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Phytochemical Screening and Antioxidant Studies of *Trigonella foenum-graecum* Seed Extract

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Abstract

Drugs are available to combact free radicals and other health related diseases. Fenugreek is one of the medicinal plants to check radicals and other health related diseases. The bioactive compounds in *Trigonella foenum-graecum* seed were extracted using Methanol through cold merceration technique. The phytochemical screening and antioxidant activities were carried out on the seed using standard methods. The results of phytochemical analysis carried out on the Methanol seed extract of Fenugreek indicates flavonoid, phenol, terpenoid, alkaloids, tannin, Glycoside, Fatty acid, phenols and saponins. However, the IC_{50} details was evaluated using DPPH free radical. The result of antioxidant activity of the ethylacetate extract exhibited high inhibition at low concentration of 0.1520 mg/cm^3 of DPPH while low inhibition of chloroform extract at high concentration of 0.4816 mg/cm^3.

Keywords: Fenugreek; Methanol; Antioxidant; DPPH; radicals.

INTRODUCTION

Medicinal plants are priceless gifts from nature for food and medicine. In the scientific world, the diverse medicinal potentials of these plants have aroused the interests of researchers across disciplines worldwide. Medicinally, isolated natural products after been subjected to clinical trials can be used as drugs for the treatment of cancer (Crow, 2008), antimicrobial agents (Woldemichae *et al*., 2003; Jagessar *et al*., 2008; Prasad *et al*., 2008), antitumor (Flores *et al*., 2010), antioxidant agents (Manga *et al*., 2006; Kukic *et al*., 2008; Nile and Khobragade, 2010).

The seed commonly known as Fenugreek (English), Hulba (Arabic), Alholva, Feno-greco (Spanish), Eru (Yoruba), Kimba (Hausa) which belong to the Family Fabaceae has been used as a flavoring agent and as a medicinal plant for a long time. Fenugreek is a leguminous plant. The seeds are brownish yellow in colour but the colour may change to dark yellow with difference in environmental conditions e.g. temperature. It is also used in post-natal care by nursing mothers. Nowadays, fenugreek is widely cultivated as a drug plant.

The seed has been widely noticed to possess multipotent antioxidant, antimicrobial, anticancer, gastroprotective and antidiabetic properties by Zameer *et al*., (2017) and Basch *et al*., (2003). It was indicated that the seed extract might help in alleviating hyperglycemia (Pundarikakshudu *et al*., 2016). The fenugreek seed possess antidiabetic effect (Renuka, 2009). *Trigonella foenum-graecum* showed strong antioxidant activity (Pawar and Hagar, 2012).

Antioxidants have been identified as major compounds found in various medicinal plants (Szymanska *et al*., 2016).

The compounds derived from secondary metabolism of Fenugreek are phenolic compounds (Pang *et al*., 2018). The quantitative analysis of the aqueous extract showed 73.54 mg of gallic acid and 70.30 mg of quercetin per gram of extract (Kumar *et al*., 2021). Fenugreek showed a positive effect on physiological aspects of libido and testosterone levels (Megha *et al*., 2012). The seed extract inhibited the DMBA-induced mammary hyperplasia (Amr Amin *et al*., 2005). Cholesterol level was increased from reactions between bile acid and saponin in the seed extract (Yadav *et al*., 2011).

The GCMS analysis of Fenugreek extract showed 14 different compounds, including Hexadecanoic acid, a methyl ester ($C_{18}H_{36}O_2$) at 18.81% in area (Moniruzzaman *et al*., 2015). The main constituents were found to possess antioxidant properties (Akbari *et al*., 2019). Palmitic acid is used as a food additive (Benzidia *et al*., 2018). Results showed that the major compounds in fenugreek seed oil were linoleic acid, palmitic acid,

pinene, 4-Pentyl-1-(4-propylcyclohexyl)-1-cyclohexene, and linoleic acid methyl ester (Akbari *et al*., 2019).

MATERIALS AND MAETHODS

Materials

Apparatus includes Round bottomed flask, Water-bath, Glass wool, Heating mantle, Anti-bumping chips, pre-coated silica gel plate, rotary evaporator and Weighing balance, beakers, separating funnel and thin-layer chromatographic plate.

