

Antagonism activity of citrus fruit juices on some pathogenic bacteria

الفعالية التضادية لثمار الحمضيات Citrus fruit juice كمضادات حيوية لبعض أنواع البكتريا

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Abstract:

An experiment was conducted in the Biological Dep. College of Science, Kufa University to determine the antagonism activity of Lemon and Lime juices against some bacteria e.g. *Esherichia coli*, *Klebsiella pneumonia*, and *Shigella flexneri*.

Results of this experiment revealed that all juices concentrations have inhibiting effects against bacteria due to the presence of citric acid and some volatile oils e.g.(lamonine) and some materials e.g. linalyl acetate, linalool, turpinol and cymen. Results also showed a reduction in the value of pH. Both juices from lemon and lime were found to be responsible for inhibiting the studied bacterial growth parameters with inhibition zone ranging from 8-29 mm. Mean while, there were significant differences between the two juices towards the inhibition of tested bacterial effects.

The antagonism effects of these two juices were compared with the antagonism effects for some antibiotics. The isolated bacteria were resistant to the most antibiotics except, for Ciprofloxacin, Gentamicin, Chloramphenicol and Cefotaxime. The tested inhibition zone bacteria were 25-30 mm Ciprofloxacin and Chloramphenicol, respectively, compared with the inhibition zone that ranging from 5-29 mm and 25 mm for lemon and lime juices, respectively. The experiment proved that citrus fruit juices were more effective than antibiotics in bacteria growth inhibition. These results were encouraging to prepare special pharmacological formulas from citrus fruit juices.

الخلاصة:

هدفت التجربة التي أجريت في قسم علوم الحياة/ كلية العلوم، جامعة الكوفة لتحديد الفعالية التضادية لكل من عصير الليمون الحامض Lemon و ليمون بنزهير (نومي بصرة) Lime ضد بعض البكتريا الممرضة المختارة والتي شملت كل من *Esherichia coli* ، *Shigella flexneri* و *Klebsiella pneumonia* . نتائج هذه التجربة بينت إن جميع تراكيز ثمار الحمضيات المختبرة ذات فعالية تضادية ضد البكتريا لوجود نسبة كبيرة من حامض الستريك و volatile oils (lamonine) و بعض المواد مثل linalyl acetate ، linalol ، turpinol و cymen. النتائج أوضحت إن انخفاض الأس الهيدروجيني pH كان له تأثير قوي في مقاومة البكتريا بسبب حساسية البكتريا له.

النتائج أظهرت إن كل من تراكيز عصيري ثمار الليمون و ليمون بنزهير تأثيرا تثبيطيا في نمو البكتريا المدروسة وبنطاق تثبيطي تراوح بين (5-29) ملم ، إذ كانت هناك فروقا معنوية بين بكتريا الاختبار تجاه النوعين lemon و lime. التأثيرات التضادية لكل من تراكيز هذه العصائر قورنت مع التأثيرات التضادية لبعض المضادات الحيوية، فكانت العزلات البكتيرية مقاومة لأغلب المضادات الحيوية عدا المضادات التالية ciprofloxacin, gentamicin, Chloramphenicol and Cefotaxime ، ذات النطاق التثبيطي كان (25-30) ملم للمضاد الحيوي Chloramphenicol و Ciprofloxacin ، على التوالي لمقارنة مع نطاق التثبيط الذي تراوح بين (5-29) ملم و 25 ملم لكل من عصيري lime و lemon ، على التوالي.

وقد بينت هذه التجربة إن لهذه العصائر تأثيرا مضادا للبكتريا أكثر من تأثير المضادات الحيوية في تثبيط البكتريا، هذه النتائج تشجع على الاهتمام بتحضير جرعات دوائية من ثمار هذه العصائر Citrus fruit juices.

Introduction:-

Lemon (*Citrus limon*) and Lime (*Citrus aurantifolia*) are popular citrus fruits and food ingredients for flavoring and adding acidity. Lemon and Lime juices have been reported to exhibit antibacterial activity against *E. coli* O157:H7 and other food born bacterial pathogens in cooked foods (1; 2).

