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Antioxidant Scavenging Potential and Screening of Bioactive Constitutes of Trigonella foenumgraecum L. Using GC-MS and FTIR Technique

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Abstract:

The diploid, annual plant Trigonella foenum-graecum (TF) L. experiences cleistogamy and is a member of the legume family. Trigonella foenumgraecum L.'s dried, ripe seeds are commonly referred to as fenugreek or just seeds. Its strong aroma makes it a popular ingredient for flavoring cuisine. The primary compounds isolated from Trigonella foenumgraecum L. seeds are linoleic acid, octanoic acid, bis(2-ethylhexyl) ester, carbonic acid, nonanedioic acid, 2,6,6-trimethylcyclohexene-1-methanol, linoleic acid, and 9,12-octadecadienoic acid (Z,Z)-. Ethyl Oleate-d5, 6-isopropylquinoline, dihydrocinchonamine, 4,4-dimethylcyclohexyl) methanamine, vitamin E, and 10-trans,12-cis-Linoleic acid methyl ester are all components. Trigonella foenumgraecum L. fruit extract (methanolic crude extract, ethanol fraction, and standard) and its antioxidant activity, including scavenging of peroxynitrite, hydroxyl, and nitric oxide radicals. Crude, ethyl acetate fraction, ethanol fraction, and standard (712.00±36.14, 656.45±33.00) and Gallic acid (standard) (874.00±38.09) were the different types of extracts that were employed for peroxynitrite scavenging, respectively. Mannitol (standard) has a hydroxyl radical scavenging potential of 520.90±29.81, Benzoic acid (298.71±25.00) and 269.00±22.95 were also noted. The nitric oxide radical scavenging potential for Curcumin (standard) was measured at 42.00±3.05, 25.13±2.98, and 58.07±3.93, in that order.

Keywords: *Trigonella foenumgraecum L.*, GC-MS, FTIR, Antioxidant

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INTRODUCTION

Among the many valuable phytoconstituents found in Trigonella foenumgraecum L. seed are an alkaloid, diosgenin, vitamin A, tannic acid, a substance that causes the plant to turn yellow, and oils, both volatile and fixed. Not only do aromatic plants have essential oils, but they also contain a plethora of other compounds with many practical applications. These oils are used in pharmaceuticals due to their antimicrobial, antiviral, antinociceptive, antioxidant, anticancer, and antiphlogistic characteristics. When it comes to flavoring food, the same essential oils have multiple uses [1]. Oxidative stress and autoxidation of human lipoproteins and lipids produce free radicals and other dangerous compounds, which lead to many health problems. To head this issue off at the pass, researchers are ramping up their search for natural antioxidants. Natural antioxidants found in plants include phenolic, flavonoid, and alkaloids compounds [2]. Medication resistance is a major concern and obstacle in the treatment of infectious diseases. Similarly, the food industry places a premium on concerns pertaining to packaging, storage, and safety. Global scientists are always on the lookout for new ways to solve old problems, such as how to make safe and effective medicines to treat infectious diseases, enhance food quality, and lengthen the shelf life of food components [3, 4]. The development of numerous effective medications has been facilitated by or inspired by medicinal plants. One main reason for discovering molecular entities is the huge amount of unexplored chemical space they contain. Studies show that about 80% of the global population plans to rely on traditional medicines, which are made from plant extracts, for their primary healthcare needs. Herbal remedies have long been an integral part of traditional Asian medicine. In India, for example, where traditional medical systems like Siddha, Unani, Ayurveda, and homeopathy are plentiful, about 95% of prescriptions are based on these systems. Many modern chromatographic methods are often used in conjunction with these methods to improve the accuracy of the results [5-7]. Using gas chromatography and fixed compounds can be quickly and accurately identified. This helps with both phytoconstituent identification and detailed structural profiling. Therefore, by merging gas chromatography (which offers good separation capabilities) with mass spectrometry (which gives particular structure elucidation), GC-MS offers a new way to precisely identify components in plant materials that have so far remained undiscovered.