Reagents

The solvent used were of analytical grades with no further purification obtained from GH Tech chemicals, China which include; Methanol (m.mass = 34.04, m.p=-98^{0}c, b.p=64.5^{0}c, Assay= 99.50%, Density= 0.791-0.793g/cm^3), Ethyl ethanoate(Assay=99%), Density=0.899-0.902g/cm^3, Chloroform (m.mass = 119.38g/mol, b.p=61^{0}c, Assay= 99.50%, Density= 1.49g/cm^3), n-hexane (m.mass = 86.18, m.p=94.3^{0}c, b.p=69^{0}c, Assay= 97.0%, Density= 0.059-0.663g/cm^3), distilled water, Sodium Hydroxide, Meyer's reagent, Fehling Solution, Molisch's reagents, Wagner's reagents, Hydrochloric acid etc

Sampling

Fenugreek seed was bought at Kasuwar-Bacchi, a market in Kaduna state. It was identified by Mr. U. S Gallah, Biology Department, Kaduna State University, Kaduna.

Preparation of extract

The seed of Fenugreek was firstly rinsed with warm water to remove any adsorbed particles and dried at (26 °C) for a month. It was grounded to a fine powder with mortar and pestle, and also with the use of a mechanical grinder and sieved. The extraction of the seed was done using cold maceration by soaking 1200 g of dried powdered sample in 1500 cm^3 of Hexane for defatting. The filterate was collected, followed by continuous extraction until their was no observable colour inference from the sample. The Hexane filterate was collected, concentrated and weighed for further analysis. The dried residue was soaked in Methanol for 3days. At the end of extraction, the plant extracts was filtered (whatman no 10). The filtrate was then concentrated at 40°C. The yield of *Trigonella foenum-graecum* methanol crude was also calculated.

Phytochemical Screening of Trigonella fornum-graecum seed

The presence of the bioactive compounds from the seeds of Fenugreek forms a platform for further pharmacological studies (Colombo and Bosiosio, 2018; Okwute, 1989).

Methanolic extract was phytochemically analysed for secondary metabolites such as Alkaloids, Saponin Glycosides, Flavonoids, Polyphenolic compounds and Tannins. This was carried out using standard protocols (Harbourne, 1988).

Fractionation of the methanol extracts

A portion (200 g) of pulverized seed was percolated in methanol for 3 days. It was concentrated using a rotary evaporator to obtain a dried extract, yielding 61 g of methanolic extract after weighing. The 61 g of dried methanol extract was transferred into a bama bottle and about 200 cm^3 of hexane was added and the mixture was shaken for 2 minutes before standing for about 3 minutes. It was then filtered and the filtrate was collected using Bama bottles and was concentrated at 30 ^{0}C to prevent denaturation. The process was repeated many times until no colour of the solvent from the extract. The marsh was dried and same procedure was carried out using chloroform and then followed by Ethyl acetate leaving behind the residual fraction. Bottle A represents n-Hexane fraction, bottle B represents the Chloroform fraction and bottle C represents Ethyl acetate fraction. Solvent was evaporated from each fraction, the fraction weighed and its percentage yield calculated (Nwauzoma *et al*., 2013). During the TLC profiling between bottle A, B and C, bottle A gave a lot of development which can be seen on plate D under solvent system hexane and ethyl acetate (9:1) with a slow development on the Chloroform extract(B) on plate D and no development at all on ethyl acetate extract(C) on plate D. Plate E show no development on 100% Hexane solvent.

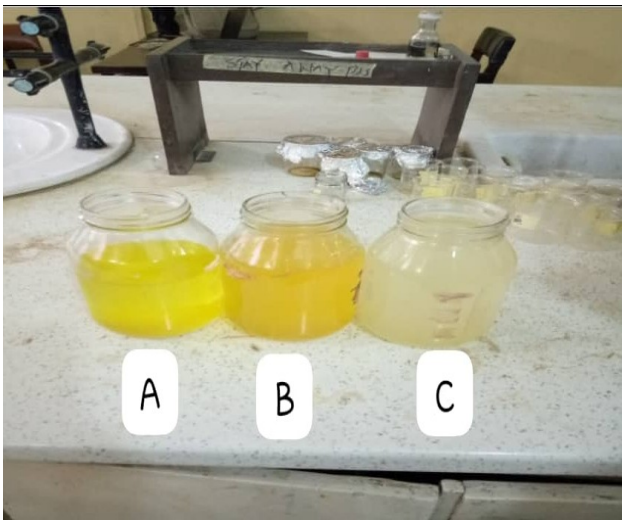


Plate A. Fractionated extracts.

