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PHYSICO-CHEMICAL, PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF BLACK CUMIN AND FENUGREEK SEED OILS

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Abstract - Plants have been used over the time as a basis of traditional cure for mankind. Plant oil components are effective against pathogenic bacteria. Lately, the utilization of phytomedicine has been increased enormously for treatment of numerous ailments because they are easily accessible and cheap. In this study, the physicochemical properties, phytochemicals constituents and antimicrobial activities of oil extracted from black cumin and Fenugreek were evaluated, also Soaps tablets were formulated using the extracted oil through cold saponification process and the activities of the soap against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Salmonella typhi* were investigated. The result of the Physicochemical analysis showed refractive indices of 1.4657 and 1.4730, specific gravity of 1.349 and 1.366 g/cm³, peroxide value of 7.03 and 10.06 meq O₂/kg, Saponification value (mgKOH/g) of 192.3 and 183.3, Free fatty acid (% oleic acid) of 7.24 and 12.4 and unsaponifiable matter of 1.2 and 1.6 % for black cumin and Fenugreek seed oil respectively. Bioactive constituents present in both seed oils were alkaloids, flavonoids, saponins, tannins, and phenolic compounds which are responsible their antibacterial activities. The results showed that soap prepared with Fenugreek seed oil have the highest activity against all bacterial strains tested in this study. However, black cumin oil is active against all strains of bacteria only at higher concentration (200mg/L).

Black cumin and Fenugreek seed oils are highly effective against skin bacteria. The oils can be used as natural antibacterial in industries for production soaps.

Keywords: Saponification, Zone of inhibition, Seed oils, Isolation

I. INTRODUCTION

The development of bio cosmetics products from natural sources is now being encouraged worldwide because it is estimated that only 15% out of the 300,000 herbal species have been explored for therapeutic purpose. Among several medicinal plants, *Nigella sativa* L. (*Ranunculaceae*) and Fenugreek seed (*foenumgraecum*) have been considered among the most treasured antioxidant-rich herb, various scientific studies are ongoing to prove the traditionally uses of these plant seeds [1, 2].

Black cumin (*Nigella sativa*) belongs to the Ranunculaceae family it is often called as “Haba al-barakah” meaning seeds of blessings. It has been used as a medicinal plant across the globe e.g for remediation of health in folklore medicine for treating various health disorders [3]. Black cumin seeds contain significant levels of iron, copper, zinc, phosphorus, calcium, thiamin, niacin, pyridoxine, and folic acid [2]. Research have shown that black cumin seed contain 26-34% fixed oil which are linoleic acid (64.6%) and palmitic acid (20.4%). The seed oil comprises 0.4%–2.5% essential oil [4,5]. According to Erkan *et al* 2008 [6] the plant seed have the potential of being used as natural sources of antioxidants as it contain flavonoids, tannins and phenolic acids etc, the seed oil also contains thymoquinone which is the major component of the essential oil and is the most bioactive compound with wide range of therapeutic benefits, it is effective against cardiovascular disorders, neurological and mental illness, , diabetes, and



infertility as well as various infectious diseases caused by fungal or bacterial, parasitic, and viral infections [7]. Black cumin seeds oil extracts have also been proven to possess antioxidant, anticancer, analgesic, anti-inflammatory and antimicrobial activities [8].

Fenugreek is a legume and it belongs to the plant family of Fabaceae. Fenugreek seeds are commonly used as spices worldwide, its leaves can be eaten as green vegetables in the daily diet. Fenugreek Leaves and seeds have been used extensively for medicinal purposes [9]. Fenugreek is reported to have anti-diabetic, anti-fertility, anticancer [10], antimicrobial, anti-parasitic and hypo cholesterolaemic effects [11]. The bioactive constituents of seeds of *Fenugreek* such as volatile oils, flavonoid, alkaloid, and phenol were reported to have been responsible for its antimicrobial activity [12]. Based on the studies carried out on fenugreek and black cumin, report shows that the seed oil of these plant possesses strong antibacterial activity [13]. However, few reports are available on the use of these seed oils as antibacterial agent in soap production. Hence, this present research was conducted out to determine the antibacterial activity of soap prepared with black cumin and fenugreek seed oils against the selected strains of bacteria.