Traditionally, people in developing countries have relied on herbal treatments as their primary method of health care [8–10]. In many industrialized countries, people are turning to herbal treatments as an additional kind of treatment. These plants come in both edible and medicinal types. These plants have a wide range of biological and pharmacological actions, such as oxytocic, antibacterial, and laxative effects. Chemicals, flavorings, additives, flavorings, flavorants, insecticides, and flavorings are just a few of the many uses for the many physiologically active compounds found in plant secondary metabolites. The United States Food and Drug Administration has designated fenugreek as "Generally Recognized as Safe" when used in small dosages, which contributes to its popularity as a spice in cooking [11, 12]. In the Middle East, North Africa, Egypt, and India. Diabetes, hyperlipidemia, inflammation, gastrointestinal difficulties, gout, and wound healing are just some of the many medical conditions that have traditionally been treated with the plant's seeds. The antihyperglycemic characteristics of fenugreek seeds and their subfractions have been demonstrated in studies involving diabetic rats, dogs, and people. Patients with hypolipidemia and cancer can both benefit from the seeds. The antioxidant capacity of both control and diabetic rats is improved by supplementing their diet with seeds. Because of its nutritive value, healing properties, and capacity to stimulate digestion, it may be useful in the treatment of gastrointestinal ulcers. Some of the pharmacological actions of fenugreek include reducing inflammation, lowering blood pressure, protecting against viruses and bacteria, and even fighting cancer [13–15]. Fenugreek seeds contain, amino acids, flavonoids, coumarins, mucilaginous fibers, nicotinic acid, arginine, galactomannan, and fenugreekine and diosgenin [16]. There are also different vitamins and minerals present. The wonderful biological actions of flavonoids are many, including blocking enzymes, modulating the activity of exhibiting antibacterial, antiviral, anti-malarial, antioxidant, anti-inflammatory, and anticarcinogenic property. Very powerful pharmacological agents include the benzo-gamma pyrone chemicals, which comprise flavonoids [17]. Many are curious about these polyphenolic compounds because of the antioxidant action they may have and the potential health benefits they could offer. The hypoglycemic effect shown in clinical trials is likely due to the fact that the mucilaginous fibers found in fenugreek seeds hinder the absorption of glucose.

MATERIALS AND METHODS

Parts of the plant and how they are extracted Those Trigonella foenum grecum seeds came straight from the local farmer's market. To remove any leftover water and particles, 12gm of powered seed material was soaked alcohol for one night. The mixture was then filtered using Whatmann filter paper with 2.5g of sodium sulphate. Before you filter the sodium sulfate and filter paper, wet them with 100% alcohol. Bubbling nitrogen gas into the mixture produced 1 milliliter of the filtrate. The extract also contains phytocomponents that are polar and nonpolar.

Gas chromatography-mass spectrometry, part of an investigation

GC/MS from Perkin Elmer was used for the analysis of the extract. An Elite-1 fused silica capillary column (100% Dimethyl polysiloxane, 30 m × 0.25 mm ID, 1 ìMdf) was added to the device to improve its performance. Gas chromatography/mass spectrometry detection was carried out using an electron ionization system with an ionization energy of 70 e. After a 2-minute isothermal run at 110°C, the temperature was increased to 200°C for 10 minutes, then 280°C for 5 minutes, and lastly 280°C for 9 minutes of isothermal run. Overall, GC ran for 36 minutes. To find its relative percentage quantity, the average peak areas of each component were compared to the overall areas. It was TurboMass Ver5.2.0 that was utilized to control the mass spectra and chromatograms.

Thermal Ion Spectroscopy with Fused Filters

The Trigonella foenum graecum L., or dry fenugreek seeds, were washed, dried in the open air, crushed, and finally passed through a 125 µm mesh screen. Then the powder was treated with KBr. To conduct the experiments in the USA, and a Nd-YAG laser source were utilized. The latter had the following specs: an excitation wavelength of 1064 nm, a power range of 0.35-1.0 W, and an exposure power of 0.6 W applied to the sample.

