

Evaluation of the Effectiveness of Essential Oils Derived from Citrus Aurantium Peels in the Treatment of Urinary Tract Infections

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ABSTRACT

Urinary tract infections (UTIs) caused by resistant bacteria are one of the most common infections in societies, which become difficult to treat with antibiotics. In recent decades, researchers have been exploring and using plant secondary metabolites as alternative antibiotics. Therefore, the current study aimed to extract essential oils of Citrus aurantium peels by using the Clevenger device and identify their chemical compounds using GC-MS analysis. Results revealed the presence of 23 compounds, and d-limonene was the most abundant compound, recorded (86.65%), while Cycloheptane, 4-methylene-1-methyl-2, was the least abundant, recording (0.04%). Then, 370 urine samples were collected from patients suspected of having urinary tract infections for both sexes, and 149 bacterial infections were diagnosed that showed high resistance to antibiotics through sensitivity testing. The infection rate was higher in women than in men, recording 50.33%, while in men it was (49.66%). Gram-negative bacteria were more prevalent than Gram-positive bacteria, recording 57.71%. Pseudomonas aeruginosa was the most prevalent compared to other species. The effectiveness of three concentrations of essential oils was evaluated. The 75% concentration showed the highest inhibition rate, recording (16.667 mm), compared with concentrations 25% and 50%. Staphylococcus aureus was the most affected, recording inhibition zone of (13.917 mm), while Enterococcus faecalis and Escherichia coli were the most resistant, recording an inhibition zone of 8.917 mm. Based on the results, Citrus aurantium peel oils can be used as a safe and alternative treatment for urinary tract infections.

Keywords: Citrus Aurantium Peels, Terpenes, UTI

INTRODUCTION

Bacteria have become increasingly adept at creating resistance mechanisms due to the increased use of antibiotics, particularly in developing nations, which has raised infection rates and mortality. According to the World Health Organisation, antibiotic resistance is the third greatest cause of death after heart and circulatory illnesses and one of the biggest risks to human health. A 2022 study estimates that antibiotic resistance caused 1.27 million deaths in 2019 and is predicted to increase to almost 10 million fatalities per year by 2050 [1]. While antibiotics are required to treat bacterial infections, Misuse causes resistant bacteria to emerge over decades and in inappropriate doses [2]. The problems of antibiotic resistance by pathogens in recent decades have led to the continued exploration and use of plant compounds as natural antibiotics. Plants produce many of these compounds as secondary receptors that they use to defend themselves against pathogens [3]. Essential oil, one of the most important natural substances produced by medicinal plants, aids in defending against illnesses and preserving the health of plants [4]. This leads to an increased interest in the use of essential oils in various fields [5], particularly medications and their application as anti-inflammatory, anti-fungal, and antibacterial agents. There appears to be a lot of optimism for creating novel and efficient medicines to fight infections, despite the fact that multidrug-resistant bacteria present enormous difficulties for medical professionals. Numerous studies have demonstrated the potent antibacterial and antifungal properties of the chemical makeup of essential oils isolated from citrus peels, particularly those of *Citrus aurantium* [6]. Because of its wide range of chemical composition, the essential oil of *C. aurantium* has a remarkable pharmacological impact. The kind and percentage of substances it contains vary depending on where the plant grows. Its

primary constituents are myrcene, linalool, and limonene. They are great effective against resistant infections like *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* which have diverse genetic mechanisms to acquire high resistance [7]. The effective treatment of numerous infectious diseases is at danger due to the fast rise of antibiotic resistance in bacterial populations. One of the most prominent types of infections resulting from antibiotic-resistant pathogens is urinary tract infection, which affects individuals of all ages and both sexes and is caused by bacteria that enter the urethra and infect the urinary system [8]. Although 90% of infections are caused by bacteria, about 10% of the remaining infections are caused by *Candida albicans* [9]. Since antibiotic therapy is often insufficient to eradicate bacteria due to their antibiotic resistance, researchers look for new strategies to eradicate them, such as the use of secondary metabolites in plant. One of most prominent plants used in this field is *Citrus aurantium*, also known as bitter orange [10], which has multiple therapeutic potentials, including antibacterial, anti-insect, antioxidant, and anti-inflammatory biological properties [11]. The current study's primary aim is to assess the terpenes derived from bitter orange peels' ability to suppress the growth of bacteria that cause UTIs.

