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Phytochemical and Antimicrobial Evaluation of the Volatile Components of fennel Essential Oil in Jos North, Plateau State, Nigeria

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ABSTRACT
Fennel is a flowering plant species in the carrot family. It is a hardy, perennial herb with yellow flowers and feathery leaves. This study involved extraction of the bioactive phytochemicals from fennel, seed using n-hexane, characterization of the concentrated extract using gas chromatography-mass spectrometer (GC-MS) and Fourier transform-infrared spectrometer (FT-IR), and evaluation of the antimicrobial activities of the essential oil. From the GC-MS analysis, fennel essential oil is seen to contain numerous compounds, majority of which are hydrocarbons and oxygenated hydrocarbons, they are generally unsaturated compounds. Result of the analysis revealed that the major constituents of fennel essential oil are; Anethole (65%), Estragole (15%), Vaccenic acid (7.7%), 1-Cyclohexylheptene (5.5%), 5,10-Pentadecadienoic acid (5.1%), N-Hexadecanoic acid (2.7%) and p-anisic aldehyde (1.6%). Antimicrobial evaluation revealed that fennel oil is active against both gram-positive bacteria. However, the results obtained indicate that the antibacterial effects of fennel essential oil against the test organisms varied as follows; Escherichia coli > Salmonella typhi > Staphylococcus aureus > Streptococcus Pneumoniae respectively. Therefore, the result implies that fennel seed essential oil has more effect on gram-negative bacteria as compared to gram-positive bacteria.

Keywords: Fennel, Essential oil, Antimicrobial, Phytochemicals, Fourier Transform Infrared Spectroscopy (FT-IR), Gas Chromatography-Mass Spectrometer (GC-MS)

INTRODUCTION

Spices are high value, export-oriented crops used extensively in food and beverage flavorings, medicines, cosmetics as well as perfumes production (Calucci *et al*., 2017). However, there is a growing interest in the theoretical and practical aspects of the biosynthetic mechanisms of the active components in spices as well as the relationship between the biological activity and chemical structure of these secondary metabolites (Vihiior *et al*., 2026). Fennel (*Foeniculum vulgare* Miller) is a flowering plant species in the carrot family. It is a hardy, perennial herb with yellow flowers and feathery leaves. It is indigenous to the shores of the Mediterranean but has become widely naturalized in many parts of the world, especially on dry soils near the sea-coast and on riverbanks (Nassar *et al*., 2010).

The fennel available in katako market Jos north is imported from Egypt. An analysis of fennel shows it to consist of moisture 6.3%, protein 9.5%, fat 10%, minerals 13.4%, fiber 18.5% and carbohydrates 42.3%. It's mineral and vitamin contents are sodium, calcium, phosphorous, iron, potassium, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 370 (Ali *et al*., 2016). Fennel volatile oil is a mixture of at least a dozen of different chemicals and the main ingredients are anethole (40 - 70%), fenchone (1 - 20%) and estragole (2 - 9%). The fennel oil quality is determined by the amount of anethole content. The fennel oil has a better quality if it has higher content of anethole (Kanadea and Bhatkhandeb, 2016). Research of fennel essential oil in Indonesia which is from Boyolali, Cipanas, and Bintang stated that they have different oil content of fennel. The highest of fennel seed oil content is from Bintang, which is 6.15%. The highest oil production of Fennel is 4.21% while the content of anethole is 26.17% (Fahmi, 2011).

Table 1: Taxonomy of Fennel (*Foeniculum Vulgare* Miller)

Kingdom	Plantae
Subkingdom	Tracheophytes
Phylum	Angiosperms
Subphylum	Eudicots
Class	Asterids
Order	Apiales
Family	Apiaceae
Genus	Foeniculum
Species	F. vulgare
Common name	Fennel

