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EFFECT OF *FOENICULUM VULGARE* FRUIT VOLATILE OILS EXTRACT AS AN ANTIBACTERIAL AGAINST RESPIRATORY TRACT INFECTION PATHOGENS ACTIVITY

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Abstract

This study investigate the role of volatile oils extract of *Foeniculum vulgare* fruits as antibacterial against respiratory tract infection RTI pathogens. The chemical analysis of the extract was performed by GC-MS Analysis. The antibacterial ability was carried of using agar well diffusion method and Minimum inhibitory concentration method. Result of GC-mass showed D-Limonene 7.98%, Fenchone 14.94% Estragole 20.78%, Benzaldehyde, 3-methoxy- 1.92%, Anethole 46.41% Eugenol 1.86%, 1-(4-Methoxyphenyl)propane-1, 2-diol 1.67%, 1-(4-Methoxyphenyl)propane-1, 2-diol 2.43 and n-Hexadecanoic acid 2.01 the extract exhibit antibacterial activity against RTI pathogens with excellent and broad spectrum activity as compared with antibiotics. The highest inhibition zone was 16.2 mm for concentration 100 mg of *Klebsiella pneumonia* and the lowest 5.13 mm for concentration 5mg of *Klebsiella pneumonia* MIC between 3 and 50 mg.

Key words: Medicinal plants, Antibacterial, RTI.

Introduction

The respiratory tract can be classified into two parts: the upper respiratory tract (URT) consists of a nose, a nasal cavity, sinuses, pharynx, epiglottis, larynx and the lower respiratory tract (LRT) consists of trachea, bronchi and lungs. (Hassan and Mosa, 2019). Pneumonia is a common disease of the respiratory tract in lung contamination alveoli (air sacs) and can be brought about by organisms, including bacteria, viruses and fungi (Aggarwal, 2016). Other respiratory tract infection include cystic fibrosis, chronic obstructive pulmonary disease (COPD) and pneumonia produces OMVs carrying virulence factors (Kahlon, 2016). *Moraxell catrrhalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoni* the most common microorganisms cause RTI (Griffith and Aksamit, 2016). *Foeniculum vulgare* (Apiaceae) commonly known as fennel is a well known and important medicinal and aromatic plant widely used (Rather *et al*., 2016). Fennel (*Foeniculum vulgare* Mill.) is a small genus of annual, biennial or perennial herbs distributed in central Europe and Mediterranean region (Foroughi *et al.*, 2016). Fennel (*Foeniculum vulgare* Mill.) is a biennial plant with a thick rootstock, erect, much-branched smooth, often 1 meter or more in height. Leaves are 2-, 3-, or 4-pinnate and about 20 centimeters long, the segments are filiform and 2 to 4 centimeters long. Umbels are 5 to 10 centimeters (Al-Snafi, 2018). Phenols, phenolic glycosides and volatile aroma compounds such as transanet hole, estragole and fenchone have been reported as the major phytoconstituents of this species Foeniculum vulgare and other plants were cultivated, but not on large scale (Liquorice *et al*., 2005). Other compounds fatty acids; and amino acids. Compiled data indicate their efficacy in several *in vitro* and *in vivo* pharmacological properties such as antimicrobial; antiviral; anti-inflammatory, antimutagenic, antinociceptive, antipyretic, antispasmodic, antithrombotic, apoptotic, cardiovascular, chemomodulatory, antitumor, hepatoprotective, hypoglycemic, hypolipidemic. (Badgujar *et al*., 2014).

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Materials and Methods

Collection

It was obtained from the local markets of the city of Nasiriyah in southern Iraq in November of 2019 and cleaned of impurities well and was ground into a fine powder using the electric mill, preserved in sterile glass bottles until use.

Preparation Volatile oils

The oils were isolated by hydro distillation using a Clevenger-type apparatus for 4 h. (Rabbani *et al*., 2011). Briefly, 30 to 40 g of the plant was introduced in the distillation flask (250ml), which was connected to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oil were released from the plant material and evaporated into hot steam. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system to condense the steam. The steam was applied for 4h. After settling the recovered mixture. Afterward, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the essential oil were done using dimethyl sulfoxide (DMSO). (Foroughi *et al*., 2016).

Gas Chromatography-Mass Spectroscopy

The analysis of the essential oil was carried out in a gas chromatograph (Bernuci *et al*., 2016). The EOs were filtered by a 0.45 µm filter membrane and subjected to gas chromatography-mass spectrometry (GC-MS) for analysis using a DB-17 MS capillary column (30 m × 0.320 mm, film thickness, 0.25 µm). A sample of 0.8 µL of EOs was injected manually, and the GC split ratio used was 10:1. Helium was the carrier gas at a flow rate of 1 mL/min. The mass-selective detector was operated in an electron-impact ionization (EI) mode with a mass scan range from m/z 50 to 550 at 70 eV. Injector and MS transfer line temperatures were both set at 250°C. The oven temperature was programmed as in the GC-FID analysis. (Xiang and Han, 2017).