To our knowledge, there is report on antibacterial activity of citrus fruits against bacteria, an important food-borne enteropathogen, or other pathogenic bacteria.

The antimicrobial properties of plants have been investigated by a number of researchers worldwide, especially in Latin America. In Argentina, a researcher tested 122 known plant species used for therapeutic treatment (3).

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes (4). Citrus fruit juice has been reported to inhibit the growth of bacteria such as *Vibrio cholerae*, *E. coli*, and other (1; 5).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the further is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate aim is to offer appropriate and efficient antimicrobial drugs.

Materials and methods:-

1. Lemon and Lime were purchased from a supermarket, the pH values of Lemon and Lime juices were 2.2, and 2.1, respectively.

2. Tested bacteria

The tested bacteria used in this experiment were collected from the Microbiology / Department of Medicine College/ Kufa University, these are *Esherichia coli*, *Klebsiella pneumonia*, and *Shigella flexneri* were confirmed using standard bacteriological methods according to (6; 7).

3. Nutrient agar plates were separately flooded with different test bacteria already in sterile nutrient broth by culturing at 37°C for 24 hr. Muller-Hinton agar plates were drained and allowed to dry at 37°C for 30 min before wells of 6 mm in diameter were punched using cork borer at different sites on the plates, five concentrations of 2, 4, 8, 16, and 100% Limon and 2, 4, 8, 16, 20% Lime, were separately placed in the different punched wells and the plates were incubated at 37°C for 24 hr. (8) the diameter of the inhibition zones were measured and recorded.

Muller-Hinton agar plates which had been flooded separately with different tested bacteria, were allowed to dry at 37°C for 30 min. before, placing conventional antibiotics discs shown in table (1) by the disc diffusion technique on it, the plates were incubated at 37°C for 24 hr. based on the method of (9), the diameter zones of inhibition was measured and recorded according to the (10).

Table (1): Antibiotics disc (Bioanalysis, Turkey)

No.	Antibiotics	Symbol	Concentrations (µg)/disc
1	Cephalexin	CL	30
2	Cefixime	CFM	5
3	Cefotaxime	CTX	30
4	Trimethoprim	TMP	5
5	Tetracycline	TE	30
6	Ciprofloxacin	CIP	5
7	Gentamicin	CN	10
8	Chloramphenicol	C	30

Note: All antibiotics disc to be stored between 0-8 °C (For prolonged use store below -20 °C).

4. Statistical analysis:

The data were analyzed statistically, using the least significances differences test (LSD), T- test and analysis of variance (ANOVA) at the P- value were 0.05 (11)

Results and Discussion:

The two juices tested showed strong inhibitory activities against *Sh. flexneri* strain (Table 2, 3, 4 and Fig 1, 2).

Table (2): Antimicrobial activity of conventional antibiotics on tested bacteria

No.	Tested bacteria	CL	CFM	CTX	C	CN	CIP	TE	TMP
1	<i>E. coli</i>	R	15	R	25	R	30	R	R
2	<i>Sh. flexneri</i>	R	10	R	10	13	25	R	R
3	<i>K. pneumoneae</i>	R	R	R	23	R	R	R	R

CL: Cephalexin, CFM: Cefixime, CTX: Cefotaxime, TMP: Trimethoprim, Te: Tetracycline, CIP: Ciprofloxacin, CN: Gentamicin, C: Chloramphenicol, R: resistance to antibiotics

Table (3): Antibacterial activity of Limon on tested bacteria

Tested bacteria	Lemon juice concentration					
	control	2%	4%	8%	16%	100%
<i>E. coli</i>	0	0	0	0	0	22.3
<i>Sh. flexneri</i>	0	0	0	6.6	17.3	23.3
<i>K. pneumoniae</i>	0	0	0	0	0	15
LSD 0.05 (17.2)						

