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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} value. These results has potential of conferring protective property of Fenugreek oil against certain diseases which could be due to its high radical scavenging and antioxidant activities.

Key words: Fenugreek, DPPH Radical scavenger, Nutritional value, Ascorbic acid

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 in Acharya *et al*,[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' by Petropoulos[2]. It is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Fabaceae. Fenugreek (*T. 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 as found out by Basch *et al*,[3].

Many wonderful functional and medicinal values of fenugreek like hypocholesterolemic, antibacterial and hepatoprotective effects 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 (trigonolline, 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 component for its various biological effects by Trivedi *et al,*[4].

It has been shown that fenugreek has anti-diabetic, anticancer, hypocholesterolemic, anti-inflammatory, antioxidant and chemo preventive activity due to its useful chemical constituents as found by Madhava *et al.*[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 IC_{50} of both extracts relative to the standard used

MATERIALS AND METHODS

MATERIALS

Methanol, DPPH (2,2-Diphenyl-1-picrylhydrazyl), n-hexane (Sigma Germany), ascorbic acid, beaker, test tubes, aluminum foil paper, reagent bottles, spatula, jar, syringe and needles, refrigerator (thermocool T- 200), rotary evaporator, analytical balance (Mettler Toledo), Blender (Crown star 300w), spectrophotometer Jen way 6405 uv/vis).

SAMPLE COLLECTION AND HANDLING

The seeds of mature Fenugreek (*T. 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 ^{o}C before the extraction process.

METHODS

EXTRACTION OF FENUGREEK OIL

Fenugreek seeds were blended to obtain powder before the oil extraction process. 350g 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 ^{o}C) at 37 ^{o}C for 24 hrs using a Soxhlet extractor. After extraction, the solvent was evaporated with a rotary evaporator at 40 ^{o}C. The seed oil was transferred to brown glass vials, flushed with nitrogen, sealed, and stored in the refrigerator until use [6].

Proximate composition

The percentage of moisture, ash, protein, fat 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 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 0.3 mmol 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 517nm 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.

$$\mathbf{AA} = \frac{\boldsymbol{Ao - Ac}}{\boldsymbol{Ao}} \times \mathbf{100}$$

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:-

$$\mathbf{DPPH^{*} + RH \rightarrow DPPH_2 + R^{*}}$$

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

Determination of IC_{50}

IC_{50} is the effective concentration of sample required to scavenge DPPH radicals by 50%.

The IC_{50} 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 IC_{50} was then calculated.

Statistical analysis

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

Results and Discussion

Table 1: Proximate composition of Fenugreek oil and powder extracts

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

Fenugreek (*T. foenum-graecum*) is well known for imparting flavor to several traditional foods. It also provides tremendous amount 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 reported similar 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, 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 IC_{50} 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	

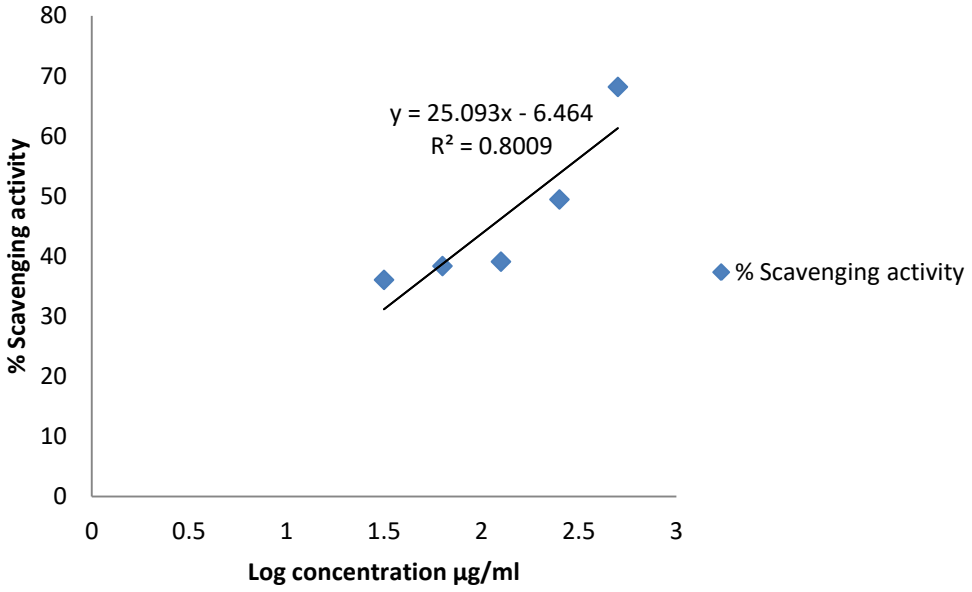


Fig. 1: IC_{50} correlation of Fenugreek powder

Table 3: DPPH radical scavenging activity and IC_{50} 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	