MATERIALS AND METHODS

Plant Collection

Citrus aurantium was cleansed with tap water after being collected from Baghdad fields, then placed in a basket. The plant was identified in the herbarium of the biology department. College of Science, Bagdad University. Peels were removed manually, then cut into little pieces, and dried in an electric oven at 40°C for 48 hours. Then it is

ground by an electric grinder into a fine powder and stored until required [12].

Preparation of Essential Oils

Essential oils were extracted from the peels using hydro-distillation in a Clevenger apparatus. 100 grams of bitter orange peel powder were placed in 1000 ml of distilled water. oils were distilled for 8 hours, then condensed and collected in a collection tube, then carefully separated and placed in a dark glass container and stored at 20 °C until analysis and use [13].

Analysis of Essential Oil

Active compounds of essential oil extracted from the peels were determined by using a GC-MS analysis in Samarra University, College of Applied Sciences, Central Laboratory, at a temperature of 60°C.

Collection of a Urine Sample

During the period from January to June 2024, a total (370) urine samples were collected from patients suspected of urinary tract infection at Imam Ali General Hospital; 162 samples were positive. placed in sterile 100 ml containers for diagnostic and examination.

Isolation and Identification of Bacteria

Urine samples were transferred to blood agar, MacConkey agar, and mannitol salt agar, which are differential and selective media for isolation, purification, and identification of infection bacteria and yeast which caused infection by determining the ability of each isolate to hemolyze blood, ferment lactose and menthol. Then examined shape, colour, and pigment production. Plates were incubated at 37°C for 24 hours, single pure colony was transferred to nutrient agar for storage. Then the Vitek test was performed to confirm and identify the isolates [14].

Antibiotic Sensitivity Test

About five colonies were removed by sterile swab from the culture plate and placed in a tube containing 5 ml of saline until the turbidity level reached 0.5 McFarland. sterile swab was dipped in the bacterial suspension and spread over the entire surface of the Mueller-Hinton agar. the antibiotic discs were picked up with sterile forceps and gently pressed onto the surface of the agar. Five discs were placed on each plate, all plates were incubated at 37°C for 24 h. inhibition zones

around antibiotic disk were measured with a ruler in millimetres and sensitive of resistant bacteria were determined using antibiotics ampicillin 10 µg, augmentin 10 µg, cefotaxime 30 µg, ceftriaxone 30 µg, ceftazidime 30 µg, ciprofloxacin 5 µg, nitrofurantoin 300 µg, amikacin 30 µg and voriconazole 1 µg [15].

Evaluation of the Effectiveness of Terpenes Against Some Bacteria

Activity of three concentrations from essential oil (25%, 50%, and 75%), respectively against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* was evaluated using the agar well diffusion method on Mueller-Hinton medium, with Tween 20 as a negative control. Mueller-Hinton agar plates were inoculated with bacterial isolates, three replicates for each isolate, under sterile conditions. Each well was filled with 50 µL of each concentration and incubated at 37°C for 24 hours. The growth inhibition zone was measured in mm with by ruler and recorded the results [16].

Statistical Analysis

Results were analysed by using Genstat v12 program for an experiment applied according to the CRD design with triplicate the averages were tested according to the least significant difference (LSD) test at the 0.05 probability level.

Results and Discussion

Compositional Analysis of Essential Oils

The chemical constituents of bitter orange peel essential oil were identified using GC-MS. Results indicated that D-Limonene had the highest percentage compared to the other compounds, which recorded (86.65%), with a retention time 5.105, followed by β -myrcene, which recorded (6.46%) with a retention time 4.292. Nerolidyl acetate had the lowest percentage, which was recorded (0.05%) with a retention time 14.765 (Table1).

