Physico-chemical, GC-Ms Spectrometry Analysis and Antimicrobial Activity of *Foeniculum vulgare* Seeds Oil

ABSTTRACT

The aim of this study is to evaluate the physicochemical properties, to investigate the chemical constituents of the Fixed Oil from *foeniculum vulgare seed*s and to evaluate its potential antibacterial activity against six microorganisms (*Bacillus subitus, Staphylococcus aureus, Escherichia coli, Salmonella, Penicillium* and *Aspergillus niger*). The chemical constituents of *foeniculum vulgare* seeds oil were identified and quantified by GC-MS technique, where disc diffusion assays were employed to evaluate the antibacterial activities and physicochemical properties using standard methods. Results from the physicochemical analysis showed that the seed oil was green in colour and liquid at room temperature with the acid, iodine, saponification, peroxide values and free fatty acidat (2.01± 0.01mgKOH/g, 78.36±0.1 gI_2/100 g, 15.42±0.02mgKOH/g, 18.2±0.01 meqH_2O_2, 5.2mg KOH /g) respectively. The relative density, specific gravity, viscosity and refractive index of the oil were at 0.8808±0.0001 (g/ml), 0.918±0.01, 35.14±0.1 and 1.471±0.1respectively. The GC-MS analysis revealed six components which had been identified and detected revealing the following major components 10-Nonadecanone (79.28%), Estragole (8.61%), 6-Octadecenoic acid methyl ester (7.63%). The oil showed partial activity against *Bacillus subtilis* and *Staphylococcus aureus*, inactive against *Escherichia coli*, *Salmonella* and *Aspergillus niger,* but more active against *Penicillium* as a potential source of natural antibacterial and justified its uses in folkloric medicines.

Keywords: Antimicrobial Activity,*Foeniculum vulgare*,*Bacillus subtilis*, GC-MS analysis.

INTRODUCTION

Fennel (*Foeniculum vulgare*) is herbaceous plant that grows annually with seeds that are helpful to humans (Hosseini et al, 2021). According to botanists, F. vulgare has two varieties, one is sweet fennel (F. Vulgare Var. Dulce), which is almanacs or biennials with small sweet-tasting fruits. The other is unpleasant fennel (F. vulgare var. vulgare), which is a returning with fruits having an unpleasant taste (Cosge et al., 2008; Miraldi, 1999). They are generally used as medicine, both as homemade remedies as well as in the medicinal industry (Li et al, 2004). They indicate that the *Foeniculum vulgare* seeds secrete certain yellowish or sometimes clear oil that is used in the manufacture of perfumery drinks through carminative and stimulant act (Malhotra, 2012). Recently, much attention has been focused on

Foeniculum vulgare due to the nutritional and health protective value of their seeds that are rich in vegetable and volatile oils (Roby, 2013). *Foeniculum vulgare* seeds are considered also as source of various health useful compounds including minerals, vitamins, and others which describe their applications for pharmaceutical, cosmetic, perfumery and food industries (Nassar et al., 2010). Traditionally *Foeniculum vulgare* is suggested for gastrointestinal and neurological disorder, kidney stones, vomiting and diarrhea. It also has antispasmodic, antiseptic, carminative and anti-ulcer properties. There are many nations around the world which deal with the *Foeniculum vulgare* as a beneficial material for the treatment of many diseases for example, Basilicata, Italy treats mouth ulcer by tender leaves, and digestive system by fruits; Ecuador treats cancer, conjunctivitis and gastritis by using leaf and flower (Pieroni and Cattero, 2019) .*Foeniculum vulgare* was known as an excellent source of natural antioxidants and contributes daily antioxidant diet (Shahat et al., 2011). The volatile oil exhibited strong antioxidant activity compared with hydroxyisoleum butyrate (BHA) and butylhydroxytoluene (BHT) (Singh, 2006). The Ethanolicaqueous extract of *Foeniculum vulgare* revealed that the antioxidants were lower in comparison with the essential oil (Diaz-Maroto et al., 2005).

