

## **Evaluation of the inhibitory effect of some citrus juices in bacteria *Klebsiellae pneumonia* in vitro**

### **تقيم التأثير المثبط لعصائر بعض الحمضيات في بكتريا *Klebsiellae pneumonia* في المختبر**

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#### **Summary**

The aim of this research is study the effect of some citrus juices such as lemon *Citrus limon* , bitter orange *Citrus Aurantium* and orange *Citrus sinensis* in bacteria *Klebsiellae pneumonia* in vitro, four concentrations usage (25%, 50%, 75%, 100%) compared to some industrial antibiotics (Bacitracin, Erythromycin, Novobiocin ), as the results show the juices have important impact in inhibiting the growth of bacteria and the lemon greatest influence in the growth with concentration 100%, where the inhibition rate after 24 hours was 30 mm and decreasing with low concentration reaching 15 mm at 25%, while the bitter orange had the second-level after lemon , where the inhibition rate was 23 mm with concentration of 100% and 5 mm with 25% concentration . and at increasing the incubation period to 48 hours , lemon inhibition with concentrations (75% and 100%) was 19 mm and decrease to 16 mm with a concentration of 100% after 72 hours , while the bitter orange has not had any significant inhibition when increasing incubation period , while the orange did not have any mention Influence of all concentrations and for three incubation period ,and the same results obtained with industrial antibiotics .

#### **المستخلص**

إستهدف البحث الحالي دراسة تأثير عصائر بعض الحمضيات منها الليمون *Citrus limon* ، النارنج *Citrus Aurantium* و البرتقال *Citrus sinensis* في بكتريا *Klebsiellae pneumonia* في المختبر باربعة تراكيز ( 25% ، 50% ، 75% ، 100% ) بالمقارنه مع بعض المضادات الحيائية الصناعية ( Bacitracin , Erythromycin , Novobiocin ) ، اذ اظهرت النتائج ان للعصائر تأثير مهم في تثبيط نمو البكتريا و كان لعصير الليمون التأثير الاكبر في النمو بتركيز 100% حيث بلغ معدل التثبيط بعد 24 ساعة 30 ملم و تناقص المعدل مع انخفاض التركيز حيث بلغ 15 ملم عند تركيز 25 % ، اما النارنج فكان له المستوى الثاني بالتاثير بعد الليمون حيث بلغ معدل التثبيط 23 ملم بتركيز 100% و 5 ملم بتركيز 25% ، وعند زيادة فترات الحضان الى 48 ساعة تثبيط النمو للليمون كان مع التركيزين ( 75% و 100% ) 19 ملم ليتناقص و يبلغ 16 ملم مع تركيز 100% بعد 72 ساعة ، بينما النارنج لم يكن له اي تاثير عند زيادة فترات الحضان ، اما البرتقال فلم يكن له اي تاثير يذكر بكل التراكيز و لفترات الحضان الثلاثة كما كان الحال بالنسبة للمضادات الحيائية الصناعية المعتمدة ضمن البحث .

#### **Introduction**

*Klebsiellae pneumonia* is an opportunistic, Gram-negative bacterium , responsible for both communal and hospital acquired infections , if it repeated will cause of urinary tract infections , pneumonia and subsequent systemic infections. The percentage of death rate of *K.pneumoniae* range from 20%– 50% , with no improvement in result within the last decade [1,2,3]. It is also involved in surgical site infections, peritonitis, pyogenic liver abscess and thoracic empyema [4]. *K. pneumoniae* is known worldwide for fast acquisition of traditional and new anti-microbial drug resistance [5] .

Threat to life caused by microorganisms has become an important reason in the mortality rate of patients [6] . Increased resistance of bacterial species to different treatments showed new strains low sensitivity to antibiotics , For this reason it became necessary to look for new sources more safer for infection control [7, 8] . Plant products become more important sources in influencing human health , Effectiveness of antibacterial branded as widely and many of them used as a healing alternative [9] . In developing countries there are many uses of plant material because of its effectiveness against microorganisms as secondary metabolites such as alkaloid , phenolic compound , etc. The practice session of complementary and alternative medicine is now on the increase in reaction to Macrocosm Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific base for the efficacy of many plants used in folk medicine to treat infections [10 , 11] . In spite of the presence of antibiotics and powerful anti-fungus, but resistant strains constantly show which requires the search for new drugs is always [12] . Plant sources are cheaper alternative and safer antibiotics [13, 14, 15].

Fruits are known to be an integral part of diet and are consumed fresh and as juices, salads or fruit based drinks . Besides their delicious taste and flavor, the fruits are known to reduce the risk of several chronic diseases including cancer. The protective nature of fruits is due to the presence of phytoconstituents such as polyphenolic compounds . Among various fruits that are consumed, citrus fruits are widely used in almost all countries [16, 17].

The Citrus is the most important fruits group , which rich with phenolic compounds , minerals , vitamin , dietary fiber , requirement oils and carotenoid . It has strong commercial value for the market of fresh produce and food industry. Moreover, produced citrus produces a large amount of wastes that are an important source of biologically active compounds [18].

Citrus fruits are richer sources of bioactive compounds having beneficial effect on human health such as vitamin C, carotenoids, flavonoids, limonoids, essential oils , alkaloids , minerals and vitamin B complex . Majority of citrus fruits are eaten fresh [19, 20].

## **MATERIALS AND METHODS**

### **Preparation of bacteria**

The bacteria was obtained from the central health laboratory in holy Karbala city.

