

Original Research Article

Antioxidant, Anti-Diabetic (A-Amylase and A-Glucosidase) Inhibitory Potential and Screening of Bioactive Secondary Metabolites of Seed Extract of *Trigonella Foenum-Graecum*

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Abstract: Spices and herbs function as antioxidants to extend lipid peroxidation protection in biological environments. Fenugreek (*Trigonella foenum-graecum*) stands as an essential spice because its dried seeds serve both culinary purposes in beverages and food additives and medicinal applications. The Soxhlet extraction method yielded fenugreek extracts through extraction with the solvents methanol, ethanol, hexane and ethyl acetate. FT-IR peak values of solid analysis of *Trigonella foenum-graecum* were Peak (Wave number cm^{-1}), Intensity, Corr. Area, Type of Intensity, Bond, Type of Vibration, and Functional group assignment (667.37, 69.147, 0.063, Strong, =C-H, Bending, and Alkenes), (894.97, 82.045, 0.030, Strong, =C-H, and Alkenes), (1029.99, 61.548, 10.810, Strong, C-F, Stretch, and alkyl halides), (1238.30, 81.092, 0.042, Strong, C-F, Stretch, and alkyl halides), (1317.38, 81.874, 0.136, Strong, C-F, Stretch, and alkyl halides), (1373.32, 81.514, 0.008, Strong, C-F, Stretch, and alkyl halides), (1519.91, 82.843, 0.127, Medium, C=C, Stretch, Aromatic), (1616.35, 77.669, 0.027, Bending, N-H, Stretch, Amide), (1743.65, 87.838, 0.667, Strong, C=O, Stretch, and Ester), (2852.72, 87.591, 0.191, Strong, C-H, Stretch, and Alkane), (2920.23, 83.176, 0.812, Strong, C-H, Stretch, and Alkane). Radical scavenging activities of *Trigonella foenum-graecum* Crude (methanolic extract), Ethanol fraction, and Quercetin (standard) recorded 23.68 ± 1.13 , 34.68 ± 1.35 and 48.11 ± 2.15 for Superoxide radical scavenging. At the same time recorded 41.07 ± 2.08 , 30.45 ± 2.67 and Curcumin (standard) 95.00 ± 4.07 for Nitric oxide radical scavenging. Anti-Diabetic (α -amylase and α -glucosidase) Inhibitory potential recorded (99.35 ± 3.71 , 44.34 ± 1.26 and 20.73 ± 0.11) respectively inhibitory potency against α -amylase. While recorded (73.45 ± 2.11 , 52.08 ± 1.96 and 17.93 ± 0.08) respectively inhibitory potency against α -glucosidase activity. Among the components responsible for fenugreek seed hypoglycemic activity stands fenugreek galactomannan. One of the beneficial antidiabetic compounds found in fenugreek seed is the fenugreek galactomannan component. Research shows that fenugreek galactomannan possesses blood glucose reducing ability yet lacks strong support from peripheral glucose uptake and antioxidant effect mechanisms. The rise in liver glycogen content through fenugreek galactomannan administration suggests enhanced glycogenesis whereas reduced glycogenolysis; therefore it likely causes liver glycogenesis increase and/or glycogenolysis decrease in diabetic rats. Studies have confirmed that seed alcoholic solutions help maintain enzyme activities from carbohydrate and lipid metabolism at near typical values.

Keywords: Bioactive Secondary Metabolites, *Trigonella foenum-graecum*, Antioxidant, Anti-Diabetic, Seed Extract.

INTRODUCTION

A wide range of species and aromatic herbs serves as natural food additives that help control diabetes. Aging and biological tissue breakdown receive delayed effects from spices and aromatic herbs which play essential roles in dietary medicine. Among both research fields and industrial sectors there is intense interest in developing synthetic antidiabetic agents instead of using natural antioxidants. Plant material obtains its antidiabetic properties from numerous active phytochemicals including vitamins alongside flavonoids and terpenoids and carotenoids and cumarins and curcumins and lignin and saponin and plant sterol among others. Fenugreek stands as one of these plant species which people in the Indo-Pak subcontinent together with oriental countries consume in traditional medicine along with food preparation as a

