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Evaluation of the Antibacterial Activity of Citrus Juices: An In Vitro Study

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الخلاصة

الأهداف: تهدف الدراسة الى تحديد الفعالية المضادة للجراثيم لثلاثة انواع من عصائر الحمضيات على ثلاث انواع من الجراثيم (المكورات العنقوديةالذهبية والمتقلبات والزائفة الزنجارية). **المواد وطرائق العمل:** تم قياس الفعالية المضادة للجراثيم لعصائر الحمضيات (الليمون والنارنج والكريب فروت) ضد بعض انواع الجراثيم الموجبة والسالبة لصبغة كرام مستخدمين اربعة تراكيز مختلفة من هذه العصائر. وتم تحديد مستوى التأثير بطريقة الأنتشار بالأقراص داخل المختبر بقياس قطر منطقة التثبيط. **النتائج:** اوضحت الدراسة وجود فعالية مضادة للجراثيم لهذة العصائر حيث ان اكبر قطر للتثبيط وصل الى(18) ملم لعصير الكريفون بتركيز(10٪) على المكورات العنقودية الذهبية ولم يلاحظ اي تاثير لهذا العصير على الانواع الاخرى من الجراثيم. بينما لوحظ ان لعصير الليمرن والنارنج بتراكيز(10%)و(5%) تاثير قاتل على المكورات العنقوديةالذهبية والمتقلبات والزائفة الزنجارية المستخدمة حيث بلغ قطر مناطق التثبيط لعصير الليمون وصلت الى(10)و(12) و(13)ملم لتركيز(5%)و (15,21)،و(20) ملم لتركيز(10%)على التوالي. أما عصير النارنج بتركيز(5%) فقد بلغت اقطار مناطق التثبيط الى(18,19 و 20 ملم) اما عند تركيز(10%) فقد بلغت اقطار مناطق التثبيط الى (16,19 و 19ملم) على التوالي. أثبتت هذه الدراسة بأن تأثير هذه العصائر اعطى نتيجة افضل من المضادات الحيوية عند استخدامها كمجموعة سيطرة. **الاستنتاجات:**ان استخدام مستخلصات عصائر الحمضيات وبتراكيز مختلفة له تأثير مضاد للجراثيم (المكورات العنقوديةالذهبية والمتقلبات والزائفة الزنجارية).

الكلمات الدالة: الفعالية المضادة للجراثيم، عصير، الليمون، النارنج، الكريب فروت.

ABSTRACT

Aims: This work aimed at determining the antibacterial activity of three Citrus Juice on *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. **Materials and Methods:** The antibacterial activities of juices of three types of Citrus (C.) fruit, *C. limon (lemon)*, *C. aurantium (bitter orange)* and *C. paradisi (grapefruit)*, against some gram positive and gram negative bacteria using four concentrations of these juices by agar diffusion method with measurement of diameter of the zone of inhibition around the extracts. **Results:** The results confirmed the presence of anti bacterial activity of the Citrus juice. The highest inhibition zone (18 mm) was observed with a 10% concentration of *C. paradises* juice on *Staphylococcus aureus*, but *Proteus vulgaris* and *Pseudomonas aeruginosa* were resistant. *C. limon* and *C. aurantium* juice at 10% and 5% concentration gave positive results with bactericidal effects on the three tested bacteria (*Staphylococcus aureus*, Proteus *vulgaris* and *Pseudomonas aeruginosa*). The zones of inhibition of *C. limon* were 10, 12 and 13mm for 5% and 15, 21 and 20 mm for 10% respectively. For *C. aurantium*, they were 18, 19 and 20 mm for 5% concentration and 16, 19 and 19 mm for 10% concentration respectively. Those effects were better than those produced by standard antibiotic disc used for comparison. **Conclusions:** The use of different concentrations of Citrus juice extracts had an effective antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*.

