

PROXIMATE COMPOSITION AND DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACTS OF FENUGREEK (*Trigonella foenum-graecum* L.) POWDER AND OIL

ABSTRACT

Seeds of *Trigonella foenum-graecum* L. (Fenugreek) were blended to powder and oil was extracted by soxhlet extraction method. Proximate composition of both powder and oil was then carried out to determine the profile. DPPH radical scavenging activity of the powder, oil and the standard [ascorbic acid](#) were also evaluated and their IC₅₀ established. Obtained results of the proximate analysis showed the following composition for powder: moisture content 3.54±0.42%, ash content 3.37±0.08%, protein 19.3±0.35%, fat 4.14±0.11% and crude fiber 7.05±2.24%. The oil extract contains moisture content 9.70±0.41%, ash content 3.80±0.61%, protein 22.3±0.71%, fat 7.20±0.33% and crude fiber 8.16±0.64%. The DPPH radical scavenging activity revealed an IC₅₀ value of 177.83 µg/ml for the powder and 133.66 µg/ml for the oil, while that of the standard, ascorbic acid, was found to be 89.13 µg/ml. Since a lower IC₅₀ value translates to more potency of the extracts to scavenge free radicals, it then means that with reference to the standard used, the oil is more potent than the powder since it has a lower IC₅₀ value. These results confirm the cardio-protective property of Fenugreek oil which could be due to its high radical scavenging and antioxidant activities.

Key-words: Fenugreek, DPPH, Radical scavenger, IC₅₀

INTRODUCTION

Trigonella foenum-graecum L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. Around 260 species of *Trigonella* are diffused worldwide [1]. The genus name *Trigonella* means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name *foenum-graecum* means 'Greek hay' [2]. It is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family ~~Fabaceae~~ Fabaceae. Fenugreek (*Trigonella foenum-graecum*) is a plant from the family of Leguminosae that grows annually and is widely cultivated in Mediterranean countries and Asia. The seeds have horny and relatively large layer of white and semi-transparent endosperm encircling central hard, yellow embryo [3].

Many wonderful functional and medicinal values of fenugreek are attributed to its chemical composition (20-25% proteins, 45-50% dietary fiber, 20-25% mucilaginous soluble fiber, 6-8% fixed fatty acids and essential oils, and 2-5% steroidal saponins). Moreover, some minor components such as alkaloids (trigonelline, cholin, gentianine, carpaine, etc), free unnatural amino acids (4-hydroxyisoleucine), and individual spirostanols and furastanol like diosgenin,

gitogenin and yamogenin have also been identified and determined as the main components [responsible](#) for its various biological effects [4].

Regarding the composition of fenugreek seeds, husk and cotyledons, it has been reported that endosperm has the highest saponin (4.63 g/100 g) and protein (43.8 g/100 g) contents, whereas husk has higher total polyphenols (103.8 mg of Gallic acid equivalent/g, and total dietary fiber (TDF) (77.1 g/100 g) [contents](#), comprising insoluble dietary fiber (IDF) (31.9 g/100g) and soluble dietary fiber (SDF) (45.2 g/100 g). It is observed that 200 microgram concentrations, extracts of husk, fenugreek seed, and endosperm showed 72%, 64%, and 54% antioxidant activity, respectively, by free radical scavenging method [5]. It has been shown that fenugreek has anti-diabetic, anticancer, hypocholesterolemic, anti-inflammatory, antioxidant, and chemopreventive activity due to its useful chemical constituents [5].

Specific objectives of this research were to:

- Carry out methanol extraction of fenugreek oil from its powder using the soxhlet extraction method
- -Carry out proximate analysis of fenugreek powder and oil extracts using standard procedures
- Determine the free radical scavenging potential of the above extracts
- -Compare IC₅₀ of both extracts relative to the standard used.

MATERIALS AND METHODS

MATERIALS

Methanol, DPPH (2,2-Diphenyl-1-picrylhydrazyl), [n-h](#)Hexane (Sigma Germany), [a](#)Ascorbic acid, [b](#)Beaker, [t](#)Test tubes, [a](#)Aluminum foil paper, [r](#)Reagent bottles, [s](#)Spatula, [j](#)Jar, [s](#)Syringe and needles, [r](#)Refrigerator (thermocool T- 200), [r](#)Rotary evaporator, [a](#)Analytical balance (Mettler Toledo), [b](#)Blender (Crown star 300w), [s](#)Spectrophotometer Jen way 6405 UV/Vis).