Antioxidant scavenging peroxynitrite

A process that was described by Beckman et al. [18] was followed in order to create peroxynitrite (ONOO-). The capacity to scavenge peroxynitrite was measured by means of an Evans Blue bleaching assay. The experiment was conducted by modifying a commonly used approach [19]. The following components were used in the reaction mixture, plant extract doses ranging from 0 to 200 µg/ml, and 1 mM peroxynitrite. After incubating at 25°C for 30 minutes, the absorbance was measured at 611 nm. By comparing the findings of the test and blank samples, we were able to determine the proportion of ONOO-scavenging. The tests were repeated six times each. Gallic acid was the material used as a benchmark.

Protection from hydroxyl radicals

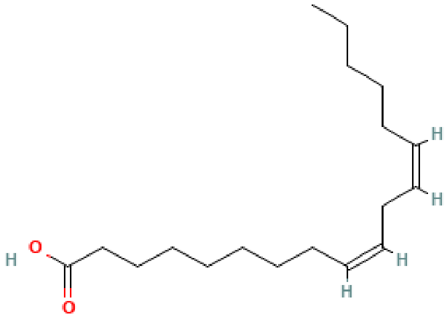
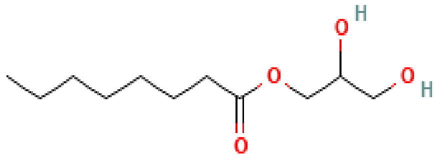
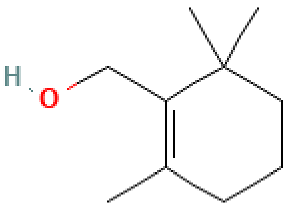
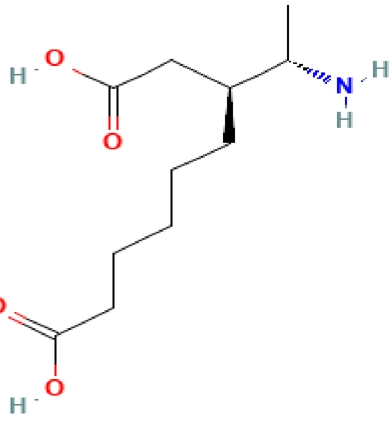
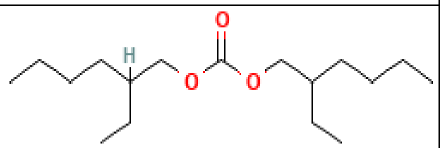
The test is based on quantifying the breakdown product of 2-deoxyribose by condensation with TBA. Hexavalent iron, ascorbate, EDTA, and hydrogen peroxide were utilized in a mechanism known as the Fenton reaction to generate hydroxyl radicals. The reaction mixture, which had a final volume of 1 ml, consisted of the following components: ascorbic acid (100 µM), and varying amounts (0-200 µg/ml) of the original chemical or reference sample. The reaction mixture was mixed with 1 milliliter of 2.8% TCA after the first hour of incubation had elapsed at 37°C. The percentage inhibition was determined by comparing the test and blank solutions [20, 21].

