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ISOLATION AND BIOACTIVITY OF ESSENTIAL OIL OF ELETTARIA SP AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

The current study included volatile oil extraction from *Elettaria sp* plant and diagnosis composite Using some chemical spectral techniques such as Infra-Red spectrum (IR and Gas Chromatography Mass (GC\Mass) 34 compound were identified in essential oil extract of leaves of Elettaria sp and evaluated the antibacterial activity against six bacterial species *E.coli*, *Staphylococcus aureus*, *Psedomonas aeurginosa*, *Streptococcus pneumoniae*. *Klebsiela pneumoniae* and *Bacillus cerrus*, which showed the antibacterial activity of the against bacterial high and inhibion of different diameters ranged between (12.5-30.0mm) exception *Ps.erugenosa* was resistance for essential oil teste. The current study also included identifying Minimum Inhibition Concentration (MIC) to essential oil, Where was less minimum inhibitory concentration is ranged between (50-400µl).

KEYWORDS: *Elettaria sp*, Essential oil, Antibacterial, bacteria.

INTRODUCTION

Essential oils are the volatile liquids of the secondary metabolism of aromatic plants. They are termed “essential” because they represent the most important part of the plant. They are synthesized by all plant organs such as flowers, leaves, stems,[1] seeds,[2] barks,[3] fruits,[4] roots,[5] peels[6] and are stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes.[7,8] Essential oils are not limited to a particular class or family of plants but they are widely distributed in all plant kingdom. The essential oils are found in plants belonging to thefamilies .The essential oils are complex mixers comprising of many single compounds. Chemically they are derived from terpenes and terpenoids (isoprenoids) and aromatic and aliphatic aldehydes and phenols, all characterized by low molecular weight.[9] Each of these constituents contributes to the beneficial or adverse effects.[10] There are many methods of

extraction of essential oils. They can be obtained by steam distillation, mechanical expression, hydro distillation, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. During distillation, water condensate and is separated by gravity leaving a very small amount of volatile liquid that is the essential oil.[11] Due to their extraction procedure, they contain a variety of volatile molecules such as terpenoids, terpenes, aromatic compounds and aliphatic components.[12] Essential oils and their constituents significantly inhibit a wide range of microorganisms including human and phytopathogens, food spoilage and poisoning bacteria. Essential oils and extracts from several plant species are able to control microorganisms related to skin, dental caries and food spoilage, including Gram-negative and Gram-positive bacteria, fungi and viruses.[13,14,15] The essential oils are very well known for their bactericidal, bacteriastatic, virucidal, fungicidal activity due to their medicinal properties against the wide range of pathogenic microorganisms.[16] However, the spectrum of antimicrobial activity is dependent on the tested pathogens, measurement conditions and the source of the antimicrobial compounds.[17] Antimicrobial effects of different species of herbs and spices have long been known and used to increase the shelf-life of food. Thus the essential oils and their components, currently used as food flavorings are also known to possess antimicrobial activity.[18] Now-a- days, clinically important microorganisms are characterized not only by single drug resistance, but also by multiple drug resistance. It is now common practice to use a combination of two or more antibiotics with different mode of action in an effort to prevent the expansion of antibiotic resistance and improve the outcome of therapy[19] Sun *et al.*,[20] reported antibacterial activity of root essential oil of *Dictamnus angustifolius* against the *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Burt and Reinders,[21] analyzed the antibacterial properties of five essential oils bay (*Pimenta racemosa*), clove bud (*Eugenia caryophyllata, Syzygium aromaticum*), oregan (*Origanum vulgare*), red and light thyme oils (*Thymus vulgaris*) against *Escherichia coli* O157:H7strain. Oregano and light thyme essential oils had the potential of preventing the growth of *E. coli* O157:H7 in foods Sousa *et al,*[22] reported antimicrobial activity of essential oil and its four fractions from *Eugenia calycina* Cambess. leaves against oral bacteria. The results showed that essential oil exhibited strong antibacterial activity against anaerobic Gram-negative bacteria *Prevotella nigrescens* and *Porphyromonas gingivalis* and Fraction 3 and Fraction 4, composed of oxygenated sequiterpenes showed higher activity against all the bacteria.[23] Observed the anti-*Helicobacter pylori* effect of *Satureja bachtiarica* Bunge essential oil. The results showed that essential oil significantly inhibited the growth of clinical isolates of *Helicobacter pylori*.

