A study of Antibacterial Activity of the Fruitpeels of *Citrus sinensis* & *Punica granatum*

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Abstract

The aim of this study was to determine the antibacterial activity of the fruitpeels of *Citrus sinensis* (Portuqal) and *Punica granatum* (Rumman) on *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Two extraction methods were used hot water and cold water. The screening of antibacterial activity was done by agar diffusion technique. Minimum inhibitory concentrations (MICs) studies were made by agar dilution technique .The extracts of *Punica granatum* were statistically more effective than that of *C. sinensis.* Likewise,. cold extracts exhibited statistically a large degree of antibacterial activity than hot extracts .The MICs were (12.5), and (50) mg \ ml for *S. aureus* and *P. aeruginosa* respectively . *K* . *pneumoniae* was resistant to all types of extracts . Upon these results, the cold extracts of the fruitpeels of *Punica granatum* and to a lesser extent *C. sinensis* can be used as antibacterial agent in topical pharmaceutical, oral administration, and as food preservatives.

Introduction

The antimicrobial activity of plants had been received attention many years ago as one of the most effective mechanism for the control of microorganisms . In Iraq , many studies have been attempted to evaluate the antibacterial activity of some plant extracts (AL-Thahab,1998 ; AL-Ani *et al* ., 1996 ; AL-Gazaly *et al* . ,2002). Peels of *Citrus* sp and *Punica* sp were commonly used for this purpose (Jayaprakasha *et al*. , 2001; Prashanth *et al* . 2001 ; deCastillo *et al* .,2001). This work has been carried out to evaluate the antibacterial activity of fruitpeel extracts of *Citrus sinensis* and *Punica granatum* on some pathogenic bacteria including S*taphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.]

Materials & Methods

This study was done in Babylon University – College of Medicine with the following information:

1- **Extraction**

Hot water and cold water extracts were prepared from fresh fruitpeels of *C. sinensis* and *P. granatum* according to Okanla *et al* . (1994) .

2- **Bacterial isolates** :

Three clinical isolates were included in this work :-
S. aureus , *P. aeruginosa* and *K. pneumoniae* . These isolates were diagnosed according to (Baron *et al* . , 1994) .

3- **Studing of antibacterial activity :-**

Agar diffusion technique was used to determine the growth inhibition zones of bacterial growth. Agar dilution technique was used to determine the minimum inhibitory concentrations (MICs), (Harborne , 1984) . Müller – Hinton agar was used as culture media in both techniques with stock solutions of 250 mg / ml . Distilled water was used as a control.

4- **Statistical analysis :-**

The experiment consisted of three replications in a complete randomize design with 3×2×2 factorial experiment was conducted. The computations for the analysis of variance for test of significance and the least significance difference (LSD), for comparisons of the differences between the treatment means were used, (Daniel, 2000).

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Results and Discussion

Distilled water control revealed nil activity, whereas hot and cold extracts gave up to 10 mm inhibition zones (table – 1).

S . *aureus* was sensitive to the hot and cold extracts of *P. granatum* as well as to the cold extacts of *C. sinensis* , *P. aeruginosa* was sensitive to the hot and cold extracts of *P. granatum*,but exhibited resistance to the hot and cold extracts of *C. sinensis. K. pneumonia* was unaffected by all .types of extracts (table B). MICs for *S* . *aureus* were (12.5)mg /ml with the hot and cold extracts of *P. granatum* and cold of extract of *C. sinensis* whereas MIC was 50 mg /ml with the hot extract of *C. sinensis.* MICs for *P. aeruginosa* were (50) mg/ml with the hot and cold extracts of *P. granatum* and (100)mg /ml of with hot extracts of *C. sinensis* . MICs for *K. pneumonia* were 250 mg/ml with hot extract of *C. sinensis* ,150 with cold extract of *C. sinensis,* hot and cold extracts of *P. granatum* gave 100 mg /ml MICs for *K. pneumonia* (table B).The antibacterial activity of *P.granatum* is due to the presence of tannin, mucilage and resin in their fruit peels. Whereas fruit peels of *C* . *sinensis* contain essential oils , acids and bitter resin (Al – Rawi & Chakravarty , 1988). The resistance of *K* . *pneumoniae* is due to capsule permeability masking effect . Such finding is similar to that observed by (Al- Gazaly et al ., 2002) and Abdul-Latif et al * .