A raw fennel bulb (235 g) consists of 212 g of water, 2.91 g of protein, 0.47 g of fat, and 17.2 g of carbohydrate (including 7.28 g of dietary fiber and 9.24 g of sugars), providing a total of 72.8 Calories (kcal) of energy (Ghanem *et al*., 2012). The 235g bulb provides 115 mg of calcium, 1.72 mg of iron, 40 mg of magnesium, 188 mg of phosphorus, 973 mg of potassium, 122 mg of sodium, trace amounts of zinc, copper, and selenium, 28.2 mg of vitamin C, as well as choline, several B vitamins, folate, beta-carotene, lutein, zeaxanthin, vitamin E, and vitamin K (Astrilia and Eko, 2012). Dried fennel fruits are typically used as a spice and normally eaten only in minute quantities. A 100-gram reference amount of fennel fruits provides 1,440 kilojoules (345 kilocalories) of food energy, and is a rich source (20% or more of the Daily Value, DV) of protein, dietary fiber, B vitamins and several dietary minerals, especially calcium, iron, magnesium and manganese, all of which exceed 100% DV. Fennel fruits are 52% carbohydrates (including 40% dietary fiber), 15% fat, 16% protein and 9% water (Nyerges *et al*., 2016). Fennel is one of the three main herbs used in the preparation of absinthe, an alcoholic mixture which originated as a medicinal elixir in Europe and became, by the late 19th century, a popular alcoholic drink in France and other countries. Fennel fruit is also used in the production of akvavit. Fennel is also featured in the Chinese Materia Medica for its medicinal functions (Badgujar *et al*., 2014).
The aromatic character of fennel fruits derives from volatile oils imparting mixed aromas, including trans-anethole and estragole (resembling liquorice), fenchone (mint and camphor), limonene, 1-octen-3-ol (mushroom), other phytochemicals found in fennel fruits include polyphenols, such as rosmarinic acid and luteolin, among others in minor content (Dua *et al*., 2013).

MATERIALS AND METHODS

Collection of Plant Material

Fennel (*Foeniculum vulgare* Miller) seed were purchased on the 20th of May 2021 at katako market Jos north, Plateau State, Nigeria. The plant material was deposited in the postgraduate research laboratory of chemistry Department, University of Jos.

Extraction of Plant Materials

The procedure employed in this research was cold extraction. Exactly 370 g of Fennel seed was weighed on the weighing balance and pounded using clean mortar and pestle. The pounded material was transferred into a wide neck reagent bottle with the using spatula. 500 ml of N-Hexane was measured and added to the powdered plant material. The sample (mixture) of the pounded plant material was mixed using glass rod and stirred daily for three days (72 hours), after which the liquid phase was decanted from the chaff into a conical flask and was properly filtered with filter paper into clean conical flask, the extract was concentrated using laboratory rotary evaporator in a clean, dried and weighed empty (103.9 g) quick fit round bottom flask. After concentration the extract was successfully carried out, the extract was allowed to cool and then weighed (114.85 g). The concentrated extract was stored in the quick fit round bottom flask until for future use.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

Gas chromatography-mass spectroscopy (GC-MS) was carried out to identify the chemical constituents of fennel (*Foeniculum vulgare* Miller) seed essential oil, at Yobe State University Damaturu, Faculty of Sciences, Department of Chemistry, Chemistry analytical laboratory on GC 7890B, MSD 5977A, Agilent Tech.

Fourier Transformed-Infrared Spectroscopy (FT-IR) Analysis

The plant extract was analyzed using Fourier transformed-infrared spectroscopy (FT-IR) so as to determine the functional groups present in the identified chemical components of fennel (*Foeniculum vulgare* Miller) seed essential oil, at Yobe State University Damaturu, Faculty of Sciences, Department of Chemistry, Chemistry analytical laboratory.

Determination of Antibacterial Activity

The antimicrobial activities of the concentrated extracts (essential oils) was evaluated at National Veterinary research institute (NVRI) Vom, Jos Plateau State.

Preparation of Nutrient Agar

Exactly 20g of the nutrient agar was accurately weighed and dissolved in distilled water (1 litre). The mixture was heated in boiling water with frequent agitation until it dissolved completely. The resultant solution was autoclaved for 15 minutes at 121 ^{0}C and poured into the petri dishes (plates). The plates were left over night at room temperature to check for any contamination to appear.

Preparation of the Plates

The agar well diffusion assay method was used. Sterilized petri dishes containing the nutrient agar was incubated with the microbes using a sterile swab stick; the plates were labeled clearly with various microbes. Wells were then bored into the agar using a sterile 6 mm diameter corn borer. Approximately 100 µl of the essential oils (100, 50, and 25 %) were introduced into the wells. The plates were allowed to stand on a laboratory bench at room temperature for 2 hours to allow for proper diffusion of the essential oil into the media and then incubated at 37^0 C. The plates were observed for zones of inhibition after 24 hrs. Ciprofloxacin was used as standard drug against the bacterial infection, 20mg/ml was used and the zone of inhibition in millimeter (mm) was considered as the positive control (100%).

