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Invitro Antidiabetic activity of Ethanolic Extracted Leaves in *Trigonella foenum gracum.L*

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ABSTRACT

The present study was aimed to investigate *In-vitro* Antidiabetic activity of Ethanolic extract Leaves in Trigonella *foenum gracum. L* and investigate Phytochemicals constituents responsible for the lowering blood glucose levels. Other pharmacological effect also produced by TFG is Reduced Respiratory Infections, optimation of cholesterol levels, lower Inflammation, etc. The Preliminary Phytochemical Investigation of the Ethanolic Leaves Extract of TFG was performed. From the observation we identified Alkaloids, Terpenoids, Flavonoids, Sterols saponins, Glycosides and proteins. TFG Ethanolic extracted leaves potent inhibited pancreatic α-amylase and α-glucosidase enzymes. In conclusion, the above phytochemical constituents used in controlling Type-II Diabetes Mellitus.

Keywords: Trigonella foenum gracum, α-amylase, α-glucosidase enzymes, Type-II Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus is a multipart and a diverse group of disorders that interrupts the metabolism of carbohydrate, fat and protein. Diabetes mellitus was divided into two major types, Type 1 (Insulin Dependent Diabetes Mellitus) and Type 2(Non-Insulin Dependent Diabetes Mellitus), and about 90% of diabetes patients in the world are Type 2 diabetes[1]. Daily injection of insulin, the main treatment method employed to management of Type 1diabetes, is administered and also brings great pain to patients. Alpha-amylase, Alpha-glucosidase inhibitors focusing on reducing the digestion of carbohydrate is the most common and efficacious agents utilized for the treatment of Type 2 diabetes[2]. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2019, the world health organization estimated a total of 1.5 million deaths from diabetes mellitus, and this report projected to increase to 366 million by 2030[3]. With a long course and serious complications often resulting in high death rate, the treatment of diabetes spent vast amount of resources including medicines, diets, physical training and so on in all countries. Thus searching for an Improve the Herbal Medicines for treatment of diabetic complication. There is continuous search for alternative drugs[4].

Trigonella foenum gracum Linn. (Fabaceae) is commonly known as Fenugreek in English.

Trigonella foenum gracum Linn leaves are used in the treatment of Reduced Respiratory Infections, optimation of cholesterol levels, lower Inflammation, etc. The leaves showed hypoglycemic, Inhibition of alpha- amylase and alpha-glucosidase enzymes can be an important strategy in management of post prandial blood glucose level in type 2 diabetes patient[5]. Thus, objective of the present study is to investigate the phytochemical, invitro antidiabetic activity of ethanolic extract of Trigonella foenum gracum Linn leaves.

MATERIALS AND METHODS

Materials and Reagents

Alpha-Amylase, Alpha-Glucosidase Purchased in National Scientific Laboratories, Acarbose, Glibenclamide, 0.02 M sodium phosphate buffer, α-amylase solution, dinitrosalicylic acid, p-nitro phenyl-β-D- glucopyranoside (PNGP), potassium phosphate buffer, p- nitro phenyl-β-D-glucopyranoside were purchased from Aman scientific Labs. In UV determinationsUV–vis spectrophotometer, Elico was used.

Plant Material

Leaves of *Trigonella foenum-graecum L.* was collected from surrounding areas of Tenali, Guntur Dist and authentication of the plant was done by Mr.T.S.Chandra Saker Raju. A herbarium specimen of the plant was kept in Department of Pharmacognosy (NSK/Ph.cog/herb/07/2021), NRK&KSR Guptha College of Pharmacy, Tenali, Guntur Dist,Andhra Pradesh. The collected material was washed with running water. The Leaves were chopped into small pieces and dried under shade. Dried Leaves were coarsely powdered and used for extraction.