DPPH Antioxidant Analysis

As described by Alara *et al*., (2017) with some modification, the stock solution of 0.1 mmol DPPH was prepared in analytical grade methanol (99.50% purity) and stored until analysis. This was done by dilutiion of 10 cm^3 stock solution with 90 cm^3 of methanol. The stock solution of extracts was prepared in methanol by dissolving 0.001 g of the extracts in 2 cm^3 to give a concentration of 1000 µg/cm^3 which was diluted to give

concentration of 500 µg/cm^3. Concisely, 2 cm^3 of various concentrations (250, 125, 62.5, 31.25, and 15.62 µg/cm^3) in methanol was added to 2 cm^3 of DPPH solution and incubated for 30mins in dark. The absorbance of the sample was measured at a wavelength of 517 nm using a uv/visible spectrophotometer (Hitachi U-1800, Japan), in triplicate at different molarity and the average absorbance was determined. Ascorbic acid was used as a standard and compared to the percentage inhibitions of the serial concentrations of the methanolic antioxidant extracts.

The Inhibition Concentration at 50% was determined from the graph of % inhibition against concentration.

RESULTS AND DISCUSSIONS

Results

Table 1. Phyto-screening of the Methanol crude of Fenugreek seed.

Phytocompounds	Inference
Alkaloid	+ve
Flavonoid	+ve
Glycosides	+ve
Saponins	+ve
Steroids	+ve
Tanins	+ve
Phenols	+ve
Carbohydrate	+ve

Table 2. Percentage yield of T. foenum-graecum seed extract in various fractions.

Extracts	Yield (g)	Percentage Yield (%)
Hexane fraction	9.3	15.25
Chloroform fraction	2.2	3.61
Ethylacetate fraction	1.9	3.11

Table 3. Percentage inhibition of Ascorbic Acid.

Conc(mg/cm^3)	Absorbance1	Absorbance2	Absorbance3	Average	%IAA
0.5	0.0802	0.0805	0.0801	0.0803	66.98
0.25	0.0852	0.0851	0.085	0.0851	65.01
0.125	0.0901	0.0909	0.0904	0.0905	62.79
0.0625	0.128	0.129	0.129	0.1287	47.08
0.03125	0.131	0.133	0.136	0.1333	45.19

The $A_{control}$ is 0.2432

Key: %IAA: Percentage Inhibition of Ascorbic Acid

Table 4. Percentage inhibition of Ethyl acetate fraction.

Conc(mg/cm^3)	Absorbance1	Absorbance2	Absorbance3	Average	%IEA
0.5	0.072	0.067	0.068	0.069	71.63
0.25	0.084	0.099	0.084	0.089	63.4
0.125	0.123	0.134	0.127	0.128	47.37
0.0625	0.163	0.161	0.16	0.161	33.8
0.03125	0.175	0.179	0.174	0.176	27.63

Key: %IEA: Percentage Inhibition of Ethylacetate

Table 5. Percentage inhibition of Chloroform fraction.

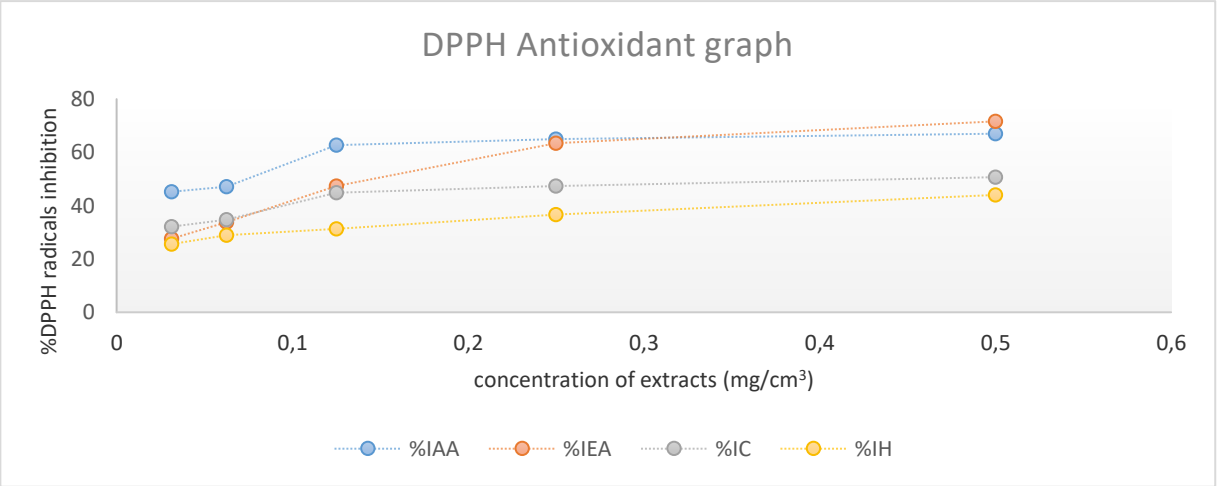
Conc(mg/cm^3)	Absorbance1	Absorbance2	Absorbance3	Average	%IC
0.5	0.119	0.119	0.121	0.12	50.66
0.25	0.128	0.127	0.129	0.128	47.37
0.0125	0.133	0.135	0.134	0.134	44.9
0.0625	0.158	0.159	0.159	0.1587	34.75
0.03125	0.165	0.164	0.165	0.165	32.16

Key: %IC: Percentage Inhibition of Chloroform

Table 6. Percentage inhibition of Hexane fraction.

