

COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY BETWEEN THE CITRUS LIMON (LEMON) & CITRUS AURANTIUM (BITTER ORANGE) EXTRACTS ON PSEUDOMONAS AERUGINOSA ISOLATES FROM BURNS INFECTIONS

BASAM BASIM MOHAMMED, SOMAYA YASSIN NAJIM, ZAINAB OWAIID SHATTI, ENAS IBRAHIM JASIM AND WISSAM ADNAN DARI

Department of Biology/College of Science/Al-Mustansiriyah University/Baghdad- Iraq. Email: basamalrehany@yahoo.com

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ABSTRACT

Aims: This study to the compared antibacterial activity of the Citrus Juices (lemon & bitter orange) in vitro by the well diffusion assay against *Pseudomonas aeruginosa* isolated from burned patients in Baghdad, Iraq. **Materials and Methods:** Freshly squeezed lemon and bitter orange juices diluted with water; Four different concentrations of each extract, (25%, 50%, 75%, 100%) were prepared of C. limon (lemon) & C. Aurantium (bitter orange) juices; in order to check their the antibacterial activities against 10 isolates of *Pseudomonas aeruginosa* gram-negative bacteria by agar diffusion method with measurement of diameter of the zone of inhibition around the extracts. **Results:** The highest inhibition zone (45 mm) was observed with 100% concentration of C. limon on *Pseudomonas aeruginosa*, while the highest inhibition zone recorded by C. Aurantium was (34 mm). Less concentration used in C. limon and C. aurantium juices at 25% concentration had antibacterial effects on the bacteria isolates tested. **Conclusions:** The different concentrations of *Citrus limon* juice had an effective antibacterial activity more than citrus aurantium juice against all *Pseudomonas aeruginosa* isolates. The citrus fruits extracts have lesser side effects than the synthetic medicines used against pathogens & can be used in vitro to treat surface problems of the skin.

Key Words: Lemon, Bitter orange, antibacterial activity, pseudomonas aeruginosa, well diffusion agar, burns infections.

INTRODUCTION

Pseudomonas aeruginosa as an important life-threatening nosocomial pathogen plays a prominent role in serious infections in burn patients [1]. The *Pseudomonas aeruginosa* is a Gram-negative aerobic bacilli and the natural flora of the skin and intestinal tract that is also found in water and soil. It is an opportunistic pathogen of the immunocompromised individuals, including patients with malignancy, cystic fibrosis & burns, etc. [2, 3].

Infections caused by *P. aeruginosa* difficult to treat with conventional antibiotics also which are resistant to different antibiotics [4, 5]. In general, bacteria have several genetic mechanisms to transmit and acquire resistance to antibiotics. The resistance genes in the bacterial populations are rapidly spreading [6].

Plants with medicinal effects are important compounds of traditional medicine in virtually all cultures. Medicinal plants are of great benefit in the field of curing diseases and act as important sources of materials for a wide variety of human ailments. Some researchers have shown that using plants with medical effects are better than common antibiotics used for the treatment of patients with different diseases [7]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs [8].

Citrus fruits have a high percentage of chemical compounds so appeared high antimicrobial capacity in this plant more than other plants. There is a relationship between the chemical structures of the most abundant compounds in the plant extracts and microbial activity of tested plants due to compounds synthesized in the secondary metabolism of the plants [9]. Plants have been used as medicines over hundreds of years, constitute an obvious choice for study, active components from plants of different parts (fruit seeds, roots, stems, leaves, flowers, peels, juices and essential oils) have potential antibacterial in crude extracts [8]. The aim of this study was to assess the antimicrobial effects of *Citrus aurantium* & *Citrus limon* extracts against different *Pseudomonas aeruginosa* isolates from patients with burns infections.

MATERIALS & METHODS

Bacterial Isolates

The study was carried out in April 2017 in the department of microbiology, College of Science, Al- Mustansiriyah university the *Pseudomonas aeruginosa* isolates from burns infections were obtained from high studies laboratories and identified again according to their colony morphology, microscopic gram stain investigation, and biochemical tests.

Rod - gram-negative, on MacConky agar appear as pale colonies (Non-lactose fermenting) and large flat dark greenish colonies on nutrient agar when incubated aerobically at 37 °C for 24 h. The biochemical tests: oxidase positive, urease positive, Simon-citrates positive.