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flavoring agent [1, 2]. The plant contains multiple vitamins alongside calcium and iron with β -carotene along with other nutritional compounds. The family diet should incorporate both seeds and leaves because their blood-forming properties will benefit all household members who are pregnant or growing up but adolescent girls and senior citizens in particular. The milk-producing property of fenugreek seeds makes them commonly chosen by nursing mothers to boost insufficient lactation [3-5]. The therapeutic components of fenugreek seeds include lysine and L-tryptophan rich proteins and mucilaginous fiber along with other uncommon chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, saponin, phytic acid, scopoletin and trigonelline which researchers believe lead to its therapeutic benefits by potentially blocking cholesterol absorption and helping decrease sugar levels [6]. Medical science uses fenugreek seeds as a natural remedy to manage diabetes alongside hypercholesterolemia conditions. The substance demonstrates healing functions and nourishes the body in addition to activating digestive processes which help treat multiple conditions of digestive tract ulcers. Fenugreek exhibits pharmacological properties which include antitumor behavior together with antiviral and antimicrobial and anti-inflammatory characteristics and hypotensive β -Co effects as well as antioxidant capabilities. The modified version of existing assays set forth the method to calculate antioxidant activities through Folin-Ciocalteu reagent-based total phenolic content determination followed by total flavonoids content evaluation utilizing 2, 2'-bipyridyl competition assay and free radical scavenging analysis utilizing DPPH[•] in conjunction with reducing power measurement. Progress in medical research has not impacted the widespread use of herbal agricultural products to prevent and treat different diseases because of their therapeutic and nutraceutical properties. Since ancient times *Trigonella foenum-graecum* L [7, 8]. (fenugreek) has been used medicinally as a food spice because of its various healing properties. Fenugreek seeds stand out as the main application of this plant but the stems and leaves are also believed to have curative properties. The substance characteristics emerge from secondary metabolites found in fenugreek plants called phytochemicals. saponins, Alkaloids, phenols and tannins along with other elements act as examples of these metabolites [9-11]. Historical medical practice demonstrates that fenugreek serves different traditional functions such as laxative treatment as well as labor assistance during childbirth and nursing support. Research shows fenugreek stands as a suitable plant species that shows promise for medicinal development into new pharmaceutical products. Scientific reports indicate that fenugreek exhibits strong phytochemical content along with seven known biochemical effects: anti-diabetic properties alongside antioxidant strength, antineoplastic action, protection for the stomach, prevention of liver damage and elevated cholesterol levels and regulation of blood sugar.

MATERIAL AND METHODS

Plant Material and Extraction procedures

A comprehensive fenugreek seed sample acquisition occurred at the Hyderabad local market in Pakistan. Different treatments were applied to fenugreek seed in order to analyze antioxidant properties. A waring blender operated to divide 10 g of dry fenugreek seed into small pieces before sieving the product through a 1-mm aperture. Six hours of soxhlet extraction obtained the following extraction solutions: methanol, ethanol and dichloromethane, acetone, hexane and ethyl acetate at 150 ml concentrations. The extracts were filtered. Continuous re-extraction of the residue multiple times with identical conditions served to achieve complete component extraction from the sample. The scientists combined the collected extracts before using filtering methods and evaporating them under reduced pressure at 60 Co using a rotary evaporator. Dark bottles contained the extracts before their storage at -8 °C until analysis was ready.

Fourier transform infrared spectrophotometer [technique known as the FTIR]

The FTIR spectral analysis took place at Shimadzu, IR Affinity, Japan for *Haloxyton salicornicum* powder samples. Analysis of the sample involved scanning at the infrared wavelength region [13, 14].