The aim of this study is to extract the Fixed Oil from *Foeniculum vulgare* seeds and evaluate the physicochemical properties, to investigate the chemical constituents by GC-MS technique, and to evaluate its potential antibacterial activity.

MATERIALS AND METHODS

Plant material

Seeds sample (5 kg) of species, *Foeniculum vulgare* were purchased from the local market called Al-Anaqreeb in Omdurman, Sudan. The seeds sample was further identified and authenticated by the Medicinal and Aromatic Plants Research Institute and ground to powder using a grinder prior to oil extraction. All chemicals reagents used in the study were of analytical grade and used without further purification.

Oil Extraction

A quantity (250 g) of the dried milled sample was put into the thimble and the materials were continuously extracted for (6) hours using n-hexane (76°C -80°C) as solvent, and by the end of the extraction, the thimble was removed and the solvent was allowed to evaporate, the flask and the content were dried. The flask containing the oil was cooled in the desiccators, weighed and subjected to the drying process repeatedly until a constant weight was obtained.

Determination of physicochemical properties of the oil:

Determination of Specific Gravity and Refractive Index:

The tests of Specific Gravity and Refractive Index were determined by the manual methods of analysis food, (FSSAI, 2015).

Determination ofAcid Value

The Acid Value was determined by using the method described by Ronald (1991). Equal volumes (25 ml) of diethyl ether and ethanol were mixed together and 1 ml of 1% phenolphthalein indicator solution was added and was then neutralized with 0.1M potassium hydroxide solution. The oil sample (between 1 to 10 g) was dissolved in the neutralized solvent mixture and titrated with 0.1 M potassium hydroxide solution with constant shaking until a pink color which persists for (15) seconds is obtained. The Acid Value is given as:

$$\text{Acid Value} = \frac{\text{Titer value(ml)x5.61}}{\text{Weight of sample used (g)}}$$

Determination of Percentage Free Fatty Acids (FFA)

This was carried out using the method described by AOAC (1990). One gram of the oil sample was accurately weighed into a conical flask, followed by the adding 10 cm^3 of neutralized 95% ethanol and Phenolphthalein. This was then titrated with 0.1 M NaOH, with constant shaking until a pink color persisted for 30s. The percentage free fatty acid was calculated from the equation below:

$$\text{Free Fatty Acid (FFA)} = \frac{\text{V x M x 2.82}}{\text{Weight of oil (g)}}$$

Determination of Peroxide Value

One gram of the oil was weighed into a clean dry boiling tube, 1g of powdered potassium iodide and 10cm3 of the solvent mixture were added. The mixture was allowed to boil vigorously for 30 seconds. The tube was washed twice with 25cm3 portions of water and the washings were added to the titration flask. This was then titrated with 0.002M Sodium thiosulphate using starch indicator.

The relation for peroxide value is given as;

$$\text{Peroxide value} = \frac{\text{V x Molarity of titrant x 100(meq KOH/g)}}{\text{Weight of oil (g)}}$$

Determination of Saponification Value

This was carried out using the method described by AOAC (1998). Two grams of the oil sample were added to a flask with 30 cm3 of ethanolic potassium hydroxide solution and

were then attached to a reflux condenser and heated on a water bath for 1 hour with occasional shaking to ensure the sample was fully dissolved. After the sample had cooled, 1cm3 of phenolphthalein indicator was added and titrated with 0.5M hydrochloric acid until a pink endpoint was reached. A blank determination was also carried out omitting the oil and saponification value was calculated using the equation:

$$\text{Saponification Value} = \frac{(b - a) \times M \times 56.1}{\text{Weight of Sample (g)}}$$