Formatted: Font: Italic

SAMPLE COLLECTION AND HANDLING

The seeds of mature Fenugreek (*T. [figonella](#) foecum greacum*) plant were collected from a local market in Lokoja, Kogi state, Nigeria. The seeds were transported back to Biochemistry laboratory facility in Kogi state university, Anyigba, where it was kept in the oven for 24 hours at a temperature of [60 °C](#) before the extraction process.

METHODS

EXTRACTION OF FENUGREEK OIL

Fenugreek seeds were blended to obtain powder before the oil extraction process. 350_g of the finely ground powder was weighed for oil extraction. Soxhlet extraction of ground fenugreek seeds was conducted by using the method of *Aliyu et al.* [6], with some modifications. Extraction was done with 500_ml of *n*-hexane (60 – 90 °C) at 37 °C for 24_hrs using a Soxhlet extractor. After extraction, the solvent was evaporated with a rotary evaporator at 40 °C. The seed oil was transferred to brown glass vials, flushed with nitrogen, sealed, and stored in the refrigerator until use [6].

Formatted: Font: Italic, Highlight

Formatted: Font: Italic

Proximate composition

The percentage of ~~of moisture content~~, ash ~~content~~, protein, fat ~~content~~ and crude fiber contents of fenugreek powder and oil extracts were evaluated using standard procedures of the Association of Official Analytical Chemists [7].

Determination of DPPH radical scavenging activity

The hydrogen or radical scavenging property of the extracts was determined using the stable radical (DPPH) 2,2-diphenyl-1-picrylhydrazyl hydrate according to the method of *Blois et al.* [8], and as described by *Brace et al.* [9]. One ml of different concentrations (500, 250, 125, 62.5, and 31.25) µg/ml of the extracts or standard was added to one ml of 0.3mMol of DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured at 517_nm against a DPPH control containing only one ml of methanol in the place of the extract. The antioxidant activity, AA, was then calculated using the formula.

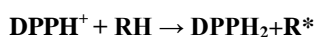
Formatted: Font: Italic

Formatted: Font: Italic

$$AA = \frac{A_o - A_c}{A_o} \times 100$$

Where A_o = Absorbance without extract and A_c = Absorbance with extract

When DPPH react with an antioxidant compound which can donate hydrogen, it is reduced as indicated by the following equation:-



The change in color from deep violet to light yellow was measured at 517_nm on a spectrophotometer.

Determination of IC₅₀

IC₅₀ is the ~~inhibition concentration at 50 percent, it is the~~ effective concentration of sample required to scavenge DPPH radicals by 50%. The IC₅₀ was determined from a scatter plot of the percentage inhibition on y-axis and logarithmic value of concentration on x-axis. Using the equation of a straight line graph, $y = mx + c$, the IC₅₀ was then calculated.

Statistical analysis

The results of this investigation are presented as mean \pm standard deviation (SD) of three replicate measurements. Statistical analyses among treatments were determined at the significance level of $p < 0.05$.

Formatted: Font: Italic

Formatted: Line spacing: Multiple 1.15 li

RESULTS AND DISCUSSION

Table 1: Proximate composition of Fenugreek oil and powder extracts

Parameters	Fenugreek powder (%)	Fenugreek oil (%)
Moisture content	3.54 \pm 0.42	9.70 \pm 0.41
Ash content	3.37 \pm 0.08	3.80 \pm 0.61
Protein	19.3 \pm 0.35	22.3 \pm 0.71
Fat	4.14 \pm 0.11	7.20 \pm 0.33
Crude fiber	7.05 \pm 2.24	8.16 \pm 0.64

Fenugreek (*Trigonella T. foenum-graecum*) is well known for imparting flavor to several traditional foods. It also provides tremendous amounts of active ingredients for health promotion and disease prevention.

Obtained results of the proximate analysis of fenugreek powder extract showed a low moisture content, as shown in Table 1, which explains the long shelf-life of fenugreek seeds. Values are significantly consistent with those in literature, like that of Mahfouz *et al.* [10] which was reported similaras; moisture content (8.30%), ash content (2.92%), crude protein (24.60%), crude fat (6.11%), crude fiber (7.72%) and nitrogen free extract (50.35%) for fenugreek seed powder,

Formatted: Font: Italic

and [Yaser et al. \[11\]](#), also found that fenugreek seeds contain 6.57% moisture, 4.03% total ash, 26.78% proteins, 6.35% lipids, 6.75% crude fiber and 49.52% carbohydrates.

Table 2: DPPH radical scavenging activity and IC₅₀ of fenugreek powder extract.

