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Original Reserach Article

Fenugreek [*Trigonella graecum*] Methanolic Seeds Extract for Antioxidant (Superoxide and Singlet Oxygen Radical Scavenging), Antifungal Effect and Screening of Its Phytochemical Constitutes Using GC-MS

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Abstract: One of the plants that have antioxidant properties is known as Fenugreek with a scientific name of Trigonella foenum-graecum. This can be attributed to polyphenol content of the herb which consists of: flavonoids, phenolic acids, and tannins. Culturally fenugreek is used in traditional medicine because it has antioxidant properties that are applied in the treatment of blood sugar and cholesterol levels. A small market was taken to get the fresh seeds of Trigonella (*foenum grecum*). A diluted alcohol was applied to remove the sediment or traces of water as 10 grams of powdered seed was soaked in 80 percent alcohol. The so obtained filtrate was filtered through Whatmann filter paper No. 41 taking 2 grams of sodium sulphate. Phthalate -D 4, Methyl D-glucopyranoside; M ethyl, 2-Furanmethanol, a-ethyl, 3-O-Methyl-d -glucose, Adenine arabinoside, 1-Aziridineethanamine, 4-Tridecyne, palmitic acid, Pentanal, 2-methyl, gamma -Tocopherol, Piperid In singlet oxygen scavenging, the antioxidant activity was the following; crude (methanolic extract)- 53.18±4.39, ethyl acetate- 53.18±4.39, ethanol- 53.18±4.39, hexane fraction- 53.18±4.39, water fraction- 53.18±4.39, and Lipoic acid (standard)- 53.18±4.39. In superoxide radical scavenging, the antioxidant activity was 19.60±1.01, 23.76±1.30, 26.36±1.39, 21.46±1.07, 29.31±1.42, and 39.82±2.07. The antifungal activity of methanolic crude extract and ethanol fraction was compared to the standard and found to be bioactive natural component. The concentrations of itraconazole and flucytosine in the Fenugreek seeds extract (*Trigonella graecum*) against *Penicillium digitatum* were; 22.78±0.32, 16.88±0.26, 32.08±0.58 and 26.00±0.53 respectively and against *Penicillium italicum* 12.27±0.20, 13.97±0.21, 30.05 Against the *Tricophyton rubrum*, the antifungal activity came to be 11.05±0.17, 17.63±0.26, 23.13±0.28 and 28.89±0.41. Recorded 10.00l±0.14, 08.65l±0.10, 34.00l±0.59 and 29.16l±0.54 respectively against *Microsporum Canis*.

Keywords: Superoxide and Singlet Oxygen Scavenging, Antifungal, Antioxidant, GC-MS, Fenugreek.

INTRODUCTION

A leguminous herb of the family Fabaceae popularly known as Trigonellafoenum-graecum L. the seeds of the plant have found medical, aromatic, cosmetic, beverage usage, industrial, as well as, a staples food in many of the countries of Asia, Mediterranean, and Africa. White hard embryo of the fenugreek seeds is enclosed by corneous wide layer of endosperm white and enclosed by yellow hard embryo. Steroidal sapogenins are amongst the number of phytochemicals that are contained in fenugreek. Diosgenin is a familiar steroid precursor and is present in the oil seed of an herb known as fenugreek. Most of the total polyphenols are lodged in the husk/zest, and the endosperm is rich in saponin/protein [1]. The seeds of fenugreek comprise

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many alkaloids including coumarins, trigonelline, gentianine and carpaine among others. It is considered that the bio-chemical components that play the main role in the fenugreek seeds are polyphenol chemicals including rhaponticin and isovitexin. Among the most profound reasons as to why Fenugreek is SO effective in treating medical conditions is that they are antioxidants. Antioxidants promote in curbing the event of oxidative stress by getting rid of the reactive oxygen species (ROS) and the existence of free radicals in the body. It is in this manner that the body protects enemy side effects of oxidation. In fenugreek, it has polyphenols, an antioxidant. One of the ingredients of fenugreek which helps it accomplish the task of medicine is antioxidants. Antioxidants promote in curbing the event of oxidative stress by getting rid of the reactive oxygen species (ROS) and the existence of free radicals in the body. Fenugreek Plant and other plants contain polyphenols that help as antioxidants. Polyphenol components of raisins include tannins, phenolic acids and flavonoids [2]. They gained their popularity because of the antioxidant properties and they have been attributed with some of their health benefits. Fenugreek has very many polyphenols that include tannins, phenolic acids, and flavonoids. Flavonoids belong to polyphenolic compounds which are wide spread in flora. They have gained notoriety in the beer circles because of their antioxidant properties and there are even some health benefits attributed to them. Nature has provided a remedy to most illnesses especially diabetes and menstrual cramps together with gastrointestinal problems since our use of it dates back to such early times. Fenugreek has only recently drawn the attention of the scientific community as a possible anti-oxidant and polyphenol. Chemicals known as antioxidants can even exert injurious effects on the cells and lead to the occurrence of chronic diseases like heart diseases and cancer. Fruits, vegetables, and Herbs like Fenugreek contain antioxidants such as polyphenols [3, 4]. Certainly, quercetin is an active and highly-active antioxidant that is a flavonoid and found in fenugreek and may form a protective mechanism against chronic diseases, such as cancer and heart disease. Fenugreek is replete with tannin, a poly-phenol. They are famous because of the capacity to fight against inflammations and preventing the growth of dangerous bacteria and viruses. Tannins can also provide some form of cancer prevention. One of the types of polyphenols that contain anti-cancer principles is lignans. Fenugreek has such types of lignans as matairesinol and pinoresinol. The laboratory test has demonstrated that these lignans inhibit the nourishment of breast cancer. Other substances that attribute fenugreek with its antioxidant abilities besides polyphenols include some vitamins and especially vitamin C and E [5, 6]. These vitamins are antioxidant and they prevent motor division on free radicals.