Determination of antibiotics resistant and fruits voltiles oil activity

Diffusion methods were used to determine the sensitivity of isolates to 8 antibiotics mentioned in Blindness among gar Muller Hinton, as mentioned (Howell *et al*., 2015). A bacteriological population of isolates attended to test their sensitivity to antibiotics by transferring a small number of pure colonies at the age of 24 hours from the McConkey medium in (3) ml the physiological solution, the density was measured using a densichek meter and compared with a fixed turbid solution standard. (0.5 McFarland turbidity standards ^{8}10), Spread some of the microbial traces using a cotton swab on the surface of the Muller Hinton agar medium, Transfer by sterile forceps, several antibiotic tablets, the dishes were incubated at 37°C for 24 hours. The results were read by observing the inhibiting zones formed by the disk

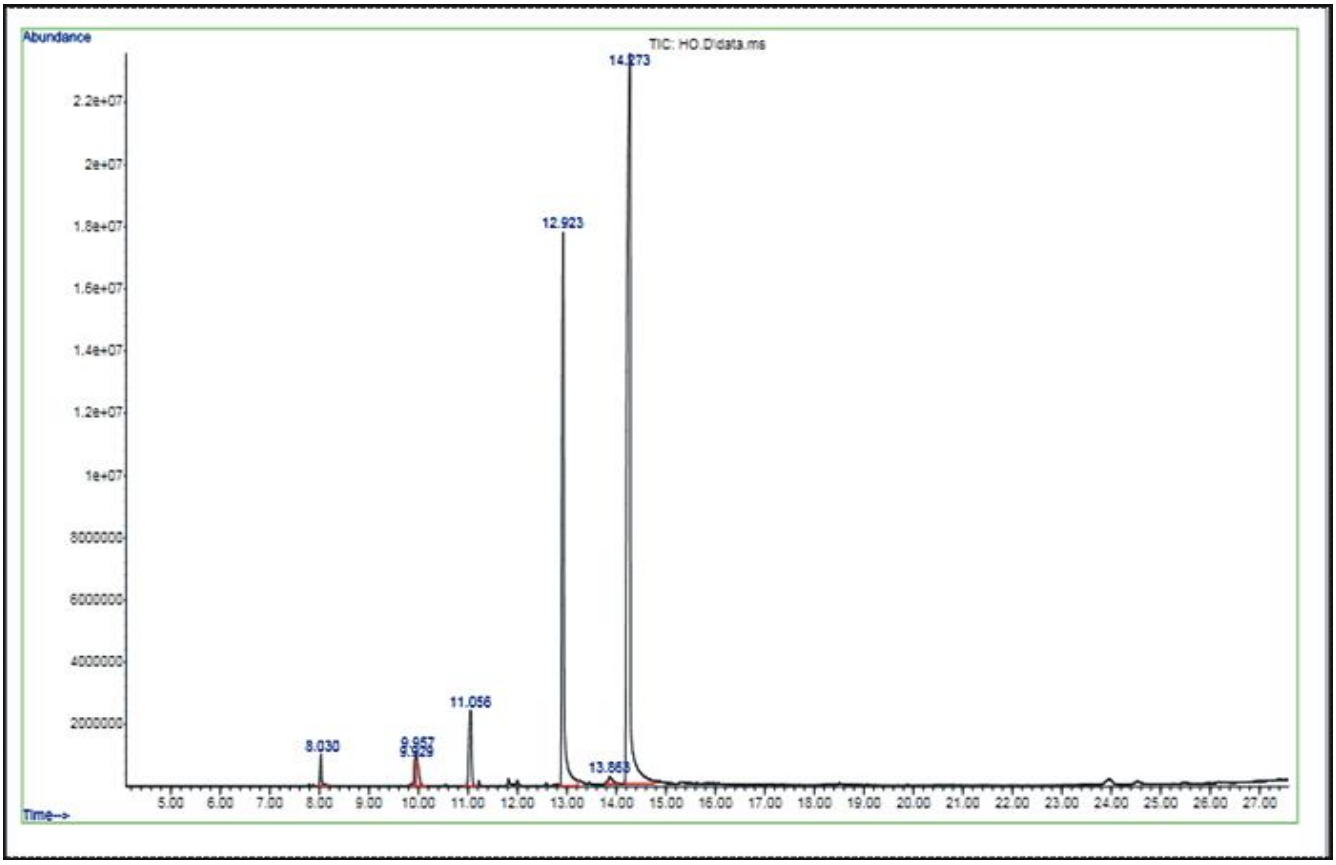

Fig. 1: Chromatogram of chemical compounds of volatile oils of *Foeniculum vulgare*.

