Chemical Composition of essential oil and antibacterial Activity against some pathogenic bacteria of Citrus aurantium leaves extracts

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**ABSTRACT:** This study was conducted in 2018-2019 Aleppo City, Syria. The volatile oil extracted a gas chromatography device attached to the unit mass. The results showed that the yield of Citrus aurantium was 1.92% (fresh peel). The diameters of the aura of inhibition zone ranged between 32 mm against staphylococcus aureus and 18mm against Pseudomonas aeruginosa for the Ethyl acetate extract and from 22mm to 14mm respectively for the same studied bacteria for the aqueous extract. The antibacterial activity of Citrus aurantium leaf extract was evaluated and the ethyl acetate extract showed greater efficacy than the aqueous extract.

**Keywords:** Citrus aurantium, essential oil, antibacterial activity.

**I. Introduction**
There are many plants have an inhibitory effect against pathogenic bacteria to find resistance to them compared to antibiotics used as a treatment for these pathogenic agents [1]. Increased resistance of microorganisms (bacteria, viruses and fungi) to antibiotics has led to search for new sources such as natural products to counteract the effect of drugs in the killing, inhibition and spread of pathogens with the least cost and possible side effects [2]. The efficiency of these extracts varies according to the method of their extraction, the type of solvent used for the extraction and the experimental microorganism) [3].

Citrus aurantium which has many uses, especially the use of medical purposes because it contains many active compounds, so was selected on the basis of its use in folk medicine.
as rich in effective substances against pathogenic bacteria.
This study aimed at to prepare the ethyl acetate and hot aqueous extracts of it and tested its effect on some bacterial isolates, and to be used as an alternative to antibiotics used in the treatment, which show bacterial high resistance to them [4]

Many active phytoconstituents such as flavonoids have a large spectrum of biological activity including antibacterial, anticancer, antiviral, and antifungal. [5, 6]

Parts used from it: fruits, peels and flowers.
The chemical composition of Citrus aurantium:
The fruits, peels and flowers of the Citrus aurantium contain many chemical compounds of medicinal and application interest because they contain a large group of acids, the most important of which are:
Acetic acid, ascorbic acid, benzoic acid, formic acid, Geranic acid, citronellic acid, Malic acid, Methanol, Ethanol, terpineol, acetaldehyde: tannic acid, Cinnamic acid, hesperidin, geraniol, furfurol, citronellal, citronellol, citral, Naringin, Naringenin, and limonene [7].

This plant grows naturally in India and is widely cultivated in the subtropics. Its most important commercial sources come from Spain, Sicily, and hot regions in South America [8].

Its peels are used as a stomach medicine and repellent gas and as an insecticide, while the oil extracted from flowers and leaves is used as a treatment for skin diseases [9] and nervous conditions[10].

Its fruits are used in Chinese medicine to remove fat and reduce weight by increasing heat generation in muscles without stimulating the central nervous system, which leads to burning a large number of calories [11].

Its fruits also release fatty acids, as it contains alkaloids at a rate of 0.8%. It is believed that there are no side effects of the alkaloids of the Citrus aurantium because these alkaloids do not reach the brain due to their high specialization towards fat cells [12].

Citrus aurantium is used as an anti-insomnia and an anti-seizure, as a disinfectant [13], hypnotic and restorative of skin cells [14].

The aim of this study was to determine the antibacterial activity against some pathogenic bacteria

2. Materials and methods

2.1. Sample preparation

Fresh, fully ripe fruits were picked from citrus trees grown in home gardens in Aleppo.
The collected plant samples were classified according to the most important available fluorescence [15].

Table No. (1) shows the scientific and Arabic names of the plant, the place of collection

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Place of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus aurantium</td>
<td>Bitter orange</td>
<td>Aleppo</td>
</tr>
</tbody>
</table>

2.2. Preparation of plant extract

The dried leaves were crushed very finely, and 50 g of plant powder was soaked with about 250 ml of solvent for 24 hours and three times in a row, in 1-liter opaque flasks, the extract was filtered and the solvent was evaporated each time at a temperature less than 50 °C and under pressure. Diluted with rotary evaporator to obtain dry extracts.
The percentage of each extract was calculated.
by dividing the dry extract by the weight of the plant powder multiplied by 100.