Conc(mg/cm^3)	Absorbance1	Absorbance2	Absorbance3	Average	%IH
0.5	0.136	0.135	0.137	0.136	44.08
0.25	0.153	0.155	0.154	0.154	36.68
0.125	0.168	0.167	0.166	0.167	31.33
0.0625	0.173	0.172	0.174	0.173	28.87
0.03125	0.182	0.181	0.181	0.181	25.58

Key: %IH: Percentage Inhibition of Hexane



Appendix A: A graph of % Inhibition of Hexane, Chloroform, Ethyl acetate fractions and standard Ascorbic acid of DPPH radical versus concentrations (mg/cm^3).

Keywords:

% I H = Percentage inhibition of Hexan
% I = Percentage inhibition
% I AA = Percentage inhibition ofAscorbic standard
% I EA = Percentage inhibition of Ethyl acetate
IC = Inhibition Concentration
%IC = Percentage inhibition of Chloroform

Ethyl acetate extract seed of *Trigonella foenum-graecum* showed higher antioxidant activity at a lower concentration compared to chloroform in figure above. Chloroform extract showed 0.4816 mg/cm^3 while ethyl acetate of *Trigonella foenum-graecum* seed revealed high inhibition at lower concentration of 0.1520 mg/cm^3.

CONCLUSION

The phytochemical investigation showed the presence of vital natural products. The fractions were evaluated for its antioxidant potentials and was observed that ethyl acetate fraction contains considerably high amount of antioxidants. This indicates that Fenugreek is a significant source of natural antioxidant.

Competing Interests: The authors declare that there are no competing interests.

REFERENCES

Akbari, S., Abdurahman, N.H., Yunus, R.M., Alara, O.R. & Abayomi. O.O (2019). Extraction, characterization and antioxidant activity of fenugreek (*Trigonella-Foenum Graecum*) seed oil. *Materials Science for Energy Technologies*. 2: 349-355.

Alara, O.R., Abdurahman, N.H., Ukaegbu., & Abdulmudalip, S. K. (2017). Effect of drying methods in the free radicals scavenging activity of *Vernonia amgdalina* in Malaysia. *J King Saud Univ-Sci*.

Amr Amin, AyshaAlkaabi, Shamaa Al-Falasi, Sayel, & Daoud, A. (2005). "Chemopreventive activities of T. foenum-graecum (Fenugreek) against breast cancer *Emirates" Cell Biology International*. 29, pp.687-694

Basch, E., Ulbricht, C., Kuo, G., Szapary, P. & Smith M. (2003). Therapeutic applications of fenugreek. *Alter Medi Rev*;8(1):20-27.

Benzidia, B., Barbouchi, M., Hammouch, H., Belahbib, N., Zouarhi, M., Erramli, H., Ait Daoud, N., Badrane, N. & Hajjaji, N. (2018). Chemical composition and antioxidant activity of tannins extract from green rind of *Aloe vera* (L.) Burm. F., *J. King Saud Univ.* - Sci, https://doi.org/10.1016/j.jksus.2018.05.022.

Colombo, M.L. & Bosiosio. E. (1996). "Pharmacological Activities of *Chelidonium majus*" L (Papaveraceae). *Pharmacological Resource*. 33: 127-134

Crow, J. M. (2008): Cancer drugs for the next decade unveiled. *Chemistry World*, 32 (12): 21.

Flores, G. R., Martínez, H. H., Tamez, P. G., Guerra, R. T., Licea, R. Q. & Ramiro. (2010). Antitumor and immunomodulating potential of *Coriandrum sativum*, *Piper nigrum* and *Cinnamomum zeylanicum*. *Journal of Natural Products (India)*, 3: 54-61.

Harbourne, J. B. (1988): Phytochemical Methods, A guide to Modern Techniques of Plant Analysis", *2nd ed, New York, Chapman and Hall*, 4-193.

