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In Vitro Antidiabetic, Antioxidant Activities and GCMS Analysis of Crude Extracts of *Trigonella foenum-graecum* Seeds

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Abstract: *In vitro* antidiabetic and antioxidant activity and an evaluation of phytochemicals and the Gas chromatography Mass spectroscopic analysis of *Trigonella foenum graecum* (fenugreek) seed extracts were performed in the present investigation. Different seed extracts such as aqueous, methanol and ethanol extracts of fenugreek seeds were extracted and the qualitative presence of tannins, alkaloids, flavonoids, terpenoids were determined in all the extracts along with the other metabolites tested in the present study. The GCMS analysis revealed the presence of many bioactive compounds such as Chliefenapyr, hexadecylamine-N allyl, 4-(1 indanyl) phenol trimethylsilyl ether, Dasycarpidan 8 (16H)-etanol,3,18-didehy, n-proppyl 9,12-octa decadienoate were recorded. DPPH free radical scavenging activity of the crude extracts showed potential antioxidant activity in comparison with the standard antioxidant agent. Alpha amylase and alpha glucosidase enzyme inhibition studies revealed the potential activity of the methanol (IC_{50} = 56.46 µg/ml) and ethanol (IC_{50} = 77.05 µg/ml) extracts in controlling the postprandial glucose level than the standard drug acarbose (IC_{50} = 71.52 µg/ml). The *in vitro* analysis of inhibition of enzymes responsible for the rise in glucose level implied the potential role of these crude extracts in the medicinal use of fenugreek seeds. Further investigations are warranted for the identification of the bioactive compound responsible for the activity and *in vivo* validation of the pharmacological properties of the fenugreek seeds.

Keywords: Diabetes mellitus, DPPH, α-Amylase, α-Glucosidase, Antioxidant, Fenugreek, *Trigonella*

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Introduction

Modern lifestyle and availability of pleasurable junk foods contributes to a gene-environment interaction with many serious health complications such as cardiac disorders and various types of cancers (Saad *et al*., 2017). Diabetes mellitus a metabolic disorder has been described as one of the medical emergencies around the globe in the 21st century (IDF, 2015). Around 75% of people with diabetes were lived in low- and middle-income group of countries

(IDF,2015). Impaired production of insulin by pancreas or improbable response of target tissues such as liver and muscle to the circulating insulin results in diabetes. Type 2 diabetes was referred as a complex metabolic disorder with the complications like β cell dysfunction, insulin resistance, abnormal metabolism, increased oxidative stress which leads to micro and macrovascular complications.

The onset of diabetes is due to the lack of insulin production or insulin resistance (Tahrani *et al*., 2016). The prolonged uncontrolled diabetes mellitus leads to major complications such as cardiovascular disease, nephropathy, neuropathy and retinopathy (Yuen *et al*., 2019). The diabetic patients especially type I patients are treated with synthetic drugs with more of side effects. Diabetes mellitus can be managed by administering subcutaneous dosage of insulin and several oral hypoglycemic agents like insulin sensitizers, insulin secretagogues and α-glucosidase inhibitors etc. However, the administration of synthetic drugs to the patients leads to untoward side effects such as acidosis, abdominal discomfort, cholestasis, severe allergic reactions and renal failure (Baliga *et al*., 2017). The synthetic drugs effectively maintain the hypoglycemic condition while it fails to control the progression of the disease and associated complications. The challenge ahead of managing diabetes is to treat without any side effects and it is required to explore alternative source with no or less side effects. It is essential to develop a new drug to provide long lasting metabolic effects to avoid β cell damage. Chronic hyperglycemic condition adversely affects both the secretion and action of insulin (Rosetti *et al*., 1993).

The α-amylase hydrolyses polysaccharides into oligomers such as maltose (Kashton and Baek, 2023). α-glucosidase located in the brush border hydrolyses the oligomers and saccharides into glucose into the lumen of intestine (Butterworth *et al*., 2011). The antidiabetic enzyme α-amylase secreted by the pancreas and salivary glands are involved in the digestion of insoluble oligosaccharides into glucose and simple monosaccharides in the intestinal mucosa (Zinjarde *et al*., 2011). α-amylase hydrolyses the alpha bonds of alpha linked polysaccharides and thereby increases the amount of available glucose for absorption which results in hyperglycemic condition. The inhibition of the activity of alpha amylase is considered as one of the major strategies for the management of diabetes and obesity (Sales *et al*., 2012).

α- glucosidase inhibitors like acarbose working on the mechanism by inhibiting the enzyme action in the small intestine and thereby reduces the available glucose for absorption (Chang *et al*., 2004). α- glucosidase enzyme is secreted on the brush border of enterocytes of jejunum (Lebovitz *et al*., 1997). α-glucosidase enzyme binds with di and oligosaccharides and cleaves the 1,4 alpha bonds to enable the formation of single α glucose molecules. It is known that mild hyperglycemic condition leads to the impaired secretion of insulin in humans.