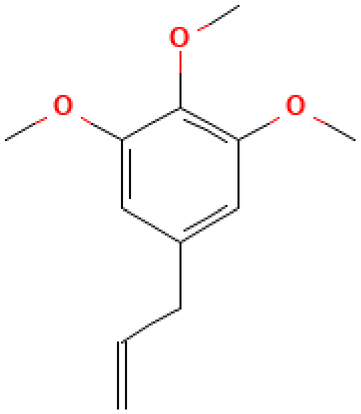
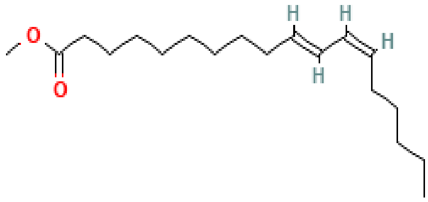
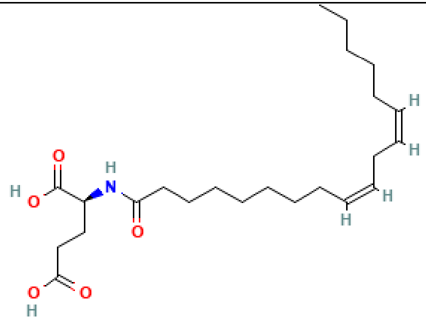
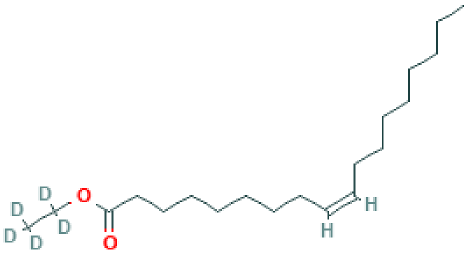
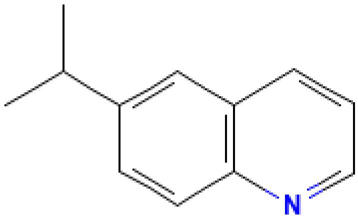
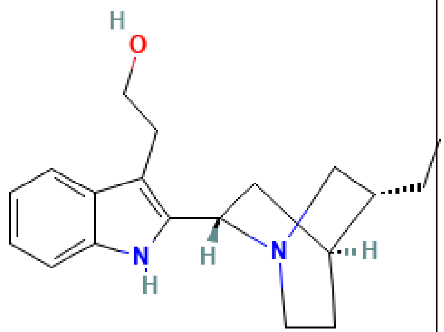
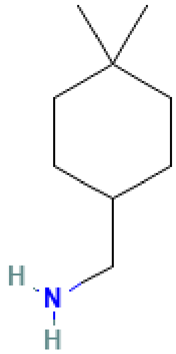
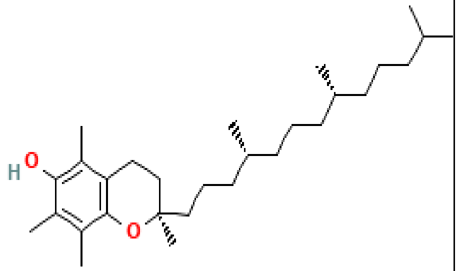
Consuming nitric oxide free radicals

When oxygen combines with nitric oxide in an aqueous sodium nitroprusside (SNP) solution at a physiological pH, the Griess-Illosvoy reaction can be utilized to quantify the amount of nitrite ions generated [22, 23]. The reaction mixture contained various concentrations of the test solution ranging from 0 to 70 µg/ml, 10 mM SNP. The mixture was then allowed to stand for five minutes. The pink chromophore, which was formed by diazotizing nitrite ions with sulphanilamide and then coupling them with NED, was determined by spectrophotometer readings taken at 540 nm in comparison to a blank sample. The tests were repeated six times each. Turmeric was a gold standard.