This is consistent with [17], who indicated that the main chemical groups of bitter orange peel oils are terpenes. A previous study by [18] showed that D-Limonene inhibited bacterial growth at a concentration of 20 %. It caused destruction of *S. aureus* cell morphology and cell wall integrity, leakage of biomolecules, and inhibition of ATP synthesis.

Table 1: Major Compounds in *Citrus Aurantium* Peel Essential Oil by using Gas Chromatography -Mass Analyzed

No	Compound Name	Ret Time	Area %
1	(1R)-2,6,6-Trimethylbicyclo	3.567	1.73
2	beta.-Phellandrene	4.054	0.49
3	Bicyclo-(3.1.1)-heptane, 6,6-dimethyl-2- meth	4.126	0.31
4	beta.-Myrcene	4.292	6.46
5	D-Limonene	5.106	86.65
6	beta.-Ocimene	5.214	0.70
7	alpha.-Methyl- alpha-(4-methyl-3-pentenyl	5.542	0.40
8	alpha.-Methyl- alpha-(4-methyl-3-pentenyl	5.784	0.18
9	1,6-O ctadien-3-ol- 3,7-dimethyl	.000	0.99
10	Neryl nitrile	6.396	0.06
11	endo-Borneol	7.184	0.06
12	L.-alpha.-Terpineol	7.638	0.40
13	Decanal	7.894	0.17
14	Acetic acid, octyl ester	8.081	0.07
15	D-Carvone	8.459	0.11
16	Geraniol	8.861	0.09
17	Linalyl acetate	8.990	0.40
18	Geranyl acetate	11.323	0.20
19	Caryophyllene	12.358	0.27
20	2-Dodecenal	12.868	0.06
21	beta.-copaene	13.435	0.13
22	Nerolidyl acetate	14.763	0.05
23	Cycloheptane, 4-methylene-1-methyl-2	16.313	0.04

Bacterial Isolation and Susceptibility Test

Based on the diagnosis of urine samples, 149 bacterial infections were confirmed, distributed as follows: 63 samples were

Gram-positive, which accounted (42.28%) and 86 were Gram-negative, which accounted (57.71%). Table (2). All of them were resistant to antibiotics according to the results of the sensitivity test.

Table 2: Distribution of Gram-Positive and Gram-Negative Isolates from Patients with UTIs.

Result of Urine Culture	Patients with Urinary Tract Infection	
	Number of Isolated	Percentage %
Gram-Positive Bacterial Infection	63	42.28
Gram-Negative Bacterial Infection	86	57.71
Total	149	100

Types of Pathogens are Isolated from Urinary Tract Infections

Results in Table (3) indicated the distribution of bacterial species isolated from patients with urinary tract infections, the highest prevalence being *Pseudomonas aeruginosa*, which recorded the highest percentage (46.30%) compared with other Bacterial species,

while the lowest percentage is *Enterococcus faecalis*, which recorded (7.38%).

Also, results showed that women are more susceptible to infection, as table (4) which showed that the infection rate among women reached (50.33%) compared with men (49.66%).

Table 3: Number of Isolates According to Bacterial Species

Bacteria Species	Patients with urinary tract infection	
	Number of Isolated	Percentage %
<i>E. Coli</i>	17	11.40
<i>Pseudomonas Aeruginosa</i>	69	46.30
<i>Staphylococcus Aureus</i>	52	34.89
<i>Enterococcus Faecalis</i>	11	7.38
Total	149	100

Results of current study consistent with numerous studies and research which confirmed that women are exposed to infection at a higher rate than men in study conducted by [19], infection rate was (81.8%) in women compared with men was (18.2%) researcher stated that the reason for the higher infection rate among women is due to anatomical differences between the sexes and the shorter length of the

urethra in women, which made them more susceptible to urinary tract infections.

While [20] indicated that the reason for the higher infection rate of *Pseudomonas aeruginosa*, which recorded the highest Percentage compared with other Bacterial species, is due to its opportunistic nature and high resistance to antibiotics.