Concentration (µg/ml)	Log concentration	% Scavenging activity	IC ₅₀ (µg/ml)
500	2.6990	68.17	
250	2.3979	49.45	
125	2.096	39.10	177.83 ^a
62.5	1.7959	38.37	
31.25	1.4949	36.07	

Linear equation for a = $y = 25.09x - 6.464$, $R^2 = 0.800$

Table 3: DPPH radical scavenging activity and IC₅₀ of fenugreek oil extract.

Concentration (µg/ml)	Log concentration	% Scavenging activity	IC ₅₀ (µg/ml)
500	2.6990	69.52	
250	2.399	56.98	
125	2.096	43.91	133.66 ^b
62.5	1.7959	41.14	
31.25	1.4949	34.34	

Linear equation for b = $y = 28.63x - 10.87$, $R^2 = 0.943$

Table 4: DPPH radical scavenging activity and IC₅₀ of Ascorbic acid (standard).

Concentration (µg/ml)	Log concentration	% scavenging activity	IC ₅₀ (µg/ml)
500	2.699	73.52	
250	2.3979	64.31	
125	2.096	50.0	89.13 ^c
62.5	1.7959	45.24	
31.25	1.4949	38.40	

Linear equation for $c = y = 29.66x - 7.904$, $R^2 = 0.970$

The extent of decrease in absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. These scavenging activities are usually due to the presence of different phenolic contents [12]. DPPH is scavenged by antioxidants where the DPPH is reduced (decolorized). The values for the percentages of DPPH scavenging activity of fenugreek powder and oil extracts and that of the standard (ascorbic acid) are shown above in tables 2, 3 and 4, respectively.

The IC₅₀ values in µg/ml were determined by plotting the % scavenging activity against the log concentration of the extracts. This is done to determine the concentration of the extract that can scavenge DPPH radicals activity by 50%. Lower IC₅₀ values indicate greater scavenging activities of the extracts. From the tables above, fenugreek powder extract had an IC₅₀ of 177.83 µg/ml, the oil extract had an IC₅₀ of 133.66 µg/ml, while that of the standard ascorbic acid was gotten to be 89.13 µg/ml. Since a lower IC₅₀ value indicates greater potency, the standard (ascorbic acid), in this finding, had the lowest IC₅₀ value indicating that it has the highest antioxidant capacity when compared with fenugreek powder and oil extracts. This is understandable as ascorbic acid is a powerful antioxidant capable of terminating free radical conditions. When comparing both extracts, the fenugreek oil showed a greater scavenging potency than the powder, since its IC₅₀ value was gotten to be lower than that of the powder.

This result suggests reveals a potential the cardio-protective properties of fenugreek oil in combating cardiovascular diseases which have been recorded to have the highest mortality rate in recent times. The obtained result is also in agreement with those of Sharma *et al.* [13] and Perumal *et al.* [14] who reported that fenugreek powder and oil contain high antioxidants which

Formatted: Font: Italic

Formatted: Font: Italic

are very good reducing agents, hence they are capable of scavenging free radicals effectively. Results obtained have been able to meet specific objectives of this research and reconfirmed existing reports on the subject matter.

The antioxidant property of fenugreek has been attributed to the presence of many active phytochemicals, including flavonoids, plant sterols, vitamins, coumarins, terpenoids, carotenoids, curcumins, lignin, and saponin [15]. However, the phenolic compounds has the highest contribution to this effect. It has been reported that there is a significant correlation between the polyphenolic components present in fenugreek and its antioxidant activity [16], which has been reported to range from 127.8 to 139.2 mg GAE/100 g in different varieties of fenugreek [17]. Similarly, flavonoids are a diverse group of polyphenolic components with exceptional strength to act as free radical scavenger, anti-inflammatory and antibacterial agent [18].

CONCLUSION

Free radicals have been shown to induce oxidative stress and are implicated in a wide variety of diseases. Various experimental and clinical trials have shown that plants with antioxidant activity can combat pathologic conditions especially for the treatment and prevention of life-threatening diseases, such as diabetes, cancer and gastrointestinal disorders [19]. Medicinal plants, because they contain antioxidant compounds, bioactive compounds, phenols, flavonoids, and anthocyanins, have been shown to counteract these conditions and are capable of providing drug supply in complementary medicine [20]. From this study, fenugreek was found to possess rich antioxidant activity, making it a potent ingredient for herbal drug formulation in order to prevent oxidative stress and other free radical-induced diseases like cardiovascular and neurodegenerative diseases.

