# *Evaluation of anti bacterial activity of punica granatum peels extracts, on growth of gram-positive bacteria isolated from clinical samples*

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***Received:*** *١٨/٥/٢٠٠٥*; ***Accepted:*** *١/١١/٢٠٠٥*

## Abstract

Thirty samples were collected from patients (١٠-٤٥) years old, suffered from tonsillitis,pharyngitis, infected wounds, acne & Bronchitis.

Gram –positive bacteria were isolated from these samples, and diagnosed, of which, *Staphylococcus aureus* ( ٥٠%) and *Staphylococcus epidermidis* (١٦.٦٦%), *Streptococcus pyogenes* (١٣.٣٤%), *Streptococcus pneumonia*e (١٠%) and *Micrococcus spp* (١٠%). Alcoholic and water extracts of the *punica granatum(Pomegranate)* peels as well as the dried powders were prepared, the effect of these extracts were studied against these isolates.

The antimicrobial susceptibility tests of the extracts were determined by Kirby- Bauer method and the MICs were determined. The antibacterial activity of *punica granatum(Pomegranate)* peels was determined. The alcoholic extract showed more potent inhibitory effect on the isolates than water extract, and the best effect was on the growth of *Staphylococcus epidermidis followed by Staphylococcus aureus , Micrococcus spp, Streptococcus pyogenes and Streptococcus pneumoniae*. The zone of inhibition was (١٧- ٢٣mm) for alcoholic extract and( ١٢-٢٣mm) for watery extract. The antibacterial activity of pomegranate peels extracts should make it useful for treatment of wounds, skin infections, tonsillitis and pharyngitis caused by the above bacteria.

**Key word:** *punica granatum, Streptococcus pneumonia, Streptococcus pyogenes.*

## الخلاصـــة

تم جمع ثلاثين نموذج ، من مرضى مصابين بالتهاب البلعوم واللوزتين والجروح وبعض الالتهابات الجلدية ونموذج من حالة إصابة بالتهاب القصبات ولأعمار من ١٠سنوات إلى ٤٥ سنة. عزلت من هذه الحالات جراثيم موجبة لصبغة كرام وتم تشخيصها ومنها :

*Staphylococcus aureus* ( ٥٠%) , *Staphylococcus epidermidis* (١٦.٦٦%), *Streptococcus pyogenes* (١٣.٣٤%), *Streptococcus pneumonia*e (١٠%) , *Micrococcus spp* (١٠%).

درست حساسية الجراثيم للمضادات الحيوية المختلفة بطريقة( كيربي بور) وكذلك التراكيز المثبطة الصغرى وتم تحضير مستخلصات من قشور الرمان المائية الحارة والكحولية، وكذلك مسحوق قشور الرمان المطحون، حضرت

تراكيزمختلفة من المستخلصات وتم إيجاد فعاليتها والتراكيز المثبطة الصغرى على الجراثيم المعزولة أعلاه ، ووجدت أعلى فعالية على عزلات

*Staphylococcus epidermidis followed by Staphylococcus aureus , Micrococcus spp, Streptococcus pyogenes and Streptococcus pneumoniae*.

وعلى التوالي . كانت أقطار التثبيط الجرثومي تتراوح من (١٧ -٢٣mm) للمستخلص الكحولي و (١٢-٢٣mm ) للمستخلص المائي الحار . أن الفعالية المضادة للجراثيم لهذه المستخلصات تجعلها مفيدة في علاج الالتهابات الجلدية والجروح والتهابات الحنجرة والبلعوم الناجمة من الإصابة بالجراثيم أعلاه.

## Introduction

**The common name of *Punica granatum* is Pomegranate, belong to Family *Punicaceae,* of the Order Myrtales, Subclass Rosidae,Class Magndiopsida Pomegranate has a long history as food Medicine and herbal use dating back more than ٣,٠٠٠ years[١]. Both the stem and the root barks contain unusual alkaloids, known as 'pelletierines', which paralyse tapeworms so that they are easily expelled from the body by using a laxative[٢]. The plant is also rich in tannin, the dried peels of the fruit contains about ٢٦% which makes it an effective astringent. It is used externally in the treatment of vaginal discharges, mouth sores and throat infections[٣].**

