	5.05	340
		100	6.69	
		200	12.21	
3	FTS 03	50	7.80	300
		100	16.33	
		200	20.93	
10	FTS 06	50	6.14	229
		100	15.28	
		200	24.87	
11	Standard (Acarbose)	10	17.30	34.94
		25	33.22	
		50	72.00	

DISCUSSION

In α-glucosidase inhibitory assay inhibition of **α**-Glucosidase delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [12]. **α**-glucosidase inhibition is one of the therapeutic approaches for reducing postprandial hyperglycemia [13]. Screening of plants for enzyme inhibitors like **α**-glucosidase despite their natural constituents, have been performed [14]. The **α**-Glucosidase inhibitory activity was evaluated using crude enzyme extracts. The extracts of Stem of *Ficus tinctoria* were tested against this crude enzyme at various concentrations (50-200µg/ml). Among them the Ethyl Acetate-stem extract of *Ficus tinctoria* has shown significant inhibitory activity with an IC_{50} value of 79.60µg/ml when compared with the standard Acarbose - IC_{50} 7.18ng/ml.

α-amylase inhibitory activity by 2-chloro-4-nitro phenyl α-maltotrioside (CNPG3) assay is one of such models used to study the antidiabetic effect of a test drug *in-vitro*. Alpha-amylase is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions, functioning in the breakdown of the α-1-4-glycosidic bonds in starch. Thus, this enzyme increases the bioavailability of glucose in the blood. For a substance to be anti diabetic, it should be able to reduce the amount of glucose in the blood or increase the efficacy of insulin. It has been reported that the inhibition of α-amylase reduces the bioavailability of glucose. The drugs that inhibit carbohydrate hydrolyzing enzymes have been proved to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion in Non-insulin dependent diabetes mellitus (NIDDM) patients. The study suggests that the polyphenolic compounds involved in radical scavenging activities may also be involved in the α-amylase inhibition. In α- amylase inhibitory assay the aqueous and ethyl acetate extract of *Ficus tinctoria* stem extracts showed activity with an IC_{50} value of 225µg/ml and 229µg/ml when compared with the standard Acarbose - IC_{50} value of 34.94µg/ml.

CONCLUSION

The experiment was done following a simple yet effective method with careful monitoring at each step, be it collection of authentic sample, selection of solvents and extraction procedures, purification of the products and evaluation of their anti-diabetic activity using established methods. It was a great learning experience and the results when compared with the standard drugs were satisfactory, that enabled us to believe the work was successful and has huge scope for further expansion.

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CONFLICT OF INTEREST
No interest

REFERENCES

1. Pickup JC, Williams G. Textbook of diabetes. Blackwell Science Ltd. United States, 2003, 43, 1- 43.
2. Vats RK, Ramachandran U, Jain R, editors. New trends in Harnessing Diabetes. Proceeding of the 57th Indian Pharmaceutical Congress. *A Pharmabiz exclusive*. India, 2005.
3. Kanter MM, Lesmes GR, Kaninsky LA, Ham-Saeger LJ, Nequin ND. Serum creatinekinase and lactate dehydrogenase changes following an eighty kilometer race. *Eur J Appl Physiol*, 57, 1998, 60-5.
4. Muramats U, Kongawa H, Janaka K, Okumura M, Koika K, kuga K, et al. Superoxidase Dismutase in SAS human tongue carcinoma cell line is a factor defining invasiveness and cell motility. *Cancer Res*, 55, 1995, 6210-4.
5. Ito N, Fukushima S, Hasegawa A, Shabata M, Ogiso T. Carcinogenicity of butylated hydroxyl anisole in F344 rats. *J Nat Cancer Institute*, 70, 1983, 343-7.
6. Akanksha AK, Srivastava R, Maurya R. *Indian J. Exp. Biol*., 48(3), 2020, 294.
7. Das MP, Yasmine Y, Vennila DP. *Int. J. Pharma Bio. Sci*., 6(3), 2015, 711.
8. Davis SN, Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas, Goodman and Gilman's: The pharmacological basis of therapeutics, 11th ed. McGraw-Hill Medical Publication Division, New York, 2001, 1706.
9. Marshal M, Bangret SK. Clinical Chemistry, Elsevier Ltd, 2004, 191.
10. Mycek MJ, Harvey S, Chape PC. Insulin and oral hypoglycemic drugs in Lippincott's illustrated Reviews, Pharmacology 2nd (ed) Lipincott Williams and Wilkins, USA, 2000.
11. Wild S, Roglic G, Green A, Sicree R, King H. Diabetes care, 2004, 27, 1047.
12. Stratil P, Klejdus B, Kubán V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables--evaluation of spectrophotometric methods. *J Agric Food Chem*, 54(3), 2006, 607-16.
13. Auddy B, Ferreiro M, Blasina F, Lafon L, Arredondo F *et al*., Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol*, 84, 2003, 131-138.
14. Meyer AS, Isaksen A. Application of enzymes as food antioxidants. *Trends Food Sci Tech*, 6, 1995, 300-304.