Where

a = sample titre value

b = blank titre value

M = molarity of the HCl

56.1 = molecular weight of KOH

Determination of Iodine Value

The determination Iodine Value was carried out according to the IUPAC method (IUPAC 1979). With the aid of a dropping pipette, about 0.2 – 0.5 g of the oil was accurately weighed into a glass stoppered flat bottom flask and 10 ml carbon tetrachloride added to the oil to dissolve. Exactly 20 ml Wijs' solution was added and the stopper which had been moistened with potassium iodide solution inserted. The mixture was mixed and allowed to stand in a dark cupboard for 30 minutes. 15 ml of freshly prepared 10% potassium iodide solution and 100 ml water was added and mixed. The mixture was titrated with 0.1 M standard sodium thiosulphate solution and using starch as an indicator just before the end point. A blank titration was also carried out. The Iodine Value is given as:

$$\text{Iodine Value} = \frac{(b - a) \times 1.269}{\text{Weight of sample (g)}}$$

Where a = sample titre value

b = blank titre value

GC-MS analysis

The oil was analyzed by gas chromatography – mass spectrometry. A Shimadzu GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is presented in (Table-1), while other chromatographic conditions are depicted in (Table-2).

Table (1): Oven temperature program

Rate(min.$^{-1}$)	Temperature(C^{o})	Hold Time
- 4.00	150.0 300.0	1.00 0.00

Table (2): Chromatographic conditions

Column oven temperature	150 °C
Injection temperature	300 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3 KPa
Total flow	50.0 ml/min
Column flow	1.54 ml/sec
Linear velocity	47.2 cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Testing of antimicrobial Activity

Mueller Hinton (MH) agar and sabouraud dextrose agars were used as media for growth of bacteria and fungi respectively. They were prepared according to the manufacturer instructions. The disc diffusion bioassay was used to assess the antibacterial potency of the oil. Bacterial suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of the bacterial suspension were swabbed uniformly on surface of MH-agar and allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MH-agar and soaked with (20 µl) of the test solution. The inoculated plates were incubated at 37 °C for 24h. The diameters (mm) of the inhibition zones were measured in duplicates and averaged. The above procedure was also used for antifungal activity, but instead of Muller Hinton agar, Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

RESULTS AND DISCUSSION

Physicochemical characteristics of oils

The values for Specific Gravity, Refractive Index, Acid Value, Percentage Free Fatty Acids, Peroxide Value, Saponification Value and Iodine Value) of *Foeniculum Vulgare*seed oil were presented in Table (3).

Table 3: Physicochemical properties of *Foeniculum Vulgare* seed oil

Parameter	**Result**
Specific gravity	0.918
Refractive index	1.471
Acid value (mg KOH /g)	2.01
Free fatty acid (mg KOH /g)	2.5
Peroxide number (meq KOH /g)	18.2
Saponification value (mg KOH /g)	15.42
Iodine number (gI_2/100g)	78.36

The value for specific gravity of this oil is (0.918) and the refractive index is (1.471). This result was lower than that reported by Dhia .F. (2018).The IodineValue is a measure of the degree of unsaturation of the fatty acids in an oil that reflects the oil's sensitivity to oxidation (Alireza et al, 2010); high Iodine values indicate the presence of the high amount of double/triple bonds in the fatty acids present in oils. The value obtained from this study is (78.36) lower than (149.27) that was recorded by Vallamkondu et al (2021). Acid value is an important indicator of the physical and chemical property of an oil which is used to indicate the quality, age, edibility of the oil. According to Demian (1990).The peroxide value is a predominant test for oxidative stress in oils and fats, whereby the obtained peroxide value is (18.2), and this value is higher than that reported by Dhia .F. Alfekaiki (2.3). The saponification value is used in the adulteration assay. The low saponification value obtained for the oil indicates that it is not industrially useful. The saponification value in this study is **(**15.42). This result was lower than that obtained by Vallamkondu etal (68.42). The percentage of free fatty acids in the oil indicates their level of degradation and their quality (Tagoe et al, 2012). In addition, seed duration and storage conditions are factors that may influence the value of free fatty acids (Fokou and Meier, 2009). The free fatty acid of this study is 2.5 and this value is lower than that reported by Vallamkondu et al (3.68).