Table 1: Chemical composition in the volatile oils of *Foeniculum vulgare* fruits using by Gas Mass Spectrometry analysis.

Peak	Chemical composition	Formula	RT	Area %
1	D-Limonene	$C_{10}H_{16}$	8.610	7.98
2	Fenchone	$C_{10}H_{16}O$	9.810	14.94
3	Estragole	$C_{10}H_{12}O$	11.804	20.78
4	Benzaldehyde, 3-methoxy-	$C_8H_8O_{12}$	12.727	1.92
5	Anethole	$C_{10}H_{12}O$	13.199	46.41
6	Eugenol	$C_{10}H_{12}O_2$	14.170	1.86
7	1-(4-Methoxyphenyl) propane-1,2-diol	$C_{10}H_{14}O_3$	16.942	1.67
8	1-(4-Methoxyphenyl) propane-1,2-diol	$C_{10}H_{14}O_3$	17.021	2.43
9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.049	2.01
	Total Area (%)			100

specifications (Tortora *et al*., 2010).

On Mueller-Hinton agar and punched with 6-mm diameter wells for the bacteria, respectively. Then 50 µl of plants extracts were added to the wells, while 10% DMSO was used as the negative control. The antimicrobial activity was estimated, after incubation of the plates at 37°C for 18 to 24 h, by calculating the diameter of inhibition zones (mm) (Al-Massarani and El-Dib, 2015).

Results

GC-MS chromatogram analysis appears numerous peaks, indicating the Organic compounds which classified by structural specification (Fig. 1) as following: were plentiful in terpenoids, fatty acids and other lipophilic compounds such as saturated and unsaturated free fatty acid, methyl and ethyl esters of saturated and unsaturated

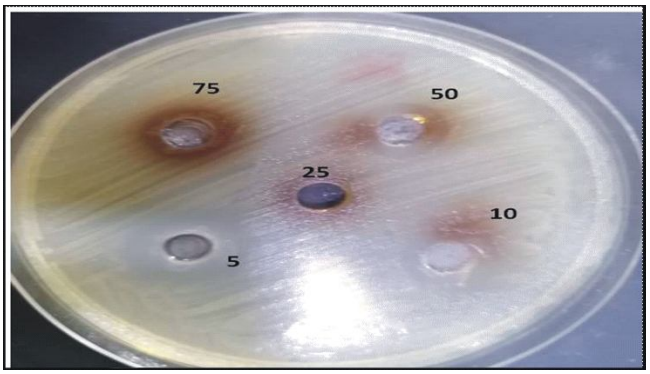

Fig. 2: Antibacterial activity of fruits of volatile oils of *Foeniculum vulgare*on *Klebsiella pneumoni.*

fatty acids, saturated triglycerides and diglycerides, sterols, triterpenes, mono- and sesquiterpenes, unsaturated monoglycerides, phytosterols and flavonoid compounds. result of GC-mass showed D-Limonene 7.98%, Fenchone 14.94%, Estragole 20.78%, Benzaldehyde, 3-methoxy- 1.92%, Anethole 46.41% Eugenol 1.86%, 1-(4-Methoxyphenyl) propane-1,2-diol 1.67%, 1-(4-Methoxyphenyl) propane-1, 2-diol 2.43 and n-Hexadecanoic acid 2.01 (Table 1). The chemical nature of compounds was identified on the basis of molecular formula, retention time (RT), molecular weight and compound name. These compounds were very effective against negative gram stain bacteria compared to the antibiotics used in the study.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The MIC and MBC values for antibacterial activity of *Foeniculum vulgare* fruits voltiles oil extract were presented in (Table 2) and (Fig. 2,3), inhibited the *K. pneumonia* were 3 mg/ml, while it was 50 mg/ml for

Table 2: The results of MIC and MBC Values of fruits of volatile oils of *Foeniculum vulgare*on pathogen isolates (At a probability level of 0.05).