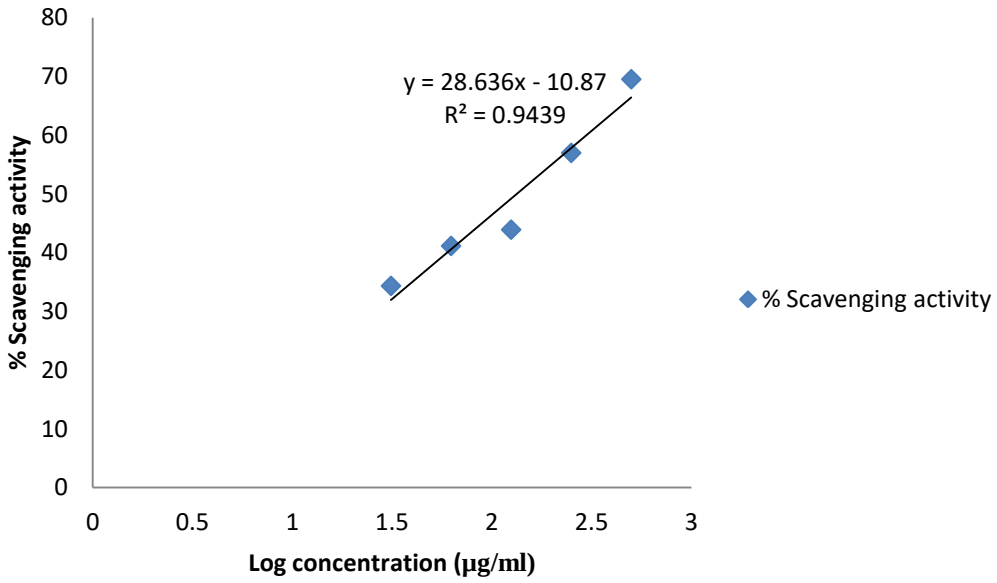
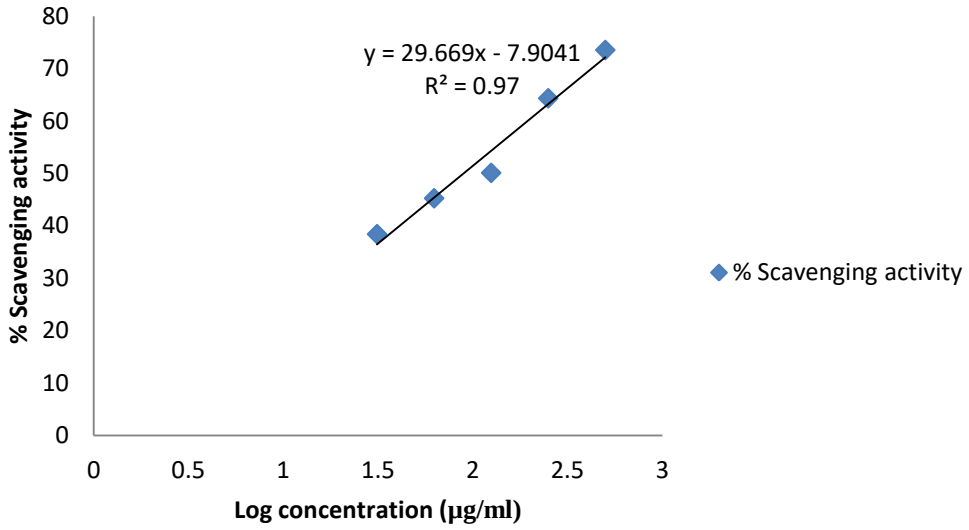


Fig. 2: IC_{50} correlation of fenugreek oil

Table 4: DPPH radical scavenging activity and IC_{50} 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	



Fig, 3: IC_{50} chart for standard ascorbic acid

The extent of decrease in absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. According to Hurrel 2003, 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 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 IC_{50} 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 by 50%. Lower IC_{50} values indicate greater scavenging activities of the extracts. From the tables above, fenugreek powder extract had an IC_{50} of 177.83 µg/ml, the oil extract had an IC_{50} of 133.66 µg/ml, while that of the standard ascorbic acid was gotten to be

89.13 µg/ml. Since a lower IC_{50} value indicates greater potency, the standard (ascorbic acid), in this finding, had the lowest IC_{50} 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 IC_{50} value was gotten to be lower than that of the powder.

This result suggests a potential 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 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, cumarins, terpenoids, carotenoids, curcumins, lignin, and saponin as agreed to by Dua *et al.*[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 as put forward by Naidu *et al.*[16], which has been reported to range from 127.8 to 139.2 mg GAE/100g in different varieties of fenugreek according to Ali *et al.*[17]. Similarly, flavonoids are a diverse group of polyphenolic components with exceptional strength to act as free radical scavenger, anti-inflammatory and antibacterial agent from the works of Premanath *et al.*[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 as shared by Hosseini-asl and Rafieian-Kopaei,[19]. Medicinal plants, because they contain antioxidant compounds, bioactive compounds, phenols, flavonoids, and anthocyanin, have been shown to counteract these conditions and are capable of providing drug supply in complementary medicine as evidenced in the research of Ghasemi Pirbalouti *et al*, [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.

Conflict of interest

Authors declare no conflict of interest.

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