Table (4): Antibacterial activity of Lime on test bacteria

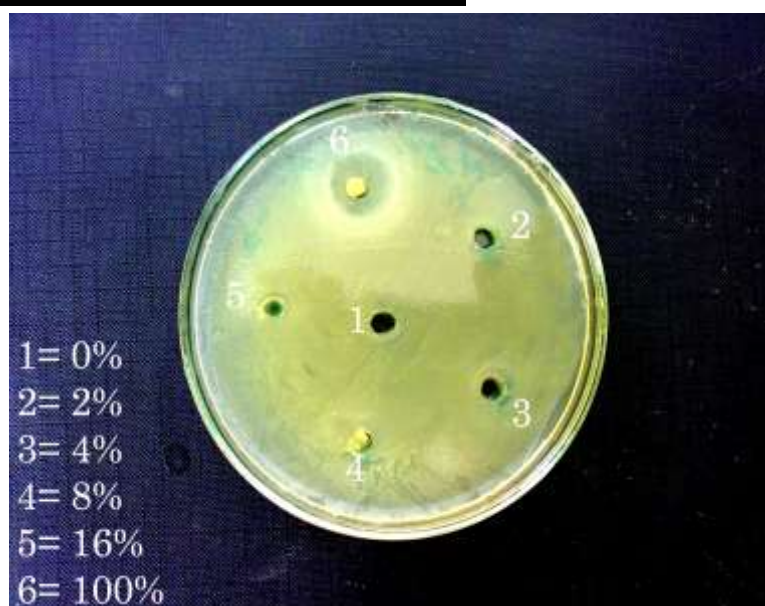
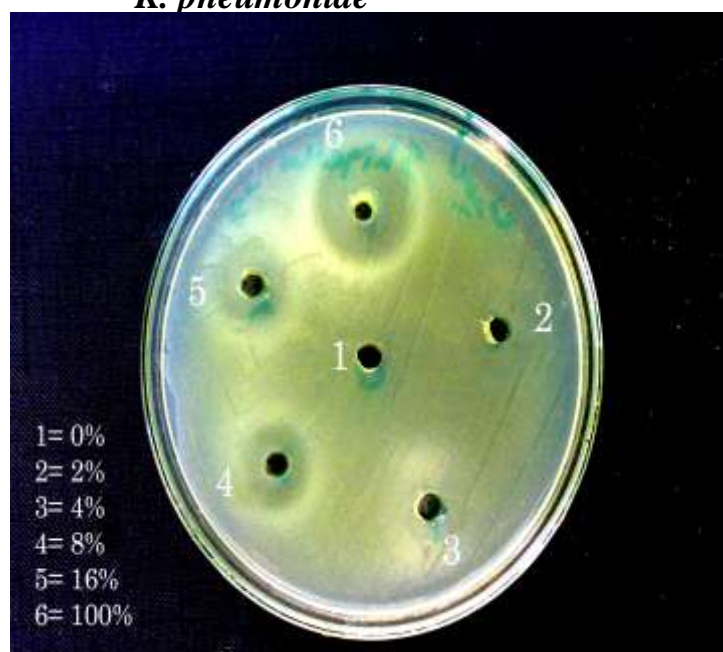
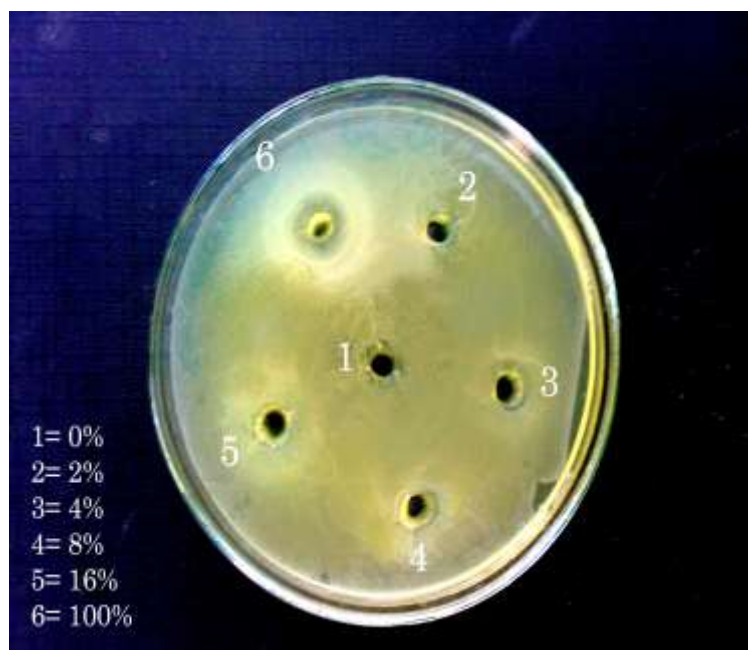
Test bacteria	Lime juice concentration					
	control	2%	4%	8%	16%	20%
<i>E. coli</i>	0	0	7	11.6	15	20.6
<i>Sh. flexneri</i>	0	0	11.6	16.3	19.5	22
<i>K. pneumoniae</i>	0	0	0	5	17.3	29
LSD 0.05 (19.46)						