**Pomegranate (*Punica granatum*) peel extracts have been shown to possess significant antioxidant activity in various in vitro models, it has already been established that antioxidant activity in pomegranate juices is higher when extracted from whole pomegranate[٤-٨]. Australian researchers found that their scientific investigation of pomegranate flower extract improved hyperglycaemia in type II diabetes and obesity in which gallic acid is mostly responsible for its glycaemic activity[٩-١١]. Concentrated pomegranate juice (CPJ) improves lipid profiles in diabetic patients with hyperlipidemia ,they concluded that (CPJ) consumption may modify heart disease risk factors in hyperlipidemic patients ,and its inclusion therefore in their diets may be beneficial[١٢,١٣]. Additionally,research findings on excess triglyceride accumulation and increased fatty acid oxidation in the diabetic heart, which contribute to cardiac dysfunction, suggested that pomegranate flower extract improves abnormal cardiac lipid metabolism[١٤]. In recent study, pomegranate juice was found to slow down cholesterol oxidation by almost half and reduce the retention of disproportionate LDL cholesterol [١٥]. Flavonoid –rich polyphenol fractions from pomegranate fruit have been shown to exert anti proliferative,anti-invasive and proapoptotic actions in breast and prostate cancer cells and other solid malignancies[١٦-٢١]. Topical application of pomegranate fruit and seed oil extract tested on mouse skin appears to posses chemopreventive activity in skin tumours[٢٢]. It has been found that the methanolic extract of pomegranate peels posses wound healing activity against an excision wound on the skin of Wistar rats[٢٣]. The whole plant, but in particular the bark, is antibacterial, antiviral Furthermore pomegranate juice provides an HIV-١ entry inhibitor by preventing the virus binding to the cellular receptor CD٤[٢٤]. The dried rind of the fruit is used in the treatment of amoebic dysentery and diarrhoea. It is a specific remedy for tapeworm infestation[٢٥,٢٦]. Pomegranate rind extract has been shown to have gastro-protective activity through its antioxidant mechanism, it posses strong antibacterial activity against different species of entropathogenes which cause diarrhoea and dysentery, *E.coli*, *Salmonella Shigella sonnei* and *Shigella Flexner*[٢٧-٣٠].Pomegranate (outer rind) extract is also screened for their antimicrobial activity against Gram-positive bacteria and yeasts, results founded that pomegranate showed good activity against *Staphylococcus aureus* and *Candida*[٣١].Plants used in Argentin folk medicine screened for antimicrobial activity against *Staph. aureus* commonly present on skin and mucous membranes which causes boils and abscesses, showed that pomegranate rind extract produced one of the more active results[٣٢]. Pomegranate peels showed also bactericidal effect on Vibrio cholerae[٣٣].**

***Aims of the study:*** This study is conducted to achieve two goals: ١- To isolate, Staphylococcus aureus. Staphylococcus epidermidis Streptococcus pyogenes, Micrococcus spps. and Streptococcus pnemoniae. from different clinical infections and determining their in-vitro susceptibility and the minimal inhibitory concentrations to different antibiotics. ٢-To evaluate the in-vitro activity of *Punica granatum L.* peels extracts against the above strains by susceptibility test and to determine their minimal inhibitory concentrations.

## Materials And Methods

***Materials:*** Nutrient agar, MaCconkey agar, mannitol salt agar, blood agar base, brain heart infusion broth, Mueller- Hinton agar, Packers agar and broth, Chocolate agar.Powders of antibiotics were also obtained from (Russell, Beecham, & Specia) *Chemical reagents* Fehling, Benidict, Dragendorfs reagent,Optochin, Bacitracin disks.Zone reader, Oven (Memmert,Germany), Pasture pipette, Vortex mixer, Balances( Sartorius), Homogenizer, Mixer, Incubator, Ultrasonic (soniprep ١٥٠HSE) at ٢٠KHZ. Centrifuge, Autoclave, Water bath, Rotary evaporator, Souxhlet apparatus, Magnetic stirrer, Shaker, Incubator.

***Bacterial strains:*** ١-*Staph. aureus* ATCC ٢٥٩٢٣ sensitive to all kinds of antibiotics used as Standard. ٣-Clinical isolates from different clinical samples collected from three hospitals

***Methods:***

***Isolation and identification of strains:*** Strains were isolated on MaCconkey agar, Blood agar, & Mannitol salt agar, Chocolate agar & Packers agar (Sodium azide ٠.٢gm,Crystal violet solution ٠.٠٥% ٤ml, Blood agar base ٣٧gm and D.W ١٠٠٠ml). All strains were identified by Api ٢٠ *Strep* and Api ٢٠*Staph* System (Biomerieux vitek, Inc), Catalase test, Oxidase test, Coagulase test and bile solubility test were done according to Jawetz methods[٣٤].

