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Original Research Paper

Chemical Study and Antimicrobial activity of *Citrus japonic* Leaves of Rutaceae Using GC-mass Technique

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Abstract: The aim of the research was to investigate the chemical compounds of *citrus* japonica leaves by GC-mass, the analysis result was the appearance of 11 curves (top) as shown in Figure (1), and Table (1) shows that the highest percentage of area was for the compound 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate(54.97%),while compound,4,6-di(1,1-dimethylethyl)-2-methyl- appeared with the lowest area(2.41%.)The Biological activity of C. japonica at methanol-acetone extraction leaves, the concentration (100 mg/ml) the lowest inhibition was 2.63 mm, while the highest inhibition at Conc. (500mg/ml) which was 17.67mm. In other hand E. coli was more sensitive than other bacteria (Staph.aureus, *P.aerognosa* and E.faecalis) also observed in this study the *Japanese orange* showed high antioxidant activity towards DPPH, at concentration(1.0mg/ml) reached (77.950%). While the lowest rate (73.091%). at concentration at (0.25mg/ml) .

Keywords: *citrus japonic*, biological activity , antioxidants , GC- Mass, methanol-acetone, secondary metabolite

1. Introduction

Human interest in plants since God created him and realized their importance in his life, as he took them as a natural source of food and medicine, also determined the beneficial and harmful [1]. Plants are a source of chemical compounds that can be used in medicine and for other purposes, as plants contain many active and bioactive compounds, such as alkaloids, tannins, volatile oils, resins, phenols and flavonoids, which are deposited in their specific parts, such as leaves, flowers, bark, seeds, fruits, roots and others [2]. Dosoky found the genus of *Citrus*, *Citrus* L. Among those plants that contain many secondary metabolic compounds and bioactive substances, they are rich in vitamins, essential oils and antioxidants [3].

and perhaps the most famous compounds found in them are limonene Pharmacologically, many plants possess [4]. Bactericidal, viral, and fungicidal activities; they are used in mummification, in food preservation, and have anti-inflammatory, antimicrobial, antispasmodic, sedative, analgesic, and local anesthetic activities [5] .

Many plant species have been reported to have pharmacological activities attributed to their plant components such as glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes [6]. To date, herbal remedies have been documented as a vital source for the discovery of new pharmaceutical molecules that have been used to treat serious illnesses [7]. The Searching and excavation were one of his priorities [8]. The diagnosis and classification of these plants and identify each type of them by studying their anatomical and chemical qualities in the process of separation and discrimination, and among the most important tropical non-climatic plants are citrus, which are consumed throughout This is due to its delicious flavor as well as possessing a wide range of other nutritional benefits [9].

Many species of the Rutaceae family are known and used in treatment and medicinal purposes for more than three thousand years, the area of origin of citrus fruits is Indochina and southern China [10]. The Rutaceae family is a family of flowering plants, including shrubs and woody trees, as well as many perennial herbaceous plants, consisting of more than 160 genera and about 2070 species, and spread throughout the world, especially in temperate regions, and the tropics, a number of these plants in particular the species of the genus Citrus L. They are important food crops, some of which are grown as ornamental plants in gardens, the seeds are endosperm that are rich in oils [11]. The citrus genus Citrus L. One of the most popular global fruit plants, as it contains active phytochemicals that maintain human health, it is rich in vitamin C, folic acid and potassium [12,13].Hesperidium Its oil glands are also used in the manufacture of excellent and rare perfumes, while leaf and fruit oils use materials that improve taste and smell such as the manufacture of liquid and dry detergents, perfumes, sanitary paper and liquid and dry cosmetics, as well as in food industries such as sweets, biscuits and juices [14].

Biological activity and Antioxidants

Free radicals are naturally produced in human body as a result of normal metabolism, different endogenous processes or exposure ,their high reactivity as an oxidant and inhibitor of enzymes, they lead to the biomolecules oxidation such as protein, lipids, DNA and amino acids which finally result into cell damage and consequently cell death [13]. Thus, for a proper physiological function in the body, free radicals and antioxidants need to be balanced to avert oxidative stress,. Antioxidant phytochemical exist widely in fruits, vegetables, cereal grains, edible accruing, microalgae, and medicinal plants [15].