MATERIALS AND METHODS

Plant Materials and Extraction Method

Seeds of Trigonella (foenum grecum) were purchased in the farmer market. \textsup\ Republican 10 grams portion of the powders seed material was then soaked in 80 percent alcohol overnight after which was filtered using whatmann filter paper No. 41 with 2 grams of sodium sulphate to remove any left over particles and water in the mixture. The volume of the bubble gas-nitrogen into the solution was then decreased down to 1 ml. The extract has phytocomponents that are polar and also nonpolar.

GC/MS Analysis

The discussion of the mechanism of action of this extract was carried out on a gas chromatograph together with a mass spectrometer (GC/MS) and a Perkin Elmer GC Claurus 500 system. The instrument had an Elite-1 fused silica capillary column (30 m length, 0.25 mm ID. and 1 0Mdf 100% Dimethyl poly siloxane). A 70 Ev electron ionization instrument was used to do the C/MS detection. A constant flow rate at 1 ml/min and injection volume of 2 ervivesno was used with 99.999 percentage helium gas as recommended by ervidalon explicit IDG (IDG). For the gas utilization amount of 30 L min (L min) 1, the temperature of the injector and ion source are set to 250 and 280 o C, respectively. The programmed specified was set up so that after a 2minutes isothermal program at 110 o C the ovens temperature was suppose to rise at a rate of 10 o C/min to 200 o C and then at an accelerated rate of 5 o C/min to 280 o C and lastly having an isothermal program of 9 minutes at 280 o C. Mass spectra were recorded with a scanning rate of 0.5 sec in the range of size pieces of 45-450 Da with 70 eV. The overall amount of time it needed GC to run was thirty six minutes. The mean peak areas per individual components was compared against the total areas to a level that we could establish the proportional percentages of the contents. We have processed our chromatograms and mass spectra TurboMass Ver5.2.0.

Antimicrobial Activity Test of the Extracts of the Plant

The mock stock suspensions were evaluated using average concentration of viable organisms per mL using surface viable counting technique. A fresh suspension of stock was made in each experiment to ensure standard experiments. Using a pipette 1ml of a young fungus mixture was poured to the center of a sterile Petri dish. The inoculum was then blended thoroughly into Potato dextrose agar (PDA) that had been cooled down in molten form in a Petri dish. 106 Well holes were pierced into the agar plates containing the inoculum when the agar was solid using a sterile cork borer of 6-mm diameter. The plates were subsequently placed in an incubator, which was adjusted to 37 o C following 24 or 48 hours. The fenugreek seed extract as the test was three times applied against the target species on sterile discs. In addition to the average and mean value, value on the diameter was obtained as growth

inhibition zones on the incubated sample. The drugs that were compared were itraconazole and flucytosine also known as the 5-fluorocytosine or 5-FC.

Superoxide Radicals

The comparison was made in terms of comparison with a reported method, which involved reduction of NBT. Nitro blue tetrazolium (NBT) is reduced by superoxide radicals generated by the nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) reaction to purple formazan [7, 8]. The amount of formazan formed was read as absorbance at 562nm against a suitable blank in the incubation of 5minutes and ambient temperature. We performed each of the tests in a series of six repeats It incorporated a positive control through quercetin