*Preparation of macfrland standard solution:* **Solution A: ١.١٧٥gm of barium chloride( BaCl٢.٢H٢O) in ١٠٠ml of distilled water. Solution B: Prepared by the addition of ١ml of concentrated H٢SO٤ to٩٩ml distilled water. ٠.٥ml of solution A was added to ٩٩.٥ml of solution B and the tube was compared with the bacterial suspension to give number of cell approximatively ١٠٨x١.٥ bacteria/ml.**

*Collection of pomegranate fruit rinds:* **The *Punica granatum. Peels* were obtained from the local market. Washed, cleaned & dried at room temperature or under the sun.**

*Spesifications of pomegranate fruit rinds:* **The rind of the fruit usually is irregular concave fragments, ١/٢٠-١/١٠inch.thick, brownish red externally and dull yellow on the inner surface, with depressions left by the seeds. The toothed calyx is present on some pieces.**

***Preparation of punica granatum peels water extract*:** A known quantity of *Punica granatum* peels was weighed and dissolved in ١٠٠ml distilled water ,boiled for ١٠-١٥minutes, soaked three hours, filtered twice, the filtrate was collected and evaporated by vacuum rotary evaporator at ٥٥C until crud extract powder was obtained. The crud extract was weighed and dissolved in distilled water to calculate the concentrations needed for different experiments[٦].

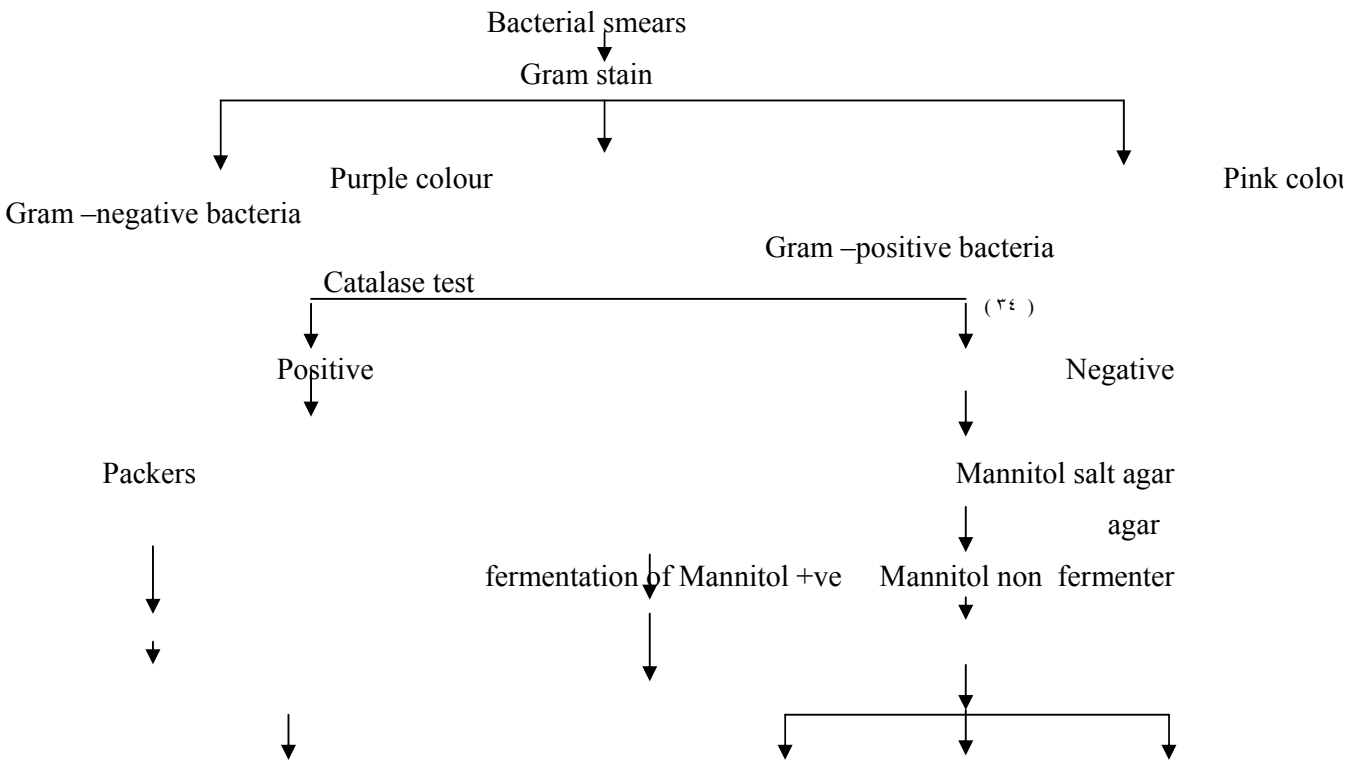
***Preparation of punica granatum peels alcoholic extract* [٣٥].**

Alcoholic (Ethanol extract was prepared by soaking the peels in ٧٥% ethyl alcohol using (Souxhlet apparatus) at ٥٠C then filtered, evaporated by vacuum rotary evaporator at ٤٥C & collected.