Key Words: antibacterial, *lemon*, *bitter orange*, *grape fru*it

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INTRODUCTION

Medicinal plants are important elements of traditional medicine in virtually all cultures. Single and poly herbal preparations have been used numerously throughout the history of treating various diseases[(1)].

Citrus fruits are acidic fruits which contain healthy nutritional content that works wonders for the body. It acts as a

fabulous source of vitamin and a wide range of essential nutrients required by the body, fresh fruits and their hand-squeezed or industrially processed juices, contain mostly flavanones and flavones [2, 3]**.** There are several *Citrus (C.)* species, of these *C. limon (lemon), C. aurantium (bitter orange)* and *C. paradise (grapefruit).*

The antimicrobial activities of Citrus plants oil and extracts were investigated [2, 3, 4, and 5]. Hammers *et al*. 1999 studied the effect of essential oils from *C. aurantium, C. limon, C. paradisi* and many other plant oils and extracts and found that the minimum inhibitory concentrations (MIC) were between 5-2% (v/v). AL- Jedah *et al*. (2000) analyzed the action of combined spices including *lemon* in its mixture and found that the spices mixture were able to exert static effect on all assayed bacteria. Hayes and Markovic in (2002) investigated the antimicrobial properties of *lemon* and found that *lemon* possesses significant antimicrobial activity against *Staphylococcus aureus, Klebsiella, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. Another study [8] found that *grape fruit* seed and pulp extracts had antibacterial and antifungal activity. Adedeji *et al.* 2007[5] found that Citrus extract form *lemon, lime* and *grape fruit* had antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, proteus, klebsiella, Escherichia coli* and that *lime* and *lemon* juice had better antibacterial activity and compared favorably to the commercial antibiotic disc.

Chemical research revealed the presence of flavonoids, ascorbic acid, tocopherols, and citric acid in those extracts [9]. In the early nineties, the presence of flavonoids in *C.* juices began to attract the attention of a number of researchers, as a result of their biological and physiological importance [1, 2, 3, 9, 10 and 11]. In general, flavonoids may contribute to fruit and juice quality in many ways, influencing the appearance, the taste and the nutritional value of the product from the plant [11]. Flavonoids are mainly present in *C.* fruits as their glycosyl derivatives. The presence of a relatively large number of flavonoids in *C.* Juices are a result of the many different combinations that are possible between polyhydroxylated aglycones and a limited number of mono- and disaccharides [12]. Correlation between the production of compounds and stress has been noticed during the course of plant infection by microorganisms, the gradual increase of that content in *C. aurantium, C. limon, C. paradise and C. sinensis,* was observed during the course of infection by *Phyetophthora citrophtora*, indicating that this process is part of the plant's responses to invading pathogens[11].

These flavonoids have been reported to act as antioxidants in various biological systems. Some studies have related the stage of growth to the production of phenolic compounds by fruits which showed to be powerful antioxidants and free radical scavengers [9, 12].

The aim of this study is to determine the efficiency of Citrus juice extract of three Citrus fruits (*C. limon, C aurantium* and *C. paradisi*) using different concentration against some gram positive and gram negative bacteria.

MATERIALS AND METHODS

1. Source of samples and Processing:

Fresh fruits of *C. limon, C. aurantium and C. paradisi* used in this study were obtained form the local market at Mosul City, Iraq. The Citrus 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 into another sterile container to remove the seeds and other tissues.

Bottles were aseptically opened and one milliliter (1 ml) of each sample from crude juice were transferred into 9 ml of sterile distilled water used as diluents to get 10%concentration, then serial dilution was carried out to prepare 5%, 3.3% and 2.5% (v/v) final concentration. All extracts were stored at 4 ℃ when not in use.

