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### Determination the antibacterial activity of *Punicagranatum* Extract towards *Streptococcus Pneumonia* and *Klebsella Pneumonia*

Ruaa Hassan Lateef

College of Education for Women , Tikrit University , Tikrit , Iraq

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##### Corresponding Author:

Name: Ruaa Hassan Lateef

E-mail: [roaaraooa@gmail.com](mailto:roaaraooa@gmail.com)

Tel:

#### ABSTRACT

This study was carried out to determine the inhibitory effect of the alcoholic and aquatic extracts of *Punicagranatum* (pomegranate peel) against multiresistant antibiotic bacteria *Streptococcus pneumonia* and *Klebsella pneumonia* bacteria with some antibiotics. These isolated from the sputum and urine samples from patients whom lies in Tikrit Teaching Hospital in Tikrit city. The results showed various effects of the water and alcoholic extracts used in the study. The results showed that the highest inhibitory effect of the alcoholic extract against the bacteria *Streptococcus Pneumonia* at concentration (150) Inhibition zone was (24) mm and this is higher than the water extract at concentration (150) which was (16) mm. The results indicated that the highest Inhibitory effect of the alcoholic extract against the *Klebsella Pneumonia* at concentration (150) was (23) mm whereas the results of the inhibitory effect of the water extract against *Klebsella Pneumonia* are similar with the inhibitory effect of the extract of *Streptococcus Pneumonia* at (150) which was (16) mm.

The results showed that the highest inhibitory diameter zone of Imipenem antibiotic on *Streptococcus pneumonia* bacteria is (38) m and is higher than the inhibitory effect of Imipenem antibiotic on *Klebsellapneumonia* bacteria which is (37) m in diameter.

#### Introduction

Natural plants have been an important source of many pharmaceuticals [1] since ancient times till today, where they are used by many people in the treatment of many diseases [2] since they contain a large number of compounds with vital effectiveness [3]. *Punicagranatum* (pomegranate) contain 25% to 28% tannins in the form of glucose, and the most important compounds of tannins are *punicalin* and *grananatine*. The pharmacological value of *Punicagranatum* (pomegranate) is that it consists of volatile alkaloids and phenol compound antioxidants [4] such as natosatine, which inhibits the oxidation of low-density lipoproteins carrying cholesterol [5], in addition to the presence of amino acids [6]. The most common causes of gastrointestinal infections and infectious ulcers are opportunistic and pathogenic bacteria, which are a group of species that are distinguished by biochemical tests. They are straight rod-shaped bacillus such as *Streptococcus* and *Klebsella*, which are a major cause and aiding of most ulcers and diarrhea and may go beyond that to urinary tract infections [7].

The aim of this study was determine the antibacterial activity of the aquatic and alcoholic extracts of *Punicagranate* (pomegranate) against *Streptococcus pneumonia* and *Klebsellapneumonia* which multi resistant to some antibiotics.

#### Materials and methods

##### 1. The isolated bacteria used in the study:

Two bacterial laboratory samples were obtained from the patients checking in the Tikrit Educational Hospital in Tikrit city. The first bacterial sample is *Streptococcus pneumoniae* isolated from people with pharyngitis. The second bacterial isolated is *Klebsiellapneumoniae* which isolated from people with urinary tract infection. The purity of bacterial isolates was confirmed through the Microbiology Laboratory of the College of Education for Women / Biology Department.

##### 2. Collection and preparation of *Punicagranate* (pomegranate peel):

An enough amount of *Punicagranate* (pomegranate peel) was collected from the local stores in Tikrit city, completely cleaned to remove all pomegranate seeds

and washed [8], dried at room temperature for seven days, and then grinded using the electric grinder to obtain a soft *Punicagranate* (pomegranate peel) powder, which was in turn placed in a glass bottle and kept for use [9].