Preparation of Herbal Extracts

The powdered Leaves were extracted in a Soxhlet apparatus with Ethanol at a temperature of 50^0C for 12 h[6]. The resultant extract was filtered. The filtered extract was then concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40^0C[7]. The dried mass was stored in a desiccators and the yield was 15 gr this extract has been termed as “TFGA”.

Phytochemical Investigation[8,9]

Table 1: Preliminary Phytochemical Investigation of Ethanolic Leaves extracts of *Trigonella foenum-graecum L*

S.No	Test	Alcoholic Extract
1.	**Alkaloids**	
	1.Mayers Test	Present
	2.Wagner’s Test	Present
	3.Dragendroff’s Test	Present
	4.Hager’s Test	Present
2.	**Flavaonoids**	
	1.Shinoda test	Present
3.	**Glycosides**	
	1.Borntrager’s test	Present
	2.Legal’s test	Present
	3.Killer-killani test	Present
4.	**Carbohydrates**	
	1.Molish test	Present
	2.Benedicts test	Present
	3.Fehling’s test	Present
5.	**Phytosterols**	
	1.Salkowski’s test	Present
	2.Liberrman Buchard’s test	Present
6.	**Phenolics and Tannins**	
	1.Ferric chlorides test	Present
	2.Gelatin test	Present
	3.Lead acetate test	Present
7.	**Saponins**	

	1.Froth test	Present
	2.Liberrman Buchard's test	Present
8.	**Proteins &Amino Acids**	
	1.Millon's test	Present
	2.Biuret test	Present
	3.Ninhydrin test	Present

Pharmacological Evaluation Tests[10,11]

Invitro Antidiabetic Activity

1. **α-Amylase Inhibitory Assay:** This assay was carried out weigh amount of PHF (1.25–10 mg/mL) was placed in a tube and 250 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was preincubated at 25∘ C for 10 min, after which 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25∘ C for 10 min. The reaction was terminated by adding 500 μL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water[12]. The α-amylase inhibitory activity was calculated as percentage inhibition

$$\% \text{ of } \alpha\text{-amylase inhibition} = \frac{Abs 100\%\ control - Abs\ sample}{Abs\ 100\%\ control} \text{X}100$$

2. **Inhibition of α-Glucosidase Activity:** In this method p-nitro phenyl-β-D- glucopyranoside (PNGP) was used as substrate and enzyme inhibitory was measured by % inhibition based on turbidity of each sample. The concept behind O.D measurement here is, more inhibition causes less conversion of substrate to product, and hence sample contains more substrate and more turbidity. A 2 mM p-nitro phenyl-β-D-glucopyranoside (0.5 mL), 0.2 mL PHF (20µg/mL) and 50 mM potassium phosphate buffer (0.3 mL) pH 5 were placed in a test tube and incubated at 37° for 10 min in a water bath. A 20 mU of enzyme β-glucosidase (Enzyme activity: 3500U/mg) was added and incubated at 37° for 30 min[13]. After completion of incubation period, the enzymatic reaction was terminated by addition of 2.6 mL of potassium phosphate buffer pH 10. The same was done with acarbose, a commercially available enzyme inhibitory drug to control Diabetes mellitus and final results were compared with this standard drug. For negative control, phosphate buffer of pH 10 was added at the beginning of the reaction to block enzyme activity. Absorbance was measured at 410 nm for each sample[14]. The percentage inhibition calculates using this formula

$$\% \text{ of} \alpha\text{-Glucosidase inhibition} = \frac{Abs 100\%\ control - Abs\ sample}{Abs\ 100\%\ control} \text{X}100$$

RESULTS AND DISCUSSION

Table 2: Observation of α-Amylase Inhibitory Assay

S.No	Sample	Concentration	% of Inhibition	IC50 (µg/ml)
1.	TFGA	100 (μg/mL)	32.44	
2.	TFGA	200 (μg/mL)	48.64	
3.	TFGA	300 (μg/mL)	59.89	233.3±4.17
4.	TFGA	400 (μg/mL)	68.62	
5.	TFGA	500 (μg/mL)	77.90	
6.	Acarbose	100 (μg/mL)	50.91	
7.	Acarbose	200 (μg/mL)	61.31	
8.	Acarbose	300 (μg/mL)	68.23	90.8±10.83
9.	Acarbose	400 (μg/mL)	74.21	
10.	Acarbose	500 (μg/mL)	89.18	