Table 4: Number of Infected According to Patient Gender

Patient Gender	Patients with Urinary Tract Infection	
	Number of Patients	Percentage %
Male	74	49.66
Female	75	50.33
Total	149	100

Effectiveness of Terpenes Against Pathogens

Antimicrobial activity of essential oils derived from *Citrus aurantium* peels was tested at three concentrations against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) using agar diffusion technique.

Tween 20 as a control. Results showed significant differences at ($p < 0.05$) between the concentrations and Bacteria species, 75%

concentration achieved the highest inhibition rate, which recorded (16.667mm) followed by the 50% then 25% concentrations, which achieved the lowest inhibition zone (12.417mm). Also showed significant differences between the bacterial species, *Staphylococcus aureus* was more sensitive, recording (18.557mm), compared with *Enterococcus faecalis* and *E. coli*, which recorded (11.889mm) (Table 5). Also showed inhibition zones for each concentration compared with the control and differences in the diameters of the inhibition zone according to bacterial sensitivity.

Table 5: Antibacterial Activity of *Citrus Aurantium* Essential Oils (Inhibition Zone in mm) $P < 0.05$

Name of Bacteria	Inhibition Zone				Mean
	25%Cons.	50%Cons.	75% Cons.	control	
<i>E. Coli</i>	9.667	11.667	14.333	0.000	11.889
<i>P. Aeruginosa</i>	14.000	16.000	17.667	0.000	15.889
<i>S. Aureus</i>	15.667	18.667	21.333	0.000	18.557
<i>E. Faecalis</i>	10.333	12.000	13.333	0.000	11.889
Lsd5%		0.930			0.465
Mean	12.417	14.584	16.667	0.000	
Lsd5%		0.465			

About 95% of urinary tract infections are caused by resistant bacteria to antibiotics, making them one of the most common diseases in many countries. Current studies indicate that the infection rate among Gram-negative bacteria is higher than that of Gram-positive bacteria. Due to the presence of a polysaccharide lipid layer in the cell walls of Gram-negative bacteria, they are more difficult to eliminate. Due to the ability of bacteria to develop defence mechanisms against antibiotics through selection and mutation, all of the microorganisms in the current study demonstrated antibiotic resistance. For example,

some bacteria have evolved metabolic "pumps" that can eliminate antibiotics before they reach their target, while others have evolved enzymes that render them ineffective [21]. Results consistent with the report of the researcher [22] in a study conducted on urinary tract infections in children, the percentage of Gram-negative was found to be higher compared to the percentage of Gram-positive. The researcher indicated that the repeated and random use of antibiotics is the reason for the high incidence, which creates severe resistance to antibiotics (Figure 1).