**3- Preparation of aquatic and alcohol extracts for *Punicagranate*(pomegranate peel):**

The water and alcoholic plant extracts were prepared using the soaked method [10]. An amount of 15 gm of *Punicagranate* (pomegranate peel) powder was taken and soaked in 100 ml of distilled and stirred water for two hours and placed in the shaking incubator for 24 hours. The solid materials were disposed of by filtering the peel of pomegranate fruits through the filter paper type Whatmann No.260 and centrifuged at 3000 rpm for 10 minutes, then the filtered material (leachate) was taken and placed in sterile bottles then kept in the refrigerator until use. The concentrations (25, 50,100,150) mg / ml were prepared from each extract by taking a quantity of the filtered material(leachate) and placing it in a certain quantity of distilled water [11]. Ethanol was used as a solvent instead of distilled water in the preparation of the alcohol extract, in which 15 gm of *Punicagranate* (pomegranate peel) powder was dissolved in 100 ml of ethanol and the same above mentioned method was followed for the water extract with the same concentrations.

**4 - Preparation of bacterial suspension:**

The bacteria were cultured in the nutrient agar medium and stored 24 hours for activation. On the

following day, the bacterial suspension of 24 hours was prepared by adding (3-5) pure colonies of the two types of bacteria used in the study to the distilled water. The bacterial suspension was diluted with the normal saline solution. The turbidity of the bacterial suspension was compared to the McFarland tubes turbidity standard.

A quantity of (0.1) ml of bacterial suspension was transferred to the Mueller Hinton Agar (MHA) medium and spread using the L- shape sterile glass spreader, and dishes incubated for (30) minutes to obtain imbibition.

After incubation, wells with each of the size (0.5) ml were made by a sterile cork borer [12]. A quantity of (0.05) ml of *Punicagranate* (pomegranate peel) extract was placed in the wells according to the pre-prepared concentrations. In addition, the antibiotic tablets were placed in the wells by sterile forceps for comparison. The dishes were incubated in the incubator at 37 ° C for 24 hours. The results were read by measuring the Inhibition Diameter zone, and compared with the inhibition diameter of the WHO-approved antibiotics [13].

**5- Antibiotics used for comparison in tests**

The ready-made antibiotic disks which are described by type, concentration and symbol in Table (3-5) below were used bearing the trademark Gokhan Laboratuvar san T.I.C.A.S. and kept in sealed packets:

**Table (1):Antibiotic disks used in the preleat study [13]**

No.	Type of Antibiotic	Disks concentration Mg/ Disks	Symbol	Sensitive	Moderate Sensitive	Resistant
1	Gentamicin	10	GM	≥ 16	13-15	≤ 12
2	Amikacin	30	AK	≥ 17	15-16	≤ 14
3	Imipenen	10	IPM	≥ 16	14-15	≤ 13
4	Amoxicillin	30	AMC	≥ 18	14-17	≤ 13
5	Cefotaxime	30	CTX	≥ 23	15-22	≤ 14

**Results and Discussion**

The results of the current study showed that the aquatic and alcohol extracts of the *Punicagranate* (pomegranate peel) have a significant inhibitory effect on the growth of the two Species of *Klebseilla pneumonia* and *Streptococcus pneumonia*, depending on the concentration of the aquatic or alcohol extract and to Species of bacteria. Table (2) shows the inhibitory diameters of the aquatic extract of

*Punicagranate* (pomegranate peel) on the two types of bacteria *Klebseilla pneumonia* and *Streptococcus pneumonia* species with four concentrations of the extract (25,50,100,150) mg/ml, which gave consistent inhibitory results respectively for *Klebseilla pneumonia* (12,13,15,16)mm, and gave similar inhibitory results for the bacteria *Streptococcus pneumonia* (12,13,14,16) mm, Figure (1).

**Table (2) : Inhibitory diameters of the aquatic extract for *Punicagranate*(pomegranate peel) against two Species of bacteria isolates (mm)**

Type of Bacteria	aquatic extract concentrations for <i>Punicagranate</i> Mcg/ml			
	150	100	50	25
<i>Klebseilla pneumonia</i>	16	15	13	12
<i>Streptococcus pneumonia</i>	16	14	13	12