Pathogens / Concentration mg/ ml		*K. pneumonia*	*E. coli*	*P.aeroginosa*	*M.catarrhalis*	P. value	LSD
		Inhibition Zone/ mm²(MBC)					
3	**Mean + S. Division**	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	<0.001	No Siginfiance
5		5.13 ± 0.2	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	<0.001	0.14
10		8.03 ± 0.2	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	<0.001	0.16
25		11.1 ± 0.5	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	<0.001	0.47
50		13.1 ± 0.5	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	<0.001	0.46
75		14.1 ± 0.1	8.10 ± 0.3	9.03 ± 0.1	6.10 ± 0.3	<0.001	0.39
100		16.2 ± 0.8	10.2 ± 0.2	12.1 ± 0.3	13.1 ± 0.3	<0.001	0.83
P. Value		<0.001	<0.001	0.001	<0.001		
LSD		0.76	0.27	0.23	0.26		
R		1.0**	0.845**	0.845**	0.845**		
Pathogens / Concentration mg/ml		**MIC/mg**					
		3	50	50	50		

Table 3: Results of antibacterial activity on pathogen RTI bacteria of fruits of volatile oils of *Foeniculum vulgare*on compared with antibiotics.

No	*Antibiotic*	*Klebsilla P.*	*E .coli*	*Pseudomonas aeruginosa.*	*MoraxellaCatrrhalis .*
1	Tobramycin	R=12.3	R=10.7	S=17.2	S=16.3
2	Ciprofloxacin	R=17.6	R=16.2	S=24.2	S=26.4
3	Ceftrixone	R=12.4	R=12.4	R=9.8	R=4
4	Cefepime	R=15.4	R=13.3	R=5.8	R=2.1
5	Gentamycin	S=15.7	S=15.6	S=16.8	S=17.5
6	Imipenem	S=23.2	S=25.1	S=24	S=25.8
7	Amikacin	IN=14.5	S=17.9	S=19.1	S=19 .7
8	Pipracillin	R=7.11	R=10.3	R=10.8	R=3.3
9	Voltiles oil extract 100mg/ml	16.2	10.2	12.1	13.1

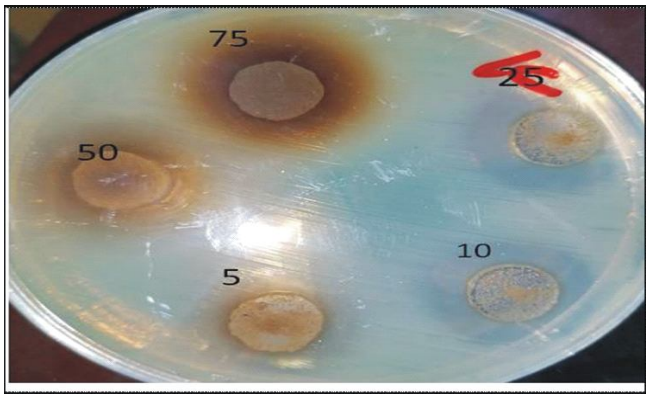

Fig. 3: Antibacterial activity of fruits of volatile oils of *Foeniculum vulgare* on *pseudomonas aeroginosa.*

both *E. coli*, *P.aeroginosa* and *M. catarrhalis* While MBC was 5 mg / ml for *K. pneumonia*, while 75 mg/ml for both bacteria *E. coli*, *P.aeroginosa* and *M. catarrhalis*.

Antibacterial Activity test

The antibacterial potential of *Foeniculum vulgare* fruits voltiles oil extract and eight antibiotics were tested against common gram negative respiratory tract pathogens (Table 3). After proper incubation the results were recorded and represented in (Fig. 2,3). The results showed that voltiles oil extract has broad spectrum activity against RT pathogens, the highest inhibition zone of voltiles oil extract was 16.2 mm of the *K. pneumonia* while there was low effect on *E. coli* was 10.2 mm..

Discussion

The development of resistance in bacteria is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones. The problem of antibiotic resistance, which has limited the use of cheap and old antibiotics, has necessitated the need for a continued search for new antimicrobial compounds. Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times mechanisms of natural adaptation to the presence of an antimic (Foroughi *et al*., 2016). The results of the analysis with the GC-Mass device showed that compounds in (Table 1). Anethole, estragole and fenchone are the highest in the extract of volatile oils and that these compounds are mainly responsible for the activity against the bacteria negatively for the gram stain, which causes respiratory diseases. These results were consistent (Diao *et al*., 2014). Essential oils are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties agreed with (Kaur and Arora, 2009). While he mentioned other compounds and attributed the reason for effectiveness against bacteria Some chemical constituents from *F. vulgare* have been identified as active antimicrobial principles such as a phenyl propanoid derivative – Dillapional was found to be the active antimicrobial principle of the *F. vulgare* fruit. Another molecule – Scopoletin which is a coumarin derivative has been isolated from *F. vulgare* and reported to possess marginal antimicrobial effect (Rather *et al*., 2016).

Conclusion

This study highlights that volatile oils extract of *Foeniculum vulgare* fruits has potential components which elicited their biological activities against RTI pathogens.

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