Single Oxygen Capturing

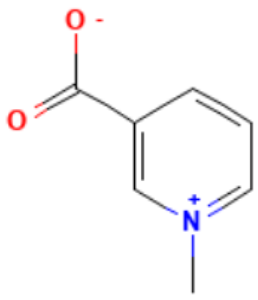
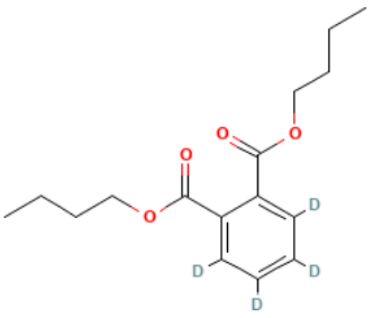
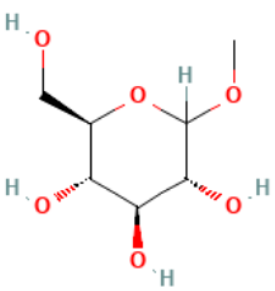
The singlet oxygen (1O 2) formation could be measured by spectrophotometric method described above, upon which the bleaching of N, N-dimethyl-4-nitrosoaniline (RNO) was monitored. By the reaction between NaOCl and H 2 O 2, singlet oxygen was produced and RNO was bleached at 440 nm. Reaction mixture included 45 mM of phosphate buffer (pH 7.1), 50 mM of NaOCl, 50 mM of H 2 O 2, 50 mM of histidine, 10 mM of RNO and sample (0-200 100 8g/ml) with a final reaction volume of 2.0 ml. The fall in the absorbance at 440 nm was recorded at 40 minutes after incubation at room temperature of 30o C. Measurement of the sample scavenging activity was compared with that of a standard chemical, lipoic acid. We administered each test clause six times.

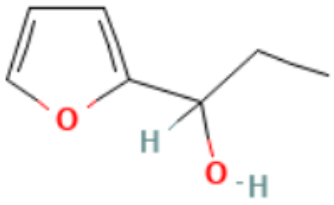
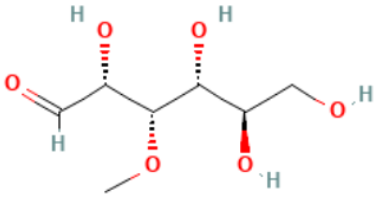
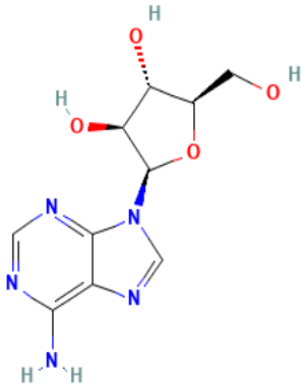
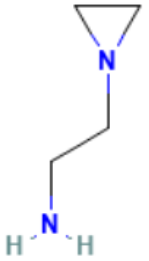
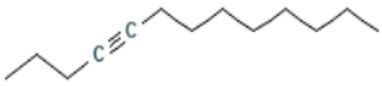
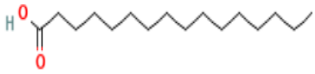
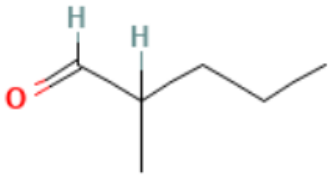
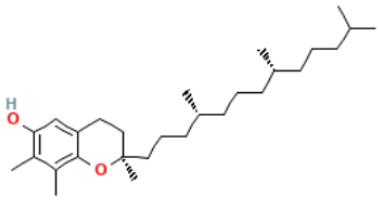
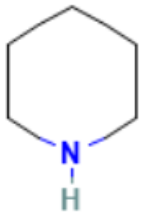
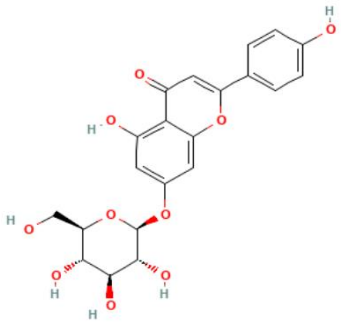
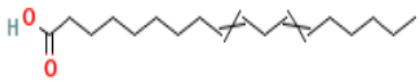
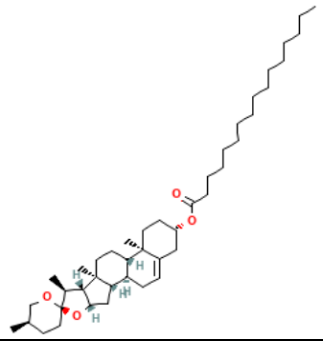
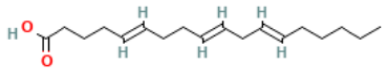
Statistical Analysis