Inhibition of α- amylase will delay the process of conversion of polysaccharides into simple sugars for the absorption into the blood stream, thereby prevents the increase in the postprandial blood sugar level. α-amylase and α-glucosidase are the enzymes responsible for the process of digestion of carbohydrates, it is known that the inhibition of these enzymes will reduce the chances of availability of glucose and prevents the increase of blood glucose level after the meal. The quest for the invention of new α-amylase and α-glucosidase inhibitors is very crucial for the maintenance and management of postprandial hyperglycemic condition in type-2 diabetes patients. *In vitro* antidiabetic and antioxidant activity and an evaluation of phytochemicals and the Gas chromatography Mass spectroscopic analysis of *Trigonella foenum graecum* (fenugreek) seed extracts were performed in the present investigation.

Materials and Methods

Seed extract preparation:

Fenugreek (*Trigonella foenum graecum*) seeds were collected and authenticated with the P.G Research Department of Plant Biology, Chennai, India. The seeds were washed thoroughly and dried at an ambient temperature for 15 days. The air dried seeds were ground into fine powder using electric blender.

The dried powder of the fenugreek seeds was extracted using soxhlet method. Water, methanol, and ethanol were used as solvents for the extraction procedure following the method of Kavitha and Karthikeyan (2023). The collected extract was concentrated using rotary evaporator under controlled pressure. The collected extracts were dissolved in DMSO with different concentration subjected to various analysis.

Phytochemical analysis:

The qualitative analysis of the phytoconstituents such as steroids, tannins, saponins, coumarins, alkaloids, flavonoids, diterpenoids, cardiac glycosides, proteins, terpenoids and anthocyanins was performed following the standard protocols (Harborne, 1973).

DPPH Radical Scavenging Assay (*Tailor and Goyal, 2014*):

2,2-diphenyl-l-picrylhydrazyl scavenging assay was performed to evaluate the antioxidant potential of the different extracts of *Trigonella foenum-graecum* seeds. 0.5 ml of the aliquots of seed extracts with varying concentration from 20, 40, 60, 80 and 100 µg/ml were taken in different test tubes other than reagent blank. 3 ml of ethanol and 0.3 ml of 0.5 mM DPPH radical solution in ethanol was added to the tubes. 3.5 ml of ethanol and 0.3 ml of DPPH radical solution was used as control solution. 30 min after the period of reaction the absorbance of the mixture was measured using UV-10 UV Vis spectrophotometer and the percentage of scavenging activity was measured using the formula:

$$\% \text{ of Antioxidant activity} = \frac{\text{OD of Test- OD of Control}}{\text{OD of Test}} \times 100$$

Alpha amylase inhibition assay (*Kwon et al., 2006*):

The α-amylase activity of the crude extracts of *Trigonella* seeds were measured by DPPH reagent method. Different concentrations of the extracts and standard drug acarbose (20 µg/ml to 100 µg/ml) were taken into separate test tubes and 1 ml of alpha amylase dissolved in 0.2 M sodium phosphate buffer (pH 6.9) was added and the tubes were incubated for 30 min at 25 °C, after the period of incubation 1% starch solution was added into the tubes and the reaction mixture was incubated at 25 °C for 3 min. The reaction was arrested by adding 1 ml of 3.5 dinitrosalicylic acid and 9 ml of distilled water was added to the reaction mixture and the absorbance was measured at 450 nm using Thermo UV-10 UV-Visible spectrophotometer. The percentage of alpha amylase inhibition was calculated as:

$$\% \text{ of } \alpha\text{- amylase inhibition} = \frac{\text{OD of Test- OD of Control}}{\text{OD of Test}} \times 100$$

Alpha Glucosidase inhibition assay (*Kim et al., 2004*):

α-Glucosidase enzyme inhibition was carried out with the standard protocol. A 100 µl of 10 µl of glucosidase was taken in different tubes and 50 µl of different extracts and standard acarbose were added into the tubes labelled as test and control and incubated for 10 min at 25 °C. 50 µl of p-nitrophenyl alpha- D-glucosidase was added, vortexed and incubated at 25°C for 5 min. The reaction was stopped by adding 800 µl of stop solution (0.1 M sodium carbonate) into the tubes and the samples were measured at 405 nm using Thermo UV-10 UV Visible spectrophotometer. The percentage of alpha glucosidase inhibition was calculated using the following formula:

$$\% \text{ of } \alpha\text{-Glucosidase inhibition} = \frac{\text{OD of Test- OD of Control}}{\text{OD of Test}} \times 100$$

Results

Phytochemical analysis of the different solvent extracts is depicted in Table 1. The results revealed the presence of tannins, coumarins, alkaloids, flavonoids, terpenoids along with the

Table 1: Preliminary phytochemical screening of different extracts of *Trigonella foenum-graecum* seeds