***Measuring** pH*

Ten grams of peels extract were dissolved in ٥٠ml of distilled water, shacked well by magnetic stirrer for ١٢ minutes, filtered and measured the pH.

The following scheme showed the method of identification:



*Streptococcus spp*

Oxidase -ve [٣٤] Oxidase +ve Hemolysis on blood agar

*Staphylococcus spp* *Micrococcuc spp.*

Coagulase test [٣٤] Beta Alpha No

Sensitive to Bacitracin ٠.٠٤I.U

Hemolysis

Positive Negative *Strept.salivarius*

*Staphylococcus aureus* Optochin(+) Optochin(-)

Other types of *Streptococcus* *Strept.pneumoniae*
*Staphylococci* *pyogenes*
*Strept. viridance*

API ٢٠ Strept. API ٢٠Staph

Lancefield group

***Scheme: of identification bacterial strains***

***Detection of punica granatum peels constituants***

***Detection of tannins*** [٣٦]

Ten gm of extract was dissolved in ٥٠ml of d.w, filtered and cooled ١% of lead acetate was added .The appearance of precipitation indicated positive reaction

***Detection of glycosides***

Equal amounts of Fehling reagent and extract were mixed and boiled for ١٠ minutes in water bath red precipitation indicated positive reaction[٣٦]

***Detection of phenoles***

Ten gm of *Punica* powder was dissolved in ٥٠ml of distilled water and boiled for ١٠minutes, filtered, cooled. ١% of iron chloride was added, greenish blue colour appeared which indicated the presence of phenol[٣٦]

***Detection of saponines***

Saponines form foam when shake with water, also

Five ml of extract was added to١-٣ml of $HgCl_2$, the appearance of white precipitate indicated positive reaction[٣٦].

***Detection of resin***

**Fifty ml of ethyl alcohol ٩٦% was added to five gm of pomegranate powder and boiled in water bath for two minutes, filtered (Ederal N٠٢) ١٠ml of acidified with HCl, was added to filtrate precipitation will occur in the case of positive reaction[٣٦].**

***Detection of alkaloides***

Ten gm of extract powder was boiled with ٥٠ml of distilled water acidified with ٤٠% HCl. The solution was filtered and cooled .٠.٥ml from filtrate was tested with the following solution:

Wagner solution- Grey precipitate positive reaction

Mayer solution- white precipitate positive reaction[٣٧]

***Detection of comuurins***

A small quantity of extract was dissolved in alcohol in a test tube covered with filtered paper moisture with NaOH in water bath boiled ٢-٥minutes. The filter paper was exposed to U.V light wave length ٣٣٦ nm the presence of yellow-green colour indicated the presence of comuurins[٣٧]

***Detection of flavones***

Solution A –١٠gm of extract/ ٥ml of ethyl alcohol ٩٦%( Filtered)

Solution B- ١٠ml of Ethyl alcohol ٥٠%. Equal quantity was mixed

Yellow precipitate indicated positive reaction[٣٦].

By exposing the spot of flavones to uv light, give fluorescent spot, or by spraying with sulfomolybdic acid solution give purpul to rose color.

*Antibiotic Susceptibility test (Disk diffusion method)*

The resistance pattern for antibiotics were determined by Kirby – Bauer[٣٨] diffusion assay on Mueller – Hinton agar (٢٠ml / plate) the inoculum was $١٠^{٤}$ – $١٠^{٥}$ bacteria / ml, of ٦ hours cultures at ٣٧C˚ for ٢٤ hours, antibiotics used were indicated in Table I,IIand III.

***Minimum inhibitory concentrations (MICs)***

Minimum inhibitory concentrations (MICs) were determined by dilutions of antibiotics in Mueller–Hinton agar. Inoculums of $١٠^{٤}$-$١٠^{٥}$bacteria/ml were spotted on agar, and incubated at ٣٧C˚ .The lowest concentration preventing growth (MIC) was estimated after ١٨ hours of incubation. As control, fully sensitive *S.aureus* strains were tested under the same conditions.

**Table (I):** *Normal values of MICs & diameters (Ø) zone of inhibition of Cephalosporins***.**

**Staphylococci & N.gonorrhoeae ---**Non enterococcal streptococci & Listeria monocytogenes.*

*١-Staphylococci; ٢-N.gonorrhoeae; ٣- Non enterococcal streptococci & Listeria monocytogenes.*

| Cephalosporins | Abbreviations | Critical concentrations In µg/ml | | Ø of * Zone of Inhibition | | Potency of disk/ µg/ml |
|---|---|---|---|---|---|---|
| First generation | | c | C | D | d | |
| Cefalexin | cfx | ٨ | ٣٢ | ١٨ | ١٢ | ٣٠ |
| Third generation | | | | | | |
| Cefotaxime | Ctx | ٤ | ٣٢ | ٢١ | ١٥ | ٣٠ |
| Ceftriaxone | Cro | ٤ | ٣٢ | ٢١ | ١٥ | ٣٠ |