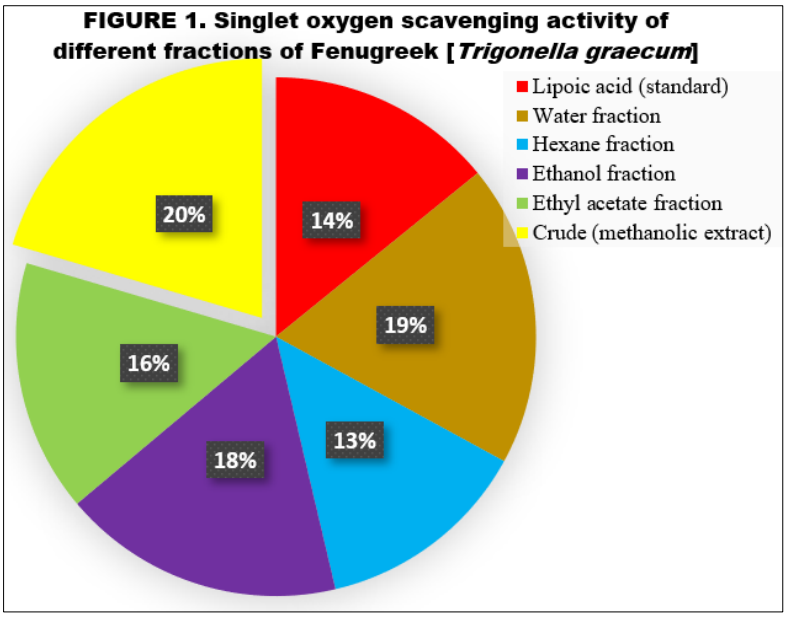
In conducting the statistical analysis, I utilised the graph pad produced by SPSS Inc. The data are expressed as mean+ SEM. Statistical test is performed using ANOVA (one- way analysis of variance). When the p value is greater than 0.05 then a difference is statistically significant.

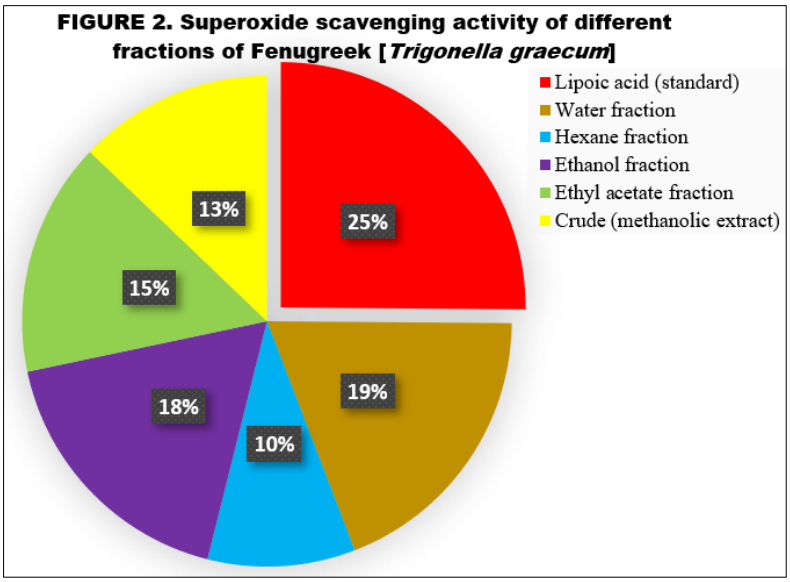
RESULTS AND DISCUSSION

Trigonelline, Dibutyl Phthalate-d4, Methyl D-glucopyranoside; Methyl, 2-Furanmethanol, alpha-ethyl, 3-O-Methyl-d-glucose, Adenine arabinoside, 1-Aziridineethanamine, 4-Tridecyne, pentanal, 2-methyl, gamma-Tocopherol, Piperidine, Apigenin 7-O-beta-D-gl Fenugreek assists in alleviating digestive disorders, openness of the bowels and various other disorders of the digestive tract. There are many biological activities of Flavonoids. These may be enzyme inhibitive effect, cell type modular effects, have an antioxidant property, anticarcinogenic property, antifungal activity, antimalarial or antibacterial properties. A group of highly pharmacogical powerful compounds known as flavonoids are benzo-gamma pyrone. The potential of these polyphenolic molecules as antioxidant has drawn much interest in such chemicals because of the potential health benefits. Fenugreek seeds have been seen in clinical studies to reduce the quantity of blood sugar and the mucilaginous fibers in the seeds are most likely guilty. Crude (methanolic extract, fractions of ethyl acetate, ethanol, hexane, and water, and lipoic acid (standard) exhibited the scavenging activities against superoxide radical of 53.18±4.39, 52.70±4.28, 54.16±4.38, 50.76±4.36, 53.68±4.39, and 41.26±1.13, respectively. It is quite evident that the DPPH (2,2-diphenyl-1-picrylhydrazyl) is among the most popular tests applied in exploring the evidence of the antioxidant activity. The anti-oxidant activity is attributed to, which is the capability of the antioxidants to reduce the concentration of DPPH in a solution after its addition. The DPPH test has been used by scores of researchers to study the antioxidant potential of fenugreek. The antioxidant became altered in the process of extraction yet in one study the extracts of the seed of fenugreek were more effective than the leaves. In another study, it was revealed that it was the strongest antioxidant level of the extract of fenugreek seed, in comparison to other spice extracts, i.e. cumin, coriander among others. Fenugreek is rich in polyphenols that make it have potent antioxidants. In one study, it was determined that phenolic acids as well as flavonoids are present in the fenugreek seeds [10, 11]. Fenugreek leaf extracts were also found to contain other phenolic compounds that have high quantities such as gallic acid that has anti-inflammatory and antioxidant properties in another study.