Test	Ethanol	Methanol	Aqueous
Steriod	-	-	-
Tanins	++	+	+
Saponin	-	-	++
Cumarin	++	++	+
Phenol	-	-	-
Alkaloid	++	+++	+
Flavonoid	++	+++	++
Diterpenoids	+	+	+
Cardio glycosides	-	++	+
Carbohydrates	++	+++	+
Protein	++	+++	+
Terpenoids	+	+++	++
Anthocyanin	-	-	-
Enodin	+	+	-

+++ Strongly present; ++ Moderate Present; + Traces

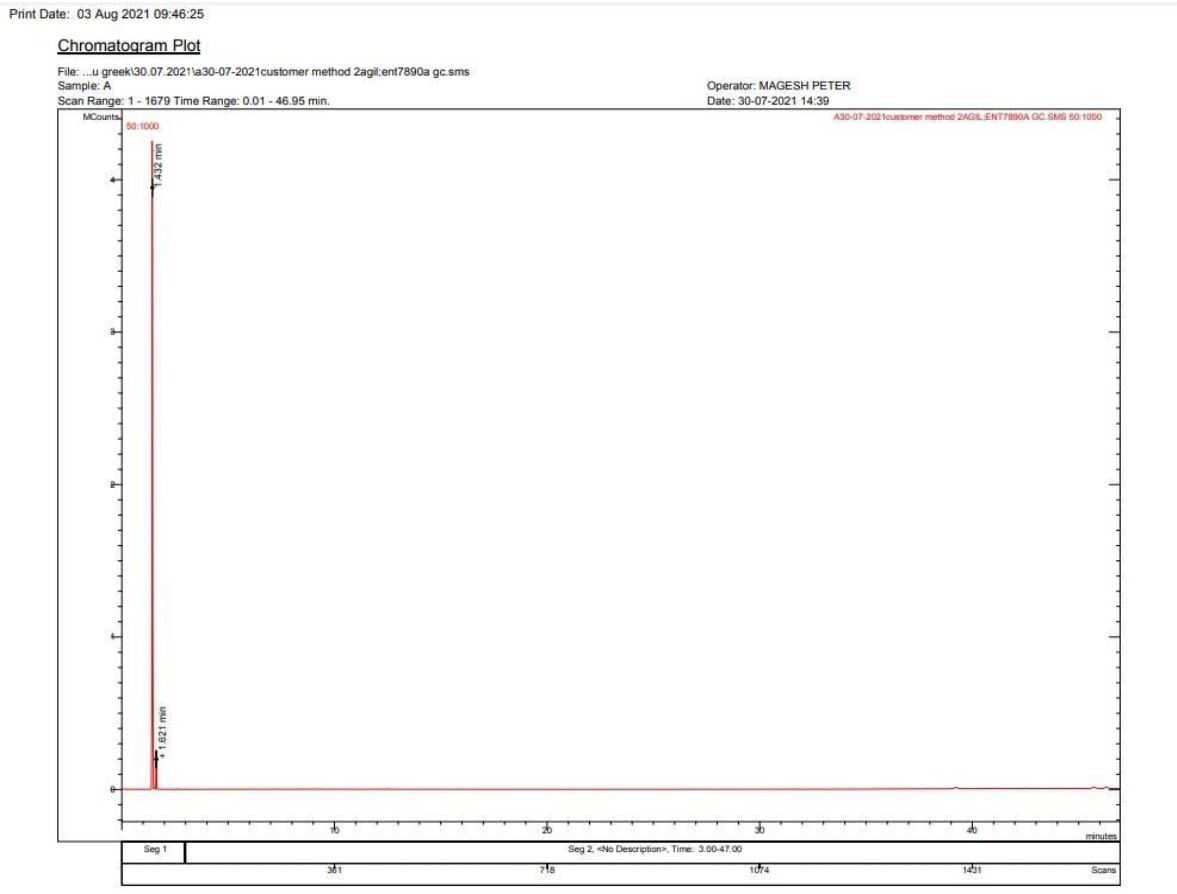

Fig. 1: GC-MS Chromatogram of aqueous extract of *Trigonella foenum graecum* seeds.

Table 2: Bioactive compounds of aqueous seed extract of *Trigonella foenum graecum*

S. No.	R.T.	Peak Name	Molecular formula	Peak area %
1	1.432	Chlorfenapyr	$C_{15}H_{11}$	95.938
2	1.552	Methane isocyanato	C_2H_3NO	0.159
3	1.621	Methane, dichloronitro	$CHCL_2NO_2$	3.903

