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

Munged Ibrahim [a*], Fath Elrahman Ahmed [b] and ELfaiz Fathi [a]

[a] Department of Chemistry, College of Education, University of Bahri, P.O. Box 1660, Khartoum, Sudan.
[b] Department of Chemistry and Biology, Faculty of Education, Omdurman Islamic University, Sudan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this research is to evaluate the physicochemical properties, to investigate the chemical components of the fixed oil from *foeniculum vulgare seed*s and to appraisal its l antimicrobial activity against six microorganisms (*Bacillus subitus, Staphylococcus aureus, Salmonella, Escherichia coli, Aspergillus niger* and *Penicillium*). The chemical components of *foeniculum vulgare* seeds oil were specified and quantified using the GC-MS technique, where disc diffusion assays were appointed to evaluate the antimicrobial activities and physicochemical properties by criterion methods. the physicochemical analysis results showed that the seeds oil was green in colour and liquid at room temperature with the iodine, acid, peroxide, saponification, values and free fatty acid at (2.01± 0.01mgKOH/g, 78.36±0.1 gI_2/100g, 15.42±0.02mgKOH/g, 18.2±0.01meqH_2O_2, 5.2mgKOH/g) respectively. The specific gravity, relative density, refractive index and viscosity 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

**Corresponding author: E-mail: fathkhir72@oiu.edu.sd;*

and detected revealing the following major components 10-Nonadecanone (79.28%), Estragole (8.61%), 6-Octadecenoic acid methyl ester (7.63%). The oil appeared partial activity against *Staphylococcus aureus*, *Bacillus subtilis* and inactive against *Escherichia coli*, *Salmonella* and *Aspergillus niger,* but more active against *Penicillium* as naturally source of antimicrobial activity and justified its uses in traditional medicines.

Keywords: Antimicrobial activity; Foeniculum vulgare; Bacillus subtilis; GC-MS analysis.

1. INTRODUCTION

Fennel (*Foeniculum vulgare*) it is belong herbaceous family which grows annually with seeds that are helpful to humans [1]. The botanists assorting *Foeniculum vulgare* in to two species, one is sweet fennel (*Foeniculum vulgare* Var. Dulce), which is almanacs or biennials with small fruits have sweet-tasting. The other is unpleasant fennel (*Foeniculum vulgare* var. vulgare), which is a returning with an unpleasant taste fruits [2] (Miraldi,1999).generally was used as medicine, both as homemade remedies as well as in the industry of medicines [3]. They indicate that the seeds of Foeniculum *vulgare* secrete sometimes clear oil or certain yellowish that is used in the manufacture of perfumery drinks through carminative and stimulant act [4,5,6]. “Recently, much attention has been focused on *Foeniculum vulgare* due to the health protective and nutritional value of their seeds that are rich in volatile oils and vegetable” [7]. “The seeds of *Foeniculum vulgare* are deemed as source of various health useful materials including vitamins, minerals, and others which describe their applications for cosmetic, perfumery, pharmaceutical and food industries” [8]. Traditionally *Foeniculum vulgare* is suggested for neurological disorder, kidney stones, gastrointestinal, diarrhea and vomiting. It also has antiseptic, antispasmodic, anti-ulcer properties and carminative [9,10]. 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 [11]. “*Foeniculum vulgare* was known as stellar source of natural antioxidants and contributes daily antioxidant diet” [12]. The volatile oil exhibited strong antioxidant activity compared with hydroxyisoleum butyrate (BHA) and butylhydroxytoluene (BHT) [13]. The Ethanolicaqueous extract of *Foeniculum vulgare* revealed that the antioxidants were lower in comparison with the essential oil [14,15,16].

The purpose of this research 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.

2. MATERIALS AND METHODS

2.1 Plant Materials

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

2.2 Oil Extraction

(250 g) of the dried desiccated seeds was put into the thimble and were extracted continuously for (6) hours by the solvent n-hexane (76°C - 80°C), and till 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.

2.3 Determination of Physicochemical Properties of the Oil

2.3.1 Determination of specific gravity and refractive index

The tests of Refractive Index and Specific Gravity were determined by the manual methods of analysis food, [17].