It is apparent from the F-tests in table (4) that a highly significant difference exists among the treatments and some interactions. From these results and LSD test, it can be concluded that:

1- The fruit peels of *P. granatum* are highly significant effect against S. *aureus, P. aeruginosa* than fruit peels of *C. sinensis.* ($P<0.01$).
2- Cold extracts is statistically more effective than hot extracts. ($P<0.01$).
3- *K* . *pneumoniae* is resistant to such treatments due to their encapsulation ($P>0.05$) .
4- Cold extracts of fruit peels of *C* . *sinensis* and *P.garantum* can be used for pharmaceutical uses and as food preservatives (vonWoedltke *et al* . , 1999) .

Table (1) The Judgment of antibacterial activity of *C. sinensis* and *P.granatum, (*fruitpeels).

Parameters	
Inhibition zones (mm)	**Conclusion**
0 – 2	**Control (distilled water)**
0 - 4	**Resistant**
5 - 15	**sensitive**

Table (2) The antibacterial activity of *C.sinensis* and *P.granatum* (diameter of inhibition zones mm)

Bacteria	**Inhibition zones (mm)**			
	C. sinensis		***P.granatum***	
	Hot extract mean	**Cold extract mean**	**Hot extract mean**	**Cold extract mean**
S. aureus	**4.33**	**7.67**	**9.0**	**9.0**
K. pneumoniae	**0.0**	**1.67**	**2.33**	**2.67**
P. aeruginosa	**2.33**	**4.67**	**7.0**	**6.67**

Standard error = 0.040

Table (3) Minimum inhibitory concentrations (MICs)

Bacteria	***C. sinensis***		***P.granatum***	
	Hot extract	**Cold extract**	**Hot extract**	**Cold extract**
S. aureus	**50**	**12.5**	**12.5**	**12.5**
K. pneumoniae	**250**	**150**	**100**	**100**
P. aeruginosa	**100**	**50**	**50**	**50**

Table (4) The analysis of variance for factorial experiment in (CRD)*

ANOVA

Source	Sum of Squares	df	Mean Square	F
Method of extract (A)	**0.134**	**1**	**0.134**	**28.471**
Type of fruitpeels (B)	**0.640**	**1**	**0.640**	**153.529**
Type of bacteria (C)	**2.069**	**2**	**1.034**	**219.059**
AB	**0.134**	**1**	**0.134**	**28.471**
AC	**0.009**	**2**	**0.004**	**0.941**
BC	**0.047**	**2**	**0.023**	**4.941**
ABC	**0.016**	**2**	**0.008**	**1.647**
Error	**0.113**	**24**	**0.005**	
Total	**3.162**	**35**		

The coefficient of determination ($R^2 = 0.964$).

* Complete Randomize Design.

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دراسة الفعالية المضادة للبكتريا لقشور ثمار البرتقال والرمان

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الخلاصة

هدفت هذه الدراسة الى تحديد الفعالية المضادة للبكتريا لقشور ثمار البرتقال والرمـان على ثلاثة انواع من البكتريا (*S.aureus, K. pneumoniae , P. aeruginosa*) . تـم تحضير نوعين من المستخلصات المستخاص المائي الحار والبارد ولدراسة الفعالية المضـادة للبكتريا تم استخدام تقنية الانتشار بالاكار اما التراكيز لدينا فتمت دراستها باسـتخدام تقنيـة التخفيف بالاكار . ظهر من خلال هذه الدراسة بان المستخلصات المائية المحضرة من قشور الرمان ذات تاثير اشد احصائية من البرتقال . وظهر ايضاً بان المستخلصـات البـاردة ذات درجة كبيرة احصائياً من الفعالية المضادة للبكتيريا مقارنة مع المستخلصات الحارة تراوحت التراكيز المضبطة لدينا من 12.5-50 ملغم/ مل لكل من *S.aureus* و *P. aeruginosa* على التوالي فيما اظهرت *K. pneumoniae* مقاومة لكل انواع المستخلصات. اعتماداً علـى هذه النتائج فان المستخلصات الباردة لقشور ثمار الرمان وبدرجـة اقـل البرتقـال ممكـن استخدامها كمواد مضادة للبكتريا في الاستخدامات الصيدلانية الجلدية او عن طريق السـبيل الفموي ويكن استخدامها ايضاً كمواد حافظة للاغذية.