All juices were effective on the growth of *E. coli* , *Sh. flexneri* and *K. pneumonia*. The table (3) and Fig (1) showed the effects of the Lemon juice was the highest on *Sh. flexneri* (23.3 mm in 100% conc., 17.3 mm in 16 % conc., and 6.6 mm in 8% conc.) followed by *E. coli* (22.3 in 100 %) and *K. pneumonia* (15 mm in 100%), while the table (4) and Fig (2) the inhibitory effects of the Lime juice was the highest on *K. pneumonia*(29 mm in 20%, 17.3 mm in 16%, and 5 mm in 8%), *Sh. flexneri* (22 mm in 20% conc., 19.5 mm in 16 % , 16.3 mm in 8% conc., and 11.6 mm in 4%) and *E. coli* (20.6 mm in 20 % conc., 15 mm in 16% conc., 11.6 mm in 8% conc., 7 mm in 4% conc.).

The growing resistance of bacteria to convectional antimicrobial agents is a source of concern to clinical microbiologists all over the world. As a result efforts are being made to develop antimicrobial agents from local sources for better chemotherapeutic effects (12), from this study, all the tested bacteria were inhibited by the two juices.

Sh. flexneri

K. pneumoniae

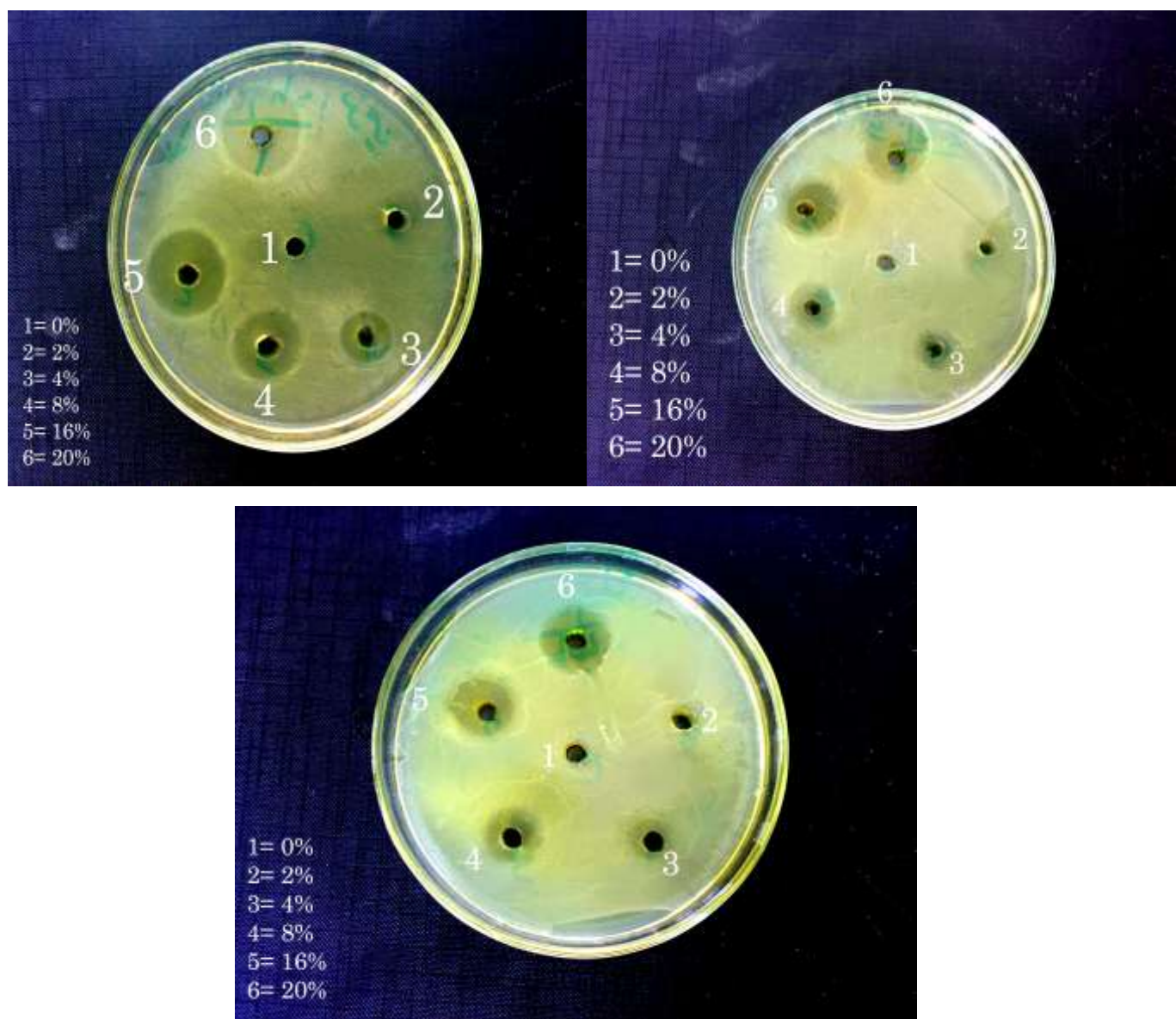


E.coli

Fig (2): Antibacterial activity of *Limon* on tested bacteria

Sh. flexneri

E.coli



K. pneumoniae

Fig (3): Antibacterial activity of Lime on tested bacteria

The inhibition of *Sh. flexneri* by the two juice used was the same in some cases with that of standard antibiotics. Lemon juice at 16% conc. give zone of 17.3 mm which was a little higher than of Cefixime (10 mm) and Lime juice at 4% gave zone of 11.6 mm which was the same as that of Cefixime 10 mm. These results suggest that the juices contain bio-components whose antibacterial activities is effective only in low pH is highly comparable bacteria with that of these two antibiotics against tested bacteria. The inability of tetracycline and other antibiotics to inhibit the growth of any these bacteria may be as a result of misuse and abuse of this drug (13)