The GC-MS spectrum of the *Feoniculum vulgera* seeds oil revealed the presence of six components. Total ions chromatograms are depicted in Fig. (1), while the different constituents of the oil are presented in Table (4). Fatty acids constituted small bulk of the oil.

The GC-MS analysis revealed the following major components: 10-Nonadecanone (79.28%), Estragole (8.61%), 6-Octadecenoic acid methyl ester (7.63%).

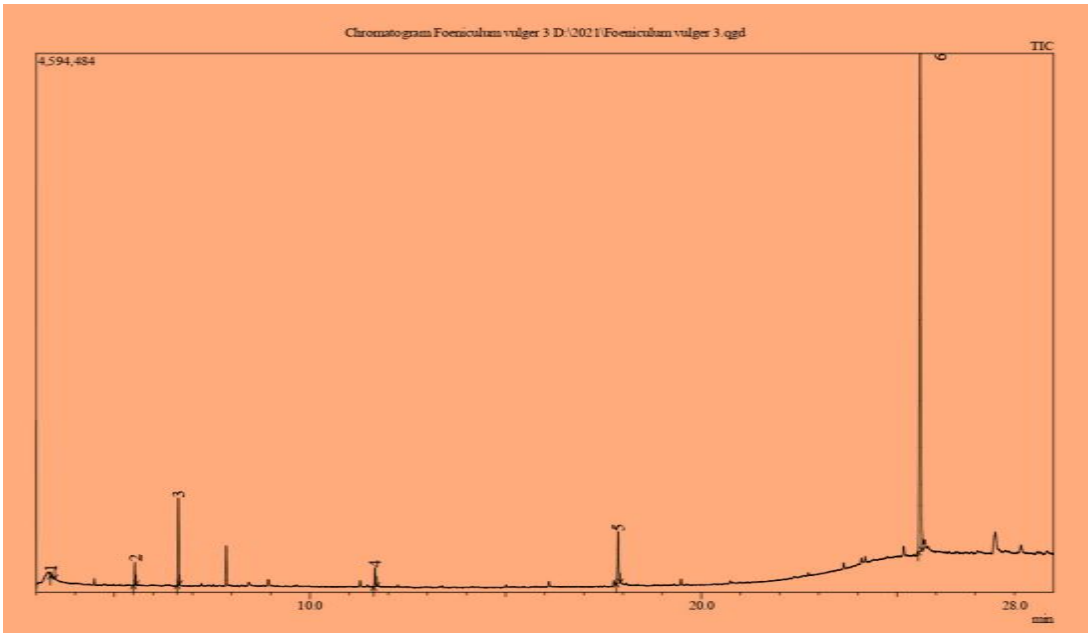

Fig. (1): Total ions Chromatogram of *Feoniculum vulgera* oil

Table (4): Fatty acids constitutions of *Feoniculum vulgera oil*

Peak	Name	R.Time	Area%
1	1-Methyldecylamine	3.380	0.29
2	Undecane	5.525	2.12
3	Estragole	6.636	8.61
4	2-Tridecenal, (E)	11.660	2.08
5	6-Octadecenoic acid methyl ester	17.872	7.63
6	10-Nonadecanone	25.589	79.28

The EI mass spectrum of 10-Nonadecanone is shown in Fig. (2). The peak at m/z 282, which appeared at R.T. 25.590 in total ion chromatogram, corresponds M^+ $[C_{19}H_{38}O]^+$, The peak at m/z 239 corresponds to loss of propene fraction because it is a neutral type.