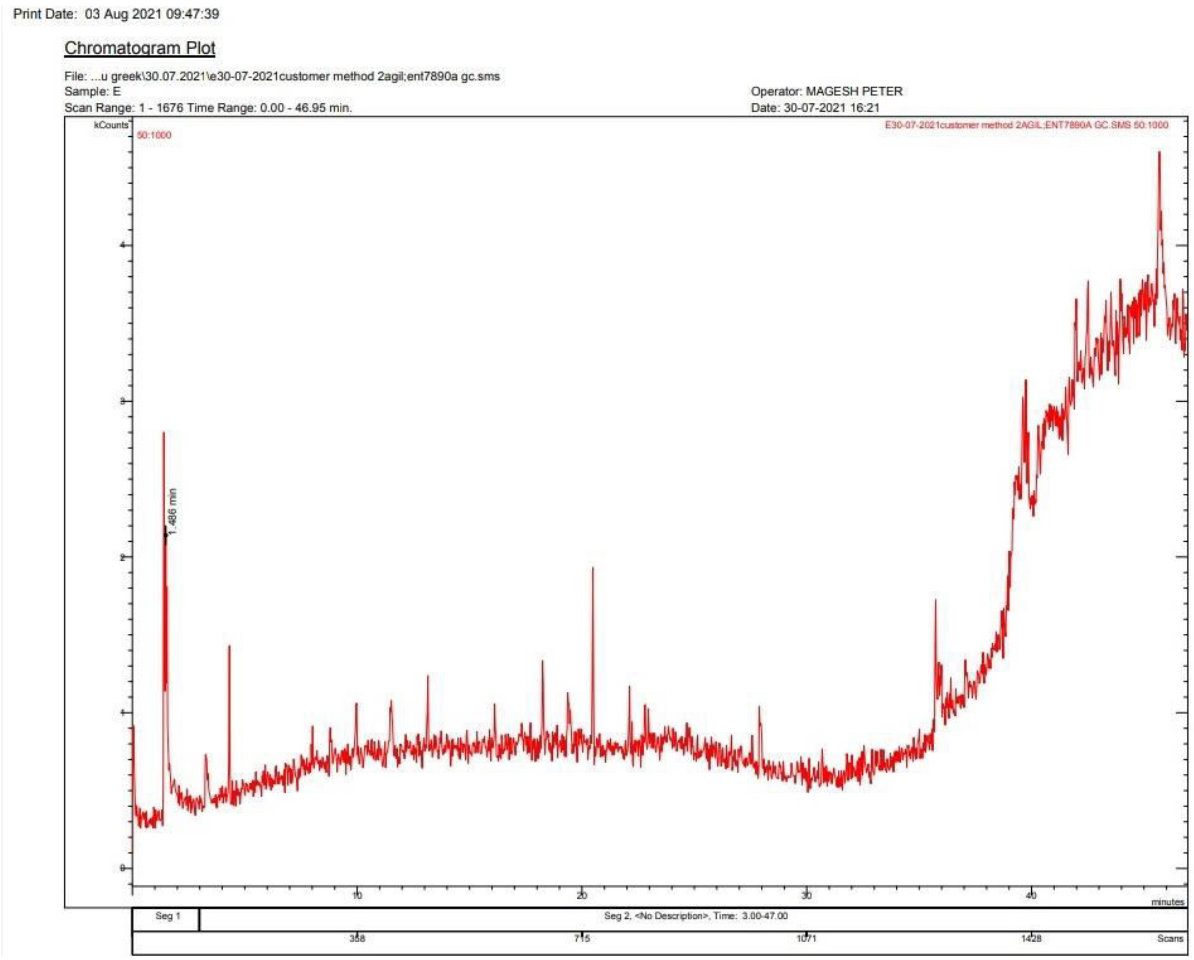

Fig. 2: GC-MS Chromatogram of ethanol extract of *Trigonella foenum graecum* seeds.

traces of diterpenoids recorded with aqueous and alcoholic extracts of fenugreek seeds. Higher amounts of alkaloids, flavonoids, proteins and terpenoids was found in methanol seed extract (Table 1). Preliminary GC-MS analysis based on the retention time and molecular mass was determined to record the bioactive compounds present in the seed extracts of fenugreek (Figs. 1-3). Compounds such as Chliefenapyr, and methane dichloronitro (Table 2) were reported with aqueous seed extract. The methanol seed extract (Table 3) analysis shows the presence of hexadecylamine, N allyl, Dasycarpidan 8(16H)-etanol,3,18-didehy, n-propyl 9, 12-octa decadienoate and Methyl eicos-11-en-14-ynoate. However, only one major compound 4-(1-indanyl)phenol, trimethylsilyl ether was recorded with ethanol seed extract (Table 4).