### **Preparation of Juices**

The fresh fruit used in this study was obtained from the local market in Karbala City, Iraq, 2016. Fresh fruit was washed in run tap water in vitro , sterilized shell with 70% alcohol , rinsed with distilled water and clear cut with sterile knife and pressure juice Into a sterile universal container and then filter (using the 0.45-millibor filter paper) into another sterile container to remove the seeds and other tissues and use them freshly without cooling .

### **Examination antimicrobial activity of plant extract in bacteria**

The method of diffusion in wells [ 21] were used to determine the inhibition effect of citrus juices in bacteria , four concentrations of juices used (25,50,75,100)% .

The bacteria activate on broth Muller- Hinton Medium for 24 hours then make series of dilutions by distilled water, and chose more adequate one . the bacteria spread on Muller- Hinton agar Medium by swab then make wells by using a sterile cork borer to produce 5mm diameter well . For each well, 40 µl of different concentration of the extract was added and allowed to be spread at room temperature for 20 minutes then transmitted to incubator in 37 °c for the three limited periods. This was compare with using three types of antibiotic include: Bacitracin, Erythromycin, Novobiocin (Bioanalyse, Turkey).

## **RESULTS AND DISCUSSION**

Like show in table, the juices of citrus had highly effects in bacteria where the inhibition zone was increased with increase concentrations, the most effect one was the juice of lemon where the inhibition zones after 24 h were (15, 22, 28, 30) mm with (25, 50, 75, 100)% concentrations respectively, after 48 h this rates reduced to be 0 with both (25, 50)% concentrations and 19 with (75, 100)% concentrations, after 72 h there are no effects just with 100% concentration reach 16 mm . the results of bitter orange different where show effects after 24 h less than with lemon (5, 13, 17, 23) mm with (25, 50, 75, 100)% concentrations respectively and effects were zero when increase periods of incubation to 48 and 72 hour. While orange juice didn't show any effects with all concentrations, with all incubation periods. This results was compare with usage industrial antibiotics (Bacitracin, Erythromycin, Novobiocin) which didn't show any effects in bacteria growth. This results same with [22] who use the water extracts of peel of the same three type of citrus, which show antimicrobial activity in *Klebsiellae pneumonia*, but *Citrus aurantium* the most effective than *Citrus limon* & *Citrus sinensis*, this may be back to deferent in part of plant which uses. [23] evaluated antibacterial efficacy of peel methanolic extracts of *C. sinensis* and *C. aurantium* against 3 bacteria, *K. pneumonia* exhibited higher susceptibility to peel extracts . Results match with [24] who found that extract of C. lemon juice has top antimicrobial activities in bacteria under study. The development of drug resistance in human pathogens against antibiotics has often required the search for new antimicrobial agents from other sources , involve plants and microbes [25]. Natural microbial inhibitors have been recovered from a wide range of foods involved onions , garlic, fruits, vegetables, grains and spices. Many of these antimicrobial foods contribute to natural resistance to degradation. Flavor components consist of compounds such as alcohol, aldehydes, esters, terpenes, phenols, organic acids, etc., some of which have not yet been determined [26] . [27] say there are high amounts of photochemical and bioactive compounds such as flavonoids, Alkaloids, Flavonoids, Tannins, Phenols, Saponins carotenoids, vitamins and minerals available in Citrus fruits. The most important cropped Citrus fruits are oranges, mandarins, grapefruits and acid Citrus fruits, namely lemons [28]. [29] When examined the properties of antimicrobial activity, he found the lemon was the most important against *S. aureus*, *Klebsiella*, *Escherichia coli*, *P. aeruginosa* and *C. albicans*. In addition [30] explained that there is good inhibition of *C. limon* against bacteria particular *S. aureus*, *P. aeruginosa* and *P. vulgaris*.

Moreover [31] said *C. limon* has a high ability to inhibit both *S. aureus* and *K. pneumoniae* with inhibition zones 17.4 mm for *S. aureus* and 13.3 mm for *K. pneumoniae* . [32] Antibacterial activity is attributed to the fact that lemon juice may contain organic acids, vitamins, secondary metabolites, as well as the interaction of these substances with each other. So the inhibitory efficacy of bacteria in citrus juices was attributed to citric acid and not to malic acid [33, 34] . Flavonoids and some phenolic compounds are known as antimicrobial agents to inhibit growth of *Staphylococcus aureus* [35]. Flavonoids and many subclasses of it found in sweet orang *Citrus sinensis* [Flavanone glycosides, Flavones glycosides, Anthocyanins, Flavone aglycones [36].

One of the most important chemicals that are given a significant importance in the bitter orange peel in the modern markets is synephrine, which was later isolated from the leaves and fruits of many citrus species, especially *C. aurantium*[37]. Synephrine is an Alkaloid [38] . Activity against bacteria for some alkaloids, flavonoids and some phenolic acids has been studied by [39] , all of which have shown active activity against bacteria . It was also found that this activity depends on specific patterns in the chemical composition of the tested materials.

Table : The effects of Juices and antibiotic in bacteria :

Juices %		Inhibition zone (mm)		
		24 h	48 h	72 h
Lemon	25 %	15±0.5	-	-
	50%	22±2.08	-	-
	75%	28±1.6	2.08±19	-
	100%	30±0.7	0.5±19	0.5±16
Bitter orange	25 %	5±0.5	-	-
	50%	2.8±13	-	-
	75%	1.6±17	-	-
	100%	0.7±23	-	-
Orang	25 %	-	-	-
	50%	-	-	-
	75%	-	-	-
	100%	-	-	-
Antibiotic				
Bacitracin		-	-	-
Erythromycin		-	-	-
Novobiocin		-	-	-

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