Trigonella foenium gracum Leaves L, IC50: Inhibitory concentration, values are expressed as mean+SEM, SEM: Standard error of the mean

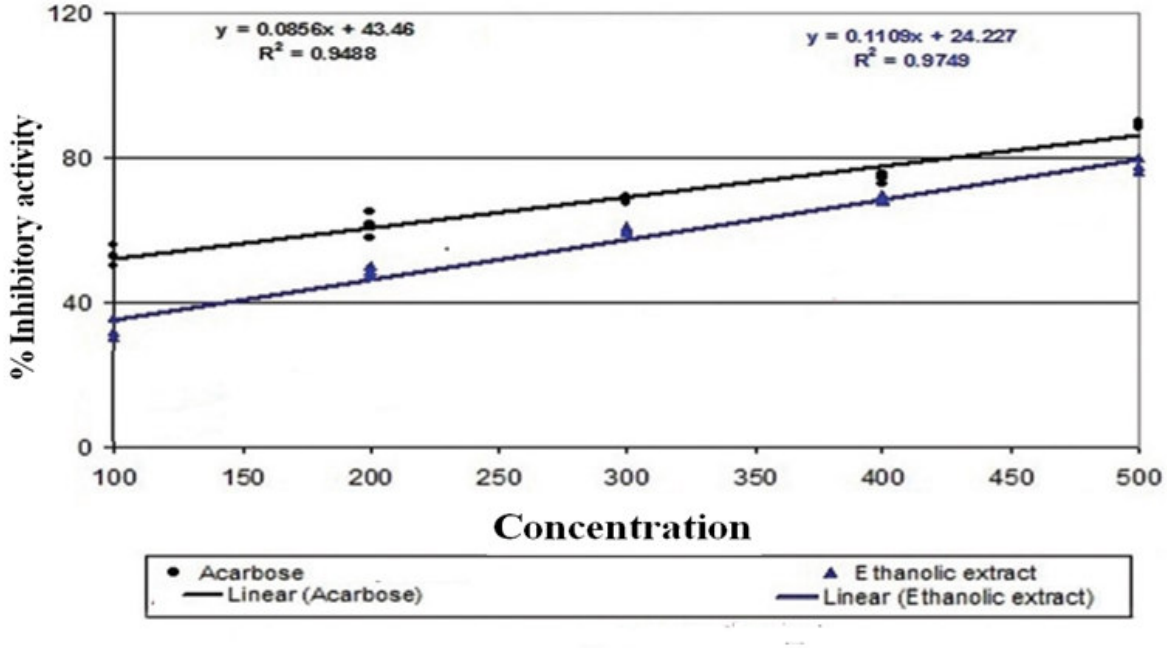

Figure 1: In vitro α-amylase inhibitory activity of *Trigonella foenium gracum Leaves L.*

Table 3: Observation of *α*-Glycosidase Inhibition Activity

S.No	Sample	Concentration	% of Inhibition	IC50 (µg/ml)
11.	TFGA	100 (*µ*g/mL)	34.90	
12.	TFGA	200 (*µ*g/mL)	49.10	
13.	TFGA	300 (*µ*g/mL)	58.22	236.7±1.67
14.	TFGA	400 (*µ*g/mL)	61.94	
15.	TFGA	500 (*µ*g/mL)	80.94	
16.	Acarbose	100 (*µ*g/mL)	47.76	
17.	Acarbose	200 (*µ*g/mL)	64.89	
18.	Acarbose	300 (*µ*g/mL)	77.63	75±14.43
19.	Acarbose	400 (*µ*g/mL)	81.27	
20.	Acarbose	500 (*µ*g/mL)	88.65	