Plant Collection

The fruits of *Citrus limon* and *Citrus aurantium* were bought from the local market in Bagdad Fig 1 – 2.

Extraction of the Plant Material

The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water and cut open with a sterile knife and the juice pressed out into a sterile universal container separately and then filtered (using Millipore 0.45 filter paper) into another sterile container to remove the seeds and other tissues which is used freshly as crude extract without refrigeration.

Preparation of Aqueous Extract

Juices extractions were prepared according to methods reported by Owhe-Ureghe *et al.* 2010 the liquid after squeezed into sterile container. The extract was marked as 100% concentrated juice extract. The other Concentrations were prepared by diluting the 100% extract with right volume of sterilized distilled water [10]. 75% solution: 75 ml juice was mixed with 25 ml of distilled water, 50% solution: 50 ml of juice was mixed with 50 ml of distilled water, 25% solution: 25 ml of juice was mixed with 75ml of distilled water, which were further used for antibacterial activity [11].

Agar Well Diffusion Method

The antimicrobial activity carried out by different concentrations (100 %, 75%, 50%, 25%) of aqueous citrus extracts against 10 *Pseudomonas aeruginosa* isolates were evaluated by using agar well diffusion method.

The medium was sterilized by autoclaving at 120°C (15 lb/in2). About 20ml of the medium (Mueller-Hinton Agar Medium) was transferred aseptically into each sterilized petri plate. The plates were left at room temperature for solidification. Bacterial suspensions prepared by reactivated the 10 isolates of *Pseudomonas aeruginosa* in brain heart infusion broth , until obtain of growth by observed the tube; about 1.5×10^8 CFU/ml of each isolate were swabbed with sterile swabs.

Each plate, a four wells of 6 mm diameter were made by using sterile borer. 50 l volume of all concentrations that mentioned above of citrus extracts was tested against *Pseudomonas aeruginosa* isolates. Antibacterial assay plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 h. . The diameter of the zones of inhibition were measured with scale



Figure 2: C. Aurantium (Bitter orange)



Figure 1: C. limon (Lemon)

RESULTS

Increase the concentrations of each lemon & bitter orange extracts may produce more inhibition to bacterium. Results of the antibacterial activity for extracts against all isolates; Observations are shown in Table 1. The results which showed that lemon was very effective at all concentrations that used in this study (25%,50%,75% and 100%) on all *Pseudomonas aeruginosa* that causes burns infections, as the concentration of *Citrus limon* increases the zone of inhibition also increases. The highest inhibition zone of lemon were 45 ,40 , 30 and 25 mm by 100 % , 75 % , 50 % and 25%(v/v) concentrations respectively. The zone of inhibitions were observed at higher concentrations of 50% , 75% and 100%. A similar study conducted by on the antimicrobial activity of *Citrus aurantium* extract , the juice was also effective against *Pseudomonas aeruginosa* isolates but not effective as *Citrus limon* juice.

The highest inhibition zone of *C. aurantium* (bitter orange) 34 , 30 , 25 , 19 mm by 100%,75%,50% and 25% (v/v) concentrations respectively. Fig-3.

Table 1 : Inhibitory Activity of Aqueous Lemon and Bitter orange Extracts against *Pseudomonas aeruginosa* Isolates From Burns Infections .

Bacterial Isolates	Diameter of zone of inhibition (mm)							
	<i>C. limon</i> (lemon)				<i>C. aurantium</i> (bitter orange)			
	Conc. 100%	Conc. 75%	Conc. 50%	Conc. 25%	Conc. 100%	Conc. 75%	Conc. 50%	Conc. 25%
<i>Pseudomonas aeruginosa</i> 1	40	35	30	25	31	28	25	19
<i>Pseudomonas aeruginosa</i> 2	39	35	29	25	33	29	24	17
<i>Pseudomonas aeruginosa</i> 3	35	30	26	22	30	26	22	15
<i>Pseudomonas aeruginosa</i> 4	45	40	30	24	34	30	25	18
<i>Pseudomonas aeruginosa</i> 5	35	29	25	22	31	26	22	17
<i>Pseudomonas aeruginosa</i> 6	41	38	25	21	33	27	24	18
<i>Pseudomonas aeruginosa</i> 7	38	32	28	23	31	25	21	16
<i>Pseudomonas aeruginosa</i> 8	37	30	25	20	31	26	20	15
<i>Pseudomonas aeruginosa</i> 9	43	40	30	24	33	28	25	18
<i>Pseudomonas aeruginosa</i> 10	40	36	30	24	31	27	24	19



Fig. 3: Comparison the effects of aqueous lemon and bitter orange extracts against *p. aeruginosa* isolates from burns infections in well diffusion method.

