Antibacterial Activities of Fenugreek Oil and Seed Extracts on Selected Pathogenic Bacteria and Proximate Composition of Fenugreek Seed

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Abstract: Fenugreek is an annually globally grown medicinal plant known for its various bioactive components, including antibacterial activities. It have been consumed medically for food as condiments. The proximate analysis of fenugreek seed showed that carbohydrates (37.79 %) had the highest value followed by fibre (22.48%), protein (20.76%), moisture (8.96%), fat (6.57%) and lowest value was recorded for ash (3.44%). The antibacterial activities of fenugreek oil and fenugreek seed extract (ethanol and methanol of 60 g/200 ml) was tested against pathogenic bacteria using open well diffusion and paper disc method at 10 µl, 20 µl, 40 µl, 50 µl and 100 µl concentration. The higher the concentrations, the more significant were the antibacterial activities of both fenugreek oil and seeds extracts. It was observed that the ethanolic extract was the most active against *Escherichia coli* with the highest zone of inhibition of 14 mm, followed by *Klebsiella pneumoniae* with 13 mm, while *Staphylococcus aureus* and *Pseudomonas aeruginosa* had 12 mm each zone of inhibition. *Streptococcus pyogenes* had the lowest zone of inhibition of 11 mm. The findings of this study revealed that fenugreek samples extracts were potent against the test bacteria.
Key word: Antibacterial, fenugreek, pathogenic bacteria, proximate composition.

INTRODUCTION

Fenugreek (*Trigonella foenum graecum)* is an annually grown crop of dicotyledon plant that belongs to the family of Fabaceae, it is capable of self-pollination by producing a golden yellow seeds, white flower, branched stem and trifoliate leaves are found growing in Africa, America, Australia, Europe and Asia as an oldest therapeutic crop (Benyagoub *et al.*, 2021). The seed is about 3-6 mm tall, 2 mm thick and 2-5 mm broad. In raw form the fenugreek seed had a bitter taste and maple flavour but when roasted the bitterness is reduced and its flavour is enhanced (Mona and Dina, 2014: Prabhat 2019). It is widely distributed across the globe, and globally consumed as food, served as food condiments, feed additive and also as herbal medicinal remedies (Benyagoub *et al.*, 2021).

Medicinal plant such as fenugreek had an extensive chemical diversity and were regarded as foundations for most of highly effective pharmaceutical synthetic drugs and the discovery of molecular compounds. The molecular compounds such as tannins, alkaloids, phenol, resins, fatty acids, flavonoids and steroids are usually the secondary metabolites capable of establishing body physiological actions (Mona and Dina, 2014; Mawahib *et al.*, 2015). Fenugreek seeds and leaves possess antibacterial, antidabetic, antihelminthic, antipyretic, antifungal and anti-inflammatory properties (Mona and Dina, 2014; Mawahib *et al.*, 2015).

Anciently the seed and leaf of fenugreek have been used to prepare an extracts and powder used medically (treatment of weakness and leg edema; labour and delivery; indigestion; baldness; hypoglyceamia; hyperlipidemia; cancer) and as nutrients (maize flour and wheat flour supplements including lactation stimulant) (Prabhat *et al.*, 2019). The fenugreek seeds are rich in mucilaginous fiber, proteinoues lysine, L-tryptophan, saponin, nicotinic acid, coumarin, phytic acid, sapogenin, calcium, scopoletin, iron, vitamins and carotene (Prabhat *et al.*, 2019). Therefore, this study focus on the antibacterial activities of the seed and oil of fenugreek on selected pathogenic bacteria and its proximate composition.

