Spectroscopic Analysis by GC-Mass Technique of Citrus Maxima leaves and Effectiveness of its aqueous extract on some Pathogenic Bacterial Isolates.

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Abstract

Citrus is an evergreen tree belonging to the family Rutaceae, mentioned in the Iraqi flora, and it is one of the plants with high medicinal values because it is used in folk medicines and is a source of many effective compounds. With interest by researchers in Iraq, a general detection of the active compounds of the ethanolic extract of Citrus maxima (Burm.) Merr leaves was conducted by GC-MS (Gas Chromatography Mass Spectrometry) method. In the plant, 13 chemical compounds were detected, which were used as an inhibitor of some species of pathogenic bacteria aeruginosa, Pseudomonas, Staphylococcous aureus, E. coli, proteus and Klebsiella, and the results showed its effectiveness as an antidote to some pathogenic bacteria. Where it reached 18 mm at a concentration of 700 mg/ml and Bacterba Klebsiella did not show effectiveness when it was used g 300 mg/ml. The results obtained in this study are due to the fact that it is possible to be among the experimental and promising treatments for bacteria or to be used as a complementary treatment instead of chemical treatments with harmful side effects for the body.

Keywords: chemical detection, Citrus maxima, gas chromatography, Mass Spectrometry.

1. Introduction

The citrus plant is one of the fruits that are rich in active compounds, and it belongs to the family Rutaceae, which consists of 140 Genus and approximately 1300 species, including bitter C.aurantium, C.sinesis and C.lemon,C.reticulata, C.paradisi, C.maxima, C.aurantifolia, C.limettioides and others [1, 2], Citrus fruits are a natural source of many active and important compounds that are considered effective natural compounds, including alkaloids, saponins, coumarins, sterols, terpenes, carbohydrates, phenols, essential oil, proteins and minerals, as well as natural antioxidants such as carotenoids, flavonoids, and vitamins in addition to the nutritional value [3].

Scientific Name Citrus maxima (Burm.) Merr. And the common name is Pomelo fruit in English: Pomelo, pumelo, or Sindhi shadock, Indian lemon or Sindhi large lemon. The Sindhi tree is characterized by being large, round in shape, reaching a height of 2-9 meters, with very large leaves. Leaves are 10-15 cm long, 5-8 cm wide, and oval in shape. Sharp at the top and rounded below, the flower is large, solitary, or can be found in axillary panicles, the ovary is spherical, and the fruit is very large when ripe and is spherical in shape or obliquely spherical or pear-like yellow in color when ripe The peel is thick and large and crumbles easily As for the seeds, they are wrinkled and yellowish, flowering season is in March and April [4]. It has been used as an antimicrobial, antioxidant, larvicide, anticancer, blood coagulant, vascular protector, antidiabetic and anti-inflammatory in folk medicine in several states [5, 6].

The pulp and peels of the fruits are used as a stomach tonic, treatment of inflammation and treatment of cough, and fruit juice has the ability to help lose weight and reduce cholesterol [7, 8].

2. Materials and Methods

1- Samples of Collection: C.maxima leaves were collected from different regions in three rounds from Diyala Governorate - Iraq (Baquba, Hoydar, Buhrz, Mandali, Shafa, Haji Sohail, Al-Abarah) in the month of April. Or any rupture, after that samples were taken, diagnosed and classified, and all these species belonged to one Genus and one family, and the classification was done by the supervisor, Prof. Dr. Ashwaq Talib Hameed, according to the "Iraqi Botanical Encyclopedia," the leaves were dried, then the leaves were ground by the electric grinder, and then placed in sterilized glass containers until use.

2- Chromatography-mass spectrometry analysis: The filtered plant samples extracted from alcohol were placed in a GC-MS device of the type (GC-MS-QP2010 plus (Shimadzu, Japan). Helium gas purity (9) is used to carry the gas at a constant flow rate of 1 ml (1m) per minute. , The temperature of the column starts from 80 °C and gradually increases every ten degrees by 10 °C until it reaches 280 °C, while the temperature of the heat source of the device is 350 °C, the initial temperature of the device is set at 80 °C and this temperature remains for two minutes. At the end of this period the temperature of the Oven is raised to 280°Cat an increase rate of 5°C per minute and remains for 9 minutes, the injection port temperature remains within 280°C and the flow rate of helium gas is l ml (1m) per minute and the ionization energy value is 70 ev (Electron volt), Separation was accomplished with Column 5MS heat for 30 minutes, Quadrupole mass detector was used to detect compounds through an opening in the column, the temperature of the detector was 280°C and the chemical compounds of the plant samples were compared with the

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chemical compounds stored in the Column Library Nist type chord connected to the GC-MS device.