2.3.2 Determination of acid value

According the method described by Ronald [18] The Acid value was determined. Equal volumes

(25 ml) of diethyl ether and ethanol were mixed together and 1 ml of 1% phenolphthalein indicator solution was added and then neutralized with 0.1 M 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 continuous shaking until a pink color which persists for (15) seconds is obtained. Then account the value such as:

$$Acid\ Value = \frac{Titer\ value(ml)\ .5.61}{Weight\ of\ sample\ used\ (g)}$$

2.3.3 Determination of percentage Free Fatty Acids (FFA)

The method described by AOAC (1990) was using to determine the percentage of free fatty acid. One gram of the oil sample was accurately weighed into a conical flask, followed by the adding 10 cm3 of neutralized 95% ethanol and Phenolphthalein. Then titrated with 0.1M NaOH with continuous shaking until a pink color which persists for (30) seconds. The percentage free fatty acid was calculated from the equation below:

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

2.3.4 Determination of peroxide value

One gram of the oil was weighed into a clean dry boiling tube, 1 g of powdered potassium iodide and 10 cm^3 of the solvent mixture were added. The mixture was allowed to boil vigorously for 30 seconds. The tube was washed twice with 25 cm^3 portions of water and the washings were added to the titration flask. This was then titrated with 0.002 M Sodium thiosulphate using starch indicator. The value of Peroxide is given as:

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

2.3.5 Determination of saponification value

This was implemented out by the method described by AOAC (1998). Two grams of the oil sample were added to the flask with 30 cm^3 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, 1 cm^3 of phenolphthalein indicator was added and titrated with 05 M 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:

$$Saponification\ value = \frac{(b-a)\ .M\ .56.1}{Wieght\ of\ Sample\ (g)}$$

Where

a = sample titre value
b = blank titre value
M = molarity of the HCl
56.1 = molecular weight of KOH

2.3.6 Determination of iodine value

The determination of 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 weighted 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. Then was titrated the mixture with 0.1M 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:

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

Where

a = sample titre value
B = blank titre value

2.4 GC-MS Analysis

The gas chromatography-mass spectrometry was using to analyze to the oil. A Shimadzu GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25µm, 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
-	150.0	1.00
4.00	300.0	0.00

Table 2. Chromatographic conditions

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

2.5 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 108 cfu/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 (20µl) of the test solution. The inoculated plates were incubated at 37^{o}C for 24h. The inhibition zones diameters 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" [19]. Above same concentrations were used for the samples here.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of oils

The values for Refractive Index, Specific Gravity, Acid value, percentage of free fatty acid, Peroxide value, Saponification value and Iodine value) of *Foeniculum vulgare* seeds oil were presented in Table 3.

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 [20]. The Iodine value is a measure of the degree of unsaturation of the fatty acid 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 [21]. The acid value is an important indicator of the physical and chemical property of an oil which is used to indicate the edibility, age, quality 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 et al. (68.42). The free fatty acids percentage 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 [22]. The free fatty acid of this study is 2.5 and this value is lower than that reported by Vallamkondu et al (3.68).

Table 3. Physicochemical properties of *Foeniculum vulgare* seed oil

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

The GC-MS spectrum of the *Feoniculum vulgare* seeds oil gained a six components. The chromatograms total ions are shown in Fig. 1, while the different components of the oil are depicted in Table 4. The GC-MS analysis detected the following major components: 10-Nonadecanone (79.28%), Estragole (8.61%), 6-Octadecenoic acid methyl ester (7.63%).

