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### Determine the antibacterial activity of lemon (*Citrus limon*) extract against some bacteria isolated from different sources

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#### ABSTRACT

The antibacterial activity of lemon extract (*Citrus limon*) was investigated. The aim of the study was to determine antibacterial activity of lemon juice against some pathogenic bacteria isolated from different clinical samples. Agar well diffusion assay was used to detect antibacterial activity of different concentration of lemon juice (25, 50, 75, and 100)%, and some antibiotics against *Staphylococcus aureus*, *Klebseilla pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The results revealed, all isolates were inhibited by 100% lemon juice with Diameter of Inhibition Zone (DIZ) of (13-23) mm. The inhibitory activity of lemon juice was found to be higher than the effects exerted by some used antibiotics except MEM, and NOR which inhibited *Pseudomonas aeruginosa* with DIZ of (19-30)mm, and (18-19)mm respectively. Also, RA, and NV which were inhibited *Staphylococcus aureus* with DIZ (25-28)mm, and (27-31)mm respectively. In addition to CIP that produced DIZ of (19, 23, and 28) mm against *Escherichia coli*, *Klebseilla pneumoniae*, and *Proteus mirabilis* respectively. it can be concluded that lemon juice extract has substantial antibacterial activity, and possible to be used for health promotion, accessory to the antibiotic therapies, since it is inexpensive, natural, and had less side effects. further studies regarding antibacterial activity of lemon juice against pathogenic bacteria in vivo is recommended.

#### Introduction

The overuse use of antibiotic therapy cause an increase microbial resistance to these drugs. To overcome this problem, it is necessary to exploit novel, and more effective antimicrobial agents with less side effects [1]. Many researchers were look into the possibility of using medicinal plants as an alternative therapy [2].The lemon (*Citrus limon* ) had been attracted the attention of many investigators. It is important medicinal plant, and belong to the family Rutaceae. The antimicrobial activity *Citrus limon* might be attributed to the lemonoids [3], and Citrus flavonoids [4]. In addition to the protopine, polyacetylene, corydaline lactons, hypericin, pseudohypericin, acyclic sesquiterpenes, and essential oil compound are effective against various bacterial strains [5].The antibacterial effects in lemon juice on

clinically significant bacterial strains have been reported [6,7]. Hence the aim of the study was to evaluate the antibacterial activity of Lemon juice against some pathogenic bacteria isolates from clinical samples, and compare it with the effects of available antibiotic therapies.

#### Materials and methods

##### Bacteriological study:

A total of 10 bacteria isolated from stool and urine samples of patients attending Tikrit Hospital in Tikrit city during 1<sup>st</sup> of February to 1<sup>st</sup> of March, 2018. The samples transferred to the laboratory for culturing in Brain heart infusion broth, on Blood agar, Nutrient agar and MacConkey agar, then incubated at 37°C for 24 hours. The grow colonies were further sub cultured on other selective media as Cetramide, and

Manitol salt agar. The pure colonies were tested with routine microscopic and biochemical tested for identification of bacteria according to Bergey's manual of determinative bacteriology [8] and confirmed by Api system (Biomeraux, France). The cultures were maintained on Nutrient agar slants, then stored at 4°C in a refrigerator and sub cultured every 15 days .

#### Preparation of plants' extracts:

The Fresh lemon fruit was purchased from markets in Tikrit city, Iraq. They washed in running tap water, then the surface of the fruit was sterilized with 70% alcohol. Later rinsed with sterile distilled water. The fruit was cut with sterile knife, then the juice pressed out, and filtered with Millipore filter 0.45µm pore size. The crude juice was stored at 4 °C for subsequent use (Abdulhusin, *et.al.*, 2018). Different concentration (Serial dilution of the juice (25, 50, 75, and 100)% was prepared by adding sterile distilled water.

#### Antibiotic susceptibility test:

The isolated bacteria were subjected to antibiotic susceptibility testing on Mueller-Hinton agar using Kirby-Bauer disc diffusion method. The susceptibility of bacterial isolates evaluated according to recommended National Committee for CLSI (Clinical Laboratory Standards Institute) guidelines (9).