α -amylase inhibitory assay

The standard procedure for α -amylase inhibitory activity measurement of extract and fractions required additional modifications. The assay began with two international units of α -amylase present in 20 millilitres before adding extract and fraction samples which contained amounts of 0.5 milligramme per millilitre to 200 millilitres followed by 500 millilograms of 6.8 phosphate buffer having one hundred millimolar phosphate concentration. The solution was distributed into a 96-well plate before spending 20 minutes at 37 degree Celsius. The preincubation period required a temperature setting at 37 °C for incubator use. The experimentation solution was shifted from the first incubator to a different one kept at 37 degrees Celsius for thirty minutes. The following solution received twenty litres of one percent soluble starch dissolved in 100 mM phosphate buffer at pH 6.8. The mixture received DNS colour reagent at 100 litres before the liquid boiled under controlled pressure during ten minutes. The Multiplate Reader known as Multiska Thermo Scientific version 1.00.40 produced an absorbance measurement at 540 nanometres. The measurement process served to determine the absorbance values of the final mixture solution. The researcher utilized standard acarbose solutions ranging from 0.1 to 0.5 mg/ml as their reference levels. A material was simultaneously synthesized through no experimental procedures while extracting and fractioning components. Each experiment was carried out three times. The expression used the applied formula to calculate results that showed percentages of inhibition. The IC₅₀ values came from visual analysis of enzyme activity inhibition through different fraction dosages.

The percentage of inhibition could be determined by applying the following formula:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}) / \text{Abs}_{\text{control}} \times 100$$

α -Glucosidase Inhibitory Assay

The assessment procedure determined how the extract and fractions affected α -glucosidase action. The standard procedure with multiple minimal deviations conducted the analysis. The 96 well plate contained pre-cooled serum which was stored at 37 degrees centigrade for 15 minutes. A total reaction mixture encompassed twenty liters of nine different extracts and first nine fractions adjusted to 0.500 mg/mL together with ten liters of one unit/mL purified alpha-glucosidase and fifty liters of 6.8 phosphate buffer solution at 100 mM concentration. The pre incubation step took place at the temperature of 37 degree centigrade. The incubation was conducted using a thirty-seven degrees centigrade environment for twenty additional minutes. Subsequent to the twenty liters of P-NPG solution at five millimolar were added as the new substrate. The researchers added 50 liters of 0.1 M sodium carbonate solution to terminate the reaction process. The multiplate reader measured absorption of recently released nitrophenol at a wavelength of 405 nm in order to estimate the amount that was absorbed. During the experiment acarbose served as the standard measurement and it was detected in its tested sample at a concentration of 0.5 mg/mL. The tests were performed three times to enable evaluation of experimental data. During simultaneous control testing the investigator excluded the examination of the chemical substance. The given studies conducted three separate trials for precise assessment results. A method was employed to evaluate the inhibition activity of α -glucosidase through calculating percentage inhibition.

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}) / \text{Abs}_{\text{control}} \times 100$$

In the expression A control and A extract stand for the measure of absorptions taken from control solution and from extracted portions respectively. The values for IC50 originated from graphed data and designated the amount of each fraction that reduced enzyme activity to 50%.

Statistical Analysis: The statistical evaluation resulted from gathering data points from three distinct trials which allowed the calculation of results based on averages and standard deviations. The statistical analysis included one-way ANOVA testing followed by Tukey's multiple range tests to divide means which showed significant differences at a confidence level of 95%.