The DPPH antioxidant activity of *Trigonella* seed extract with varying concentrations of seed extracts was performed. The antioxidant activity of the fenugreek seeds was generally good, with over 50% inhibition with aqueous and methanol extracts with a concentration of 100 µg/ml (Table 5; Fig. 4). The DPPH antioxidant activity was increased with the concentration. The highest

Table 3: Bioactive compounds of methanol seed extract of *Trigonella foenum graecum*

S. No.	R.T.	Peak Name	Molecular formula	Peak area %
1	8.777	Hexadecylamine, N allyl-	$C_{16}H_{35}N$	57.662
2	20.442	Dasycarpidan 8(16H)- ethanol, 3,18-didehy	$C_{20}H_{28}N_2O_2$	6.281
3	34.853	n-propyl 9, 12-octadecadienoate	$C_{21}H_{38}O_2$	19.685
4	35.000	Methyl eicos-11-en-14-ynoate	$C_{21}H_{36}O$	16.372

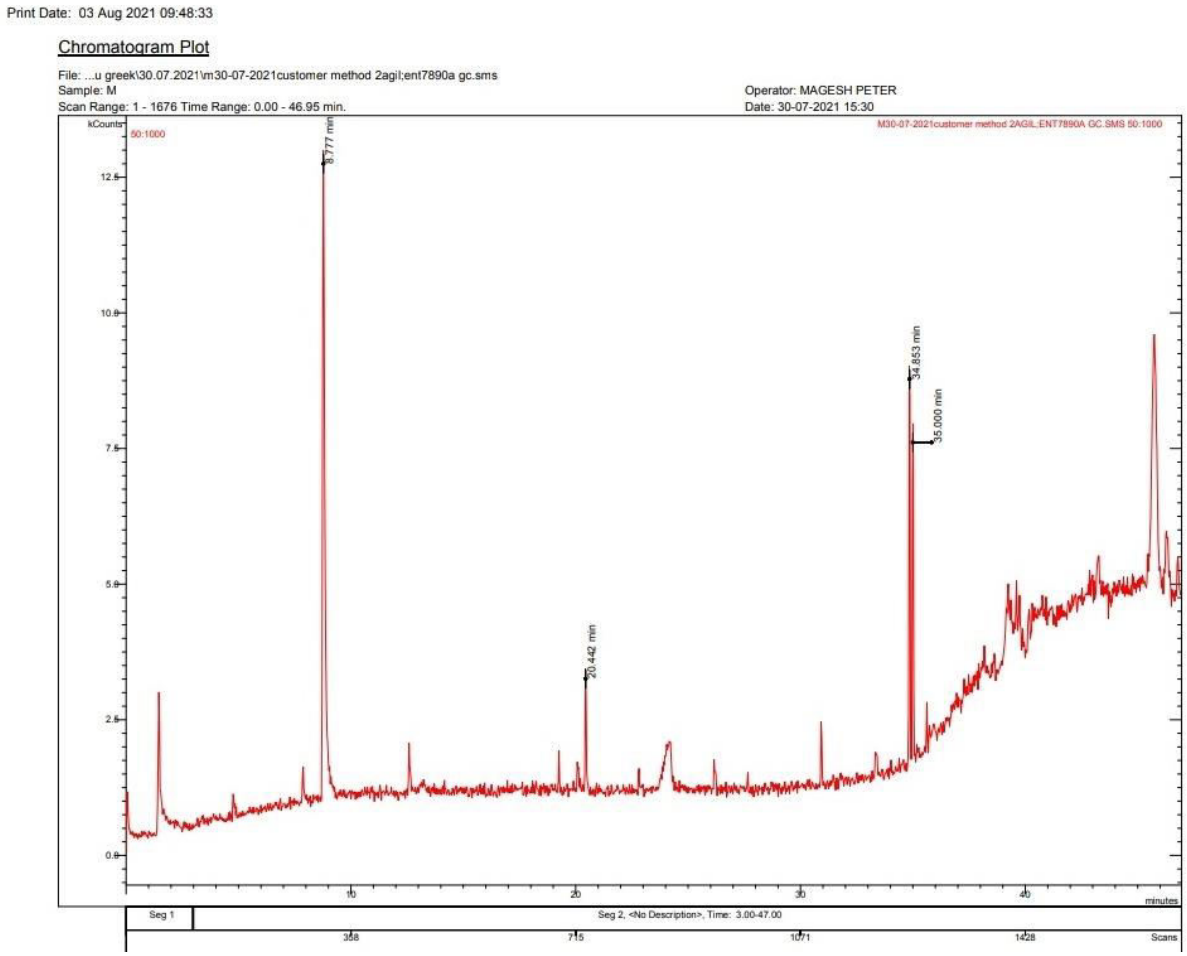

Fig. 3: GC-MS Chromatogram of methanol extract of Trigonella foenum graecum seeds.