Tetracycline and other antibiotics are one of the common antibiotics that have been greatly abused, this result agrees with the finding of (14).

This work has been able to show that Lemon and Lime juice have antimicrobial activity against bacteria that can cause many disease such as diarrhea especially the ones that are caused by *E. coli*, and *Sh. flexneri* .

It has been proposed that antibacterial activity of the organic acid in low PH is due to its undissociated structure, which is hydrophobic and has cytotoxicity as well. Because the concentrations of acid in lemon, and Lime are higher than in any other fruits juices, these juices may be antimicrobial active against a variety of bacteria (15). This antimicrobial effect of citrus fruit juices may be due to the presence of some substances with antibacterial activity including citric acid, limonene, linalool, linalyl acetate, turpinol, and cymen (16)

The antimicrobial activity of Lime juice against *V. cholerae* has been reported by (17; 5).

De Castillo *et al.*, 1998 also reported that freshly squeezed Lemon juice inhibited the growth of *V. cholerae*.

It could be concluded from this result that citrus fruit juices have great potential effects as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes, as good antibacterial effect, sterility, and no or minimal side effects of fruit juice in comparison to many antibacterial drugs.

However, it is necessary that, further investigations could be undertaken to discover the mechanisms involved in the antibacterial activity of fruit juices, and possible ways for clinical use.

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