2. Methodology

Plant specimens' collection Plant Specimen Collection

This study was carried out on samples of citrus orange leaves that were collected locally and during the month of November and December 2023, and the selection of good leaves and not infected with fungal or bacterial diseases was taken into account and placed inside paper bags and the name of the sample, the date of collection of the sample and the place of taking the sample were recorded on them, then these samples were transferred directly to the Life Sciences Laboratory at the College of Education for Girls / University of Kufa in order to conduct chemical tests.

Preparation of Methanolic-Acetone citrus plant *leaves extracttion*

Harborne [16] prepared cold alcoholic extract of citrus leaves based on the method washed leaves distilled with distilled water to get rid of dust residues and other plankton and then dried at room temperature, grinded dry leaves with an electric grinder and then took from each sample 5.0 g of dried leaf powder and added to it 2 ml of acetone and 4 ml of methanol alcohol (99.99%) and placed in a test tube and closed tightly and left the tubes for 24 hours in Vibrating incubator at laboratory temperature and then filtered. GC-Mass solution by means of filter papers and then take the filtrate for the examination of chemical compounds with the technique of GC-Mass.

GC- Mass Chemical Compound Inspection

The chemical compound examination analysis of the extract prepared according to the previous paragraphs was carried out in the GC-MS Unit / Ibn Al-Bitar Laboratory / Ministry of Industry / Iraq using the Agilent system (7820A) USA GC MS Spectrometer and gas chromatography connected to the mass spectrometer (GC-MS) and applying the following analysis conditions: Gas Chromatography Device Type: Agilent (7820A) USA GC- Spectrometer MS, Analysis and separation column: (length 30 meters × diameter 250 μm× inner diameter 0.25 μm), Use of helium gas 99.999% as a carrier gas with a constant flow of 1 ml/min. The volume of the injected sample is 1 μ liter. Pressure 11.933 psi Programmable temperature 250°C for injector, 310°C for ionic source. Injection Type: Splitless Furnace temperature is programmed from 80°C to 180°C, 8°C/min, from 180°C to 280°C by 8°C/min, and from 280°C to 300°C by 3°C/min. The total operating time of the GC device is 32 minutes.

Characterization of crude chemical compounds

Based on the results of the mass spectrometry of the unknown component, the chemical compounds were diagnosed by comparing them with the data recorded and approved by the National Institute of Metrology and Technology through the identity of the compound, its molecular weight, and its formula National Institute of Standards and. Technology (NIST) Structure and Molecular.

3. Results and discussion

Detection of Active Chemical Compounds in Citrus japonica.

Figure (1) shows the curves (peaks) of the C. japonic japonic plant (raring), as the figure shows the emergence of 11 curves (peak), and Table (2) hows that the highest percentage in terms of area was for the compound 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate, reaching 54.97%, while the compound Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl- appeared with the lowest area percentage, reaching 2.41%.

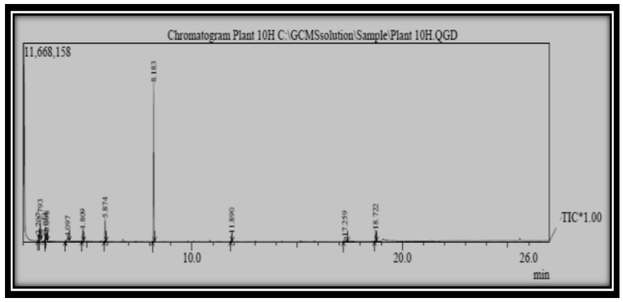
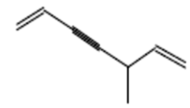
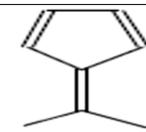
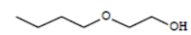
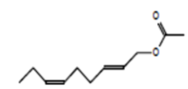
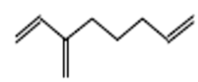
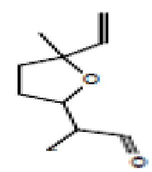
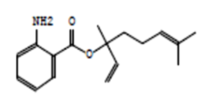
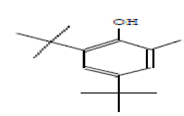
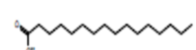
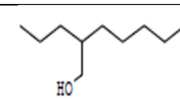


Fig 1: Curve of chemical compounds of extract (methanol-acetone) for C. japonic leaves using GC-Mass technique.