Table 2: Common Uses, Parts Used Traditionally and Bioactive Components of *Citrus aurantium* & *Citrus limonum* Juices.

Species (family)	Common uses	Parts used traditionally	Bioactive Components
<i>Citrus aurantium</i>	Antidepressant, treating all types of negative emotional conditions, states of anxiety, menopause, insomnia, improves elasticity and antiseptic effects, used in related cosmetic products, Antiobesity, It aid in digestion , Cardiovascular health, Anti-Cancer.	flowers, peel, leaves, fruit seeds, essential oil.	Tannins++, flavonoids+, polyphenols, saponins+,terpenoids alkaloids, steroids, carbohydrate protein , water soluble vitamins ascorbic acid, citric acid.
<i>Citrus limonum</i>	Treat throat infections , for acne and spots, for bone-related diseases , brain nerve food, blood purifier , blood sugar balance, for weight loss by stimulates the metabolism, prevents kidney stones, lemon is rich in vitamin C can help in lighting the skin, useful for UTI patients.	Flowers, peel, leaves, fruit seeds, essential oil.	Tannins +++, Flavonoids ++, Polyphenols++, Limonoids ++, Saponins ++, Alkaloids +, Steroidal glycosides, limonoids, Carbohydrate++, Protein, Water soluble vitamins, Cyanogenetic glycosides , Cardiac glycosides. ascorbic acid++, citric acid++.

+, low concentration; ++, medium concentration; +++, high concentration

DISCUSSION

A lot of studies proved that extracts of plant origin have the antimicrobial activities in vitro. This is especially useful for people in rural communities in developing countries who don't depend on synthetic antimicrobial drugs. Our results show that the lemon and bitter orange juice concentrations may be alternative sources of antimicrobial agents for *Pseudomonas aeruginosa* [12]. Hand washing with lemon fruit juice in hospital settings may reduce the spread of nosocomial infection and provide a reasonable alternative to alcohol-based hand washers [13].

Many studies revealed the presence of active constituents that either singly or combination in lemon & bitter orange by phytochemicals analysis shown in Table 2. such as phenolic compounds, flavonoids, Limonoids , organic acids, tannins, saponins, alkaloids, steroids, terpenoids and other substances have antimicrobial traits ; by function as capacity to alteration metabolism of microorganisms (enzymatic activities) or disrupt of bacterial membranes & cell walls so lead to cells destruction. In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses [14, 15, 16, 17, 18, 19, 20].

In general, Gram-negative bacteria are more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [21] . But in this study, the effects of limon & bitter orange juices gave a obvious effects on all Gram negative isolates at different concentrations .

The juice of *C. aurantium* less inhibited while the juice of *C. limon* was more effective on *Pseudomonas aeruginosa* isolates. This could be due to the acidic pH of this juice that will affect the charges of the amino acids that constitute the peptidoglycan, and it may affect the active sites of enzymes leading to defect in activity of *Pseudomonas aeruginosa* , furthermore the antimicrobial ingredients in *C. limon* are more concentrated as mentioned in the Table 2 [22].

There are clinical studies found that daily usage of pure hand gel among healthcare workers which contain *C. limon* to prevent risk of transmitting infections , most skincare products add to them lemon components such as cleansers, soaps, toners, skin whitening creams, moisturizers, lotions, etc. These are a good indicators to use citrus fruits on skin as natural agents without side effects [23].

CONCLUSION

Lemons and bitter oranges are natural, cheap, safe, and have bactericidal and bacteriostatic effects so it possible to use it in the treatment of burns patients from *Pseudomonas aeruginosa* infections.

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