#### Antibacterial activity of lemon juice test

All the 10 bacterial isolates were subjected to different concentration (25, 50, 75, and 100) % of lemon juice using the agar well diffusion method. They prepared with distilled water. Inoculum from tested bacterium was prepared. A single colony was transferred to 5ml nutrient broth then incubated at 37°C for 24hr. The inoculum was adjusted to 0.5 McFarland turbidity standard solution, then spread evenly with sterile swab on the surface of agar. Cork borer used to make uniform wells on the inoculated agar. Later, filled with 20 µl of antibacterial agents; lemon juice, and the standard antibiotic discs were placed at some points in the same Petri dishes with the test antibacterial agents ; Lemon juice for them to undergo the same conditions. They were standed for 45minutes. Later incubated at 37°C for 24hr. The diameter of zones of inhibition were measured and interpreted according to [10].

#### The results:

Table (1) illustrate the results of agar well diffusion assay in which all selected bacterial isolates were subjected to (25, 50, 75, and 100) % lemon juice. It had revealed that all tested bacteria were inhibited by 100% lemon juice, whereas they were not affected by 25 % lemon juice. In addition the (50, and 75) % lemon juice had less inhibitory activity. However the two isolates of *K. Pneumoniae* were not inhibited by them. The maximum antibacterial effect of 100% lemon juice was detected against *P.aeruginosa-1* with Diameter of Inhibition Zone (DIZ) of 23 mm. Followed by *E.coli*, *Staph. aureus -1*, *Staph. aureus-2*, and *P.aeruginosa-2* with DIZ of 20mm.

Moreover, *S. aureus -3*, and *P.aeruginosa-3* were inhibited with DIZ of 19mm. While the minimum inhibitory of the 100% lemon juice was exerted on the *K. Pneumoniae-1*, and *K. Pneumoniae-2* with DIZ of (13-15)mm respectively.

**Table 1. The antimicrobial effect of lemon juice on selected isolates**

Bacterial species	100 %	75 %	50 %	25 %
<i>S.aureus-1</i>	20	19	16	-
<i>S.aureus-2</i>	20	18	16	-
<i>S.aureus-3</i>	19	18	17	-
<i>K. pneumoniae-1</i>	13	-	-	-
<i>K. pneumoniae-2</i>	15	-	-	-
<i>P. mirabilis</i>	20	18	17	-
<i>E.coli</i>	20	16	14	-
<i>P.aeruginosa-1</i>	23	20	15	-
<i>P.aeruginosa-2</i>	20	17	13	-
<i>P.aeruginosa-3</i>	19	17	12	-

Table (2): shows the antibacterial effect of 100% lemon juice, and some types of antibiotics on the three isolates of *S. aureus*. It had been found 100% lemon juice all *S. aureus* isolates with DIZ of 19-20. This finding was relatively similar to the inhibitory effect of most of the tested antibiotics against these bacteria, except Ra, and NV which were produce wider inhibition zone, they produced DIZ of (25-28)mm, and (27-31) mm respectively.

**Table (2) The antibacterial effect of some types of antibiotics on isolates of *Staph. aureus***

Bacteria	NV mg	RA mg	CN mg	OX mg	E Mg
<i>S.aureus-1</i>	31	28	15	15	20
<i>S.aureus-2</i>	27	25	15	20	
<i>S.aureus-3</i>	28	26	16	20	20

Table (3): demonstrates the antimicrobial effect of 100% lemon juice, and some types of antibiotics on the *K. Pneumoniae-1*, *K. Pneumoniae-2* , *E.coli* , and *P. mirabilis*. The DIZ produced by 100% lemon juice was (13-20) mm, which consider wider than all antibiotics except CIP with DIZ (19-28) mm.

**Table (3): The antibacterial effect of some types of antibiotics on isolates of gram negative bacteria.**

Bacteria	CIP mg	AK mg	C mg	CN mg	CTX mg	PRL mg
<i>K. pneumoniae-1</i>	21	12	12	-	-	-
<i>K. pneumoniae-2</i>	23	14	10	9	12	14
<i>P. mirabilis</i>	28	15	-	10	-	-
<i>E.coli</i>	19	13	-	10	-	-

Table (4): shows the antibacterial effect of 100% lemon juice, and some types of antibiotics on the three isolates of *P. aeruginosa*. Although, they were multidrug resistant except to MEM, NOR, but they were inhibited by the lemon juice With DIZ of (19-23)mm.