Trigonelline MF: $C_7H_7NO_2$ MW: 137.14 g/mol	Dibutyl Phthalate-d4 MF: $C_{16}H_{22}O_4$ MW: 282.37 g/mol	Methyl D-glucopyranoside; Methyl MF: $C_7H_{14}O_6$ MW: 194.18 g/mol
		

2-Furanmethanol, alpha-ethyl MF: $C_7H_{10}O_2$ MW: 126.15 g/mol 	3-O-Methyl-d-glucose MF: $C_7H_{14}O_6$ MW: 194.18 g/mol 	Adenine arabinoside MF: $C_{10}H_{13}N_5O_4$ MW: 267.24 g/mol 
1-Aziridineethanamine MF: $C_4H_{10}N_2$ MW: 86.14 g/mol 	4-Tridecyne MF: $C_{13}H_{24}$ MW: 180.33 g/mol 	palmitic acid MF: $C_{16}H_{32}O_2$ MW: 256.42 g/mol 
Pentanal, 2-methyl MF: $C_6H_{12}O$ MW: 100.16 g/mol 	gamma-Tocopherol MF: $C_{28}H_{48}O_2$ MW: 416.7 g/mol 	Piperidine MF: $C_5H_{11}N$ MW: 85.15 g/mol 
Apigenin 7-O-beta-D-glucoside MF: $C_{21}H_{20}O_{10}$ MW: 432.4 g/mol 	9,12-Octadecadienoic acid MF: $C_{18}H_{32}O_2$ MW: 280.4 g/mol 	Diosgenin palmitate MF: $C_{43}H_{72}O_4$ MW: 653 g/mol 
5,9,12-Octadecatrienoic acid MF: $C_{18}H_{30}O_2$ MW: 278.4 g/mol 		





Fenugreek is rich in plenty of benefiting flavonoid and polyphenol compounds. Whole plant extracts are rich in flavonoids: quercetin, luteolin, vitexin and 7, 40 -dimethoxy flavanones are present in alcohol extracts. The aglycones kaempferol, quercetin, tricin and naringenin have also been reported to be present by other laboratories. It has been observed that fenugreek seeds contain antioxidants. This experiment has proven the antioxidant exercising capacity of three varieties of fenugreek seed on flours, that being soaked, germinated and roasting. The outcomes were recorded wherein antioxidant activity ranged between 32.7 and 73.89 with maximum antioxidant activity observed in raw fenugreek seed flour as 18.1. A study found that as a result of germination, flavonoids and polyphenols polyphenols present in fenugreek seeds produce significant antioxidant activity [12, 13]. A supplementation of fenugreek may have its impacts through multiple avenues it is through this that causes the occurrence of oxidative stress due to the formation of an imbalance between reactive oxygen species (ROS) and the antioxidants. Bioactive natural compound of the methanolic crude extract and ethanolic fraction exhibited similar antifungal activity in comparison to the standards Itraconazole and Flucytosine (5-fluorocytosine 5-FC), Fenugreek seeds extract against *Penicillium digitatum* were 22.7820.32, 16.8810.26, 32.0810.58 and 26.0010.53 respectively whereas exhibited 12.272 Antifungal was 11.05 +/- .17, 17.63 +/- 0.26, 23.13 +/- 0.28, 28.89 +/- 0.41 against Tricophyton rubrum. Whereas Water recorded 10.00 +/- 0.14, 08.65 +/- 0.10, 34.00 +/ - 0.59 and 29.16 +/ - 0.54 respectively against *Microsporum canis* (FIGURE 3-7).