3- Radical scavenging test using DPPH: The radical scavenging activity was tested according to the method described by [9], using DPPH (1,1-Diphenyl-2-picryl-hydrazyl) root, 0.04gm of it It was dissolved in 100 ml of absolute ethanol to reach a concentration of 400 µg/ml, and the tube was shaken using Vortex mixer and kept in clean test tubes and closed with aluminum foil to prevent photo-oxidation, while Ascorbic acid was prepared by dissolving as a positive control and a control sample 0.5 in 100 ml of (methanol 50 and water). 50) After that, different dilutions were prepared from each sample of plant extracts (4/1, 3/1, 2/1) (volume unit) with three replicates for each dilution, as 500 microliters were taken from each dilution and 500 substances were added to it. DPPH and complete the volume 2 ml by adding 1 ml of absolute ethanol and keeping it at a temperature of 37 °C for half an hour and then the color intensity was measured at the wavelength 517 nm compared with the control sample DPPH, the experiment was repeated three times for each sample and the average for the values of the replicates was found.

The percentage of free radical inhibition, DPPH, is calculated using the following equation

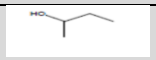
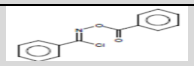
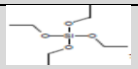
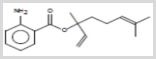
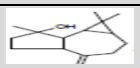
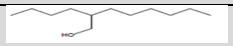
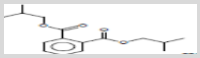
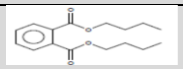
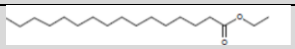
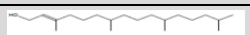
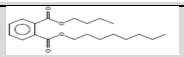
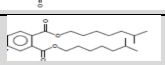
Inhibition= (A DPPH-A sample)/A DPPH x 100

Anti-bacterial activity: Citrus maxima leav powder was taken and distilled water was added to it, then it was mixed well and then left for 24 hours, then filtered by filter paper in a laboratory funnel to obtain the concentrations (700, 500, 300) mg / ml, the Using five types of bacteria obtained from the laboratory department of Ramadi General Hospital, they are Gram-negative Klebsiella,E.coli, Pseudomonas aeruginosa, Protus,Gram-positive and Staphylococcous aureus, the bacterial isolates were grown on media at a concentration of 0.5 ml/100 ml of each isolate. This process was done in sterile conditions, after the hardening of the media, the perforation process was carried out by a cork piercing and four holes were made in each dish that is prepared for each genus selected in the study from bacteria, then the four concentrations of the holes in the dishes for each species were added and three replicates were made Then these dishes were kept in the incubator for 24 hours at room temperature, after that the measurement of inhibition diameters in mm and tetracycline 15µg proved as a positive control [10].

3. Results and Discussion

The results of the chemical study showed the richness of the species Citrus maxima (Burm.) Merr. In the chemical content of plant chemical compounds, which were detected using GC-MS (Gas Chromatography Mass Spectrometry), a technique that helps in diagnosing chemical compounds, isolating species and classifying plants, and among the results of our study, an abundance of content in quantity and quality, the study concluded. The current group was reduced to 13 chemical compounds, including -Octadien-3-ol,3,7-dimethyl-,2-aminobenzoate and Hexadecanoic acid, ethyl ester, and 1,2-Benzenedicarboxylic acid, diisooctyl ester was the highest compound in terms of time. Butan-2 is the least compound in terms of time, as it reached 3.204.

Table (1) Chemical Compounds in species C. maxima.

No.	compound	Structural formula	Retention time	Height	Percentage
1	Butanol-2		3.204	825496	9.217
2	O-Benzoylbenzohydroximidoyl chloride		3.507	2046458	14.482
3	Tetraethyl silicate		5.441	1507065	9.079
4	1,6-Ocatadien-3-ol,3,7-dimethyl-2-aminobenzoate		9.004	77135	1.023
5	Tetradacane		10.929	168113	3.669
6	1H-CYCLProp[e]azulen-7-ol, decahYdro-1,1,7trimethylene-		13.348	323244	6.849
7	1-Octanol,2-butyl-		15.603	86059	0.739
8	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl)ester		16.413	265719	2.464
9	Dibutyl phthalate		17.350	503982	5.029
10	Hexadecanoic acid,ethyl ester		17.571	150743	1.341
11	Phytol		18.642	4186363	43.131
12	1,2-Benzenedicarboxylic acid,butyl ester		19.591	151481	0.934
13	1,2-Benzenedicarboxylic acid,diisooctyl ester		21.438	222799	2.043

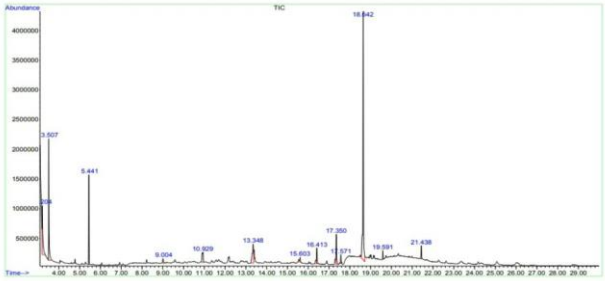


Figure 1. GC (chromatographic) mass spectrum of C. maxima.