2.3. The antibacterial activity of bacteria by the Agar Well. Diffusion Method
A bacterial suspension was prepared for the studied microbial species (staphylococcus aureus, Pseudomonas aeruginosa) at a concentration of 0.5 McFarland (1.5 x 10^8 cfu / ml). Muller-Hinton solid medium was inoculated with the studied bacteria. Wells of 8 mm diameter were made in each plate and 100 µl of extract was added. 10% in each one using sterile pipettes. Placing dishes in the fridge for two hours in order for the extract to diffuse in the agar and then incubating at a temperature of 37 °C for a whole day. The inhibition activity of the extracts was then determined by measuring the diameter of the area of inhibition formed around the pelvis in mm

2.4. Extraction of essential oil
The fruits were washed with water to remove dirt, and then peeled with a sharp knife manually. 100 g fruit peels were placed in a 1000 ml round-bottom distillation flask, and the plant material was wetted with 500ml distilled water. The essential oil were obtained by hydro-distillation using clevenger-type apparatus for 3 h. The oil were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5°C prior to analysis [16].

3. Results and Discussion
3.1. Citrus aurantium classification and description:
Citrus aurantium
common name: Bitter orange
The scientific name: Citrus aurantium
the family: Rutaceae

A tree up to 10 meters high, with dark leathery leaves, white flowers with a pleasant aromatic scent
The fruits are spherical, orange-red, rough to the touch, and sour taste.

3.2. Preparation of plant extract:
The percentage of the extracts ranged from dry weight 9.1 by ethyl acetate extract and 11.3 by aqueous extract
This increase can be explained by the high percentage of active substances in the plant

Table 2 shows the percentage of each extract from the dry weight of plant powder (50g).

<table>
<thead>
<tr>
<th>Citrus aurantium</th>
<th>Water extract</th>
<th>Ethyl acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>Weight (g)</td>
<td>Ratio</td>
</tr>
<tr>
<td>11.3</td>
<td>5.32</td>
<td>9.1</td>
</tr>
</tbody>
</table>

3.3. Test the antibacterial activity of bacteria by the Agar Well. Diffusion Method:
The ethyl acetate extract outperformed the aqueous extract with respect to both types of bacteria which were studied
The diameters of the aura of inhibition zone ranged between 32 mm against staphylococcus aureus and 18mm against Pseudomonas aeruginosa for the Ethyl acetate extract and from 22mm to 14mm respectively for the same studied bacteria for the aqueous extract.

This discrepancy can be explained by the different Solvents which were used.

3.4. Extraction of essential oil:
The results showed that the yield of C. aurantium was 1.92% (fresh peel).
The results of the current study converged with that of the researcher Al–Naser et al study in Syria in 2017 [17].
4. Conclusion:
The leaves extracts of C. aurantium clearly had inhibitory effects on the studied bacteria. We observed a higher rate of inhibition of ethyl acetate extract over the effectiveness of the aqueous extract. Based on these results, we recommend expanding the study of the anti-efficacy of these extracts against other pathogenic bacteria and fungi and purifying the active substances for use as a safe alternative to chemical antibiotics.

5. References:
[7].Agricultural Research Center (2003), Cultivation and Production of Citrus in the Valley and Delta Lands, Central Administration for Agricultural Extension, Horticulture Research Institute, Arab Republic of Egypt, Bulletin No. 850

[17]. Al–Naser, Z., Al-Abrass, N., & Al-Masri, M. S. Chemical Composition and Antifungal Activity of bitter orange (Citrus aurantium L.) essential oil against plants pathogenic fungi.
التركيب الكيميائي للزيت العطري والنشاط المضاد للبكتيريا ضد بعض البكتيريا المسببة للأمراض من مستخلصات أوراق النارنج

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الخلاصة: أجريت هذه الدراسة في 2018-2019 مدينة حلب، سوريا. تم تحليل الزيت الطيار المستخلص من قشور ثمار النارنج الكاملة النضج والطارجة وأظهرت النتائج أن نسبة الزيت كانت 1.92% على أساس الوزن الرطب.

تم تقييم الفعالية المضادة للجراثيم لمستخلص أوراق النارنج وأظهر مستخلص أوراق النارنج فعالية أكبر من المستخلص المائي حيث تراوحت أقطار هالة منطقة التثبيط بين 32 مم ضد المكورات العنقودية الذهبية و18 مم ضد الزانفة الزنجبيلية لمستخلص أسيات الإيثيل ومن 22 مم إلى 14 مم على التوالي لنفس البكتيريا المدروسة للمستخلص المائي.

الكلمات المفتاحية: النارنج، الزيت الطيار، الفعالية المضادة للجراثيم
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