**Table (II):** *Normal values of MICs & diameters (Ø) zone of inhibition of Penicillins.*

| Penicillins | | | | | | |
|---|---|---|---|---|---|---|
| | Abbreviations | Critical concentrationsµg/ml | | Ø zone of inhibition | | Potency of disksµg/ml |
| ampicillin | Amp | ٤ | ١٦ | ١٧ | ١١ | ١٠ |
| penicilling | PG | ≤٠.١*<br>≤٠.١٢** | ≥١٦*<br>≥٤** | ≥٢٩[١]<br>≥٢٠[٢]<br>≥٢٨[٣] | ≤٢٨[١]<br>≤١٩[٢]<br>≤١٤[٣] | ١٠ I.U. |
| amoxycillin | Amo | ٤ | ١٦ | ٢١ | ١٤ | ٢٥ |
| augmentin | Amc | ٤ | ١٦ | ٢١ | ١٤ | Amo ٢٠+ CA ١٠ |
| carboxypenicillin | | | | | | |
| carbenicillin | Cb | ١٢٨ | ١٢٨ | ١٥ | ١٥ | ١٠٠ |

**Table (III): Normal** *values of diameters (Ø) zone of inhibition of some antibiotics.*

| Other antibiotics | Abbreviations | Ø of * Zone of Inhibition | | Potency of µg/ disk/ |
|---|---|---|---|---|
| | | D | d | |
| Erythromycin | E | ٣ or more | ١٣ | ١٥ |
| Tetracycline | Tc | ٩ or more | ١٤ | ٣٠ |
| Gentamycin | G | ١٤-٢٠ | ١٢ | ١٠ |
| Clindamycin | Cl | ١٥-٢٠ | ١٤ | ٢ |
| Vancomycin | Van | ٢ or more | ٩ | ٣٠ |
| Viprofloxacin | Cip | ١٦-٢٠ | ١٥ | ٥ |

MIC≤ c: Sensitive strains, MIC>C: Resistant strains, C< MIC≤ C Intermediate, Ø ≥ D: Sensitive strains, Ø<d Resistant strains d ≤ Ø< D Ø=diameter

**Susceptibility test and minimal inhibitory concentrations (mics) of punica granatum peels extracts.** The activity of different concentrations of *Punica granatum.* peels extracts were determined *against Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus spp , Streptococcus pyogenes& Streptococcus pneumoniae,* by Susceptibility test, ٤٠-١٠٠ µl extracts from each concentrations (٨٠%,٧٠%, ٦٠%, ٥٠%, ٢٥%) were poured in small holes applied at equal distances in blood agar & M.H agar seeded with $١٠^{٥}$-$١٠^{٤}$/ test bacteria/ml , dried at room temperature , the inhibition zones were read ,after incubation at ٣٧C for ١٨ hours, The minimal inhibitory concentrations (MICs) were determined by dilution different concentrations of *Punica granatum.* extracts in Mueller–Hinton agar. Inoculums of $١٠^{٤}$-$١٠^{٥}$ bacteria/ml were spotted on agar supplemented by ٧% blood in case of *Streptococcus*, and incubated under $CO_{٢}$ at ٣٧C[(٣٨)].

**Table (IV):***Percentage of different isolates*

| *Type of microorganism* | number of strains | % | origen |
|---|---|---|---|
| Staphylococcus aureus | ٥ | ٥٠ | Tonsillitis |
| Staphylococcus aureus | ٤ | | Wounds |
| Staphylococcus aureus | ٦ | | Pharyngitis |
| Staphylococcus epidermidis | ٥ | ١٦.٦٦ | Skin |
| Micrococcus spp | ٣ | ١٠ | Skin |
| Streptococcus pneumonia | ٣ | ١٠ | Sputum |
| Streptococcus pyogenes | ٤ | ١٣.٣٤ | Tonsillitis |
| Total | ٣٠ | ١٠٠% | - |

**Table (V):** *Sensitivity tests of different antibiotics against different gram-positive*

| Type of microorganism | Pen G | Amp | Amo | Cb | Aug | Cfx | Ctx | Cto | Tc | E | Cln | Vm | Gm | Cip |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Streptococcus pyogenes (٤strains)* | R | R | R | R | S | R | R | S | R | R | R | R | S | R |
| *Streptococcus pneumoniae (٣strains)* | R | R | R | R | S | R | R | S | S | R | S | S | R | S |
| *Staphylococcus aureus* (١٥ strains) | R | R | R | R | S | R | R | S | R | R | R | R | R | S |
| | R | R | R | R | R | R | R | R | R | R | S | S | R | S |
| | R | R | R | R | S | S | S | S | S | R | S | S | S | S |