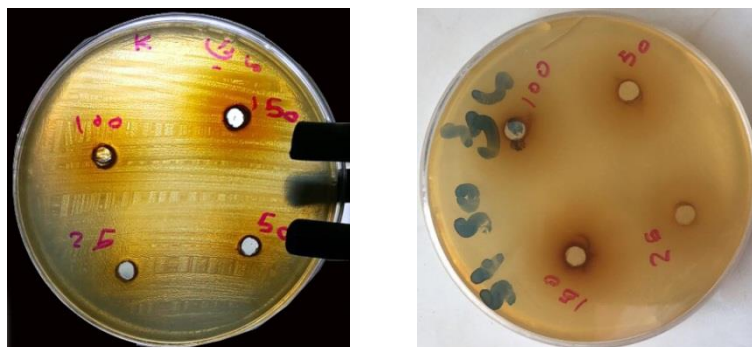


Figure (1): The inhibitory activity of aquatic extract for *Punica granate* against of two isolated bacteria

Table (3) shows that the extent of the inhibitory diameters of the alcohol extract of *Punica granate* (pomegranate peel) on the two Species of bacteria *Klebseill apneumonia* and *Streptococcus pneumonia* with the same concentrations mentioned in the

aquaticextract, and it gave higher inhibitory results than the water extract. The results were respectively (17, 19, 21, 23) mm in *Klebseilla pneumonia*, while in *Streptococcus pneumonia* the inhibitory diameters were (14, 17, 19, 21) mm, as in Fig. (2).

Table (3): The inhibitory diameters of the alcohol extract of *Punica granate* (pomegranate peel) on the two Species of isolated bacteria

Name of Bacteria	Water extract concentrations for <i>Punicagranate</i> Mcg/ml			
	150	100	50	25
<i>Klebseilla pneumonia</i>	23	21	19	17
<i>Streptococcus pneumonia</i>	24	19	17	14

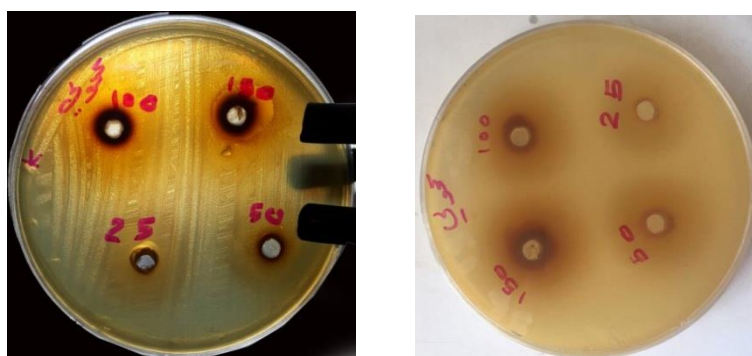


Figure (2): The inhibitory activity of alcohol extract for *Punica granate* against of two isolated bacteria

The inhibitory effectiveness varied according to the different types of antibiotics depending on the type and concentration of bacteria. Table (4) shows the susceptibility or resistance of each Species of bacteria to the antibody used. The type of bacteria *Klebseilla pneumonia* showed resistance to the antibiotics Amikacin, Amoxicillin and Cefotaxime and its

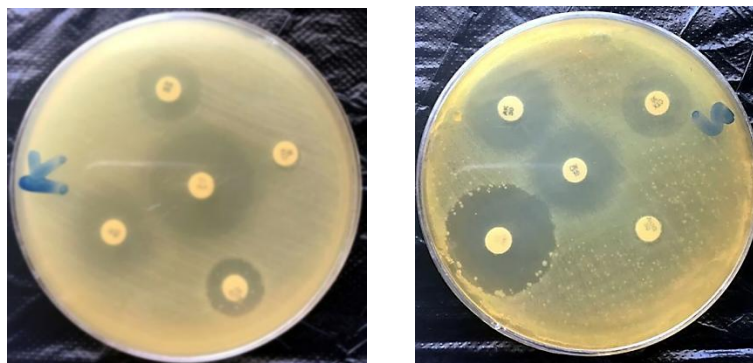
susceptibility to the two types of antibiotics Gentamicin and Imipenen. As for the type of bacteria *Streptococcus pneumonia*, it was resistant to the antibiotic Amoxicillin and of moderate susceptibility to Cefotaxime with susceptibility to the rest of the antibiotics used, as shown in Fig. (3).