MATERIALS AND METHODS

The fenugreek (*Trigonella foenum-graecum*) seed and oil were purchased in Abeokuta, Ogun State, Nigeria. Fenugreek seeds extractions was carried out using modified method of Walli *et al.* (2015) and Sharma *et al.* (2016) of which distilled water (aqueous), ethanol and methanol was used as solvent. The fenugreek seeds was ground using mechanical grinding machine into powder form. Sixty grams of ground fenugreek seed was added to each solvent at 200 ml concentration in a sterile container and was kept at room temperature for 7 days. The solutions were sieved using Whatmann No.1 filter paper into clean universal bottles and stored in refrigerator at 4^0C. The proximate analysis of fenugreek seed was carried out using modified methods of Udochukwu *et al.* (2015).

***Estimation of crude fibre content in fenugreek seeds*:** Five grams of sample was weighed with analytical balance and transferred into volumetric flask. About 100 ml of 1.25% of sulphuric acid was measured and poured into the volumetric flask containing the sample. The sample mixture was boiled under reflux for 45 minutes and a sieve was used to trap the residue of the boiled sample. The trapped residue was washed in several portion of hot water and allowed to drain. The residue was transferred to the volumetric flask and boiled again with 100 ml of 1.25% sodium hydroxide solution for another 45 minutes under the same condition. Sieve was used to trap the residue of the boiled sample. The trapped residue was also washed in several portion of hot water and allowed to drain. The residue was transferred into a weighed crucible where it was transferred into an oven to obtain a constant weight at 105°C for 3 hours. The sample in the crucible was taken into a muffle furnace where it was burnt. The ash left was weighed and crude fibre was determined and was calculated as thus:

Crude fibre value = $\frac{W2-W3}{W1} \times 100$; W_1 (weight of sample used), W_2(weight of crucible + sample after boiling, washing and drying) and W_3(weight of crucible + ash).

***Estimation of fat content of fenugreek seed*:** About 1 gram of the sample was weighed into separating funnel. 20 ml of 96% ethanol was added into the funnel and shake gently. It was allowed cooling and 10 ml of concentrated sulphuric acid was added to obtain extract to which 20 ml of petroleum ether was added and shaked for proper mixing. For emulsion to separate well, 20 ml of ethanol and 20 ml of petroleum ether was added for better extraction. The separated fat extract was decanted and all the extracts was combined and evaporated to dryness and calculated as thus:

Fat Value = $\frac{W2-W1}{W} \times 100$; W(weight of sample used), W_1(weight of empty beaker) and W_2- Weight of Crucible + Ash

***Protein content of fenugreek seed*:** One gram of the sample was weighed into a Kjeldahl digestion flask and 15 g of potassium sulphate and 0.5 g of copper (II) sulphate pentahydrate were added followed by the addition of 30 ml of concentrated sulphuric acid was added. The sample was heated in a fume cupboard to digest at 50°C until frosting ceased and then boiled at 80°C until it is cleared. About 200 ml of distilled water and 25 ml of sodium thiosulphite was added and mix. Anti-bumps were added and 50% of 110 ml of sodium hydroxide was carefully added. The flask was connected to the distillation apparatus and boiled at 80^0C and about 150 ml of the distillate was collected. About 5 drops of methyl red indicator was added to the distillate and titrated with 0.1 M of hydrochloric acid. The readings were taken and calculated

Burrette Reading

Final burrette reading (V_2)

Initial burrette reading (V_1)

Volume of Titre

Calculation:

%Protein= $\frac{\text{Tire value x 0.0014x 6.25 (Jones Convertion Factor)}}{\text{Weight of Sample Used (g)}} \times 100$

***Moisture content present in fenugreek seed*:** Two cooled crucible were weighed and recorded and 1 gram of the sample was weighed in duplicate. The crucibles with its contents were transferred into a hot air oven set at 105^0C to dry for 3 hours. Using a pair of tongs, the crucible were transferred into a desiccator, allowed to cooled, weighed, recorded and calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Loss of weight and drying}}{\text{Weight of Sample Used (g)}} \times 100$$

***Estimation of ash content of fenugreek seed*:** One gram of the sample was weighed in duplicate into crucibles and recorded. The crucibles with its content were transferred into a muffle furnace set at 550℃ until fully ashed for 5 hours using a pair of tongs, the crucibles were transferred into a desiccator and allowed to cooled, weighed and recorded and calculated thus:

$$\% \text{ of Ash} = \frac{\text{Loss of weight on drying}}{\text{Weight of Sample Used (g)}} \times 100$$

The carbohydrate content was calculated as follows: 100- (Crude fibre+ Protein+ Ash+ Moisture content+ Fat).