Table 4: Bioactive compounds of ethanol seed extract of *Trigonella foenum graecum*

S. No.	R.T.	Peak Name	Molecular formula	Peak area %
1	1.486	4-(1 indanyl) phenol, trimethylsilyl ether	-	100

radical scavenging activity was recorded with methanol seed extract with the value ranges from 3.40% to 43.12% with the concentrations ranging from 20 µg to 100 µg/ml. The values were not significantly defferent from the positive control. The calculated IC_{50} value of acarbose was found to be 71.52 µg/ml, which represents the concentration of drug required to inhibit 50%

Table 5: Antioxidant activity of aqueous, ethanol and methanol extracts of *Trigonella foenum graecum* seed

Conc.	STD	Aqueous	Ethanol	Methanol
20	41.214 ± 0.875	27.427 ± 1.072	9.923 ± 0.972	3.407 ± 0.759
40	58.179 ± 1.427	38.223 ± 0.98	19.101 ± 1.767	21.058 ± 1.017
60	70.592 ± 0.86	44.591 ± 0.755	32.838 ± 1.235	34.786 ± 1.027
80	73.985 ± 0.86	51.956 ± 1.018	33.805 ± 1.3	37.733 ± 1.232
100	79.642 ± 0.555	64.203 ± 1.022	36.262 ± 1.571	43.121 ± 0.75
IC_{50}	28.23	70.82	151.58	102.36

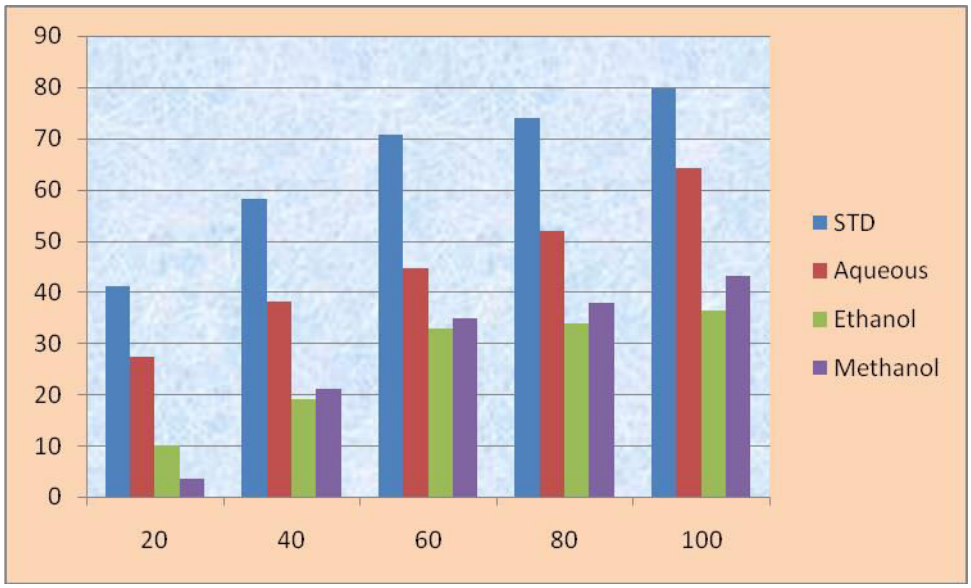

Fig. 4: Antioxidant activity of Aqueous, Ethanol and Methanol extracts of *Trigonella foenum graecum* seed.

Table 6: Percentage of α-amylase inhibitory activity of aqueous, ethanol and methanol extracts of *Trigonella foenum graecum* seed

Conc.	STD	Aqueous	Ethanol	Methanol
20	9.807 ± 0.980	26.026 ± 1.00	13.658 ± 1.40	33.915 ± 0.221
40	37.120 ± 1.405	33.283 ± 0.741	24.414 ± 0.477	45.147 ± 0.251
60	39.831 ± 1.288	46.016 ± 0.491	34.202 ± 0.288	52.973 ± 0.186
80	56.527 ± 1.114	52.967 ± 1.099	42.291 ± 0.388	55.744 ± 1.017
100	68.373 ± 0.505	57.022 ± 0.92	49.916 ± 0.835	60.872 ± 1.023
IC_{50}	71.52	77.055	97.84	56.46

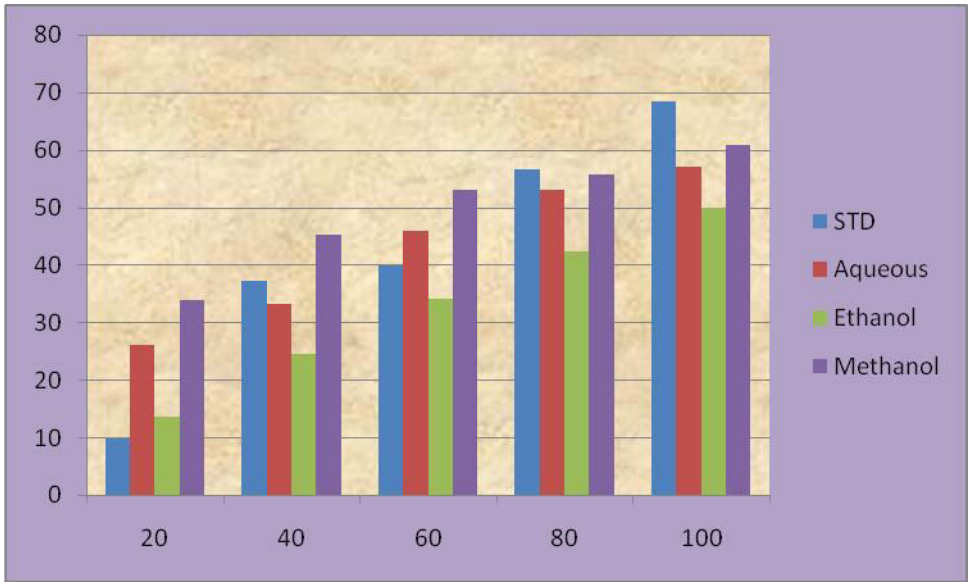

Fig. 5: Alpha amylase inhibitory activity (percentage of Inhibition) of *Trigonella foenum graecum* seeds.