2. Microbiological study:

The study was conducted in microbiological laboratory at Dentistry College. Pure isolate of three different types of bacteria were used in this study (*Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). The screening of antimicrobial activities of each Citrus extract on the tested bacteria used in this

investigation were determined by the agar diffusion techniques using modified Kirby-Bauer disc method [13]. Commercially prepared antimicrobic doses in milligram (mg) contained in compound susceptibility disc which were ampicillin 10 mg /disc, trimethoprim 10 mg /disc, nalidixic acid 30 mg /disc and gentamicin 10 mg/ disc were used as control for the susceptibility test. All plates were further incubated at 37°C for 24 hour after the incubation period; the diameters of the growth inhibition around the samples were measured and expressed in millimeters (mm). All tests were done in triplicate.

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RESULTS

Results of comparative antibacterial activity of Citrus extracts with standard commercial antibiotics disc were shown on Table (I). The antibacterial activity was observed in 10% and 5% (v/v) concentrations only but there were no antimicrobial activities in 3.3% and 2.5% concentrating for the three types of Citrus juices.

Table (1): Comparative antimicrobial activity of the three *Citrus* juices with commercial antibiotics disc.

C. juices	***C.limon***				***C. aurantium***				***C. paradisi***				**Antibiotic disc**			
C Concentration * / Types of Bacteria**	10 %	5 %	3.3 %	2.5 %	10 %	5%	3.3 %	2.5 %	10 %	5 %	3.3 %	2.5 %	Am	Tr	Na	Ge
Staphylococcus aureus	15*	10	_**	_	16	18	_	_	18	_	_	_	12	_	_	_
Proteus vulgaris	21	12	_	_	19	17	_	_	_	_	_	_	_	_	18	_
Pseudomonas aeruginosa	20	13	_	_	19	20	_	_	_	_	_	_	_	_	_	13

*Diameter of zone of inhibition in mm ﴾Data represented as mean of three reading﴿; **No Inhibition zone (-).; *** C. juice concentration in (%) (v/v); Disc diameter = 6mm; Hole diameter = 6mm; Am=ampicillin 10 mg /disc; Tr= trimethoprim 10 mg /disc; Na= nalidixic acid 30 mg /disc; Ge= gentamicin 10 mg/ disc.

The best antimicrobial activity was observed in juices of C. *limon* and *C. aurantium*, they were active against the three types of bacteria used in this study. *C. paradisi* were active against *Staphylococcus aureus* only but *Pseudomonas aeruginosa* and *Proteus vulgaris* were resistant.

C. limon produced a bactericidal activity on *Staphylococcus aureus*, with a zone of inhibition of 10mm to 15mm by 5% and 10% (v/v) concentrations respectively as shown in figure (l), *C. aurantium* produced more wider zone ranging from 16mm to 18mm by 10% and 5% (v/v) concentrations respectively on same bacteria. *C. paradisi* were effective at 10% concentration only with a zone of inhibition equals' to 18mm on these bacteria. All three juices extract produced wider zone than ampicillin antibiotic (12mm), but trimethoprim did not produce any effect when used as control (Figure 1).

Figure (1): The inhibition zone of *C.* Juice on *Staphylococcus aureus*

a. The effect of *C. aurantiu* (right) in comparison with trimethoprim; The effect of *C. limon* (left) in comparison with ampicillin; The effect of *C. paradisi* (middle) in comparison with ampicillin; The numbers 1, 2, 3and 4 denotes 10%, 5%, 3.3% and 2.5% (V/V) concentrations respectively.

On *Proteus vulgaris* and *Pseudomonas aeruginosa*, the C. paradisi showed little or no significant antimicrobial activity against the tested organisms, while C. limon and *C. aurantium* were both active against those bacterial strains as shown in figures (2 and 3)and compared favorably to the commercial gentamicin and nalidixic acid antibiotic discs (Table 1) . The zone of inhibition were about 21, 20 mm for 10% concentrations of *C. limon* on *Proteus vulgaris* and *Pseudomonas aeruginosa* respectively, while10% concentrations of *C. aurantium* produce an equal zone of inhibition (19mm) on both types of bacteria.