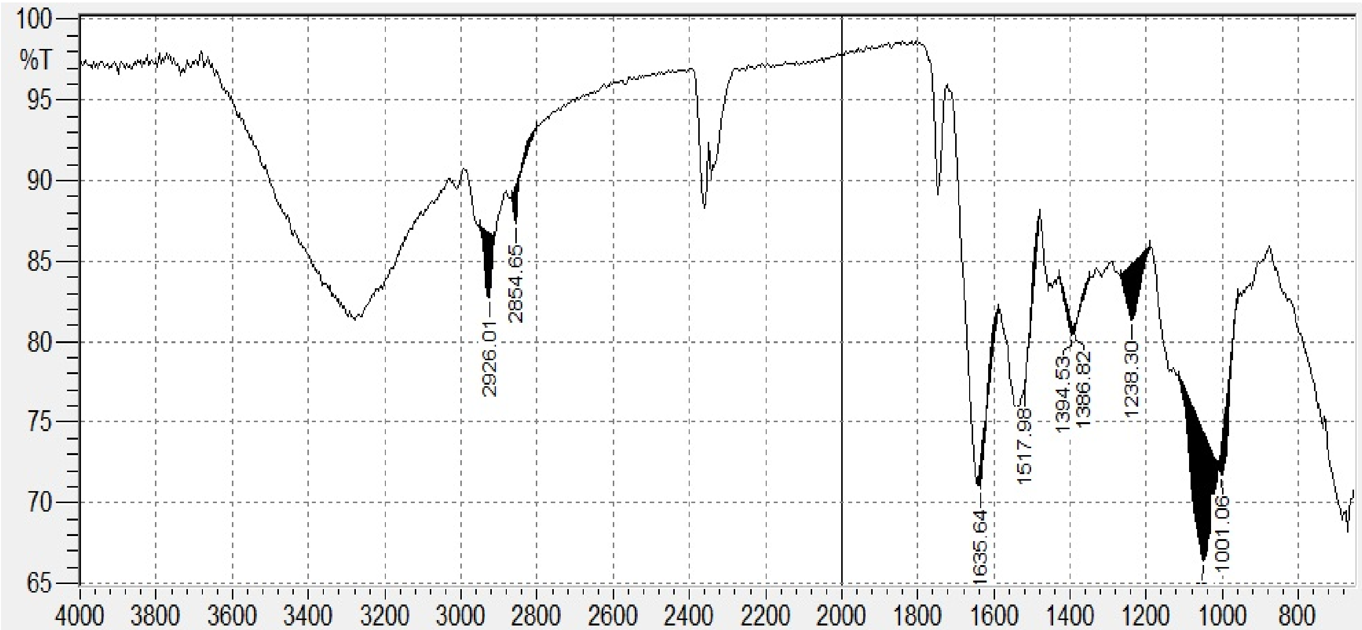
RESULTS AND DISCUSSION

Trigonella herb, belongs to the legume family. One of the many positive pharmacological effects of fenugreek is its ability to reduce inflammation, which in turn reduces bacteria, inflammation, lipidemia, lithigenic effects, liver protection, fungal infections, ulcers, cancer, and indigestion. The traditional medicinal herb Trigonella foenumgraecum L. Seeds' n-hexane extract was analyzed using gas chromatography-mass spectroscopy to determine its identity and properties. From fenugreek seed extracts, twenty-five distinct chemical compounds were extracted using gas chromatography-mass spectrometry, encompassing a wide range of functional groups [24, 25]. Although phenolic chemicals account for the vast majority of the plant's bioactivity, the primary components also include six acids, one ester, six phenols, and one ether. It is well-known that these phenolic compounds have antioxidant, anti-inflammatory, antibacterial, and antiproliferative properties. Among the most abundant chemicals, two key bioactive compounds often associated with fenugreek are trigonelline (with a retention time of 9.33 minutes) and diosgenin (with a retention time of 23.13 minutes). Among the many pharmacological effects demonstrated by the quaternary alkaloid trigonelline are anti-inflammatory, neuroprotective, and antidiabetic effects. Linoleic acid, octanoic acid, 2,3-dihydroxypropyl ester, 2,6,6-trimethylcyclohexene-1-methanol, and 9,12-octadecadienoic acid (Z,Z)-are among the major compounds found in Trigonella foenumgraecum L. seed extract. "Carnicid bis(2-ethylhexyl) ester-“ Eliminin, a ten-trans carbon lynoleic acid methyl ester, glutamic acid linoleamide, vitamin E, 4-(2-methylcyclohexyl)methanamine, 6, isopropylquinoline, dihydrocinchonamine, and ethyl oleate-d5.

 **linoleic acid** **MF: $C_{18}H_{32}O_2$, MW: 280 g/mol**	 **9,12-Octadecadienoic acid (Z,Z)-** **MF: $C_{26}H_{48}O_2$, MW: 392 g/mol**	 **Octanoic acid, 2,3-dihydroxypropyl ester** **MF: $C_{11}H_{22}O_4$, MW: 218 g/mol**
 **2,6,6-Trimethylcyclohexene-1-methanol** **MF: $C_{10}H_{18}O$, MW: 154 g/mol**	 **nonanedioic acid** **MF: $C_{11}H_{21}NO_4$, MW: 231 g/mol**	 **Carbonic acid, bis(2-ethylhexyl) ester** **MF: $C_{17}H_{34}O_3$, MW: 286 g/mol**