II. MATERIALS AND METHODS

Black cumin and Fenugreek seeds were purchased from the market. 50g each of the seeds were weighed and placed at shaded area to prevent it from being exposed to sunlight in order to avoid evaporation of active constituent such as phenol, the seeds were allowed to dry at room temperature for 7 days. After 7 days the seeds were crushed into powdered form using the crusher. The powdered sample was put into a porous thimble and placed in a Soxhlet extractor and then extracted with n-hexane (GG-17, SHUNIU; with boiling point of 65°C) as extracting solvent for 6 hours repeatedly until required quantity was obtained. The solvent in extracted solution was removed using a water bath at 70°C and the extracted plant oil was stored in refrigerator at 4°C to prevent oil degradation prior further analysis. The oil yield was calculated using equation 1

$$\text{Oil yield (\%)} = \frac{V}{W} \times 100 = \frac{\text{Amount of oil extracted in mL}}{\text{Weight of dry sample used in gram}} \times 100 \quad (1)$$

A. Physicochemical Analyses of the Black Cumin oil samples.

The specific gravity and refractive index of the oils were determined as described by Pearson [14]. Peroxide value was determined as described by AOAC [15] using titration method. Saponification value, free fatty acid value, and percentage of unsaponifiable matter of the seed oils were determined according to AOCS [15].

B. Phytochemical Analysis

The phytochemical analysis was evaluated with different reagents as described by Bourgo et al. [16]; Mbatchou et al. [17] and Kazemi [7] to determine the existence or nonexistence of saponins, alkaloids, flavonoids, glycosides and tannins in seed oil extracts

C. Test for tannins

Five milliliter (5ml) of the oil extract was measured into a test tube and then 1ml of 5% ferric chloride solution was added, the mixture was allowed to react for a few seconds, a dark green color was observed indicating the presence of tannins.

D. Test for saponins

One milliliter (1ml) of oil extract was diluted with 5 ml of distilled water. The mixture was shaken in the test tube for 15 min the formation of stable foam confirmed the presence of saponins.

E. Test for flavonoids

Few drops of 10 percent Sodium hydroxide (10% NaOH) was added to 3 ml of the oil extract in a test tube, the mixture was warmed and 4 drops of dilute HCl was added to the mixture. An intense yellow color was formed which turned colorless on addition of few drops of dilute acid indicating the presence of flavonoids.

F. Test for alkaloids

Two milliliter (2ml) of oil extracts was warmed with 4.0ml of 2.0 % HCl for 3 minutes. The solution was filtered and few drops of Dragendorff's reagent were added to the acidic mixture. There was appearance of Orange red precipitate which indicated the presence of alkaloids.

G. Test for steroids

Salkowski test was used to determine the presence of steroids in the plant oil extracts. 2ml of chloroform was mixed with 2ml of concentrated sulphuric acid, then 8 ml of oil extract was added to the solution. The mixture was shaken for a few seconds and then a red color was observed at the interphase, at the chloroform layer; which confirmed the presence of steroids in the oil extracts.

H. Test for terpenoids

Two milliliter (2ml) of oil extracts was mixed with 3 ml of chloroform, 1ml of concentrated H₂SO₄ was carefully added to the mixture to form a layer. A reddish-brown coloration was formed at the interface this indicates the presence of terpenoids.

I. Test for Phenols

One milliliter (1ml) of the oil extract was measured into a test tube, few drops of 10.0% Lead acetate was added to the oil. White precipitate was formed which confirmed the presence of phenols.