Through the above results and the difference in the emergence of compounds between the studied species, as well as the difference in the emergence of the number and type of compounds from other studies of the same genus of citrus due to the different use of solvents, each solvent has its own distinctive characteristics, as well as many effective chemical compounds associated with the emergence of the type of solvent used in the extraction process, there are compounds with high polarity, non-polar compounds, compounds with high affinity for alcoholic solvents and others with affinity for other organic solvents, and this converges with what Baeshen he proved that during his experiment in which he used several solvents to extract chemical compounds from plants, he found that each solvent that appeared in compounds did not appear in other solvents, as well as the number of compounds differed according to the solvent used[17]. These reasons also converge with what Indonesia found where it was found that the type of solvent has an impact on the number, quality and nature of the extracted compounds, as it was found that methanol is the best type of solvent used in its study [18].

Table 1: GC-Mass Analysis of Methanol-Acetone Extract of C. japonic Leaf Leaves

NO	Name	Peak No.	R.T.	Area %	Classification	Mol. Weight	Mol. Formula	Structure
1	**1,6-Heptadien-3-yne, 5-methyl-**	**1** **3**	**2.707** **3.034**	**2.56** **2.10**	**Ketone**	**106**	**C_8H_{10}**	
2	**1,3 Cyclopentadiene, 5-(1-methylethylidene)-**	**2**	**2.793**	**10.51**	**terpenoid**	**106**	**C_8H_{10}**	
3	**Ethanol, 2-butoxy-**	**4**	**3.098**	**2.55**	**Alcohol**	**118**	**$C_6H_{14}O_2$**	
4	**trans,cis-2,6-Nonadienyl acetate**	**5**	**4.097**	**3.38**	**Terpenoid**	**182**	**$C_{11}H_{18}O_2$**	
5	**1,7-Octadiene, 3-methylene-**	**6**	**4.809**	**4.98**	**Terpenoid**	**122**	**C_9H_{14}**	
6	**Lilac aldehyde C**	**7**	**5.874**	**8.68**	**aldehydel**	**168**	**$C_{10}H_{16}O_2$**	
7	**1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate**	**8**	**8.183**	**54.97**	**hydrocarbons**	**273**	**$C_{17}H_{23}NO_2$**	
8	**Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-**	**9**	**11.890**	**2.41**	**Phenol**	**220**	**$C_{15}H_{24}O$**	
9	**n-Hexadecanoic acid**	**10**	**17.259**	**3.42**	**fatty acid**	**256**	**$C_{16}H_{32}O_2$**	
10	**1-Heptanol, 2-propyl-**	**11**	**18.722**	**4.45**	**Alcohol**	**158**	**$C_{10}H_{22}O$**	

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Cendrowski found that when using several solvents to extract chemical compounds, they found that the best solvent for extracting phenolic compounds in high proportions is the use of acetone as a solvent [19].
In the extraction process, solvent selection is an important point, as the solvent will affect the chemical composition and functional properties of the extract as well as the efficiency of the extracted compounds [20]. Also, the difference in the presence of compounds may not only be due to the type of solvent used in extraction, as the environmental conditions surrounding plants may have a significant impact, as [21] found when studying extracts of some types of citrus the effect of environmental conditions such as light and temperature on the oxidative stability of secondary metabolic compounds in citrus. It was found that flavonoids, terpenoids, phenols, limonids, alkaloids and essential oils are among the most important secondary metabolic compounds in citrus fruits, in addition to that each part of citrus fruits contains a different composition of secondary metabolic compounds [22].

These results are consistent with what Singh found in the GC-MS analysis of the leaves of the plant of the genus citrus, as this analysis revealed the presence of many active chemical components such as esters, hydrocarbons, fatty alcohols, ester compounds and flavonoids [23]. Oyeniran instigate that Citrus limon leaf extracts are rich in alkaline substances [24].Analysis of GC-MS by Mohammed of citrus paradise, Citrus aurantifolia and Citrus sinensis showed that monoterpene and tetraterpene sesquiterpene hydrocarbons occupied the bulk of the chemical composition of plant extracts of the three species [25].