 **Elemicin** **MF: $C_{12}H_{16}O_3$, MW: 208 g/mol**	 **10-trans,12-cis-Linoleic acid methyl ester** **MF: $C_{19}H_{34}O_2$, MW: 294 g/mol**	 **Glutamic acid linoleamide** **MF: $C_{23}H_{39}NO_5$, MW: 409 g/mol**
 **Ethyl Oleate-d5** **MF: $C_{20}H_{38}O_2$, MW: 315 g/mol**	 **6-Isopropylquinoline** **MF: $C_{12}H_{13}N$, MW: 171 g/mol**	 **Dihydrocinchonamine** **MF: $C_{19}H_{26}N_2O$, MW: 298 g/mol**
 **4,4-dimethylcyclohexyl)methanamine** **MF: $C_9H_{19}N$, MW: 141 g/mol**	 **Vitamin E** **MF: $C_{29}H_{50}O_2$, MW: 430 g/mol**	

FTIR can be used to study fenugreek, whose scientific name is Trigonella foenumgraecum L., and its bioactive compounds, functional groups, and its medical and industrial applications. Functional group detection via Fourier transform infrared spectra is possible in extracts. Alkaloids, flavonoids, and saponins are examples of phytochemicals that fall within these categories. It is also used in nanomaterial production research, for example, ZnO-NPs generated from fenugreek extract [26, 27]. The absorption band at 3365 cm-1 is caused by the N-H stretching vibrations of protein amide A and the O-H stretching vibrations of starch fiber, respectively. Approximately 3130 cm-1 is another potential secondary amide band that surrounds the 3100 cm-1 band; this band also features a shoulder. A possible explanation could be the Fermi resonance of the N-H stretching bonds with the overtone of the amide II band in trans-amides or the combination band of C=O stretching and N-H in-plane binding in cis-amides. Around 2960, 2927, and 2845 cm-1, there are bands that are thought to be symmetric and asymmetric C-H stretching vibrations. It appears that lipids are vibrating because of C=O stretching because a medium-intensity band at 1742 cm-1 appears. A few of techniques to identify amides are by looking for N-H deformation due to a specific mixed vibration (amide bands) or by looking for absorption bands induced by N-H and C=O stretching vibrations. The amide I band is mainly caused by the stretching of the C=O bond. While NH2 deformation is the main source of the amide II band in primary amides, a mix of N-H bending and C-H stretching vibrations contributes to the band in secondary amides. The amide I and II bands are identified, along with several additional bands that point to the amide group. Amides' spectra are very helpful because of the proteins in which they are engaged. Proteins in their solid state can be identified by the powerful bands observed. A starch absorption band at 1080 cm-1 and a C-O band at 1040 cm-1 are located in the 1200 to 900 cm-1 range, respectively. These bands are visible in fibers and other materials that contain cellulose. While the carbohydrate and fat content decreases, the protein, lipid, fiber, ash, and soluble sugar content increases in fenugreek as it germinates.



Wavenumber (cm-1)

Figure 1. FT-IR profile solid analysis of Trigonella foenumgraecum L.

Table 1. FT-IR peak values of *Trigonella foenumgraecum* L.

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.	1001.06	71.703	1.860	1006.84	958.62	5.657	0.368	Strong	C-F	Stretch	alkyl halides	1000-1400
2.	1049.28	66.405	8.020	1112.93	1008.77	15.701	2.657	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1238.30	81.325	3.466	1267.23	1190.08	6.139	0.706	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1386.82	80.732	0.218	1388.75	1348.24	3.361	0.008	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1394.53	80.345	0.743	1427.32	1390.68	3.187	0.090	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1517.98	76.852	0.526	1519.91	1479.40	3.266	0.006	Medium	C=C	Stretch	Aromatic	1400-1600
7.	1635.64	70.901	1.581	1641.42	1589.34	5.984	0.132	Bending	N-H	Stretch	Amide	1550-1640
8.	2854.65	87.336	2.458	2866.22	2802.57	2.612	0.134	Strong	C-H	Stretch	Alkane	2850-3000
9.	2926.01	82.720	4.003	2951.09	2912.51	2.761	0.392	Strong	C-H	Stretch	Alkane	2850-3000