RESULTS AND DISCUSSION

The FTIR spectral analysis for specific chemical or biochemical end products involves tracking characteristic functional group bands that appear or disappear or monitoring band position changes when reactions cause structural modifications. The FTIR spectra recorded from compost processes exhibit dual signals resulting from products together with remaining unused starting reagents. The chemical structural components from original plant materials acted as building blocks for producing humic acids in peat soil, agricultural soil and lake sediment based research according to studies by [14] and [15]. Analysts use FTIR as one of the foremost analytical methods to analyze various substrates in present-day testing practices. The main benefit of this approach enables the analysis of every sample in its original state. Different sampling approaches enable FTIR analysis of all sample types ranging from liquids to solutions, pastes, powders, films, fibres and gases and surfaces. FTIR as a technology supports diverse IR sampling methods that include attenuated total reflection (ATR) and diffuses reflectance infrared Fourier transform (DRIFT) spectroscopy. FT-IR peak values of solid analysis of *Trigonella foenum-graecum* were Peak (Wave number cm^{-1}), Intensity, Corr. Area, Type of Intensity, Bond, Type of Vibration, and Functional group assignment (667.37, 69.147, 0.063, Strong, =C-H, Bending, and Alkenes), (894.97, 82.045, 0.030, Strong, =C-H, and Alkenes), (1029.99, 61.548, 10.810, Strong, C-F, Stretch, and alkyl halides), (1238.30, 81.092, 0.042, Strong, C-F, Stretch, and alkyl halides), (1317.38, 81.874, 0.136, Strong, C-F, Stretch, and alkyl halides), (1373.32, 81.514, 0.008, Strong, C-F, Stretch, and alkyl halides), (1519.91, 82.843, 0.127, Medium, C=C, Stretch, Aromatic), (1616.35, 77.669, 0.027, Bending, N-H, Stretch, Amide), (1743.65, 87.838, 0.667, Strong, C=O, Stretch, and Ester), (2852.72, 87.591, 0.191, Strong, C-H, Stretch, and Alkane), (2920.23, 83.176, 0.812, Strong, C-H, Stretch, and Alkane) Figure 1. Radical scavenging activities of *Trigonella foenum-graecum* Crude (methanolic extract), Ethanol fraction, and Quercetin (standard) recorded 23.68 ± 1.13 , 34.68 ± 1.35 and 48.11 ± 2.15 for Superoxide radical scavenging. At the same time recorded 41.07 ± 2.08 , 30.45 ± 2.67 and Curcumin (standard) 95.00 ± 4.07 for Nitric oxide radical scavenging Figure 2 and Figure 3. The addition of radical scavengers allows termination of peroxide radicals through direct reactions which enhances both the quality and stability of food products. Researchers used stable DPPH radical to check antioxidant radical quenching capacities while understanding antioxidant mechanisms through DPPH radical scavenging tests of each fenugreek extract. The oxidation of radical DPPH generates a decrease in absorbance through the reaction between an antioxidant molecule and radical wherein the radical becomes scavenged through hydrogen donation. The cultivation of microgreens produces small salad greens that show their complete non-senescent cotyledons at maturity stage with one or

two developing true leaves. These tiny leaves of vegetable gained recent popularity among consumers though they remain tender natured. The combination of low calories and abundant antioxidants and essential nutrients makes this present. Usually, they are harvested rootless [16-19]. Microgreens have become popular culinary ingredients during the last two to three decades because they enhance food dishes through improved texture and color and excellent taste and scent as well as visual appeal. The market now promotes microgreens as nutritional dietary supplements. Researchers have studied multiple plant species that belong to Amaranthaceae, Brassicaceae and vegetable and herb families for their microgreen production potential. Bioactive compounds lower power levels because they possess antioxidant capabilities. Demonstrating phenolic substance reducing capability provides essential knowledge about their antioxidant properties. Research indicates that the reducing power in the extracts directly corresponds to the amount of used extract. The ethanol extract of fenugreek contained high quantities of phenolic compounds and this explained the observed similar reducing power activity results [20, 21]. Our research shows that total phenolic content seems to exhibit a possible connection to the reducing power effects found in the study.

Anti-Diabetic (α -amylase and α -glucosidase) Inhibitory potential of Seed Extract of *Trigonella foenum-graecum* Crude (methanolic extract), Ethanol fraction and acarbose (Standard) According to the type of extract Crude (methanolic extract), Ethanol fraction and Acarbose (Standard) recorded $(99.35 \pm 3.71, 44.34 \pm 1.26$ and $20.73 \pm 0.11)$ respectively inhibitory potency against α -amylase. While recorded $(73.45 \pm 2.11, 52.08 \pm 1.96$ and $17.93 \pm 0.08)$ respectively inhibitory potency against α -glucosidase activity. The inhibition strength of methanol and ethanol fraction proved to be statistically meaningful ($P < 0.05$) more potent than that observed with acarbose in percent inhibition of α -glucosidase. The metabolic disorder diabetes produces hyperglycemia because patients experience issues with insulin regulation and insulin response. Long-term high blood sugar levels trigger microvascular conditions including neuropathy and nephropathy together with macrovascular complications mainly affecting the cardiovascular system through elevated reactive oxygen species generation and damaged antioxidants. Throughout history herbal treatments became common methods for medicine resulting in numerous disease treatments [22]. Scientists continue to study natural products because they want to determine their positive health outcomes and negative effects while researchers have not yet fully understood these substances' exact mechanisms or patterns in nature. Patient preference is directed towards botanical therapies because they choose them instead of traditional diabetes medications because these pharmaceuticals come with excessive costs and adverse effects. Plants including cinnamon and Ginseng act on glucose and lipid levels by promoting insulin release yet slowing gastric emptying while reducing glucosidase activity and activating AMP-activated protein pathway and GLUT4 expression and inhibiting gluconeogenesis. Various plants with medicinal properties which treat diabetes include fenugreek, cumin, garlic and ginger. Traditional medical practitioners use this plant to treat diabetes together with obesity-related symptoms. This natural compound demonstrates functionality as an antioxidant substance and displays antihyperlipidemic and antibacterial and antifungal and antiinflammatory and galactagogic features. The active compound known as Diosgenin saponin stands as the major bioactive element found in fenugreek plants. The compound exhibits antioxidant properties and serves as a critical factor that improves diabetic state conditions through multiple pathways [23]. Two key processes through which diosgenin function include stimulating insulin secretion and renewing β -cells. Besides, diosgenin elevates the mRNA transcription levels of CCAAT/enhancer-binding protein (C/EBP δ) and peroxisome proliferator-activated receptor- γ (PPAR- γ).