Biological activity

Type (C. japonica) at the concentration (100 mg/ml) the lowest inhibition was 2.63 mm , while the highest inhibition at Conc. (500mg/ml) which was 17.67mm.thus the respectively difference. In other hand E. coli was more sensitive to the extract 10.21mm with significant difference among the lowest inhibition was Saph.aueus at the diameter of the inhibition 70.08 mm followed E.faecalis (7.17 mm) diameter area so both non-significant (Table 2). The interaction bacteria and the concentration, was 500gm/ml with E. coli of the highest inhibition area 19.67 mm as the respectively difference to the lowest was the control as followed with 100mg/ml (0.00) mm for bacteria (Staph.aureus and E.faecalis) (Table 2) . The amount of hydroxyl groups found in the phenols are related to their relative toxicity towards microorganisms , with evidence that increased hydroxylation is directly proportion to toxicity and It was also reported that more highly oxidized phenols show more inhibitory activity, Many active compounds were produced by plants which contained these active groups (secondary metabolites). [26]. Also, carboxylic acids were found to be linked with many antimicrobial and antifungal activities which are found to exist in various plant metabolite molecular structures, which had been reported as a strong antibacterial agent [5] . The Citrus limon has biological activity of ethanolic plant extract might be due to multiple compounds including phenolic, terpenoids, and alkaloids, which it the most severely damaged when used [27].

Table2: Inhibition zones (mm) of C. japonic (methanol-acetone) leaves extract.

Conc. Of extract(mg/ml)	*Staph.aureus*	*E.coli*	*P.aerognosa*	*E.faecalis*	Mean of Conc.
500	16.00	19.67	19.00	16.00	17.67
250	12.33	15.67	15.33	12.67	14.00
100	0.00	5.50	5.00	0.00	2.63
control	0.00	0.00	0.00	0.00	0.0
Mean of Bacteria	7.08	10.21	9.83	7.17	34.33
L.S.D. 0.05 Conc. = 1.381, Bacteria = 1.381, Interaction = 2.579					

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Antioxidant

It is observed in this study The type (Japanese orange) showed high antioxidant activity towards DPPH, where the highest rate at concentration) reached (77.950%). While the lowest rate at concentration was (0.25ppm) where it was (73.091%). Compounds were isolated from leaves which have the highest concentration of volatile oils this leads to more scavenge . phenol was also reported that phenolic compound are effective hydrogen donor, making them very good antioxidant [5, 26].

Table 3: Antioxidant percentage

_Conc. (mg/ml)	***C.japonic %***
0.25	73.091
0.50	76.365
1.00	77.950
Mean	**75.802**
LSD = 1.009	

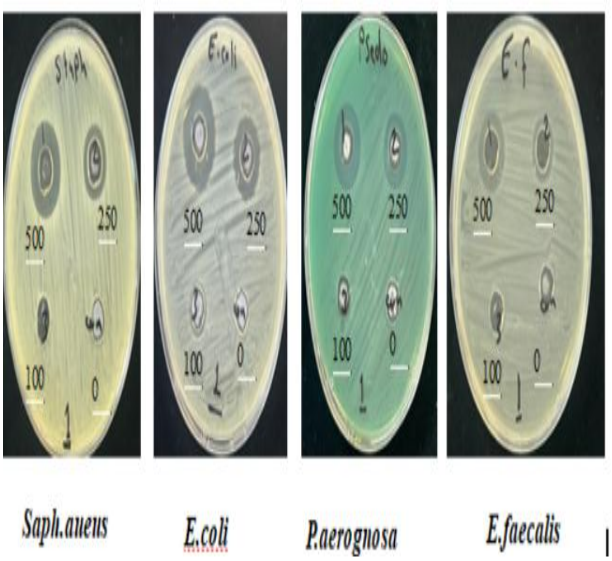

Fig 2. Antibacterial activity of Methanol-Acetone Extract of *C. japonic*

Conclusion

The GC-Mass Analysis of Methanol-Acetone Extract of C. japonic leaves ,their constituent of phytochemicals including phenols ,triterpens and Alchohol , the antibacterial activity of Methanol-Acetone Extract of C. japonic against G+ve and G-ve , showed higher inhabtion zone against MDR of G-ve E.coli , P.aerognosa and E.faecalis than the G+ve Saph.aueus. In our study the arngment depending on the persentage free ridical scavenge as the high Conc. .The antioxident effects of extract leaves.

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