Jagessar, M. R.C. & Gomes A. G. (2008): An evaluation of the antibacterial and antifungal activity of leaf extracts of *Momordica Charantia* against *Candida albicans*, *Staphylococcus aureus* and *Escheria coli*. *Nature and Science*, 6 (1): 1-14

Kukic, J., Popovic, V., Petrovic, S., Mucaji, P., Ciric, A., Stojkovic, D. & Sokovic, M. (2008). Antioxidant and antimicrobial activity of *Cnaracar dunculu s*ex tracts. *Food Chemistry*, 107 (2): 861-868.

Kumar, N., Ahmad, A.H., Singh, S.P., Pant, D., Prasad, A. & Rastogi, S.K. (2021). Phytochemical analysis and antioxidant activity of *Trigonella foenum-graecum* seeds. *Journal of Pharmacognosy and Phytochemistry*; 10(1): 23-26

Manga, H.M., Haddad, M., Pieters, L., Penge, A. & Leclercq, J.Q. (2006): Anti-inflammatory compounds from leaves and root bark of *Alchornea cordifolia. Journal of Ethnopharmacology,* 115 (1): 25-29

Megha, D., Anissa, M., Balkrishna, U., & Rohini K. (2012). Effect of *Trigonella foenum-graecum* (Fenugreek/ Methi) on Hemoglobin Levels in Females of Child Bearing Age. *Biomedical Research*; 23(1):47-50.

Moniruzzaman, Shahinuzzaman, Haque, A., Khatun, R. & Yaakob, Z. (2015). Gas chromatography mass spectrometry analysis and *in vitro* antibacterial activity of essential oil from *Trigonella foenum-graecum* S. *Asian Pacific Journal of Tropical Biomedicine*. http://dx.doi.org/10.1016/j.apjtb.2015.09.010

Nwauzoma, A.B., Chondhary, M.I., Ali, Z. & Olaiya, O,C. (2013). Biological activity of crude extracts of citrus species from Nigeria. *Nature and Science*: 11(7): 135-139.

Nile, H. S. & Khobragade, C.N. (2010): Antioxidant activity and flavonoid derivatives of *Plumbago zeylanica. Journal of Natural Products*, 3 (16): 130-133.

Okwute, S.K. (1989). Unusual sources of antibacterial substances. A survey of some Nigerian plants. *Nigeria Defence Academy journal*. 1:133-141.

Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y. & Bao J. (2018). Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. *Food Chem*; 240:212 -221

Pawar, V.S. & Hagar, S. (2012). Adaptogenic activity of *Trigonella foenum-gracum* (Linn) seeds in rodents exposed to anoxia and immobilization stress. *Asian Pacific Journal of Tropical Biomedicine.* 2(1); S208-S211

Prasad, N. R., Viswanathan, S., Devi, R. J., Nayak, V., Swetha, C.V., Archana, R.B., Rajkumar, P, J. & Rajkumar, J. (2008): Preliminary Phytochemical screening and antimicrobial activity of *Samanea saman. Journal of Medicinal Plants Research*, 2 (10): 268-270

Pundarikakshudu, K., Shah, D.H., Panchal, A.H., & Bhavsar, G.C.(2016). Anti-inflammatory Activity of Fenugreek (*Trigonellafoenum-graecum* Linn) Seed Petroleum Ether Extract. *Indian Journal of Pharmacology*; 48(4):441-444.

Renuka C. (2009). Evaluation of the antidiabetic effect of *Trigonellafoenum-graecum* seed powder on alloxan induced diabetic albino rats, *International Journal of Pharmaceutical and Technical Research.*;1:4:1580-1584.

Szymanska, R., Pospisil, P. & Kruk, J. (2016). Plant derived antioxidants in disease prevention. *Oxid Medi Cell Longe* ID: 1920208.

Woldemichael M.G., Wachter G., Singh P. M., Maiese M., William, T. & Barbara, N. (2003). Antibacterial Diterpenes from *Calceolaria pinifolia. J. Nat. Prod.*, 66 (2): 242-246.

Yadav, R., Kaushik, R., & Gupta, D. (2011). "The health benefit of *Trigonella foenum graecum*: *A Review" International Journal of Engineering Research and Applications (IJERA)*. 1, pp.032-035.

Zameer, S., Najmi, A. K., Vohora, D. & Akhtar M. (2017). A review on therapeutic potentials of *Trigonella foenum graecum* (fenugreek) and its chemical constituents in neurological disorders: Complementary roles to its hypolipidemic, hypoglycemic, and antioxidant potential. Nutr Neurosci;21(8):539-545.

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