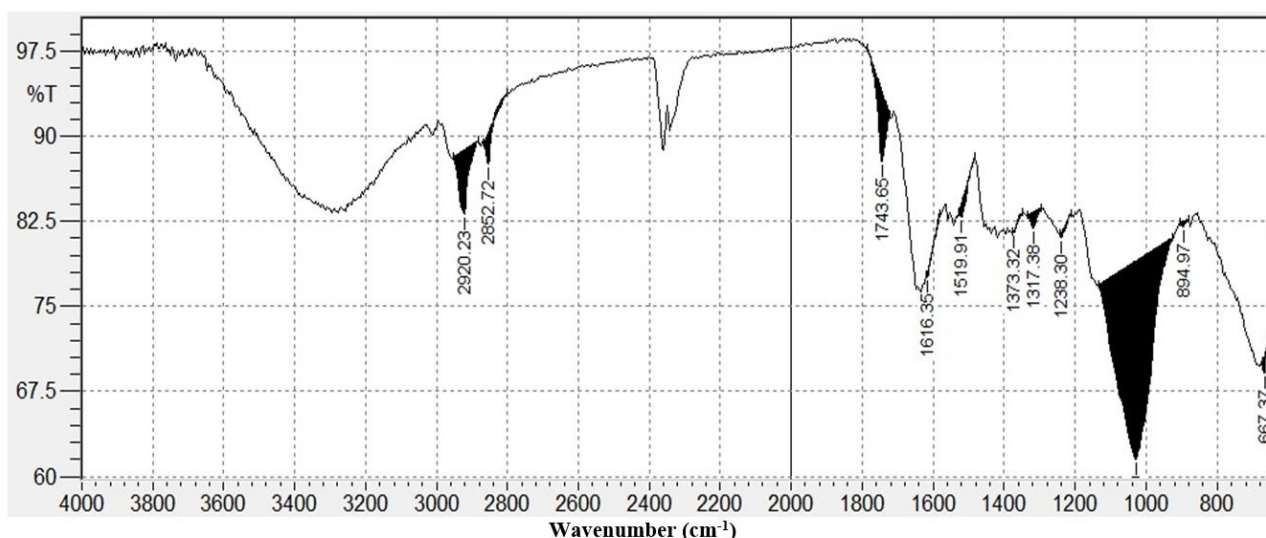
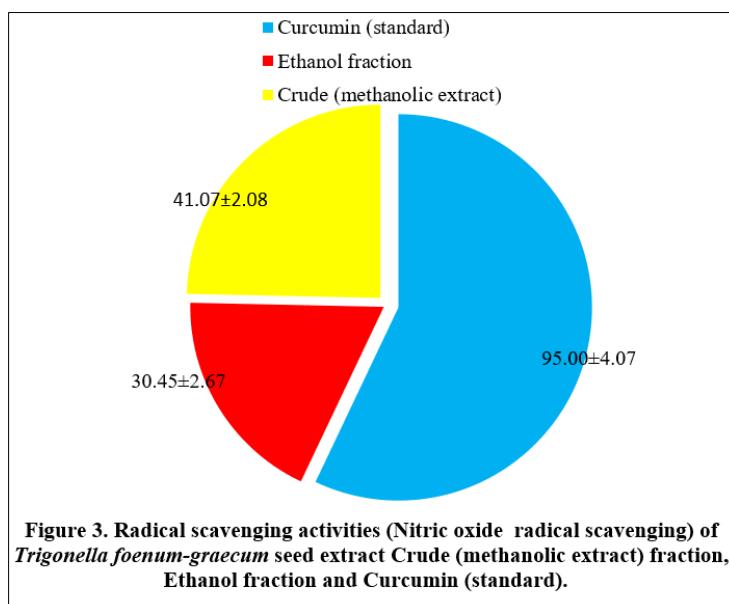
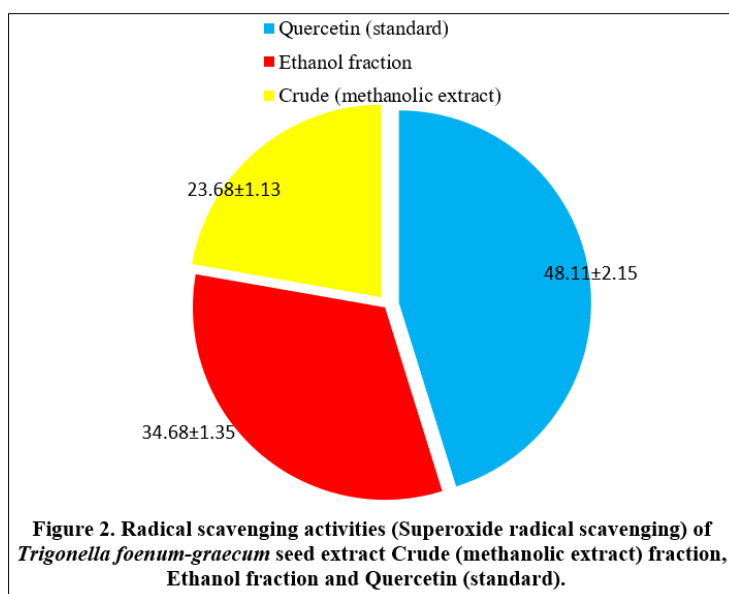