Antibacterial activity of the extracts: The antibacterial activities of fenugreek oil and fenugreek seed extract was carried out using open well diffusion and paper disc modified method of Walli *et al.* (2015). Cultured plates were seeded with test organisms and allowed to solidify, punched with sterile cork borer at about 7.0 mm diameter to make a defined wells in the agar plates. The open wells were filled with 10 µl, 20 µl, 40 µl, 50 µl and 100 µl of fenugreek oil, ethanolic and methanolic extracts of fenugreek seeds. The plates were incubated in laboratory incubator at 37ºC overnight. In the paper disc method, sterile filter paper discs (7.0 mm diameter) were soaked in the ethanolic and methanolic extract of fenugreek seed and fenugreek oil at room temperature of 30- 40^0C for 30 minutes prior to introduction on the nutrient agar seeded with tests organisms done by streak plate methods. The prepared nutrient agar plates were seeded with each of the bacteria and the impregnated filter paper disc of fenugreek oil were placed on each well labeled plate. Gentamycin was used as control and all inoculated plates were incubated at 37°C overnight. The diameters of zone of inhibitions were measured using ruler (Walli *et al*., 2015).

RESULTS

Proximate composition of fenugreek seeds

Table 1 shows the proximate composition of fenugreek seeds with carbohydrate had the highest value, followed by crude fibre, crude protein, moisture content, fat content and ash content was the lowest value.

Antibacterial activities of fenugreek seed and oil extracts

Table 2 shows the antibacterial activities of ethanol and methanol extract of fenugreek seed and oil of which the highest zone of inhibition occurred at 40 µl while the use of 10 µl and 20 µl was insignificant. The ethanol extract of fenugreek seed had the exhibited highest inhibition on *Escherichia coli* (10 mm), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (9 mm each), *Klebsiella pneumoniae* (8 mm) and *Streptococcus pyogenes* (7 mm), followed by fenugreek oil on *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* (7 mm each) and methanol extract of fenugreek seed on *Staphylococcus aureus* and *Streptococcus pyogenes* at 7 mm each. Table 3 shows antibacterial activities of ethanol and methanol extract of fenugreek seed and oil at 50 µl concentration. The ethanol extract of fenugreek seed showed the highest zone of inhibition, followed by methanol extract of fenugreek seed then the fenugreek oil. *Staphylococcus aureus* recorded the highest zone of inhibition by ethanol extract of fenugreek seed (12 mm), followed by methanol extract of fenugreek seed and oil (11 mm each). *Streptococcus pyogenes* was inhibited by ethanol and methanol extract of fenugreek seed and zone of inhibition was 11 mm each and fenugreek oil had lowest zone of inhibition of 8 mm. *Klebsiella pneumoniae* showed zone of inhibition of 13 mm and 7 mm for ethanol and methanol extract of fenugreek seed with no detectable zone of inhibition with

fenugreek oil against this pathogen. *Escherichia coli* showed zone of inhibition of 14 mm which was higher than 10 mm (fenugreek oil), followed by 8 mm recorded in methanol extract of fenugreek seed. *Pseudomonas aeruginosa* showed the highest zone of inhibition with ethanol extract of fenugreek seed (12 mm), followed by fenugreek oil (11 mm) and methanol extract of fenugreek seed (8 mm) had the lowest zone of inhibition. The gentamycin was only effective against *Klebsiella pneumoniae.*

Table 1: Proximate composition of fenugreek seeds

Parameters	Proximate composition (g/100 g)
Crude fibre	22.48
Fat content	6.57
Crude protein	20.76
Moisture content	8.96
Ash content	3.44
Carbohydrate	37.79