Fenugreek, or Trigonella foenum-graecum, has powerful radical scavenging action in its seeds and extracts, which means it protects cells against damage, aging, and chronic diseases, according to studies. The plant's abundance of antioxidants, such as flavonoids and polyphenols, makes it effective against oxidative stress and free radicals. These compounds, along with the healthy fatty acids found in fenugreek, make it an excellent source of natural antioxidants. Because of its anti-inflammatory, anti-diabetic, and liver-protective characteristics, it has a lengthy history of use as a medicinal. Trigonella foenumgraecum L. fruit extract (methanolic crude extract, ethanol fraction, and standard) and its antioxidant activity, including scavenging of peroxynitrite, hydroxyl, and nitric oxide radicals. A range of extract types were recorded for peroxynitrite scavenging, including crude, ethyl acetate fraction, ethanol fraction, and standard, with respective measures of 712.00±36.14, 656.45±33.00, and 874.00±38.09 for Gallic acid (standard). Mannitol (standard) had a hydroxyl radical scavenging potential of 520.90±29.81, whereas 298.71±25.00 and 269.00±22.95 were measured correspondingly. Nitric oxide radical scavenging potential was recorded at 42.00±3.05, 25.13±2.98, and 58.07±3.93 for Curcumin (standard), respectively.

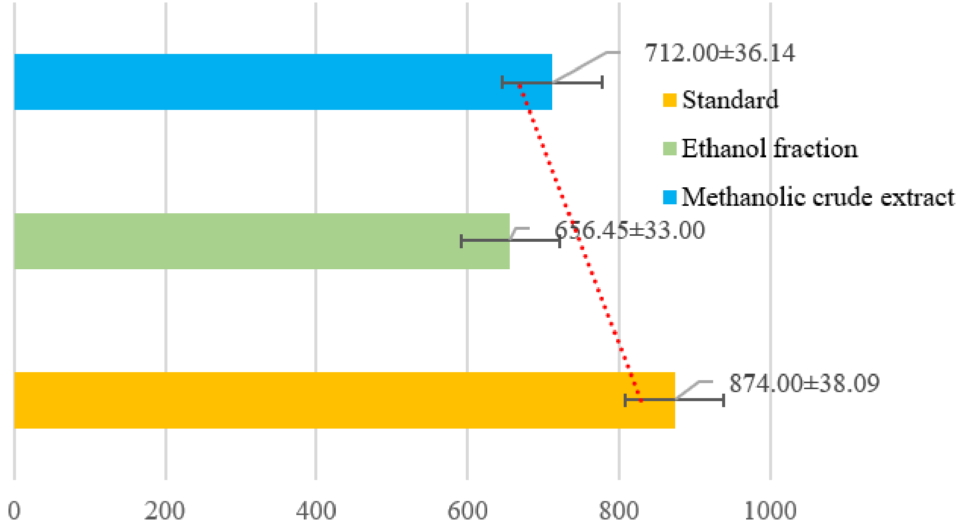


Figure 2. Antioxidant activity (Peroxynitrite scavenging) of *Trigonella foenumgraecum* L

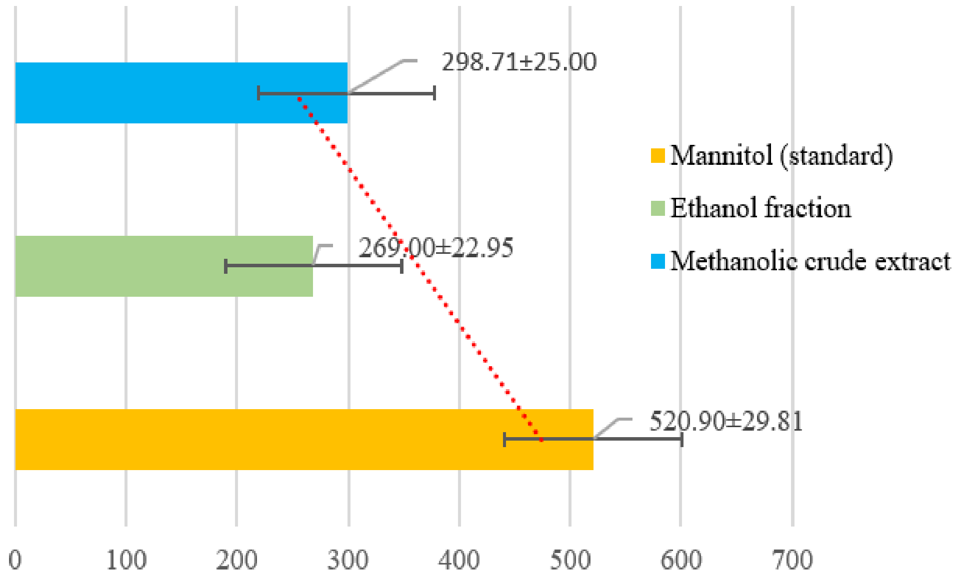


Figure 3. Antioxidant activity (Hydroxyl radical scavenging) of *Trigonella foenumgraecum* L