J. Test for cyanogenic glycosides

Two milliliter (2ml) of distilled water was added to 1ml of the plant oil in a test tube, then 6 drops of chloroform were added and the mixture stirred. The test tube was stoppered with a cork containing a strip of picrate-impregnated paper hanging down from the stopper, and incubated at ambient temperature for 2 h. A color change of the paper was observed to confirm the presence of cyanogenic glycosides. No color change was observed after 48 hours which indicated that plant oils does not contain cyanogenic glycosides.

K. Soap Preparation

The soap was produced using cold saponification process. The plant seed oils were mixed with 100 grams of sodium hydroxide in 500 mL of distilled water. The alkaline solution was mixed with plant seed oils in the ratio 2:2v/v. The mixtures were then stirred vigorously until the reaction reach equilibrium i.e. until soaps were formed at room temperature. The mixtures were then poured into the mold and allowed to cure for 48 h.

Antibacterial Activity Screening

L. Test Organisms

The test organisms used for antibacterial activity of soap prepared from the plant oil were, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. The organisms were procured from the Federal University Gusau. Identified as described by Cheesbrough [18].

M. Maintenance of Bacterial Cultures

The bacterial cultures were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4 °C

N. Preparation of Inoculum

The suspension of each organism was prepared by picking a fresh and pure colony of the test organism from an overnight culture (18 hours of incubation at 37 °C) with a sterile wire loop and suspended in 10mL of nutrient agar to form a density of approximately 1.0×10^8 , until a moderate turbidity was developed. This was carried out by matching the suspension with 0.5 McFarland turbidity standards (NCCL) [17].

O. Determination of Antibacterial of the Formulated Soap

Antimicrobial activity screening for the antimicrobial activity of prepared soap were determined according Aqil and Ahmad [13] and Abdel-massih *et al* [19] using agar well diffusion method, which is generally used to determine the antibacterial activity of microbial extracts. The test bacterial isolates were inoculated on to a plate surface by using a sterile swab to spread microbial inoculum over the entire agar surface. Then, 6mm diameter hole was punched aseptically with a sterile cork borer, and the Soaps prepared with plant in three different concentrations (100 mg, 150 mg and 200 mg) were introduced into the well. Then, agar plates were allowed to stand for 30 min and then incubated at 37 °C for 24 h. The antibacterial activity

was determined by measuring the diameter of the zone of inhibition (in mm) and compared with the standard antibiotics (Chloramphenicol).

III. RESULTS AND DISCUSSION

Table 1. Physicochemical analysis of the seed oils

Parameter	Black cumin	Fenugreek
Acid value (mg/KOH/g)	12.971	6.413
Saponification value(mg/KOH/g)	192.290	183.321
Free fatty acid (% oleic)	7.244	12.432
Peroxide Value (meq/O ₂ /Kg)	7.03	10.06
Unsaponifiable matter (%)	1.2	1.6
Specific gravity (g/cm ³)	1.349	1.366
Refractive index	1.466	1.473
Percentage yield (%)	30.32	35.26

A. Physicochemical analysis of the seed oils

The result of the Physicochemical analysis showed refractive indices of 1.4657 and 1.4730, specific gravity of 1.349 and 1.366 g/cm³, peroxide value of 7.03 and 10.06 meq O₂/kg, Saponification value (mg KOH/g) of 192.3 and 183.3, Free fatty acid (% oleic acid) of 7.24 and 12.4 and unsaponifiable matter of 1.2 and 1.6 % for Black cumin and Fenugreek seed oils respectively. Refractive index is similar to those obtained by Sulieman *et al.* [2] for fenugreek seed oil. According to Sultan *et al.* [1], Refractive index and specific gravity gives a quantitative estimation of oxidative stability of fats and oils. The peroxide value obtained is comparable with those obtained by Cheikh-Rouhou *et al* [20]. Peroxide value gives information on rancidity of fat and oils. The value obtained showed that is not rancid. The saponification value and percentage of unsaponifiable is in concordance with values obtained by Atta [21]; Abbas *et al.* [22]; Sulieman *et al.* 2008 and Cheikh-Rouhou *et al.* [20].