Table 2: Antibacterial activity of ethanol and methanol fenugreek seed extract and oil at different concentration against some pathogenic bacteria

Organisms	Zone of Growth Inhibition (mm)									
	Ethanol extract			Methanol extract			Fenugreek Oil			Gentamycin
	10 µl	20 µl	40 µl	10 µl	20 µl	40 µl	10 µl	20 µl	40 µl	10 µg
Staphylococcus aureus	R	R	9	R	R	7	R	R	R	R
Streptococcus pyogenes	R	R	7	R	R	7	R	R	7	R
Klebsiella pneumoniae	R	R	8	R	R	R	R	R	R	10
Escherichia coli	R	R	10	R	R	R	R	R	7	R
Pseudomonas aeruginosa	R	R	9	R	R	R	R	R	7	R

Key: R = resistance

Table 3: Antibacterial activity of ethanol and methanol fenugreek seed extract and fenugreek oil at 50 µl concentration against some pathogenic bacteria.

	Zone of Growth Inhibition (mm)			
Organisms	Ethanol extract	Methanol extract	Fenugreek Oil	Gentamycin
Staphylococcus aureus	12	11	11	R
Streptococcus pyogenes	11	11	8	R
Klebsiella pneumoniae	13	7	R	10
Escherichia coli	14	8	10	R
Pseudomonas aeruginosa	12	8	11	R

Key: R = resistance

Table 4 showed that the ethanol extract of fenugreek seed had the highest zone of inhibition followed by methanol extract of fenugreek seed and oil at 100 µl concentration against pathogenic bacteria. *Staphylococcus aureus* showed the highest zone of inhibition with ethanol extract of fenugreek seed (21 mm), followed by fenugreek oil (18 mm) and the lowest zone of inhibition was with methanol extract (16 mm). *Streptococcus pyogenes* was inhibited by ethanol extract of fenugreek seed (19 mm) as highest zone of inhibition followed by methanol extract of fenugreek seed (17 mm) and lowest zone of inhibition was 15 mm recorded for fenugreek oil. *Klebsiella pneumoniae* was inhibited by ethanol extract of fenugreek seed (19 mm) as highest zone of inhibition followed by methanol extract of fenugreek seed (18 mm) and lowest zone of inhibition was with fenugreek oil (17 mm). *Escherichia coli* showed zone of inhibition of 20 mm with ethanol extract of fenugreek seed which was higher than 18 mm recorded for methanol extract of fenugreek seed followed by 17 mm recorded for fenugreek oil. *Pseudomonas aeruginosa* showed the highest zone of inhibition with ethanol and methanol extract of fenugreek seed (16 mm each), followed by fenugreek oil (15 mm) which was the lowest zone of inhibition.

Antibacterial activities of fenugreek seed and oil using paper disc techniques

Table 5 showed the zone of inhibition for ethanol and methanol extract of fenugreek seed (60 g/200 ml) and fenugreek oil. The highest zone of inhibition was recorded for ethanol extract of fenugreek seed, followed by fenugreek oil and methanol extract of fenugreek seed. The highest zone of inhibition of *Staphylococcus aureus* was with ethanol extract of fenugreek seed (11 mm) followed by methanol extract of fenugreek seed and fenugreek oil (10 mm each). Also 10 mm each was recorded as zones of growth inhibition of *Streptococcus pyogenes* by ethanol and methanol extract of fenugreek seed and fenugreek oil. *Klebsiella pneumoniae* showed highest zone of inhibition of 10 mm (ethanol extract of fenugreek seed) followed by 8 mm (methanol extract of fenugreek seed) and 7 mm (fenugreek oil). *Escherichia coli* had highest zone of growth inhibition with ethanol extract of fenugreek seed (11 mm) followed by fenugreek oil (10 mm) and methanol extract of fenugreek seed (9 mm). *Pseudomonas aeruginosa* showed 10 mm each of zones of inhibition with ethanol extract of fenugreek seed and fenugreek oil and 9 mm recorded for methanol extract of fenugreek seed as lowest zone of inhibition.