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Different concentrations (100, 50, 25, 12.5, 6.25 and 3.125%) of the essential oils were added to nutrient broth tubes which were seeded with 0.1 ml of the standard bacterial inoculum. Negative control tubes with no bacterial inoculation, were simultaneously maintained. Tubes were incubated aerobically at 37 °C for 24 hours. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC. Dilutions showing no visible growth for the MIC were subcultured onto a fresh NA agar plate and incubated at 37 °C for 24 hours. The lowest concentration of the extracts yielding no growth on the NA plate was recorded as the minimal bactericidal concentration (MBC).

RESULTS AND DISCUSSION

Results

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Fennel Essential Oils

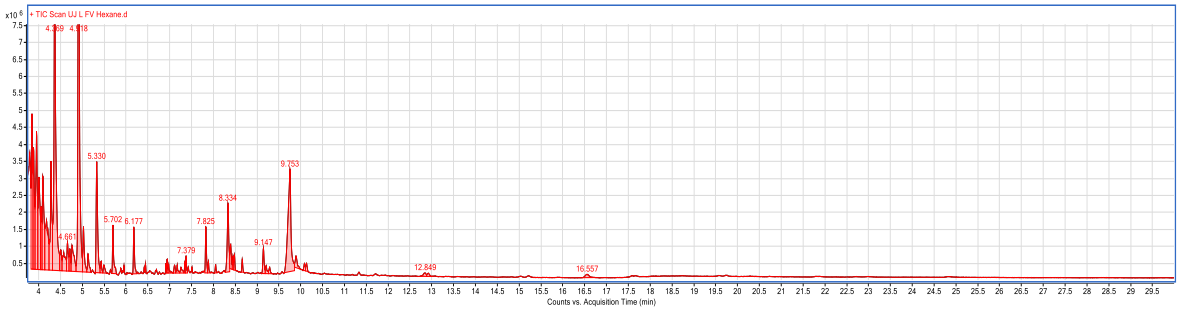


Figure 1: GC chromatogram of *Foeniculum vulgare* Miller seed essential oil

Table 2: Compounds Identified in Fennel (*Foeniculum Vulgare* Miller) Seed Essential Oil

Peak NO.	RT (min)	Name of compound	Molecular formula	Molecular weight	% Peak Area
1	3.951	1-Cyclohexylheptene	$C_{13}H_{24}$	180	5.5
2	3.848	5,10-Pentadecadienoic acid	$C_{15}H_{26}O_2$	238	5.1
3	4.06	Benzene, 2-ethyl-1,4-dimethyl-	$C_{10}H_{14}$	134	1.8
4	4.283	Estragole	$C_{10}H_{12}O$	148	15
5	4.369	2-(3-Hydroxybutyl) cyclooctanone	$C_{12}H_{22}O_2$	198	4.2
6	4.601	1,3-dicyclohexylpropene	$C_{15}H_{26}$	206	1.3
7	4.764	p-Anisic aldehyde	$C_8H_8O_2$	136	1.6
8	4.918	Anethole	$C_{10}H_{12}O$	148	65
9	5.027	1H-Indene, 1-ethylidene	$C_{11}H_{10}$	142	1.7
10	5.702	Aromadendrene	$C_{15}H_{24}$	204	1.4
11	8.334	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.7
12	7.753	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	7.7
13	31.38	10-Nonadecanone	$C_{19}H_{38}O$	282	0.8

RT = Retention time

Fourier Transform- Infrared (FT-IR) Analysis of Fennel Essential Oils

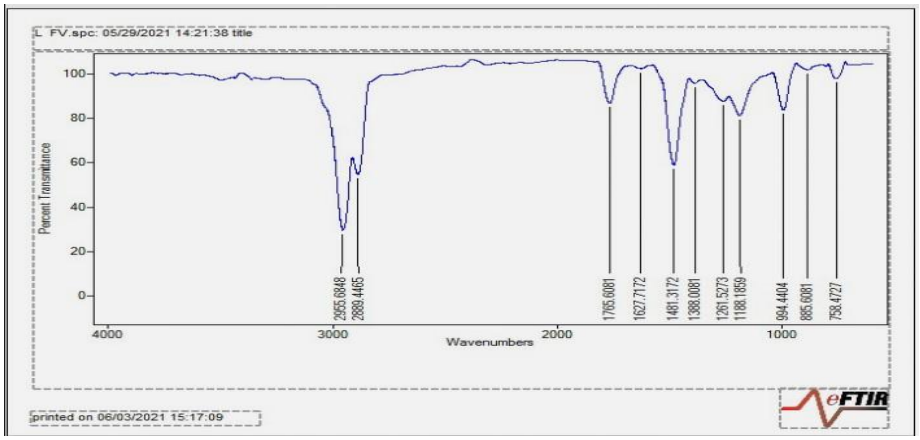


Figure 2: FT-IR absorption Spectra of Fennel (*Foeniculum vulgare* Miller) seed essential