Table 2. Phytochemical analysis

PHYTOCHEMICALS	Black cumin oil	Fenugreek oil
Tannins	+	+
Saponins	+	+



Flavonoids	+	+
Alkaloids	+	+
Steroids	+	+
Terpenoids	+	+
Phenols	+	+
Cyanogenic glycosides	-	-

B. Phytochemical analysis

Table 2 shows the results of phytochemical analysis of seed oils, the result indicates that fenugreek seed oil and black cumin seed oil consist of Tannins, flavonoids, alkaloids, terpenoids, saponins, and steroids. The presence of tannins is most expected to be accountable for the antioxidant and anti-inflammatory properties of the seed oils. Additional therapeutic properties could be due to the presence of alkaloids. Fenugreek seed oil and black cumin seed oil samples do not contain cyanogenic glycosides which make them safe to use. The result obtained is similar to those obtained by Mraihi *et al.* [23] and Ahmad *et al.* [24] for the same seed oil.

Table 3: Zone of inhibition of bacteria strains against soap formulated with Black cumin oil (*Nigella sativa*)

Soap Concentration	Zone of inhibition (mm)			
	150µL of each soap concentration used			
	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
100 mg/mL	-	17 mm	11 mm	-
150 mg/ mL	22 mm	21 mm	18 mm	12 mm
200 mg/ mL	26 mm	26 mm	25 mm	27 mm

C. Antibacterial activity

The results of the antibacterial activity of different concentrations (100 mg, 150 mg and 200 mg) of soaps prepared with Black cumin oil (*Nigella sativa*) examined against bacterial pathogens were shown in Table 3. At 100 mg/mL, antibacterial activity was only noticed in *salmonella typhi* and *Staphylococcus aureus*. No antibacterial activity against *Streptococcus pyogenes* and *Escherichia Coli*. At 150mg/ mL, zone of inhibition was recorded against all the test organisms. However, antibacterial activity was also observed against all bacteria pathogens studied at 200mg/mL and maximum zone of

inhibition was noticed against *Streptococcus pyogenes* (27 mm) followed by *Escherichia coli* and *Salmonella typhi* (26 mm) and then *Staphylococcus aureus* (25mm) which falls within the range of susceptible inhibition zone [27]. The zone observed was compared with standard antibiotic chloramphenicol (Chl).

Table 4: Zone of inhibition of bacteria strains against soap formulated with Fenugreek seed (*foenum graecum*)

Soap Concentration	Zone of inhibition (mm)			
	150µL of each soap concentration used			
	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
100 mg/mL	30 mm	14 mm	24 mm	10 mm
150 mg/ mL	35 mm	16 mm	33 mm	15 mm
200 mg/ mL	37 mm	20 mm	36 mm	18 mm

Table 4 shows antibacterial activity of different concentrations (100 mg, 150 mg and 200 mg) of soap prepared with Fenugreek seed (*foenum graecum*) against bacterial pathogens. Antibacterial activities were noticed against all the test organisms at all concentrations used in this study. The prepared soap displayed a higher inhibition zone against *E. coli* and *S. aureus*. Minimum zone of inhibition was noticed in *Streptococcus pyogenes* (10 mm), no zone of inhibition was seen in blind control. The result obtained is in concordance with those reported by Wagate *et al.* [25]; Hasan *et al.* [26] and Maryam *et al.* [27]. The result implies that the soap is more active against *Escherichia coli* and *S. aureus* than other test organism used in this study.

IV. CONCLUSION

Soaps prepared from Fenugeek seed oil and black cumin seed oil have been proven to be effective against all the bacteria strain used in this study due to the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds. Soap prepared with Fenugeek seed oil have the highest activity against all bacterial strains tested in this study. However, black cumin oil is active against all strains of bacteria only at higher concentration (200mg/L). Soaps prepared with plant seed oil are now being considered as an excellent alternative to eliminate the spread of virus and bacterial pathogens. This study recommends the incorporation of plant oil into soap production in order to stop the spread of microorganisms.



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