The results showed that there were significant differences, between the rates of the diameters of inhibition, as it was observed in Table (2), that at the concentration of the extract 300 mg / ml, the lowest inhibition was in Klebsiella bacteria, meaning there was no inhibition due to the resistance of bacteria to this concentration [11, 12], while Inhibition was observed in bacteria. Staph. aureus, and it reached 5 mm, and the inhibition began to increase in the bacteria Proteus, where the inhibition reached 7 mm and the inhibition continued to increase and reached 8 mm in the E.coli bacterium, it reached a maximum of 10 mm the inhibition in the bacteria Ps. aeruginosa; While it was observed when the concentration of the aqueous extract of Sindhi was increased to 500 mg/ml, it showed clear inhibitory results in the diameters of inhibition. There is a close inhibition of 11 mm for three species of bacteria Staphylococcus aureus, E.coli and Proteus, as for Ps bacteria. aeruginosa and Klebsiella, they showed similar behavior in increasing their ability to resist the effect of the extract, which was more than the two previous ones, and it reached 12 mg; As for increasing the extracted concentration to 700 mg/ml, it was noticed that the inhibition diameters of the studied bacteria were increased by Ps. aeruginosa and Klebsiella showed almost similar behavior between them in the diameter of inhibition was 15 mm and then the diameter of inhibition increased gradually for the rest of the bacteria, it reached 16 mm in bacteria. Staph. Aureus, then continued to increase until the diameter of the inhibition reached 17 mm in E.coli and reached a maximum of 18 mm in Protus bacteria.

We conclude from the above that the aqueous extract of C. maxima leaves had an effect at a concentration of 300 mg/ml for the species of bacteria studied, except for Klebsiella bacteria, which had no inhibition because of their resistance to the concentration, while this extract showed a positive increase in inhibition at concentrations (500, 700); The results of this study indicate that the aqueous extract has an effective effect against the growth of the species of bacteria used, and the effectiveness of this extract lies because it contains effective compounds that have an anti- and inhibiting effect on the growth of microorganisms, which gives it the character of a natural antibiotic for some inflammatory disease conditions that affect humans and that by increasing the concentrations of the substance Effectiveness leads to a direct increase by eliminating microorganisms [13].

The results obtained using the aqueous extract had a noticeable effect on the species of bacteria that were used in the study, and this may be due to the effect of phenols, flavonoids and tannins, which were characterized by their functional properties against microorganisms, including bacteria [14], or this explains the effect of the aqueous extract on Bacteria within its effect on the walls of bacteria and their penetration, and then damage to the cytoplasm and thus cell death [15] and the active substances that were recorded in C. maxima leaves Figure (2) have an inhibitory effect because they are complexes with the outer proteins of the cell wall, and types that are affinity for lipophilic flavonoids and thus lead to the destruction of cell membranes [16], suddenly the active substances against bacteria such as phenols, tannins, flavonoids and alkaloids, which were in a high percentage in sandy leaves, including the detection of chromatografie GC-MS Pandey), if in the end these extracts were able to dissolve the components in the plant Which affects the growth of bacteria by affecting the cell wall and penetrating it, or these extracts to This is related to bacterial enzymes and their effect on the DNA strand, cell ribosomes, and various activities in bacteria.

Table (2) Effect of aqueous extract of Sindhi leaves on pathogenic bacteria measured in mm.

	Bacterial isolates				
Concentration in mg/L	Ps. aeruginosa	Staph. Aureus	E.coli	proteus	Klebsiella
300	10	5	8	7	0
500	12	11	11	11	12
700	15	16	17	18	15
Tetracydclin 15 µg	9	10	7	9	11
LSD 5%	1.0223				

Our study agreed with Hindi et al. [17] and a study on the leaves of Citrus aurantefolia against types of human pathogenic bacteria, including Staphylococcus aureus, Proteus and Escherichia coli by spreading method (Will assay). Pandey et al. [18] increase the biological activity of oranges; It is due to the fact that it contains active substances with high activity and an excellent antioxidant that works on a defect in the work of enzymes associated with the wall of microorganisms and the loss of their physiological role and thus the death of the pathological bacterial cell. limetta against bacterial isolates of Pseudomons. And Escherichia showed anti-bacterial activity with inhibition zone diameter of 10, 12, 10 and 10 mm for the ethanolic extract and 8, 9, 8 and 9 mm for the aqueous extract [19].

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