*isolates*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus epidermidis (٥ strains)* | R | R | R | R | S | R | S | S | S | R | S | S | R | S |
| | R | R | S | S | S | S | S | S | S | S | S | S | R | S |
| *Micrococcus spp (٣strains)* | R | R | R | R | S | R | S | S | S | R | S | S | R | S |

**Pen: Penicillin G, Amp: Ampicillin, Amo: Amoxycillin, Cb: Carbenicillin, Aug: Amoxycillin/Clavulanate, Cfx: Cefalexin, Ctx: Cefotaxime: Cto: Ceftriaxone, Tc: Tetracyclin, E: Erythromycin, Cln: Clindamycin, Vm: Vancomycin, Gm: Gentamycin & Cip: Ciprofloxacin. R; Resistant, S: Sensitive*Disk potency: PG: ١٠ I.U, Cb: ١٠٠µg, Amp: ٣٠µg, Amo: ٣٠µg, Cfx: ٣٠ µg, Ctx: ٣٠µg, Cto: ٣٠µg.**

**Table (VI):** *Diameters zone of inhibition/ mm bacteria under test (ethanol extracts)*

| Average diameters of zone of inhibition/mm for different concentrations of *Punica granatum* | | | | | | |
|---|---|---|---|---|---|---|
| Type of microorganisms | | ٨٠% | ٧٠% | ٦٠% | ٥٠% | ٢٥% |
| ١- | Streptococcus pyogenes | ٢٠ | ٢٠ | ١٩ | ١٩ | ١٩ |
| ٢- | Streptococcus pneumoniae | ٢٠ | ١٩ | ١٨.٥ | ١٨ | ١٧ |
| ٣- | Staphylococcus aureus | ٢٣ | ٢٢ | ٢١ | ٢٠ | ٢٠ |
| ٤- | Staphylococcus epidermidis | ٢٢ | ٢٢ | ٢١ | ٢١ | ٢٠ |
| ٥- | Micrococcus spp | ٢١ | ٢١ | ٢٠ | ٢٠ | ١٩ |
| ٦- | Staphylococcus aureus ٢٥٩٢٣ | ٢٢ | ٢١ | ٢٠ | ١٩ | ١٨ |

**TABLE (VII):** *Diameters of zone of inhibition/ mm bacteria under test (water extracts)*

| Average diameters of zone of inhibition/mm for different concentrations of *Punica granatum* water extracts | | | | | | |
|---|---|---|---|---|---|---|
| Type of microorganisms | | ٨٠% | ٧٠% | ٦٠% | ٥٠% | ٢٥% |
| ١- | Streptococcus pyogenes | ١٩ | ١٨.٥ | ١٧ | ١٦.٥ | ١٣ |
| ٢- | Streptococcus pneumoniae | ١٨ | ١٨ | ١٦ | ١٥ | ١٢ |
| ٣- | Staphylococcus aureus | ٢٣ | ٢٢ | ٢٠.٥ | ١٩ | ١٨ |
| ٤- | Staphylococcus epidermidis | ٢١ | ٢٠ | ٢٠ | ١٩ | ١٨ |
| ٥- | Micrococcus spp | ٢١ | ٢٠ | ٢٠ | ١٧ | ١٣ |
| ٦- | Staphylococcus aureus ٢٥٩٢٣ | ٢١ | ٢٠ | ٢٠ | ١٨ | ١٨ |

**Table (VIII):** *Minimal inhibitory concentrations MICs µg/ml of different type of antibiotics against thirty strains.*

| Type of microorganism | PG | Cb | Amp | Amo | Aug | Cfx | Ctx | Cto |
|---|---|---|---|---|---|---|---|---|
| | Minimal inhibitory concentrations µg/ml | | | | | | | |
| *Streptococcus pyogenes (٤strains)* | ١٦ | ١٢٨ | ٦٤ | ٣٢ | ١ | ٦٤ | ٦٤ | ٠.١ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Streptococcus pneumoniae (٣strains)* | ١٦ | ١٢٨ | ١٢٨ | ٦٤ | ٠.٥ | ٦٤ | ٣٢ | ٠.٢ |
| *Staphylococcus aureus (١٥strains)* | ١٦ | ٢٥٦ | ٢٥٦ | ٦٤ | ٠.٢ | ٣٢ | ٦٤ | ٠.٤ |
| | ٣٢ | ٢٥٦ | ٢٥٦ | ١٢٨ | ٦ | ٦٤ | ٦٤ | ٦٤ |
| | ١٦ | ٢٥٦ | ٢٥٦ | ١٢٨ | ٠.١ | ٢ | ٠.١ | ٠.٢ |
| *Staphylococcus epidermidis (٥strains)* | ١٦ | ١٢٨ | ٣٢ | ٣٢ | ٠.٥ | ٦٤ | ١ | ٠.٢ |
| | ٣٢ | ١٢٨ | ٤ | ٤ | ١ | ١ | ٠.٥ | ٠.١ |
| *Micrococcus spp (٣ strains)* | ١٦ | ١٢٨ | ٢٥٦ | ١٢٨ | ٠.٢ | ٦٤ | ٠.٥ | ٠.١ |
| *Staphylococcus aureus ٢٥٩٢٣** | ٠.٢ | ١٦ | ٢ | ٢ | ٠.٢ | ٠.٤ | ٠.٤ | ٠.١ |