Table 7: Percentage of α-glucosidase activity of aqueous, ethanol and methanol extracts of *Trigonella foenum graecum* seed

Conc.	STD	Aqueous	Ethanol	Methanol
20	29.629 ± 1.416	-	14.721 ± 0.578	31.110 ± 0.699
40	52.407 ± 0.748	6.666 ± 0.555	31.388 ± 0.699	39.722 ± 0.698
60	59.814 ± 0.466	13.610 ± 0.892	48.888 ± 0.424	48.055 ± 0.578
80	70.555 ± 0.555	48.055 ± 0.699	64.722 ± 0.698	64.722 ± 0.698
100	85.184 ± 1.069	63.610 ± 0.975	80.277 ± 0.892	80.833 ± 0.833
IC_{50}	45.29	29.89	59.187	55.36

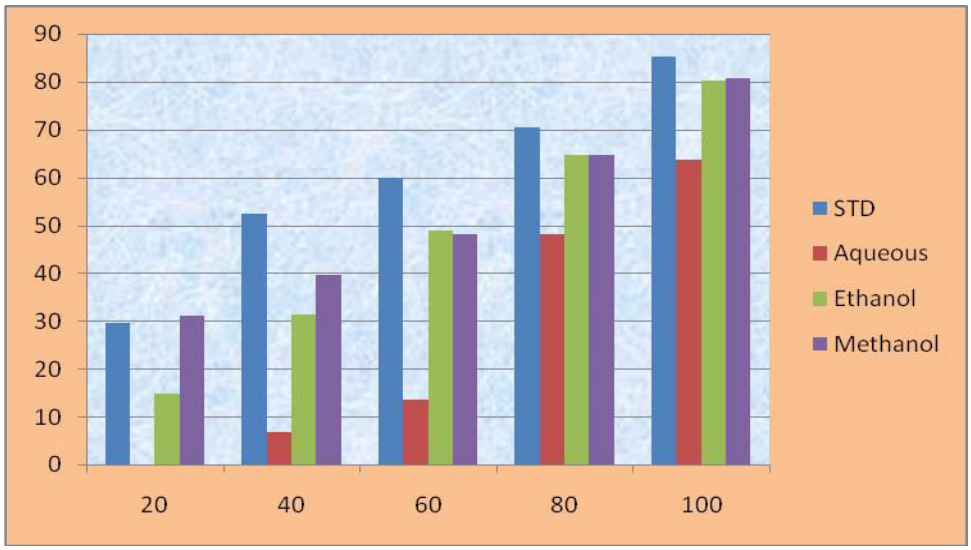

Fig. 6: α-glucosidase inhibitory activity (percentage of Inhibition) of *Trigonella foenum graecum* seeds.

alpha amylase activity. However, the fenugreek seed extract IC_{50} value amounts to methanol extract IC_{50}= 56.46 µg/ml, ethanol extract IC_{50}=97.84 µg/ml and aqueous extract IC_{50}=77.05 (Table 6; Fig. 5). The IC_{50} value of methanol is lower than the standard drug acarbose. In addition, a concentration dependent inhibition was observed with all the three different solvent extracts of fenugreek seeds tested and standard acarbose.

In vitro α-glucosidase inhibitory activity was evaluated and the IC_{50} concentration was calculated. The α-glucosidase inhibition shows a concentration gradient activity with aqueous, ethanol and methanol seed extracts. The IC_{50} value of standard acarbose IC_{50}= 45.29 µg/ml followed by the methanol and ethanol extracts. (Table 7; Fig. 6). MeoH with an IC_{50} of 55.36 µg/ml and EtOH with 59.187 µg/ml concentration suggesting the potential role of crude extracts of the tested plant fenugreek as a potent agent for the invention of novel compound to treat and manage the type 2 diabetes mellitus.