The aim of the current investigation was to study the antibacterial activities *Elettariasp* essential oils against some pathogenic bacteria and chemical compounds of essential oil test.

MATERIAL AND METHODS

Plant material

Fresh leaves were collected during the flowering stage of *Elettaria* sp leaves came from the "Basra city" (city south of Iraq).

The test microorganism

Esherichia coli from urine, *Samonellal typhimuriumm,* from blood *Streptococcus pneumonia* from Throat, *Pseudomonas aeruginosa .Klebsiella pneumoniae* from burns and *Bacillus cerrus* were isolated from clinical cases in Sadder hospital and identified in their laboratories. The organisms were cultured on maintenance media until use.

Extraction of essential oils

The essential oil extraction process was conducted using the hydro distillation method. The extraction process was conducted for a period of 3 hours the hydro late (water and oil) the essential oil was then isolated with the aid of a n. Hexan, placed in a glass bottle and stored under refrigeration.

Antibacterial Assays

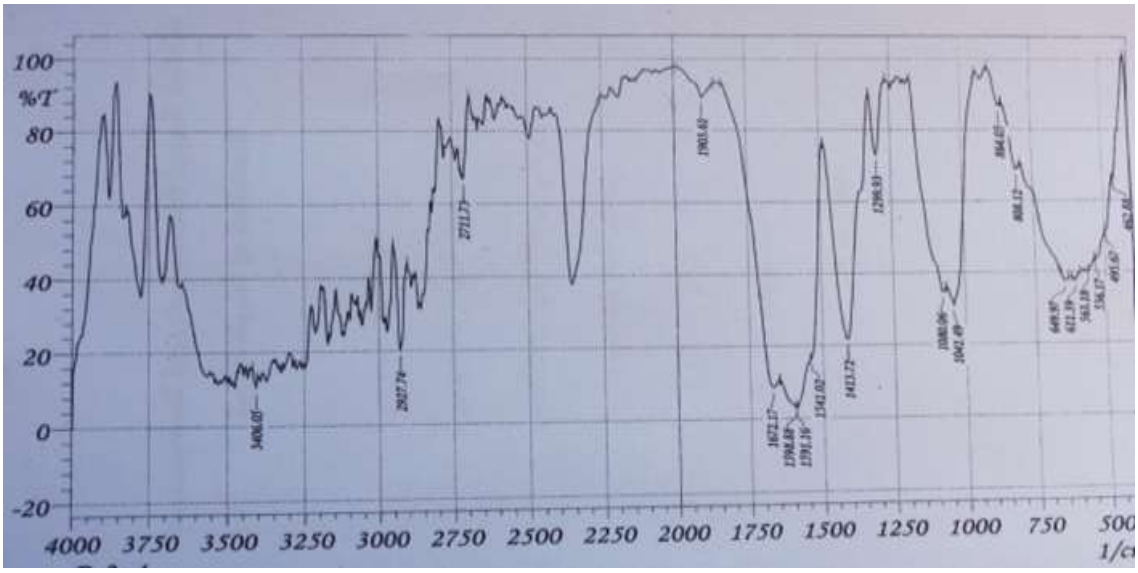
The antibacterial properties of the essential oils were done using the agar disk diffusion method.[15] Bacteria were grown in 20 ml nutrient broth at 37°C overnight. The cultures were then diluted to the McFarland No.5 standard (1.0×10^8 CFU/ml). Standard Petri dishes containing nutrient agar were then inoculated with the bacteria suspension (1.0×10^8 CFU/ml). Sterile paper disks (6 mm) were placed on the inoculated plates and 10 µl of 10 mg/ml of the essential oils in 1% DMSO were added to the paper disk. The plates were then incubated at 37°C for 24 hrs and the zone of inhibition measured.

Minimal inhibitory concentration and minimal bactericidal concentration test.

The minimal inhibitory concentration (MIC) values were determined by the standard serial dilution assay.[17] of the essential oil isolate was selected for this test. The inhibitory test was carried out on Muller-Hinton agar medium.