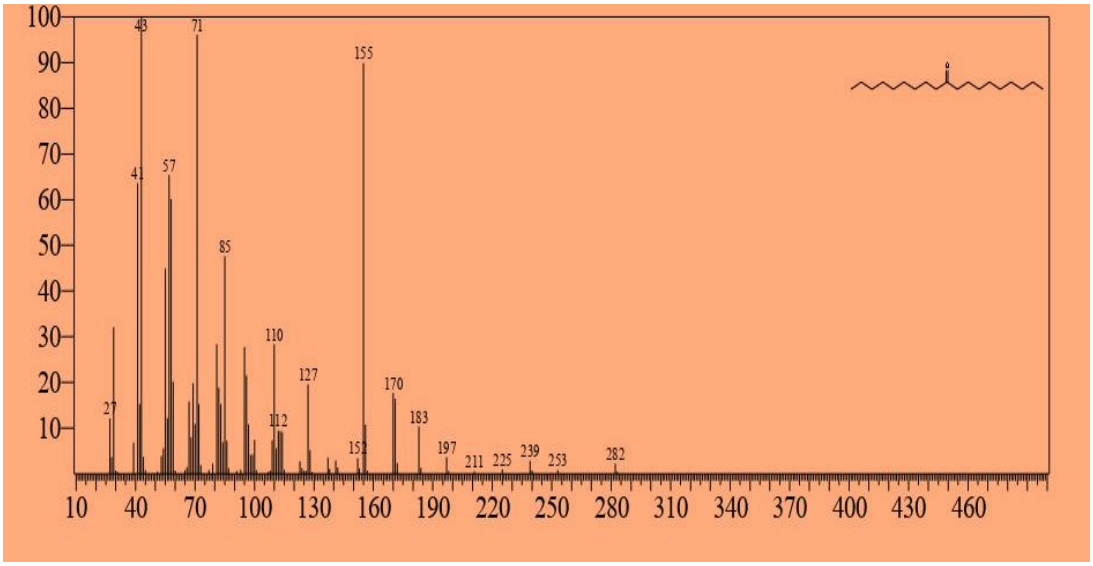

Fig. (2): Mass spectrum of 10-Nonadecanone

The mass spectrum of Estragole is presented in Fig. (3). The signal at m/z 148, which appeared at R.T. 6.636 is attributed to $M^{+}[\ C_{10}H_{12}O]^{+}$.

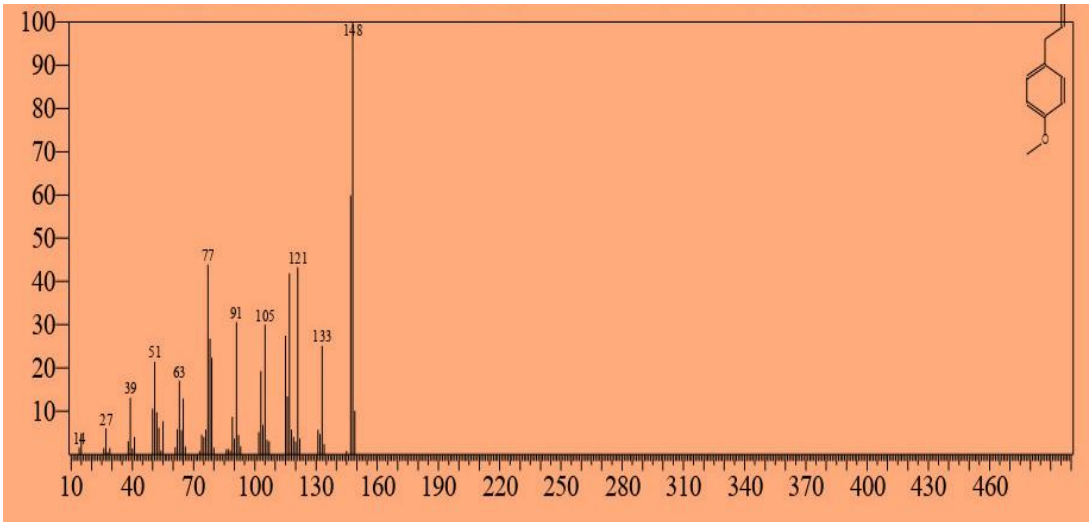

Fig. (3): Mass spectrum of Estragole

Fig. (4) shows the mass spectrum of 6-Octadecenoic acidmethyl ester. The peak at m/z 296, with R.T.17.872, corresponds the molecular ion: $M+[C_{19}H_{34}O_2]^{+}$, while the signal at m/z 265 is due to loss of a methoxyl.