Table 4: Antibacterial activity of ethanol and methanol fenugreek seed extract and fenugreek oil at 100 µl concentration against some pathogenic bacteria

	Zone of Growth Inhibition (mm)			
Organisms	**Ethanol extract**	**Methanol extract**	**Fenugreek Oil**	**Gentamycin**
Staphylococcus aureus	21	16	18	R
Streptococcus pyogenes	19	17	15	R
Klebsiella pneumoniae	19	18	17	10
Escherichia coli	20	18	17	R
Pseudomonas aeruginosa	16	16	15	R

Key: R = resistance

Table 5: Antibacterial activities of fenugreek seed and oil using paper disc

	Zone of Growth Inhibition (mm)			
Organisms	**Ethanol extract**	**Methanol extract**	**Fenugreek Oil**	**Gentamycin**
Staphylococcus aureus	11	10	10	R
Streptococcus pyogenes	10	10	10	R
Klebsiella pneumoniae	10	8	7	10
Escherichia coli	11	9	10	R
Pseudomonas aeruginosa	10	9	10	R

Key: R = resistance

DISCUSSION

The proximate composition of fenugreek seeds revealed carbohydrate to be of highest value, followed by crude fibre, crude protein, moisture content, fat content and lowest value was ash content of which the values are smaller than that reported by Sajad and Pradyuman (2018). This study of antibacterial activity of fenugreek oil and fenugreek seed extracts showed that ethanolic extract had an inhibitory effect on the growth of *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa.* This could be due to antimicrobial active ingredients (phytochemicals) present in the fenugreek oil and fenugreek seed (Mona and Dina, 2014). Pathogenic bacterial strains exhibited significant response after exposure to ethanol and methanol extract of fenugreek seed which agrees with the work of Bassetti *et al*. (2018). In this study, the antibacterial activity of fenugreek seed extract was effective against *Staphylococcus aureus, Klebsiella pneumoniae* while lowest antibacterial activity of fenugreek oil was observed which agrees with Salah *et al.* (2010) in which the authors recorded for both extraction of ethanol and aqueous extract of fenugreek seed did not exhibit any effect on test bacterial species.

The ethanol extract of fenugreek seeds reported by Mona and Dina (2014) for *Staphylococcus aureus* (0 mm), lower than 9 mm, 12 mm and 21 mm at 40 µl, 50 µl and 100 µl concentration respectively recorded in this study. The methanol extract of fenugreek seed recorded by Mona and Dina (2014) for *Staphylococcus aureus* (28.8 mm) and *Escherichia coli* (29.7 mm) was higher than *Staphylococcus aureus* (7 mm, 11 mm and 17 mm) and *Escherichia coli* (7 mm, 8 mm and 18 mm) at 40 µl, 50 µl and 100 µl concentration was recorded respectively.

The maximum zone of inhibition by methanol extract of fenugreek seed against *Escherichia coli* (20 mm) and *Staphylococcus aureus* (19 mm) reported by Sharma *et al.*, (2016), was higher than *Escherichia coli* (8 mm) and *Staphylococcus aureus* (11 mm) recorded in this study. The zone of inhibition of *Escherichia coli* (10 mm) of methanolic extract of fenugreek seed reported by Alhan *et al.* (2017) was higher than 8 mm recorded in this study due to higher concentration of the extract. The paper disc diffusion method used to test for antibacterial activities of fenugreek seed extract against pathogenic bacteria in this study agrees with antibacterial activities reported by Aklavya *et al.* (2020).

CONCLUSION

The antibacterial activities of fenugreek oil and fenugreek seed extract against selected pathogenic bacteria was relatively significant. The open well diffusion method reveals higher zone of growth inhibition than paper disc method. Therefore fenugreek oil and fenugreek seed extracts possess antibacterial potential that could be developed into drug for use against diseases and infections caused by the test bacteria.

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