RESULT AND DISCUSSION

The results of chemical composition of the *Elettaria* sp leaf essential oil by IR (Fig. 5, Tab.1) and by GC-MS analysis (Figure 6, Tab 2). The active compounds with their retention time (RT), molecular formula and molecular weight (MW) in the essential oil of leaves of *Elettaria sp* re-presented in Table 2. 34 compound were identified in essential oil extract of leaves of *Elettaria sp*, Gas chromatography (GC) is a widely applied technique in many branches of science and technology for over half a century GC has played a fundamental role in determining how many components and in what proporation they exit in a mixture. however the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced and requires a spectroscopic detection system . the most used is the mass spectrometric decector (MSD) which allows obtaining the "fingerprint" of the molecule, I, e its mass spectrum provide information on the molecular weight elemental composition, if a high resolution mass spectrometer is used functional groups present and in some cases, the geometry and spatial isomerism of the molecule.



Figur 1: Infrared (IR) of essential oil *Elettaria sp.*

Table 1: Absorption bundles and effective synthetic groups belonging to her in the spectrum of infrared (IR) of essential.

Frequency cm-1	Functional groups
3517	N-H
3338	O-H
2923	CH2 , CH
1679	C=O
1573,1419	C=O
1350	C-O

Compounds appeared antibacterial activity against bacterial species arranged between (12.5-30 mm) figure 2 table 2 because contain on some in functional groups Mechanism action of essential oils varied from different part of plant and various active constituent present in them. Different compounds present in the essential oils have different mode of action and different biological effects, i.e. antibacterial, antifungal, antiviral and cytotoxicity, effects. There are some commonly accepted mechanisms of action of essential oil in the antimicrobial interaction. The mechanism of action of essential oil involves so many targets in the cell due to large number of active constituent. The antimicrobial actions of essential oils was the inhibition of a common biochemical pathway, inactivate microbial enzyme,[24] leaking of cell membrane and increased the membrane permeability.[25,26] Essential oils may disrupt the structure of different fatty acids, polysaccharides, and phospholipids layers present in the cell wall and cytoplasmic membrane.[27] Essential oils can cause disruption of membrane in microorganisms by the action of lipophilic compounds in the oils and thereby imparting antibacterial activity.[28]

Table 2: GC-MS of essential oil *Elettaria sp*.

Peak	R. Time	Peak Area	Area%	Name
1	5.831	2245644	0.60	Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methyl
2	6.422	1293890	0.35	3-Hexenoic acid,(E)-
3	6.833	11227585	3.01	Ecalyptol
4	7.071	1015506	0.27	Benzene acetaldehyde
5	8.870	9882210	2.65	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(1s)-
6	9.153	3215177	0.86	Benzeneproanal
7	9.238	681789	0.18	Bicyclo[2.2.1]heptan-2-o1,1,7,7 trimethyl-
8	9.609	1254867	0.34	3-Cyclohexene-1-methanol,Alpha.4-
9	10.199	2045877	0.55	2,3-Dihydro-benzofuran
10	10.427	12864652	3.45	4-phenyl-2-butanone
11	10,885	1894235	0.51	Cinnamaldehyde,(E)-
12	11.041	366311	0.10	Bicyclo[2.2.1]heptan-2-o1,1,7,7 –trimethyl-
13	11.323	628908	0.17	2-propenoic acid, 3-penyl1-,methyl ester
14	11.497	1547664	0.42	2-Methoxy-4-vinylphenol
15	12.343	607787	0.16	4-Epi-cubedol
16	12.456	5758227	1.54	2-propenoic acid,3-phenyl-,methyl estar,(z)
17	12.529	947072	0.25	2-Hepten-3-o1,4,5 –dimethyl-
18	12.873	1270547	0.34	2-(1,3-Dithian-2-yl)-1,5,5 –trimethyl-3-methyl
19	12.963	233296	0.06	1,6-Cyclodecadiene,1-methyl1-5-meyhylene-&-
20	13.031	1738968	0.47	1-Hydroxymethyl-2-methyl-1-cyclohexene
21	13.280	172140	0.05	2-furanmethanethiol,5 –methyl-
22	13.325	283642	0.08	Cis-.beta. –farnesene
23	13.945	846456	0.23	Cyclopropane carboxylic acid,2,2 –dimethyl
24	14.709	514301	0.14	1,6,10Dodecatrien-3-ol,3,7,11 –trimethyl-,(E)
25	14.861	1618070	0.43	Cis-Z-.alpha. –bisabolene epoxide