REFERENCES

1. Acharya, S.N., Thomas, J. E and Basu, S. K. (2006). Fenugreek: an “old world” crop for the “new world”. *Biodiversity* 7(3-4):27-30.
2. Petropoulos, G. A. (2002). Fenugreek: The genus *Trigonella*. Medicinal and Aromatic Plants- Industrial Profiles, Taylor and Francis, London.
3. Basch, E., Ulbricht, C., Kuo, G., Szapary, P and Smith, M. (2003). Therapeutic applications of fenugreek. *Altern Med Rev.*; 8, 20
4. Trivedi, P. D., Pundarikakshudu, K., Rathnam, S and Shah, K. S. (2007). A validated quantitative thin-layer chromatographic method for estimation of diosgenin in various plant samples, extract and market formulation. *Journal of AOAC International* 90(2): 358 - 363.

5. Madhava, N. M., Shyamala, B. N., Pura, N. J., Sulochanamma, G and Srinivas, P. (2011). Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. *LWT Food Sci Tech*; **44**(2): 451 - 456.
6. Aliyu, A. B., Ibrahim, M. A., Musa, A. M., Bulus, T and Oyewole, A. O. (2011). Phenolic Content and antioxidant capacity of extracts and fractions of *Vernonia blumeoides* (Asteraceae). *International Journal of Biology and Chemistry*. (DOI:10.3923/ijbc.2011).
7. AOAC. (2000). Official Methods of Analysis. 17th Edition. The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24,992.16.
8. Blois, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*. 181, 1199-1200.
9. Brace, J. L., Van der Weele, D. J and Rudin, C. M. (2005). Svfl inhibits reactive oxygen species generation and promotes survival under conditions of oxidative stress in *Saccharomyces cerevisiae*. *Yeast*, 22(8), 641-652.
10. Mahfouz, S. A., Elaby, S. M and Hassouna, H. Z. (2012). Effects of some legumes on hypercholesterolemia in rats. *J. Am. Sci.* **8**:1453 - 1460.
11. Yaser, A. J., Muneer, A., Abdelhafid, B., Daoudi, C. S and Hammadi, L. (2013). Chemical and phytochemical analysis of some antidiabetic plants in Yemen. *Int. Res. J. Pharm.* **4**: 72 -76.
12. Hurrell, R. (2003). Influence of vegetable protein source on trace element and mineral bioavailability. *Journal of Nutrition*. **133**(9); 2973 – 2977.
13. Sharma, R. D., Sarkar, A., Hezra, D. K. (1996). Use of fenugreek seed powder in the management of non-insulin dependent diabetes mellitus. *Nutr Res.* **16**: 1331 - 1339.
14. Perumal, S and Klaus, B. (2003). *J. Agric. Food Chem.*,51, 2144.
15. Dua, A., Vats, S., Singh, V and Mahajan, R. (2013). Protection of biomolecules against *in vitro* oxidative damage by the antioxidants from methanolic extract of *Trigonella foenum-graecum* seeds. *Int J. Pharm. Sci. Res.* **4**: 3080 - 3086.
16. Naidu, M. M., Khanum, H., Sulochanamma, G., Sowbhagya, H. B., Hebbar, U. H., Prakash, M and Srinivas, P. (2012). Effect of drying methods on the quality characteristics of fenugreek (*Trigonella foenum-graecum*) greens. *Drying Tech.* **30**: 808 - 816.
17. Ali, A., Waly, M. I., Bhatt, N and N.A. Al-Saady, N. A. (2015). Proximate and phytochemical composition and antioxidant properties of indigenous landraces of omani fenugreek seeds. *Afr. J. Tradit. Complement. Altern. Med.* **12**:149-154.
18. Premanath, R., Sudisha, J., Lakshmi, N., Devi and Aradhya, S. (2011). Antibacterial and antioxidant activities of fenugreek (*Trigonella foenum-graecum* L.) leaves. *Res. J. Med. Plants* **5**:695 - 705.
19. Hosseini-asl, K and Rafieian-kopaei, M. (2002). Can patients with active duodenal ulcer fast Ramadan. *Am J Gastroenterol.* **97**: 2471-2472.

20. Ghasemi Pirbalouti, A., Momeni, M and Bahmani, M. (2013). Ethnobotanical study of medicinal plants used by Kurd tribe in Dehloran and Abdanan Districts, Ilam province, Iran. *Afr J Tradit Complement Altern Med.* **10**:368-385.

UNDER PEER REVIEW