FIGURE 3. Antifungal Effect of bioactive natural compounds of seeds extract of Fenugreek [*Trigonella graecum*] against *Penicillium digitatum*

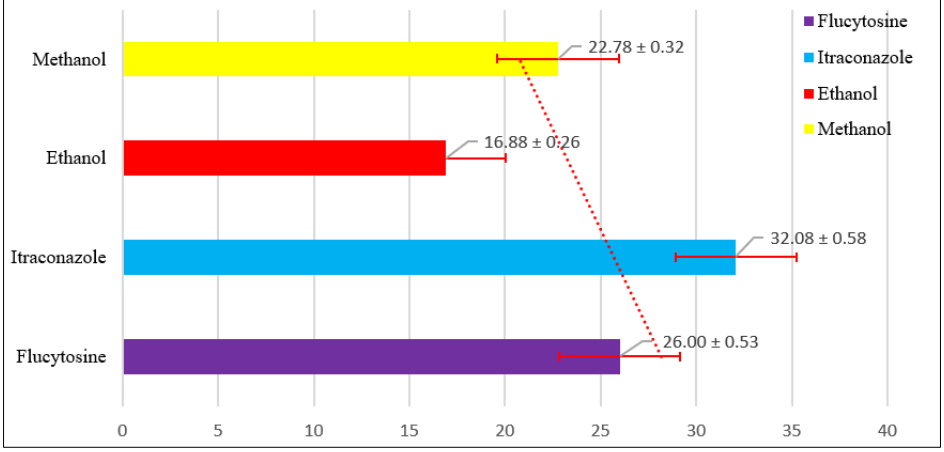


FIGURE 4. Antifungal Effect of bioactive natural compounds of seeds extract of Fenugreek [*Trigonella graecum*] against *Penicillium italicum*

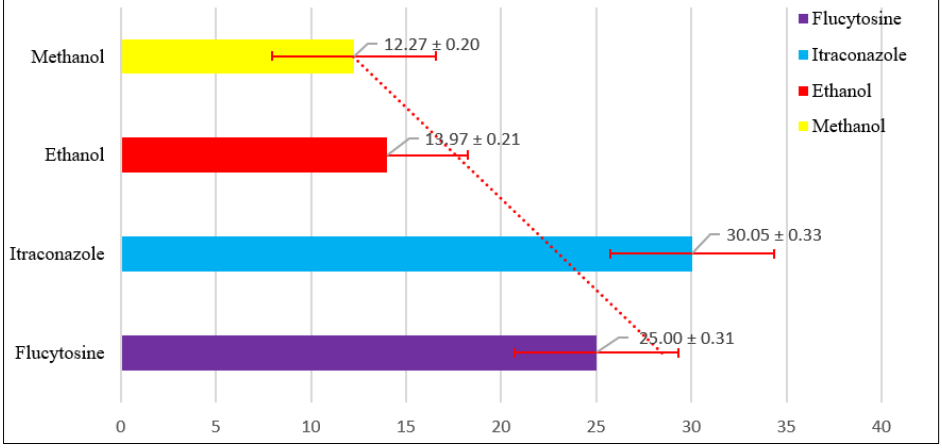
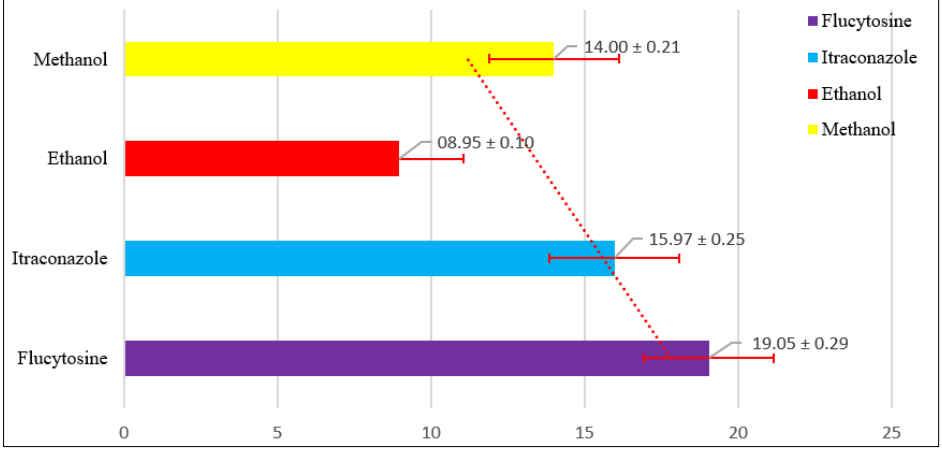


FIGURE 5. Antifungal Effect of bioactive natural compounds of seeds extract of Fenugreek [*Trigonella graecum*] against *Cryptococcus neoformans*