26	14.972	621474	0.17	9-Eicosene, (E)-
27	15.162	455187	0.12	(-)-5-Oxatricyclo [8.2.0.0(4,6)dodecane
28	15.324	5863438	1.57	3A(1H)-azulenol,2,3,4,5,8,8a-hexahydro-6,8A-D
29	15.394	3973843	1.07	Benzen,1,2,4-trumethoxy-5-(1-propenyl)-, (z)
30	15.690	1617751	0.43	Beta. –D-glucopyranose, 1,6-Anhydro-
31	16.166	2322736	0.62	2-Naphthalenemethanol
32	16.270	608336	0.16	Spiro[4.5]dec-8-en-7-one
33	16.957	1046848	0.28	Tetradecanoic acid
34	17.051	2473341	0.66	Tetracyclo [5,3,1.0e2, 6-0e8,11]undecan-4-ol,6-

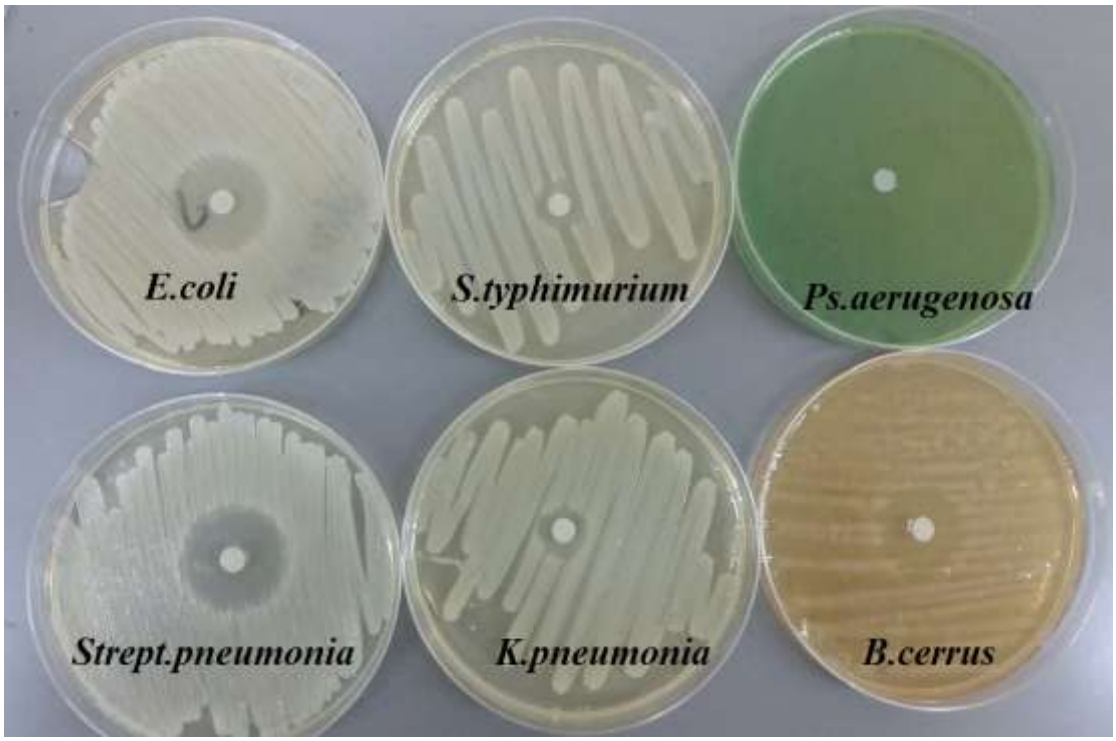


Figure 2: Iinhibion zone diameter.

Table 3: The Antibacterial activity of essential oil of *Elettaria sp.*

Bacterial isolates	Inhibition zone	MIC
E. coli	25	50
Sal. typhimurium	12.5	100
P. aerginosa	0	-
Strep. pneumoniae	30	250
Kleb. pneumoniae	12	100
B. cerrus	24	200

CONCLUSIONS

From the findings of this research, it is Conclusions that;

1- *In vitro* antibacterial activities of volatile oil extraction from *Elettaria sp* plant

2- Essential oil gave varying compounds.

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