Table (4): Susceptibility of two Species of isolated bacteria to wards five types of antibiotics

Name of Bacteria	Gentamicin GM/10	Amikacin AK/30	Imipenen IPM/10	Amoxicillin AMC/30	Cefotaxime CTX/30
<i>Klebseilla pneumonia</i>	S	R	S	R	R
<i>Streptococcus pneumonia</i>	S	S	S	R	MS

Antibiotic concentrations by Mcg/Tab

S = SusceptibleMS , MS = Moderate Susceptible ,R = Resistant



**Figure 3: Susceptibility of two Species of isolated bacterial to the five Species of antibiotics**

Thus, these results show that the aqueous and alcohol extract of *Punica granate* (pomegranate peel) have a clear inhibitory effect on the growth of the two species of bacteria *Klebsiella pneumonia* and *Streptococcus pneumonia* with a simple variation in the susceptibility of each species of bacteria used in the study, depending on the type and concentration of *Punica granate* (pomegranate peel) extract.

The results are consistent and in agreement with that reached by [14] concerning the inhibition activity of species of bacteria *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* to *Punica granate* (pomegranate peel) extract.

It is also consistent and in agreement with what [14] pointed out concerning the effect of the water extract of *Punica granate* (pomegranate peel) on the growth of the two types of bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*.

[16] noted through the chemical analyses of *Punica granate* (pomegranate peel) extracts that it contained

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Flavonoids, Triterpens and Phenols, known for its anti-bacterial efficacy.

The results were identical to those of [17] where the efficacy of *Punica granatum* was due to polyphenols such as ellagic tannins, ellagic acid, and gallic acid.

And the results are consistent with both findings [18, 19]. The effect of bacterial growth may be due to one or more of the chemical components contained in *Punica granatum*, such as Tannine, which may affect the nature of proteins in bacteria which leads to killing or may affect the plasma membrane, thus altering its functional properties, which leads to inhibiting the growth of bacteria. [20, 21, 22].

Also exist Polyphenol and flavonoids and ellagic acid in a determination *Punica granatum* peel, which has a deadly effect against bacteria [23,17].

These results were consistent with both [24,25]. These results show an extract effect of *Punica granatum* in inhibiting or killing both Gram negative and Gram positive bacteria.

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## تحديد الفعالية المضادة للبكتريا لمستخلص نبات *Punica granatum* لبكتريا الـ *Streptococcus*

### *Klebsella Pneumonia* و *Pneumonia*

رؤى حسن لطيف

كلية التربية للنبات ، جامعة تكريت ، تكريت ، العراق

#### الملخص

تأولت الدراسة مقارنة الفعالية التثبيطية للمستخلص المائي والكحولي لقشور الرمان على البكتريا *Streptococcus Pneumonia* و *Klebsella Pneumonia* مع بعض المضادات الحيوية، وهذه العزلات تم الحصول عليها من القشع والإدرار من المرضى الراقدين في مستشفى تكريت التعليمي في مدينة تكريت. وأظهرت النتائج تأثيرات متباينة للمستخلصات المائية والكحولية المستخدمة في الدراسة. وتشير النتائج إلى إن أعلى تأثير تثبيطي للمستخلص الكحولي ضد البكتريا *Streptococcus Pneumonia* عند التخفيف (150) مايكروغرام/مل هو (24) ملم أعلى من المستخلص المائي عند التخفيف (150) مايكروغرام/مل وهو (16) ملم، بينما أشارت النتائج إلى أن أعلى تأثير تثبيطي للمستخلص الكحولي ضد البكتريا *Klebsella Pneumonia* عند التخفيف (150) مايكروغرام/مل هو (23) ملم. أما نتائج التأثير التثبيطي للمستخلص المائي ضد بكتريا *Klebsella Pneumonia* كانت متشابهة مع التأثير التثبيطي للمستخلص المائي لـ *Streptococcus Pneumonia* عند التخفيف (150) مايكروغرام/مل وهو (16) ملم.

وأشارت النتائج إلى أن أعلى تأثير تثبيطي للمضاد الحيوي Imipenem على بكتريا *Streptococcus Pneumonia* هو (38) ملم وكانت أعلى من التأثير التثبيطي للمضاد Imipenem على بكتريا و *Klebsella Pneumonia* وكان قطره (37) ملم.