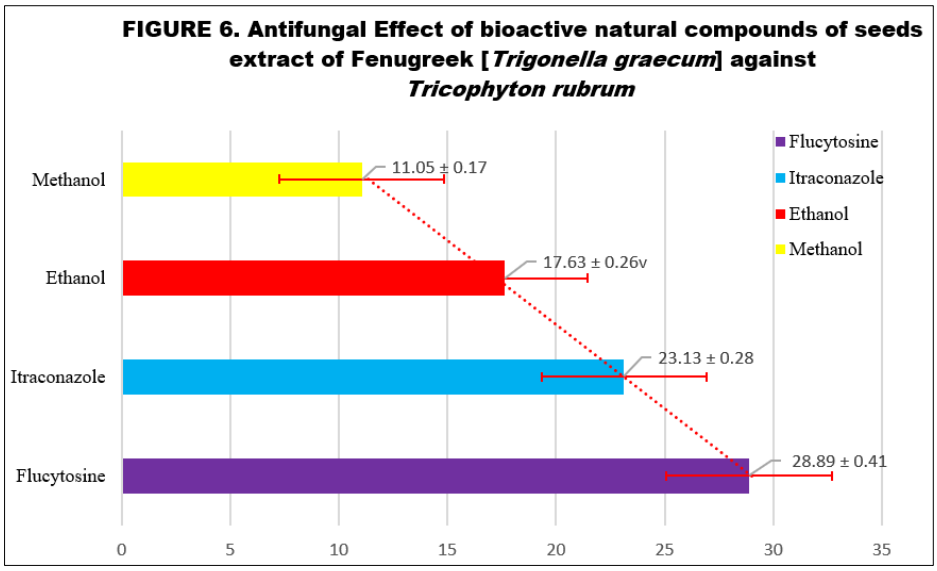


FIGURE 6. Antifungal Effect of bioactive natural compounds of seeds extract of Fenugreek [*Trigonella graecum*] against *Tricophyton rubrum*

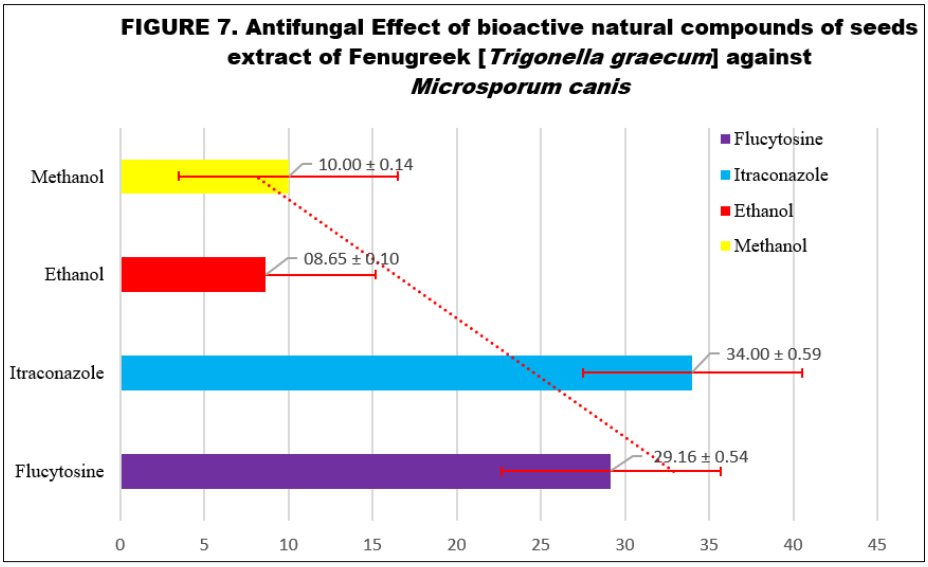


FIGURE 7. Antifungal Effect of bioactive natural compounds of seeds extract of Fenugreek [*Trigonella graecum*] against *Microsporum canis*

This indicates that the strength in the antibacterial effects of the Fenugreek seed can be presented as a proposal of a source of antibacterial herbal products. Seeds of fenugreek contains functional macromolecule antioxidative activations and improvements to the immune system. It has steroids, oils, phenolics, glycosides, flavonoids and essential fatty acids. Compared with manufactured antibiotics, its extracts are less harmful to the environment, and they are cheap. Besides the antioxidant, inhibitory, nematocidal effect, and the existence of antimicrobial actions among the constituents presented in the fenugreek seeds, 4-butyl-2(4-nitrophenyl) 9,12- octadecadiene and 9-octadecanoics were the key constituents to demonstrate productive antimicrobial actions. The notable compounds present in fenugreek seeds have been identified as 4-hydroxybenzoic acid (the phenolic) and flavonoid (Apigenin-complex), flavonoid (quercetin-complex), alkaloid (trigonelline) (the most prominent compound to be found in fenugreek), and steroidal saponin (diosgenin) [14]. The antibacterial activity of fenugreek seeds increased tremendously when these substances were added in it. In the matter of both categories of plants and fungi, the methanol extraction was more lethal when it was collected at the blooming phase. Interestingly, the antifungal activity of the various plant extracts was nonhomogenous. varying kinds of satured plant extracts in different types of chemicals, which may be an important aspect as to the varying levels of activity. The pronounced differences in chemical composition, particularly that of flavonoid chemicals played the leading role in the demonstration of the given variation when it comes to the inhibitory effect generated by organic extracts of fenugreek aerial parts that hinged on the level of ploidy. Thus flavones are involved in a variety of interactions with other species, bacteria, insect and plants [15]. Such interactions can be either detrimental and/or profitable to an injured plant. It was identified that the chemicals present in the arial parts of fenugreek were the element of flavonol glucosides further the identification was done by the help of spectral analysis.