Table 3: Infrared Absorptions for Fennel (*Foeniculum Vulgare* Miller) Seed Essential Oil

S/N	Frequency (cm^{-1})	Intensity	Bond	Class of compound
1	2955.68	Medium	C-H str.	-CH3 group (Alkane)
2	2889.44	Medium	C-H str.	Aldehyde
3	1765.60	Weak	C-H ben.	Carbonyl group
4	1627.71	Medium	C=C str.	Alkene
5	1481.31	Medium	C-C ben.	Alkene
6	1388.00	Medium	C-C ben.	Methyl
7	1261.52	Strong	C-O str.	alkyl aryl ester
8	1188.18	Strong	C-O str.	Alcohol

S/N	Frequency (cm^{-1})	Intensity	Bond	Class of compound
9	994.44	Strong	C=C ben.	Carboxylic acid
10	758.47	Strong	C-H ben.	Halo compounds

Str. = Stretching, Ben. Bending

Antimicrobial Evaluation of Fennel Essential Oils

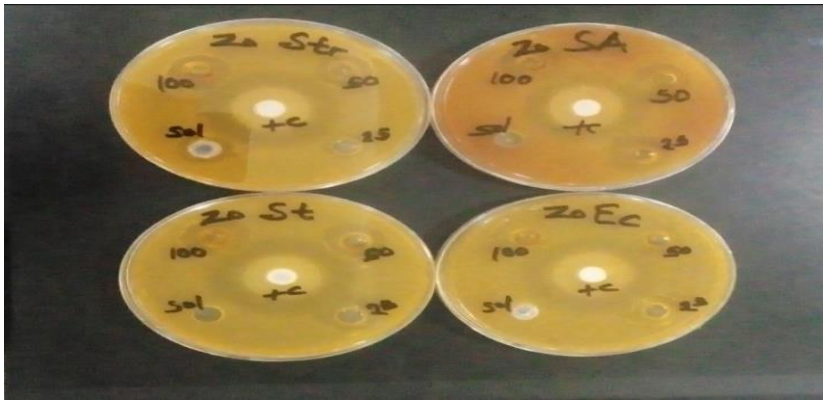


Figure 3: Antibacterial activity of ginger essential oil

Table 4: Antibacterial Activity of Fennel Essential Oil

Extract	Concentration (%)	S.a	S.p	E.c	S.t
Ginger	100	18±0.01	17±0.22	15±0.04	18±0.11
	50	15±0.00	14±0.02	13±0.01	15±0.18
	25	11±0.12	10±0.21	11±0.28	8±0.00
Solvent n-hexane	0	0	0	0	0
Positive control (ciprofloxacin)	20mg/ml	30	26	21	32

Key: S.a = Staphylococcus aureus, S.p = Streptococcus Pneumoniae, E.c = Escherichia coli, S.t = Salmonella typhi
All data were average of three values (x ±ESM)

Table 5: Minimum Inhibitory Concentration (MIC)

Test Organism	Concentration of essential oil (%)						Essential oil	MIC (%)
	100	50	25	12.5	6.25	3.125		
Staphylococcus aureus	-	-	-	-	+	+	Ginger	12.5
Streptococcus Pneumoniae	-	-	-	+	+	+		25
Escherichia coli	-	-	-	-	+	+		12.5
Salmonella typhi	-	-	-	+	+	+		25

Key: - No turbidity, + presence of turbidity

Table 6: Minimum Bactericidal Concentration (MBC)

Test Organism	Concentration of essential oil (%)						Essential oil	MBC (%)
	100	**50**	**25**	**12.5**	**6.25**	**3.125**		
Staphylococcus aureus	-	- α	+	+	+	+	Ginger	50
Streptococcus Pneumoniae	- α	+	+	+	+	+		100
Escherichia coli	-	- α	+	+	+	+		50
Salmonella typhi	- α	+	+	+	+	+		100