* Standard strain.

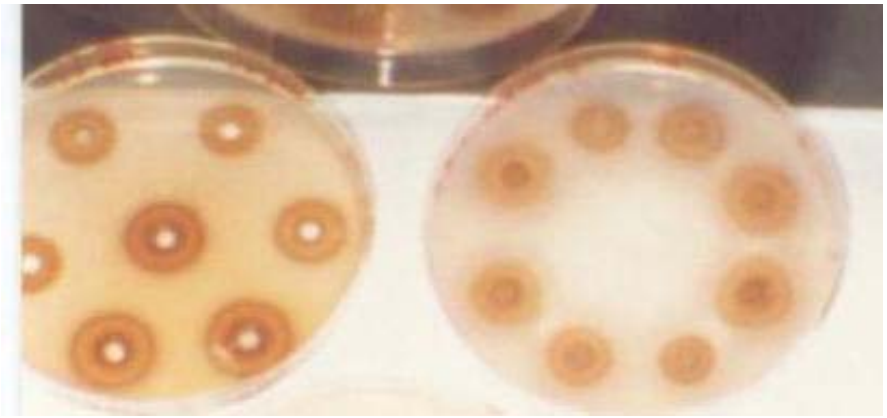


Fig (١): *Zone of inhibitions of different dilutions of punica granatum l. peels water extract against staphylococcus aureus strain isolated from case of tonsillitis.*
*١: concentrated extract, ٢ and ٣: diluted extract( ١/٢), ٤ and ٥ dilution ١/٥.*

**Table (IX):** *Minimal inhibitory concentrations/ml of Punica granatum peels alcoholic extract of different concentrations.*

| Type of microorganism | Minimal inhibitory concentrations/ml | | | | |
|---|---|---|---|---|---|
| | ٨٠% | ٧٠% | ٦٠% | ٥٠% | ٢٥% |
| *Streptococcus pyogenes(٤strains)* | ١٢٨ | ٥١٢ | ١٠٢٤ | ١٠٢٤ | ٢٠٤٨ |
| *Streptococcus pneumoniae(٣strains)* | ١٢٨ | ٥١٢ | ١٠٢٤ | ١٠٢٤ | ٢٠٤٨ |
| *Staphylococcus aureus(١٥strains)* | ١٢٨ | ٢٥٦ | ٥١٢ | ١٠٢٤ | ٢٠٤٨ |
| | ٢٥٦ | ٥١٢ | ١٠٢٤ | ١٠٢٤ | ٢٠٤٨ |
| | ٦٤ | ١٢٨ | ١٢٨ | ٢٥٦ | ٥١٢ |
| *Staphylococcus epidermidis (٥strains)* | ٦٤ | ٥١٢ | ٢٥٦ | ٢٥٦ | ٥١٢ |
| | ١٢٨ | ٢٥٦ | ٥١٢ | ١٠٢٤ | ٢٠٤٨ |
| *Micrococcus spp(٣strains)* | ١٢٨ | ٢٥٦ | ٥١٢ | ١٠٢٤ | ٢٠٤٨ |
| *Staphylococcus aureus ٢٥٩٢٣* | ٦٤ | ٦٤ | ١٢٨ | ١٢٨ | ٢٥٦ |