**Table (4) The antimicrobial effect of 100% lemon juice, and some types of antibiotics on isolates of *P.aeruginosa***

Bacteria	MEM mg	NOR mg	OX mg	CD Mg
<i>P.aeruginosa-1</i>	30	19	-	-
<i>P.aeruginosa-2</i>	19	18	-	-
<i>P.aeruginosa-3</i>	30	-	-	-

#### The discussion:

The results of the current study revealed that lemon juice had a wide spectrum of activity to word all pathogenic bacteria under investigation. However it's effect varied, depending on the bacterial species, which might be attributed to differences in nature and extend of damaging cell membrane [11].

These finding were in agreement with [6], who reported the antimicrobial activity of water extract of lemon juice against species of 5 Gram positive, and 5 Gram negative bacteria. It showed various inhibitory effects on all tested bacteria except *E.coli* and *Streptococcus epidermidis*. Also other investigators [7] suggested, the lemon juice and it's oil had better antimicrobial activity than the orange juice and its oil against different bacterial species causing wound infection including *E.coli*, *Staph. aureus*, *Strep. pyogenes*, *Strep. pneumonia*, *P. aeruginosae*, *K. pneumoniae*, and Clostridium species.

Furthermore, Al Oqaili, *et.al.* [12] found that lemon juice was effective on Methicillin resistant *Staph. aureus*. Also Kumari and Bhattacharya [13] were improved the extract showed marked inhibition of *Staph. aureus* at high concentrations. The extracts was also non-toxic to the host RBC and WBCs.

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In regard to inhibitory activity of lemon juice, and different antibiotics which illustrated in table; 2, 3, and 4, it have been showed that lemon had acceptable inhibitory effect to word tested bacterial species, and better than effect exerted by most of the tested antibiotics. This result might be contributed to overuse of these antibiotics, which caused emergence of resistant organisms [14]. Whereas lemon juice contain some active ingredients that account for its' antimicrobial properties as limonoids which had shown to be effectively decrease the growth of bacteria and fungi [15].

In addition, the antioxidant properties of Citrus flavonoids exhibit potent antibacterial activity which might be due to their ability to complex with bacterial cell wall, and disrupt its' cell membrane [3]. Furthermore, other investigator [16] suggested the antimicrobial effect of lemon might be due to the acidic PH of the juice, which affect the charges of amino acids that constitute the peptidoglycan, and may affect the active sites of enzymes, leading to defect in their functions.

According to the results of current study, it can be concluded that lemon juice has substantial antibacterial activity, and possible to be used for health promotion, accessory to the antibiotic therapies, since it is inexpensive, natural, and had less side effects. To confirm these finding, further studies regarding antibacterial activity of lemon juice against pathogenic bacteria in vivo is recommended.

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## تحديد الفعالية التثبيطية لمستخلص الليمون على بعض أنواع البكتيريا المعزولة من مصادر مختلفة

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### الملخص

الهدف من الدراسة تقييم الفعالية التثبيطية لعصير الليمون ضد بعض الجراثيم المسببة للأمراض المعزولة من النماذج السريرية. واستخدمت طريقة الإنتشار في الحفر للتركيز المختلفة من عصير الليمون (25, 50, 75, and 100) % والمضادات الحيوية ضد الجراثيم *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* أظهرت النتائج بأن كل العزلات قيد الدراسة تثبتت بتركيز 100% من عصير الليمون، حيث اعطى منطقة تثبيط بقطر مقداره (3-2-13) ملم. إن الفعالية التثبيطية لعصير الليمون كان أقوى تأثيراً من تأثير بعض المضادات الحيوية بإستثناء المضادين MEM و NOR كانتا أقوى تأثيراً من عصير الليمون على *Pseudomonas aeruginosa* واعطت منطقة تثبيط (19-30) و (18-19) ملم على التوالي. بالإضافة الى RA و NV واعطت منطقة تثبيط بقطر (27-31) و (25-28) ملم ضد *Staphylococcus aureus* والمضاد الحيوي Ciproflxacillin (CIP) التي تثبتت كل من *Escherichia coli* و *Klebseilla pneumoniae* و *Proteus mirabilis* واعطت منطقة تثبيط 19 و 23 و 28 على التوالي. نستنتج بأن عصير الليمون له فعالية تثبيطية ضد البكتيريا ومن المحتمل أن يستخدم كمعزز للصحة وملحق للمضادات الحيوية وذلك لأن الليمون رخيص الثمن وطبيعي وقلة الاعراض الجانبية الناتجة عن تناوله. يوصى بإجراء دراسات حول الفعالية التثبيطية لعصير الليمون ضد بعض البكتيريا المسببة للأمراض في الكائنات الحية.