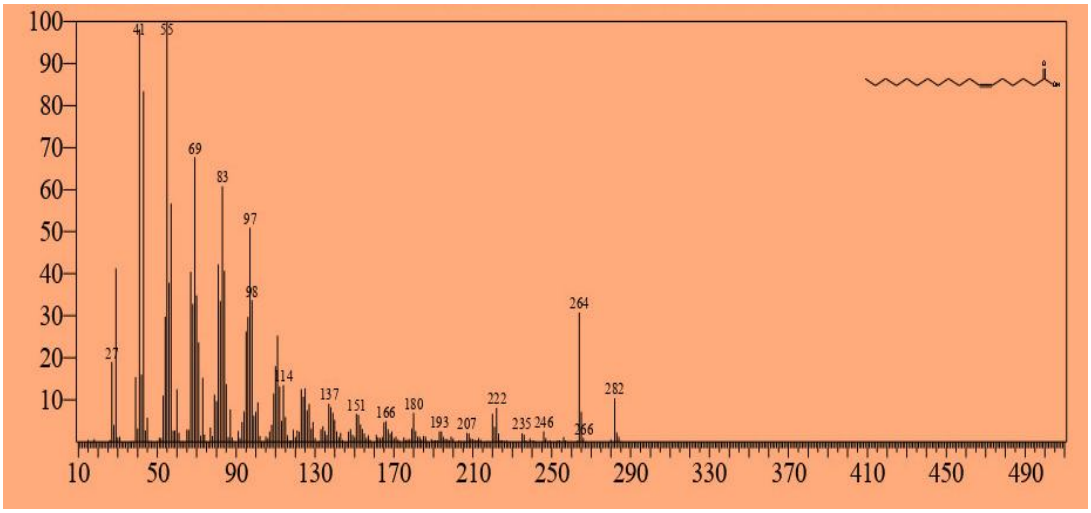

Fig. (4): Mass spectrum of 6-Octadecenoic acid methyl ester

Antibacterial activity

In cup plate agar diffusion assay, the oils were screened for antimicrobial activity against six standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (5). The results were interpreted in commonly used terms (>9mm: inactive; 9-12mm: partially active; 13-18mm: active ;< 18mm: very active). Table (6) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table (5) Antimicrobial Activity of the oil

Sample		Antibacterial activity				Antifungal activity	
		Gram-positive		Gram -negative			
	Conc.(mg/ml)	*B.S*	*St.a*	*E.C*	*Salmonella*	*Penicillium*	*As.n*
Feoniculumvulgera oil	25	11	9	8	8	15	-

B.s.:Bacillus subtilis

St.a.: Staphylococcus aureus

E.c.:Escherichia coli

As.n.: Aspergillus niger`

The *Feoniculum vulgera* oil showed partial activity against *Bacillus subtilis* ,*Staphylococcus aureus*and *Penicillium*,but inactiveagainst *Escherichia coli* and *Salmonella.*

Table (6) : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	*B.S*	*St.a*	*E.C*	*Salmonella*	*Penicillium*	*As.n*
Gentamycin	*20*	22	18	18	-	-	-
Clotrimazole	15	-	-	-	-	17	31

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CONCLUSION

The current study identified (6) fatty acids, physicochemical properties of oil and antimicrobial activity against six standard human pathogens. Therefore, the authors suggest conducting further studies to determine and identify more bioactive compounds.

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