**Table (X):** *Minimal inhibitory concentrations µg/ml of Punica granatum (Pomegranate) peels water extract of different concentrations.*

| Type of microorganism | Minimal inhibitory concentrationsµg/ml | | | | |
|---|---|---|---|---|---|
| | ٨٠% | ٧٠% | ٦٠% | ٥٠% | ٢٥% |
| Streptococcus | ٥١٢ | ١٠٢٤ | ٢٠٤٨ | ٢٠٤٨ | ٢٠٤٨ |

| | | | | | |
|---|---|---|---|---|---|
| pyogenes(٤strains) | | | | | |
| Streptococcus pneumoniae (٣ strains) | ٢٥٦ | ٥١٢ | ١٠٢٤ | ١٠٢٤ | ٢٠٤٨ |
| Staphylococcus aureus (١٥strains) | ٢٥٦ | ٥١٢ | ١٠٢٤ | ٢٠٤٨ | ٢٠٤٨ |
| | ٢٥٦ | ٥١٢ | ١٠٢٤ | ١٠٢٤ | ٢٠٤٨ |
| | ١٢٨ | ١٢٨ | ١٢٨ | ٢٥٦ | ٥١٢ |
| Staphylococcus epidermidis (٥ strains) | ٢٥٦ | ٥١٢ | ٢٥٦ | ٢٥٦ | ٥١٢ |
| | ٢٥٦ | ٢٥٦ | ٥١٢ | ١٠٢٤ | ٢٠٤٨ |
| Micrococcus spp(٣ strains) | ١٢٨ | ٢٥٦ | ٥١٢ | ١٠٢٤ | ٢٠٤٨ |
| Staphylococcus aureus ٢٥٩٢٣ | ٦٤ | ٦٤ | ١٢٨ | ١٢٨ | ٢٥٦ |

**Table (XI):** *Constituents of ponica granatum peels*.

| Constituents of pomegranate (Granatum) fruit rinds or cortex | | | |
|---|---|---|---|
| Constituents | Peels powder | Ethyl alcohol extract | Water extract |
| Tannins/ as Gallotanic acid | ٢٨% | ٢٩% | ٣٠% |
| Glycosides | + | + | + |
| Total Ash | ٥.١٤% | ٥% | %٥.٣% |
| Non soluble materials | ٣٠% | NT | NT |
| Alkaloides | _ | _ | _ |
| Phenoles | NT | ٣٠% | NT |
| Saponines | _ | _ | _ |
| Couumarins | _ | _ | _ |
| Flavones | _ | _ | _ |
| Non soluble ash in acid | ٠.٣% | ٠.٢% | ٠.٣% |
| Color | + | + | + |
| Resins | + | + | + |

## Results & Discussion

Different clinical strains were isolated and identified from patients with tonsillitis , pharyngitis, bronchitis and wound infection presented in Table IV. The results of susceptibility tests of fourteen types of antibiotics against five genera of bacteria were listed in Table V, the order of resistance was as follow: *Staphylococcus aureus*, *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus epidermidis & Micrococcus spp.*

The activity of different dilutions of *Punica granatum* watery extract *against Staph aureus strain* isolated from case of tonsillitis was shown in Fig ١.

The activity of different concentrations of *Punica granatum* Peels, aqueous and alcoholic (٧٥%) extracts against thirty strains were shown in Table VI and Table VII, the results were represented by zone of inhibition in mm which varies between ١٨-٢٣mm.for ٨٠% aqueous extract & ٢٠-٢٣mm for alcoholic extract, ١٢-١٨mm for ٢٥% aqueous extract&١٧-٢٠mm for alcoholic extract. The ethanol extract was found to be the most effective against all tested microorganisms. The minimal inhibitory concentrations of eight types of beta-lactamins against thirty strains were shown in Table VIII. Seven strains of *Staph. aureus* were resistant to Penicillin, Ampicillin,

Amoxicillin, Carbenicillin, Cefalexin, Cefotaxime and Ceftriaxone ,sensitive to Augmentin, the minimal inhibitory concentrations were ranged between ٣٠-٢I.U for PenG & ٢٥٦µg/ml for Carbenicillin & Ampicillin.

The minimal inhibitory concentrations of different concentrations of *Punica granatum* Peels, water and alcoholic (٧٥%) extracts against thirty strains were shown in Table IX and Table X.The values were ranged between ٦٤µg/ml-٢٠٤٨µg/ml. The antibacterial activity of *Punica granatum L.* Peels, water and alcoholic (٧٥%) extracts was due to the activity of Gallotanic acid[٢٥,٣٢]. Table XI show the constituents of *P. granatum Peels.* These results were in agreement with the studies of Prashanth, D, et al ٢٠٠١ [٢٥] , Holetz F B et al[٣١].and Anesini C.[٣٢]

***Conclusions:*** One can conclude, the possibility of formulations of different kind of mouth wash and gargles, as well as skin lotion and ointment for the treatment of infections due to Gram-positive bacteria. Additionally, researches undergo with Gram-negative bacteria and yeasts, however several recent researchers were reported the excellent antibacterial activity of peels extracts ,seeds and fruit juice also posses chemo preventive skin cancer efficacy, as well as breast, prostate and mouth cancer, these multifunction of pomegranate made the Scientists to formulate Capsules, lotion and suspensions[٦,١٧-١٩].

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