The 5% concentration of *C. aurantium* were very effective on *Pseudomonas aeruginosa* in producing a wider zone of inhibition equals to 20mm. *C. limon* (5%) were less active against *Pseudomonas aeruginosa* and *Proteus vulgaris* as shown in table (1).

Figure (2): The inhibition zone of *C.* Juice on *Proteus vulgaris*

a. The effect of *C. limon* (right) in comparison with nalidixic acid; The effect of *C. aurantium* (left) in comparison with gentamicin; The numbers 1, 2, 3and 4 denotes 10%, 5%, 3.3% and 2.5% (v/v) concentrations respectively.

Figure (3): The inhibition zone of *C.* Juice on *Pseudomonas aeruginosa* in comparison with gentamicin.

a. The effect of *C. aurantium* (right); The effect of *C. limon* (left); The numbers 1, 2, 3and 4 denotes 10%, 5%, 3.3% and 2.5%; (v/v) concentrations respectively.

DISCUSSION

Various industries are now looking into sources of alternative, more natural and environmentally friendly antimicrobials, antibiotics, antioxidants and crop protection agents [10]. Citrus is one of the most important commercial fruit crops grown in all continents of the world [1].

In this study, the Citrus juices of *lemon* and *bitter orange* (*C. limon* and *C. aurantium*) showed good antibacterial activities against gram positive and gram negative microorganisms. The sensitivity pattern of the organism to these extracts was comparable to the values obtained by A dedejl *et al.* (2007), similar result were obtained by Cvetnic (2004) who found that no inhibiting effect of *C. paradisi* on the growth of gram negative microorgansims. Naringin was isolated from Other clinical studies found that daily usage of purehand gel which contain *C. limon* in its ingredients is associated with reduction in the microbial load, which is important to prevent risk of transmitting nosocomal infections by healthcare workers [14, 15]. One study found that treatment of carrot samples with lemon juice caused significant reduction in *salmonella typhimurium*[16]. All these studies will support the results of this study, while Winniczuk *et al*. 1997 found that citric and lactic acids and d-limonene were less effective as anti microbial compounds. Ortunõ *et al.* (1999) studied different species of *C*. and reported the presence of nobiletin, sinesetin, and naringin at the exponential growth phase and quercetogetin and heptamethoxy flavone during the stationary phase of fruit growth. Up to now the mechanism of action and the interaction between these compounds have not been completely understood; in fact, the resulting antimicrobial effect is not always the sum of the single effect, as antagonistic and synergistic interaction s. Some studies have related the stage of growth to the production of phenolic compounds by fruits which showed to be powerful antioxidants and free radical scavengers, those compound being able to induce reactions of electron transfer and do react with vital nitrogen compound in the microbial cell such as nucleic proteins and acids [9, 12]. The complicated mixtures of those compounds represented the strongest barrier to infection and may contribute to the differences in their bactericidal activity. Flavonoids also have been reported to act as antioxidants in various biological systems. The antioxidant activities of *C*. flavonoids exhibited a potent antibacterial activity which is probably due to their ability to complex with bacterial cell walls and disrupt microbial membrane [11]. Differences in susceptibility between *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* can be explained by differences in the nature and extent of cell membrane damaged [17]. Recently these flavonoids have been implicated in cytoprotective activity [19].

Giuseppe *et al*. (2007) have recently reported the presence of limonoids in *C.* species, which can be considered responsible for activity against many clinically, isolated bacterial strains. Limonoids obtained from *C. limon*, showed good antibacterial and antifungal activity [11, 12, 16, 20]. In these studies the importance of structural features on activity is also illustrated activity [20].

CONCLUSIONS

The use of different concentrations of Citrus juice extracts has effective anti bacterial activities against *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The results indicated that the juice of the Citrus species present substantial antimicrobial properties. Antibacterial

Activities of some of the extracts was of such a level that it would probably be therapeutically useful, and it is possible that some of the extracts may be clinically applicable for the treatment of certain infections.

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