Trigonella foenium gracum Leaves L, IC50: Inhibitory concentration, values are expressed as mean+SEM, SEM: Standard error of the mean

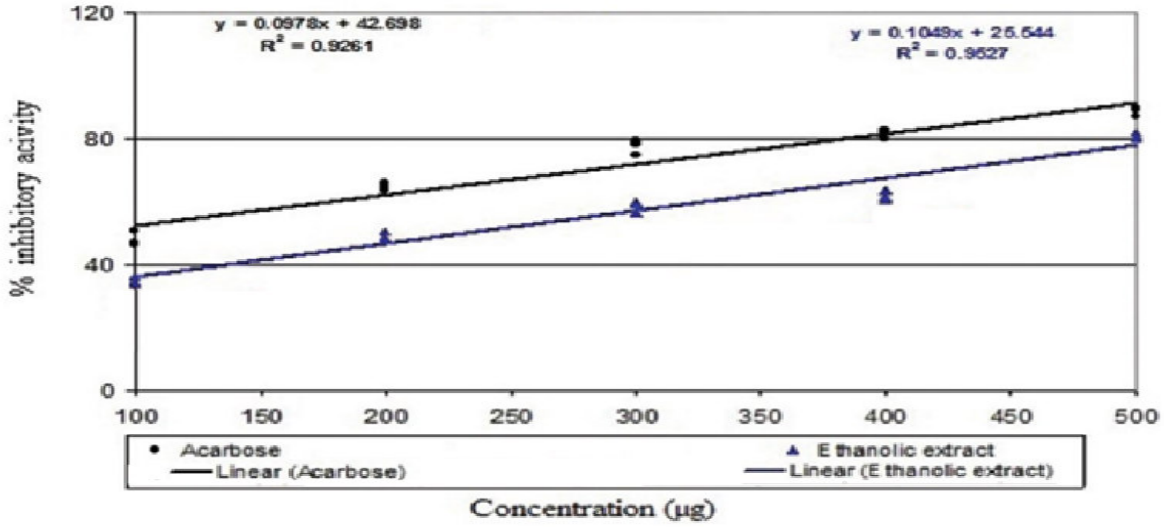

Figure 2: In-vitro *α*-Glucosidase Activity inhibitory activity of Ethanolic Leaves Extracted *Trigonella foenium gracum Leaves L.*

Statistical Analysis

All determinations were done in triplicate and values are expressed as the mean ± standard error of the mean. The result is also expressed as IC50 value. IC50 value was calculated using regression analysis.

DISCUSSION

The trend in the screening of medicinal plants for antidiabetic activity has increased, as it is important to discover novel effective drugs for the disease. However, the WHO has recommended daily exercise and healthy food intake as an effective method of controlling the diabetes type II. Therefore promoting the urban population for a healthy living style with the use of Medicinal Plants that possess anti-diabetic activity. The α-amylase inhibitory study and α-glucosidase inhibitory study performed demonstrated that the Leaves extracts of Trigonella foenum gracum had significant inhibitory potentials. The IC50 value (233.3±4.17µg/ml) of ethanol extracts Hence the above results suggest that the leaf extracts of Trigonella foenum gracum could be greatly beneficial in reducing the glucose levels in the body also can be effectively used in ayurvedic treatments

CONCLUSION

According to the result of the study on the leaf extracts of Trigonella foenum gracum exhibit α-amylase inhibitory activity and α-glucosidase inhibitory activity with remarkable activity in the crude ethanolic extract. Hence leaves of Trigonella foenum gracum has the potential to be used as a green vegetable and also be used in ayurvedic decoctions in controlling and treatment of Type II diabetes mellitus. Furthermore, this study has opened opportunities for future research in searching for novel effective drugs for diabetics that possess both anti-diabetic activities.

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