Discussion

In the present study, the efficacy of *Trigonella foenum-graecum* seed extracts on antioxidant activity, α-amylase and α-glucosidase enzymes was evaluated. *Trigonella* includes number of bioactive compounds like flavonoids, terpenoids, alkaloids and cardio glycosides which possess to have wide range of biological activities. The DPPH is one of the substrates which is used for the evaluation of the antioxidant activity of plant extracts and test the free radical scavenging activity measured by the capacity of extract to donate the hydrogen or electron in the transformation of DPPH from DPPH-H (Kaurinovic *et al*., 2011). The aqueous extract of *Trigonella* seeds showed a significant free radical scavenging activity with an antioxidant potential with an IC_{50} value of 70.82 µg/ml compared with butylated hydroxyanisle (BTH) with an IC_{50} value of 28.23 µg/ml. The antioxidant property of the seed extracts are due to the presence of flavonoids the polyphenols. *Trigonella* belongs to the family of Fabaceae which is considered for traditional medicinal herbs. Different portions of *Trigonella* species were used as food ingredients, as well as Ayurvedic medicine (Kumar *et al*., 2021). Further, *Trigonella* plants exhibit potential pharmacological effects such as antidiabetic, anti-inflammatory, antioxidant properties (Zameen *et al*., 2018). Jebur *et al*. (2021) reported that the antioxidant property of the plant is due to the presence of polyphenols. However, the alcoholic extracts show modest activity in scavenging free radicals. There is a correlation exists between the presence of phenolics and antioxidant activities (Ksouri *et al*., 2012).

Fenugreek seeds controls the elevation of blood sugar by the mechanism of stimulation of insulin synthesis, with the inhibition of amylase, glucosidase and sucrase enzyme activity (Fazlani *et al*., 2020). Substantial amount of research has been carried out to evaluate the potential therapeutic activity, it is necessary to profile the bioactive components of the seeds using GCMS method and to correlate the bioactive components of seeds and *in vitro* efficacy of seeds as an agent for the inhibition of α-amylase and α-glucosidase activity.

Fenugreek seed extract efficacy to reduce blood sugar may be due to the presence of alkaloids (Haeri *et al*., 2012). *Trigonella foenum graecum* seeds powder solution reduces the synthesis of triglycerides by inhibiting the absorption of glucose and fatty acids (Renuka *et al*., 2009). The present study evaluated the seed extract to inhibitory activity of α-amylase and α-glucosidase. The enzymes involved in the biological process especially the digestion of carbohydrates, the methanol seed extract possess to have powerful inhibition capacity against α-amylase activity (IC_{50}=56.46 µg/ml) followed by Aqueous (IC_{50} = 70.82 µg/ml) and ethanol (IC_{50}= 97.84 µg/ml). The potential α-amylase inhibitory activity of methanol seed extract (IC_{50}=56.46 µg/ml) compared to that of standard acarbose (IC_{50} = 71.52 µg/ml) suggesting the presence of

phenolic compounds in the solvent extract. Cazarolli *et al*. (2008) reported that the phenolic compounds act as antidiabetic agents by activity as α-amylase inhibitors. Terpenoids exerts their biological activity by lowering the blood glucose with high antioxidant activity in diabetic rats (Hamden *et al*., 2011). It is evident from the present findings that the seed extract possesses higher amount of flavonoids and Terpenoids.

The potential inhibition activity of the methanol extracts on α-amylase may due to the synergistic action of flavonoids and terpenoids. The results are in correlate with the work of Keskes *et al*. (2017) who reported significant antidiabetic activity of the methanol extract of fenugreek seeds and attributed this to the presence of flavonoids. Inhibition of α-glucosidase and α- amylase enzymes delay the process of breaking down of carbohydrates in the small intestine and decrease the postprandial glucose level in type 2 diabetes mellitus (Kwon *et al*., 2007). Type II diabetes mellitus can be managed by reducing post prandial glucose level by inhibiting the enzyme responsible for the breaking of oligosaccharides into glucose (Mccue *et al*., 2005).

A drug that interferes with activity of the enzymes such as lipase and amylase may help in the curing the life time diseases. The results revealed that fenugreek seed extracts exhibit α-amylase inhibition activity almost equal to the acarbose a standard drug. The anti-amylase activity results were observed with IC_{50} value of 77.05 µg/ml which is effective when compare with acarbose with 71.52 µg/ml. It is evident from the results that the antihyperglycemic potential of the plant materials depends on the presence of various phytoconstituents and their free radical scavenging activity. The present results suggest that the antidiabetic and antioxidant activity of the crude extracts of *Trigonella* can be due to the presence of one or more bioactive compounds and molecules of the seeds, the α-amylase and α-glucosidase inhibitory activity of the seeds linked with the phytocompounds. The single compound responsible for the action has not been ascertained, further studies on the isolated fractions and characterization and *in vivo* studies may provide the specificity of the compound action.

Conclusion

The present study revealed the significant inhibition of α-amylase and α-glucosidase activity with the crude extracts of *Trigonella foenum graecum* in a dose dependent manner; depending on the basis of the solvent used for extraction. The qualitative phytochemical analysis and GC-MS studies of aqueous, methanol and ethanol extract suggest the presence of various bioactive compounds which possess antioxidant and antidiabetic properties and the crude extracts can possibly be used to control the diabetes mellitus. However, further investigations are required to evaluate and elucidate the structure of the specific compound responsible for the antihyperglycemic activity.

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