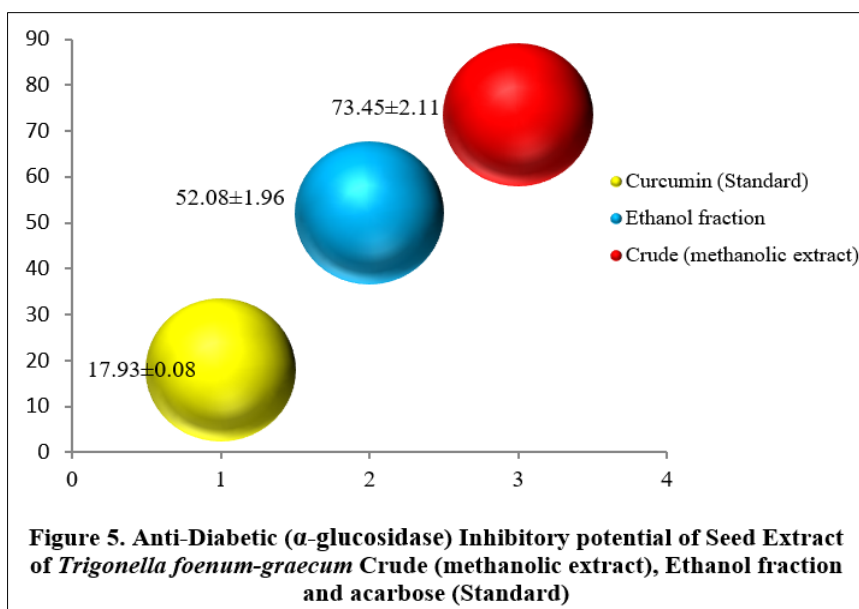
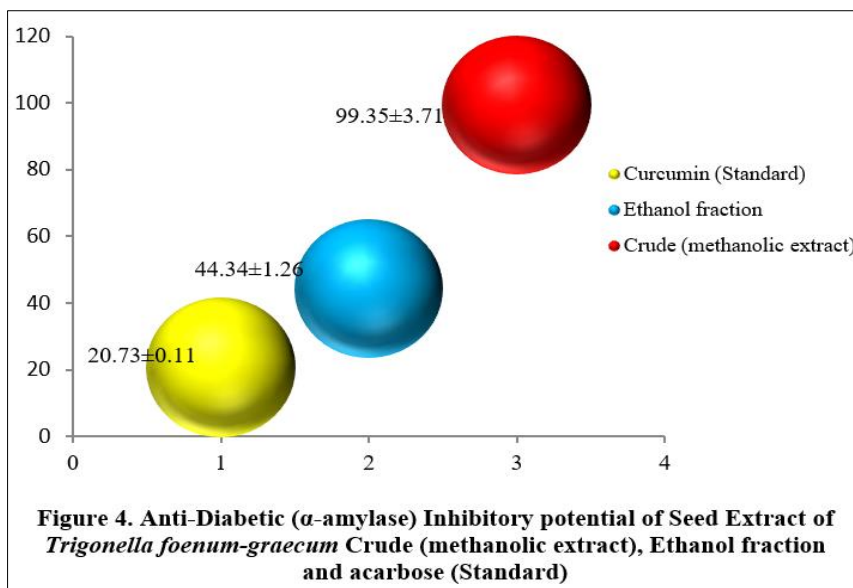