Key: - no growth, + presence of growth, α MBC

Discussion

Gas Chromatography-Mass Spectrometry of the Essential Oil

The results from GC-MS analysis of the Fennel (*Foeniculum vulgare* Miller) seed essential oils are summarized in Table 1. Forty-eight different chemical compounds were eluted from the chromatographic column at different retention times depending on the boiling points of the eluted components. Identification of the components was carried out on the basis of their retention time (Rt) and National Institute of Standards Technology (NIST) mass spectral library search. The relative amount of individual constituents was calculated based on GC peak areas (peak area X/total area). Comparison of the GC-MS spectra obtained with the instruments data bank together with computer marching with WILEY 275 and National Institute of Standards Technology (NIST3.0) libraries provided with computer controlling the GC-MS system revealed that the major constituents of fennel essential

oil are; Anethole (65%), Estragole (15%), Vaccenic acid (7.7%), 1-Cyclohexylheptene (5.5%), 5,10-Pentadecadienoic acid (5.1%), 2-(3-Hydroxybutyl)-cyclooctanone (4.2%) and N-Hexadecanoic acid (2.7%) and p-anisic aldehyde (1.6%). Estragole is used in perfumes and restricted in flavor as a biologically active principle; it can only be present in a flavor by using an essential oil (Bodsgard *et al*., 2016). Anethole contributes a large component of the odor and flavor of anise and fennel, it is distinctly sweet, measuring 15 times sweeter than sugar N-hexadecanoic acid has significant activity in the enzyme kinetic study of phospholipase A_2 (Zhang *et al*., 2018).

Fourier Transform- Infrared Spectrometry of the Essential Oil

The functional groups present in the fennel (*Foeniculum vulgare* Miller) seed essential oil were determined by comparing the vibrational frequencies in wave numbers of the sample's spectrograph obtained from an FT-IR spectrophotometer with those of an IR correlation chart. In the FT-IR spectrum of the fennel essential oil (Figure 2 and Table 2), the absorption band 2955.68 cm^{-1} medium (m), showed the presence of C-H stretching of methyl, while that at 2889.44 cm^{-1} (m), result from C-H bending vibration of methylene group, the frequencies that appeared at 1765.60 cm^{-1} and 1627.71 cm^{-1} (m) indicate C-H stretching and C=C stretching of carbonyl groups and alkenes respectively. The absorption band at 1481.31 cm^{-1} (m) revealed the presence of C-C bending of methylene, the absorbance at 1388.00 cm^{-1} (m) showed the presence of C-C bending vibration of methyl. Absorption band that was observed at 1261.52 cm^{-1} (s) indicated alkyl aryl ester and 1188.18 cm^{-1} is attributed to C-O stretching vibration of alcohols. The frequency at 994.44 cm^{-1} and 758.47 cm^{-1} (s) showed the presence of O-H bending of carboxylic acids and C-Cl of halo compounds respectively.

Antimicrobial Evaluation of Fennel Essential oils

The results showed that gram-negative and gram-positive strains of bacteria had different sensitivities to fennel (*Foeniculum vulgare* Miller) seed essential oil. *Escherichia coli*, showed maximum zone of inhibition (25mm) at 100% essential oil concentration followed by Salmonella typhi with 21mm zone of inhibition, *streptococcus aureus* 14mm, and *Streptococcus Pneumoniae* had the minimum zone of inhibition (13mm) as presented in figure 3-5 and Table 3. However, the result implies that fennel seed essential oil has more effect on gram-negative as compared to gram-positive bacteria. There are many reports establishing the anti-microbial properties of fennel seeds. Fennel essential oil has minimum inhibitory concentration (MIC) of 25% against; *Streptococcus aureus, Streptococcus Pneumoniae*, *Salmonella typhi* and 12.5% (MIC) against Escherichia coli (Table 4). The minimum bactericidal concentration of fennel essential oil was 100% except for *Staphylococcus aureus* which is 59% (Table 5). An aqueous extract of fennel seed sample inhibited the growth of *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumonia, Pseudomona aeruginosa, Salmonella typhi, Salmonella typhimurium, Shigella flexneri,* and *Bacillus cereus* (Bodsgard *et al*., 2016). Essential oil of fennel (*Foeniculum vulgare* Miller) had significant antimicrobial activities against some of microorganisms as compared to the methanolic and ethanolic extracts. Methanol extract of flower of *F. vulgare* showed significant activity against *Escherichia coli*, whereas crude and chloroform extracts failed to exhibit antimicrobial activity against *Staphylococcus aureus* (Zhang *et al*., 2018). Similar to present results among different tested bacterial strains, the methanolic fruit extract of *F. vulgare* inhibited the growth of *Staphylococcus aureus* and *Bacillus pumilus* (Dua et al., 2013).

CONCLUSION

The findings of this experiment showed that the essential oil from fennel *Foeniculum vulgare* may possess valuable antibacterial activities against gram positive and gram negative bacteria. This property however, tends to support the traditional medicinal stage in the treatment of bacterial infections. Furthermore, the study justified the use of ginger essential oil in the treatment of diseases caused by microbial agents in herbal medication.

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