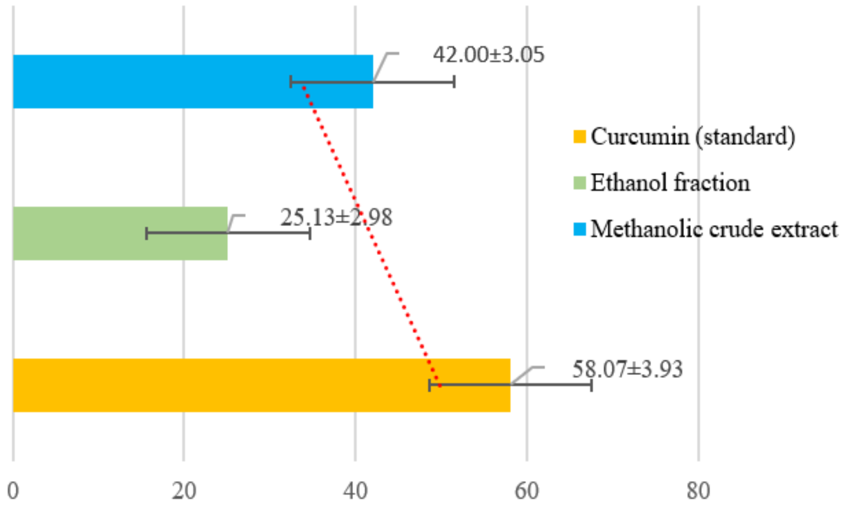


Figure 4. Antioxidant activity (Nitric oxide radical scavenging) of *Trigonella foenumgraecum* L

Having it in the extract supports the idea that Fenugreek could be useful as a medicine for diabetes and other inflammatory diseases. The plant is rich in bioactive phytochemicals that show great medical potential; one such compound is diosgenin, a steroidal saponin with estrogenic, anti-inflammatory, and anticancer properties. Not only were these crucial chemicals isolated, but so were several additional metabolites. The phenolic molecule 4-Hydroxybenzoic acid was one of them; nonetheless, our studies with Egyptian fenugreek showed that it is quite sensitive to environmental influences, despite its antioxidant and antibacterial properties. The potent antioxidant properties of α-Tocopherol are amplified by the plant's overall health benefits. These compounds provide fenugreek its antibacterial and antioxidant characteristics, making it a great option for medicinal and nutraceutical applications. These compounds may also have additional medicinal qualities. Reported beneficial effects of Fenugreek's sulfur compounds against various ailments provide more proof for the plant's broad-spectrum bioactivity. To be noted, the extract did contain dodecamethylcyclohexasiloxane, a silicone-based compound (14.2 min). There may have been changes to the chemical makeup of the sample due to processing or environmental factors since this molecule is not typically present in fenugreek. However, Fenugreek's recognized medical effects are consistent with its bioactive profile, which mostly consists of Trigonelline and Diosgenin. The fascinating discovery of β-Estradiol-3-methyl ether casts doubt on the estrogenic potential of Fenugreek. The supposed estrogenic effects of Fenugreek on hormone regulation and reproductive health require more study to determine whether these effects are due to the plant's estrogenic compounds, such as phytoestrogens.

CONCLUSION

In conclusion, the results of this study point to Trigonella foenumgraecum L. as a potential cure for some fungal infections due to its high concentration of medicinally active compounds. Aziridine, 1, 2,3-trimethyl-, trans-, one of several beneficial phytochemicals found in Trigonella foenumgraecum L., may have antibacterial properties, according to the prior research. As an additional step, it can be isolated and its biological activity studied in an invitro system.

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