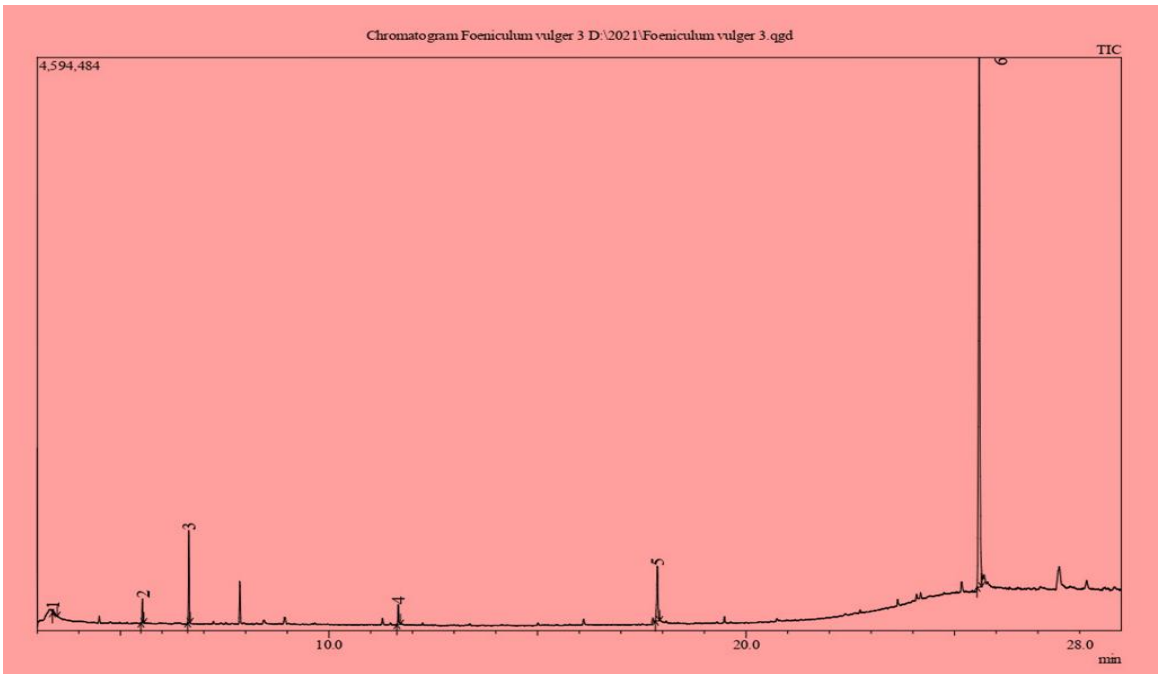

Fig. 1. Total ions chromatogram of *Feoniculum vulgare* oil

Table 4. Fatty acids constitutions of *Feoniculum vulgare* 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

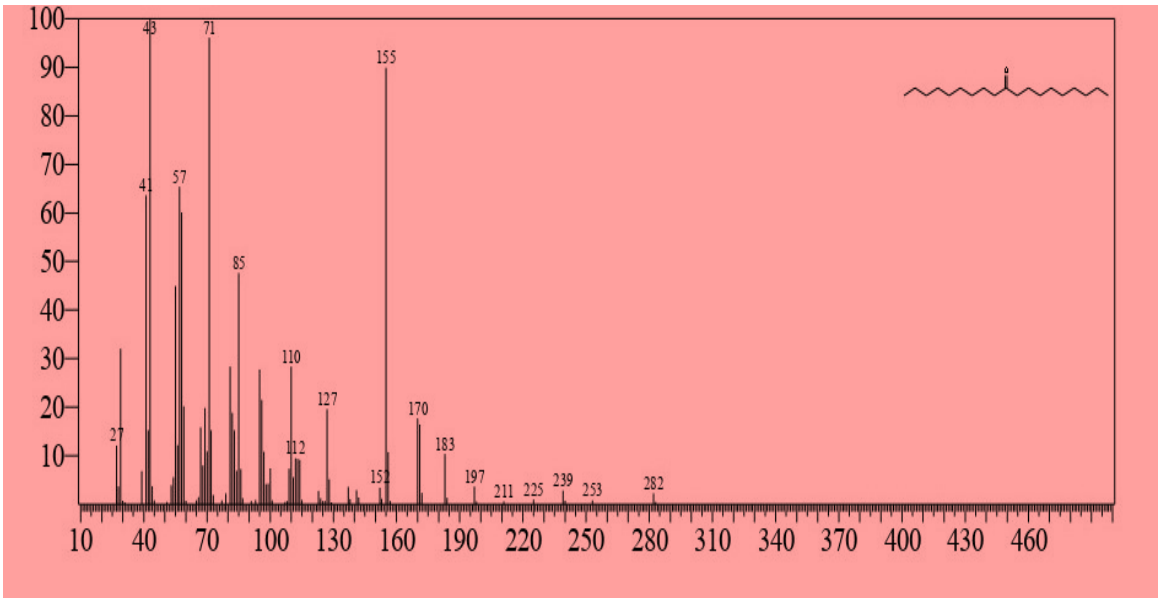

Fig. 2. Mass spectrum of 10-Nonadecanone

The EI mass spectrum of 10-Nonadecanone is appeared in Fig. 2. The peak at m/z 282, which R.T. shown at 25.590 the chromatogram of total ion in 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. 3 is the representative GC-MS chromatogram of Estragole. The signal at m/z 148, which shown at R.T. 6.636 is attributed to $M^+[C_{10}H_{12}O]^+$.

The mass spectrum of 6-Octadecenoic acid methyl ester is depicted in Fig. 4. The peak at m/z 296, with R.T.17.872, corresponds to: $M+[C_{19}H_{34}O_2]^+$, while the signal at m/z 265 is due to loss of methoxy group.

3.2 Antibacterial Activity

The antimicrobial sensitivity of the seeds oil was tested using the disc diffusion methods. The 6 standard human pathogens were used to evaluate the antimicrobial activity. The average of the diameters of the inhibition zones was measured in mm for each organism which are depicted in Table 5. The results were explicated in commonly terms (<18 mm: very active, 13-18 mm: active, 9-12 mm: partially active, >9mm: inactive). The Table 6 appears the antimicrobial sensitivity of standard antifungal and antibacterial chemotherapeutic agents against standard fungi and bacteria respectively.