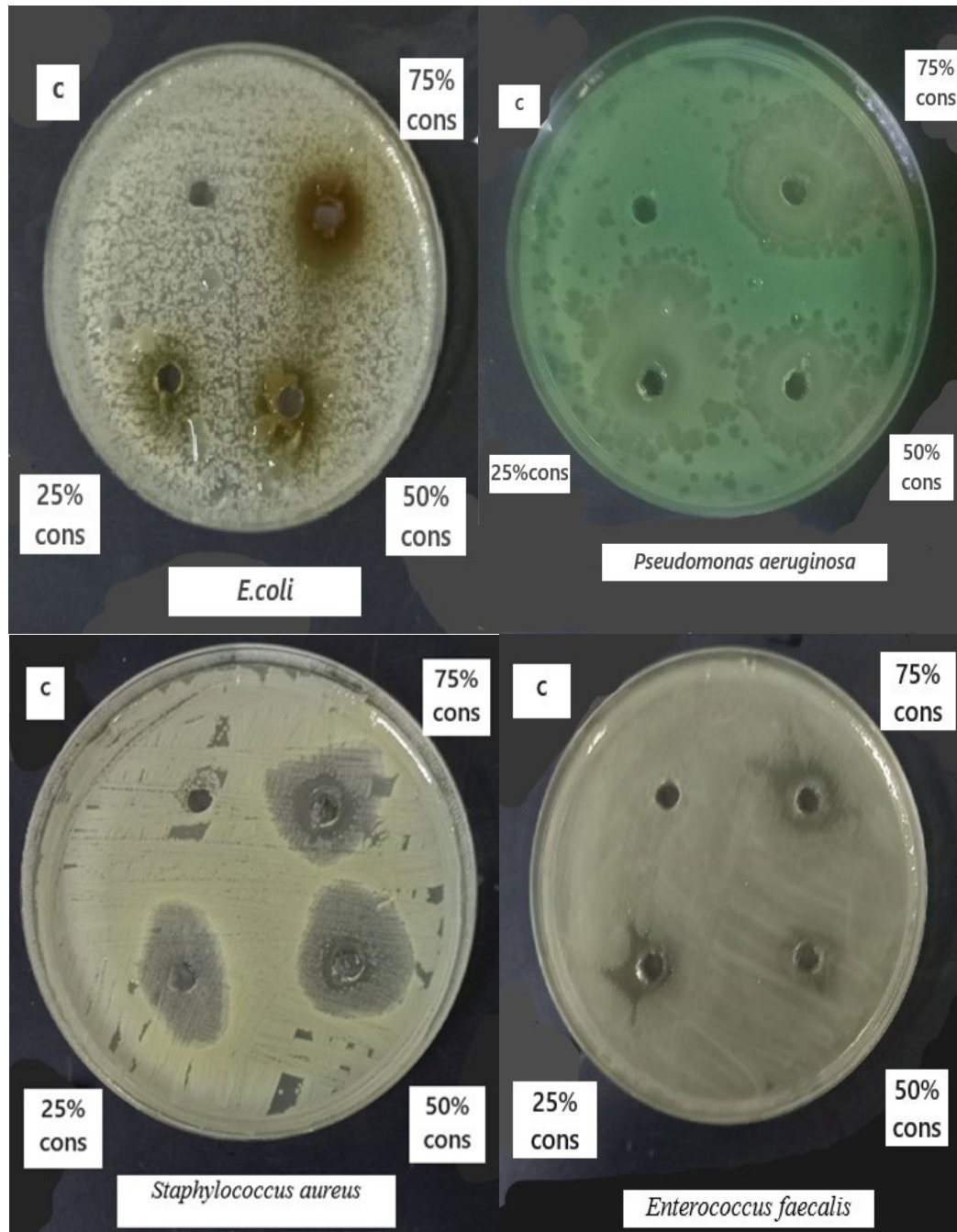


Figure 1: Inhibition Zone of Essential Oils Extracted from *Citrus Aurantium* Peels Against Bacteria (Cons: Concentration, c: Control)

Essential oils for *Citrus aurantium* peel can inhibit the growth of resistant bacteria. Which consistent with the research conducted by [23], which pointed out the presence of terpenoids which stop growth by preventing the development of biofilms. The present study also

showed significant differences between bacterial species, with *Staphylococcus aureus* being the most sensitive to the extract, while *Enterococcus faecalis* was the least sensitive. This is consistent with the results of the researcher [24], essential oils act by interfering with

the formation of membranes or cell walls, leading to their death. By degrading the peptidoglycan protein structure of the cell wall. Since Gram-positive bacteria lack a lipopolysaccharide layer to protect their membrane, essential oils can more easily damage the porin protein, leading to cell lysis [25].

Conclusion

The essential oils found in bitter orange peels contain compounds that have an effective medicinal effect against resistant bacteria and can be used in the manufacture of medicines needed as a treatment for patients suffering from (UTIs).

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Recommendations

The Present study focused on the essential oils of bitter orange peels and proved their antibacterial efficacy. recommend testing the efficacy of each essential oil compound separately, in addition to conducting a simultaneous interaction of each compound with another compound or with an antibacterial.

Ethical Approval

numbered CSEC/0225/0016, dated February 4, 2025, was obtained from the Scientific Research Ethics Committee, College of Science, University of Baghdad.



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