CONCLUSION

Trigonella (*foenum graceum*) is a possible antibacterial because there are phytochemicals present within these products. Further work should be made to isolate it in understanding its biological activities on an invitro environment further. Fenugreek seed is low in fiber, ash and moisture consumption but high in proportions of protein and carbohydrates as seen in the present study. The fnugreek oil exhibited the best efficiency against all the specimen of fungus except the Escherichia coli which was the most susceptible, based on the microbiology test. A research conducted on the *Penicillium digitatum* fungus revealed that the best way of inhibiting the growth of this fungus is through fenugreek oil. The limited activity of the oil that has shown the inhibitory effect to the examined species was the concentrated oil. There could be the possibility of antimicrobial, antifungal and antioxidant biopotential and pharmacokinetics induced. Fenugreek seed oil extends the shelf life of foodstuff products and hence a preferable commodity to chemical, medical and food industries markets.

REFERENCES

1. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006, 97, 654–660.
2. Rahmani M, Hamel L, Toumi-Benali F, Dif MM, Moumen F, Rahmani H. Determination of antioxidant activity, phenolic quantification of four varieties of fenugreek Trigonella foenum graecum L. seed extract cultured in west Algeria. J Mater Environ Sci. 2018;9(6):1656-61.
3. Afzal B, Pasha I, Zahoor T, Nawaz H. Nutritional potential of Fenugreek supplemented bread with special reference to antioxidant profiling. Pakistan Journal of Agricultural Sciences. 2016 Mar 1;53(1.
4. Baba WN, Tabasum Q, Muzzaffar S, Masoodi FA, Wani I, Ganie SA, Bhat MM. Some nutraceutical properties of fenugreek seeds and shoots (Trigonella foenum-graecum L.) from the high Himalayan region. Food bioscience. 2018 Jun 1;23:31-7.
5. Colantoni, A.; Idilman, R.; de Maria, N.; Duffner, L.A.; Van Thiel, D.H.; Witte, P.L.; Kovacs, E.J. Evidence of oxidative injury during aging of the liver in a mouse model. J. Am. Aging Assoc. 2001, 24, 51–57.
6. Pibiri, M. Liver regeneration in aged mice: New insights. Aging 2018, 10, 1801–1824. [CrossRef] 10. Smith, M. Therapeutic applications of fenugreek. Altern. Med. Rev. 2003, 8, 20–27.
7. Korycka-Dahl M, Richardson T: Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids. J Dairy Sci. 1978, 61: 400-407.
8. Tylor BS, Kion YM, Wang QI, Sharpio RA, Billiar TR, Geller DA: Nitric oxide down regulates hepatocyte-inducible nitric oxide synthase gene expression. Arch Surg. 1997, 132: 1177-1183.
9. Wani, S.A.; Kumar, P. Fenugreek: A review on its nutraceutical properties and utilization in various food products. J. Saudi Soc. Agric. Sci. 2018, 17, 97–106.
10. Naidu, M.M.; Shyamala, B.N.; Naik, J.P.; Sulochanamma, G.; Srinivas, P. Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. LWT Food Sci. Technol. 2011, 44, 451–456.
11. He, Y.; Ding, C.; Wang, X.; Wang, H.; Suo, Y. Using response surface methodology to optimize countercurrent chromatographic separation of polyphenol compounds from fenugreek (Trigonella foenum-graecum L.) seeds. J. Liq. Chromatogr. Relat. Technol. 2015, 38, 29–35.
12. Sowmya, P.; Rajyalakshmi, P. Hypocholesterolemic effect of germinated fenugreek seeds in human subjects. Plant Foods Hum. Nutr. 1999, 53, 359–365.
13. Randhir R, Shetty K. Improved α-amylase and Helicobacter pylori inhibition by fenugreek extracts derived via solid-state bioconversion using Rhizopus oligosporus. Asia Pacific Journal of Clinical Nutrition. 2007 Sep 1;16(3).
14. Hussain PR, Suradkar P, Javaid S, Akram H, Parvez S. Influence of postharvest gamma irradiation treatment on the content of bioactive compounds and antioxidant activity of fenugreek (Trigonella foenum–graceum L.) and spinach (Spinacia oleracea L.) leaves. Innovative Food Science & Emerging Technologies. 2016; (1)33:268-81.
15. Saviz A, Esmaeilzadeh Kenari R, Khalilzadeh Kelagar MA. Investigation of cultivate zone and ultrasound on antioxidant activity of Fenugreek leaf extract. J. Appl. Environ. Biol. Sci. 2015;4(11S):174-81.