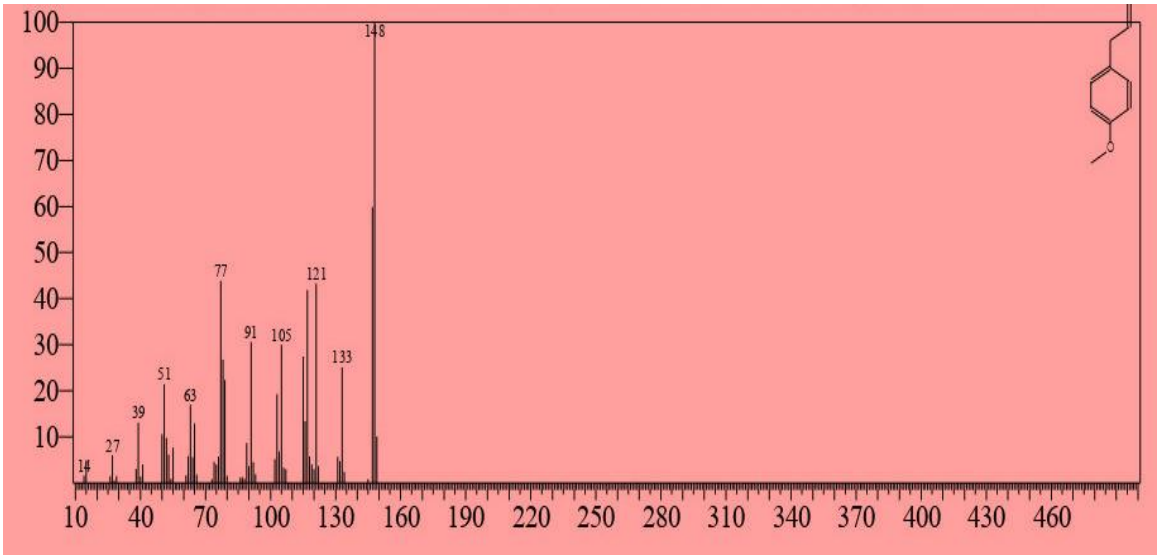

Fig. 3. Mass spectrum of Estragole

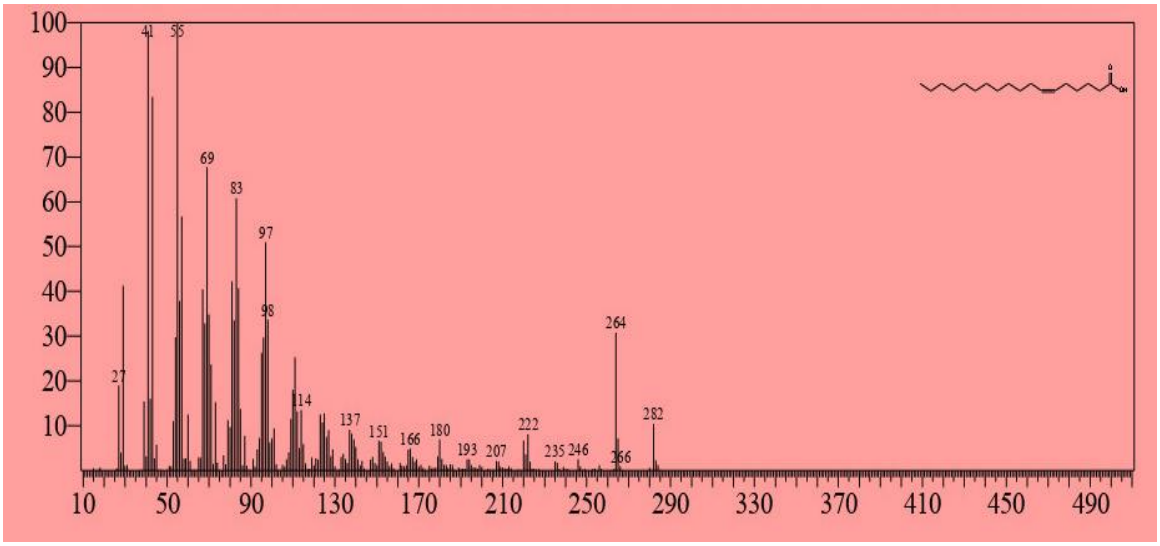

Fig. 4. Mass spectrum of 6-Octadecenoic acid methyl ester

Table 5. Antibacterial 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*
***Feoniculum vulgare* oil**	25	11	9	8	8	15	-

E.C.: Escherichia coli; B.S.: Bacillus subtilis; As.n.: Aspergillus niger; St.a.: Staphylococcus aureus
The Feoniculum vulgera oil showed partial activity against Bacillus subtilis, Staphylococcus aureus and Penicillium, but inactive against Escherichia coli and Salmonella

Table 6. Antimicrobial 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

4. CONCLUSION

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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