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Trigonella foenum-graecum*

Table 1. FT-IR peak values of solid analysis of *Trigonella foenum-graecum*

No.	Peak (Wave number cm ⁻¹)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.37	69.147	1.522	673.16	653.87	2.915	0.063	Strong	=C-H	Bending	Alkenes	650-1000
2.	894.97	82.045	0.457	904.61	881.47	1.958	0.030	Strong	=C-H	Bending	Alkenes	650-1000
3.	1029.99	61.548	17.442	1134.14	925.83	32.156	10.810	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1238.30	81.092	0.518	1242.16	1211.30	2.645	0.042	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1317.38	81.874	1.459	1334.74	1296.16	3.182	0.136	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1373.32	81.514	0.203	1375.25	1348.24	2.255	0.008	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1519.91	82.843	1.227	1527.62	1483.26	3.086	0.127	Medium	C=C	Stretch	Aromatic	1400-1600
8.	1616.35	77.669	0.321	1618.28	1579.70	3.636	0.027	Bending	N-H	Stretch	Amide	1550-1640
9.	1743.65	87.838	6.121	1786.08	1720.50	2.211	0.667	Strong	C=O	Stretch	Ester	1735-1750
10.	2852.72	87.591	2.845	2868.15	2802.57	2.629	0.191	Strong	C-H	Stretch	Alkane	2850-3000
11.	2920.23	83.176	5.651	2951.09	2883.58	4.259	0.812	Strong	C-H	Stretch	Alkane	2850-3000





CONCLUSION

The extraction process of plant constituents greatly depends on solvent selection according to results from this work. The high polarity of methanol and ethanol leads to maximum extraction of phenolic compounds during the procedure compared to all other extraction solvents. The antioxidant properties in the extract directly correspond with the polyphenolic compounds detected in its composition. Analysis methods have delivered important findings about what determines the antioxidant capacity of fenugreek seeds. The herbaceous plant *Trigonella foenum-graecum* stands within the legume family and people use it commonly as a member of this plant family. It is a multipurpose herb. Hundreds of years in Western Asian and Mediterranean territories saw fenugreek seeds used by people to prepare food and treat diseases both internally and externally. Fenugreek seed oil enables extraction to obtain antioxidants that contain linoleic acid and linolenic acid and oleic acid along with other fatty acids and properties. Many health-promoting nutrients including magnesium, iron and manganese together with fiber and other nutrients can be found in fenugreek oil. Some bacterial and fungal species show strong susceptibility to the antimicrobial properties present in this oil. The antidiabetic effects of daily fenugreek injections produced superior serum values to all other experimental groups. Every tested group demonstrated improvements in diabetic parameters and antioxidant enzymes but complete restoration of histological structure remained absent from kidney, liver and pancreas tissues. The tested low dose combined with one month experimental duration may explain the observed results. Fenugreek functions as an anti-diabetic plant that appears highly promising. To establish the mechanism of action scientists need to study and discover proper effective dose ranges. It is best to extend treatment duration to obtain histological recovery.

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