EVALUATION OF ANTIMICROBIAL ACTIVITY OF PHENOLIC EXTRACT FROM PUNICA GRANATUM *L.*PEEL

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ABSTRACT

Invegstigation of antibacterial activity of phenolic extract of *Punica granatum* Peel was carried out in this study on Gram positive and Gram negative pathogenic bacteria. The results exhibited variable susceptibilities of microorganisms for different concentration of phenolic extract. The activity of this extract was associated with high concentrations. Using plate method, phenolic extract of *P.granatum* had the highest effect and wide diameter of growth inhibition zone against *Streptococcus sp*.,and it has no effect on growth of *Burkholderia pseudomallei* and *Staphylococcus aureus* only when very high concentration is used.

INTRODUCTION

A new wave of reserarch interested in traditional practices which might be used as antimicrobial has been stimulated by the renewed attention to natural therapies. Microbiologist have two reasons to be interested in topic of antimicrobial plant extracts. First,it is very likely that these phytochemical will find their way into arsenal of antimicrobial drugs prescribed by physicians. New sources,especially plant sources,are also being investigated. Second,the public is becoming increasingly aware of problem with the over prescription and misuse of traditional antibiotics[1].

pomegranate(*Punica granatum L.*)a plant belongs to the family Punicaceae,grow as an erect shrub is native from Iron to Himalayas in northern India and has been cultivated since ancient well[2]. It has been used as avermifuge, astringent, bacteriocide, refrigerant,stimulant,stom achic,styptic,hair dye,and to alleviate the adverse effects of asthma,bronchitis,cough,cardiac problems,dysentery,diarrhea,dyspepsia,fever,inflammation,bleeding disorders,piles,wound sulcer,bruises,sores,mouthlesion,stomatitis,vaginitis,respiratory

and urinary tract infections, and as a febrifuge to ameliorate malaria and seasonal fevers[2,3,4,5]. In recent years,the biological activities of pomegranate fruit rind polyphenols have received the increased attention of reasearchers and in dustry,as well as consumers[6]. There have been a number of indication that the phytotherapeutic use of this plant might be a viable option in controlling different microbial species[7]. The purpose of this study was to investigate the antimicrobial effect of pomegranate fruit peel phenols on bacterial growth.

MATERIALS AND METHODS

Plant material and Extraction

Punica granatum were purchase from the local market of Basrah. The peels of pomegranate fruits were manually removed,sun-dried and powdered. Powder(50g)was extracted by mixing with 250ml of water at room temperature for 24hrs using a magnetic stirrer. The extract was filtered through Wattmann No.41 filter paper for removal of particles. Residue(11.8g)was extract by mixing with 150ml ethanol(70%)and filtered through Wattmann No.31 filter paper. The extract was pooled and concentrated under vacuum at 60C? by rotary evaporater and the concentrate was powdered. Powder(0.5g)were dissolved with 50ml ethanol and mixed with 25ml ethylacetate. The extract was filtered by Whatman No.1 filter paprer then left to dry to contain two layer of phenolic compound[8].

Microorganisms Test:

Seven types of pathogenic bacteria were previously isolated and identified by other works were used. To study the antimicrobial activity of phenolic extract of *Punica granatum*,Muller-Hinton agar medium was used for bacterial growth,plates were incubates at 37C? for 24-48hrs. The method of well contain extract were used and the inhibition zones were measured by scale and compared with the control[9].

RESULTS

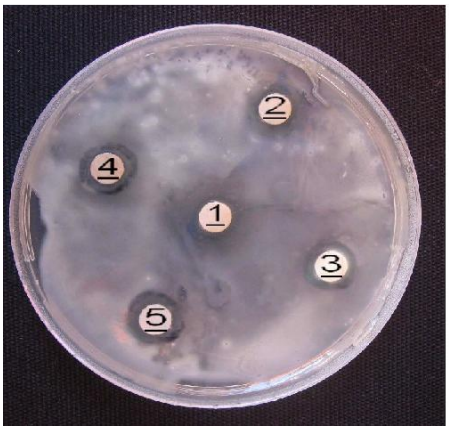
The results of the study are summarized in Table1. The table shows the means of diameter of inhibition zone induced by phenolic extract of *Punica granatum* peels on the growth of microorganisms. The inhibition zones induced by extract also illustrated by photographs which are listed in Figure1. Table1 reveals different influence of

extraction on microorganisms due to different concentration of this extract. The phenolic extract is strongly inhibit the growth of many types of bacteria above the concentrations 50 mg/ml. The growth of *Streptococcus* is inhibited with less concentration 6.25mg/ml. In contrast,the growth of *Staphylococcus aureus* and *Burkholderia pseudomallei* is less inhibited even with high concentration.

Table1: Mean of Diameter of the Inhibition zones Induced by Phenolic Extract on Microorganisms Used in This Study.

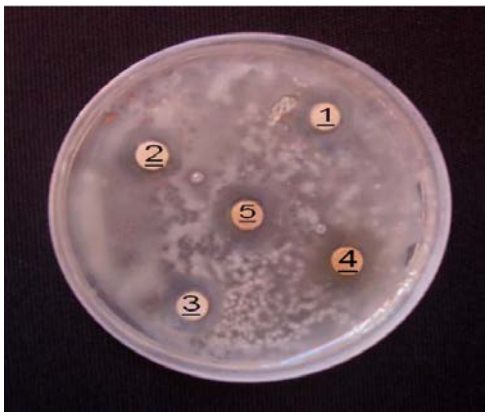
Concentration of Phenolic Extraction(mg/ml)					Microorganism
100 DIZ(mm)	50 DIZ(mm)	25 DIZ(mm)	12.5 DIZ(mm)	6.25 DIZ(mm)	Gram Positive
8	8	6	5	3	*Bacillus subtilis*
20	10	10	4	4	*Staphylococcus aureus*
15	14	13	8	8	*Streptococccus sp.*
					Gram Negative
-	8	6	4	2	*Escherichia coli*
15	15	1	9	9	*Klebsiella pneumoniae*
15	10	10	8	6	*Pseudomonas aeruginosa*
-	5	4	4	3	*Burkholderia pseudomallei*

DIZ=Diameter of Inhibition Zone Measured in Millimeter.

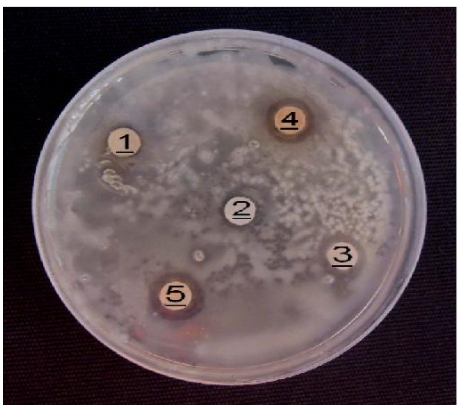


Bacillus subtilis

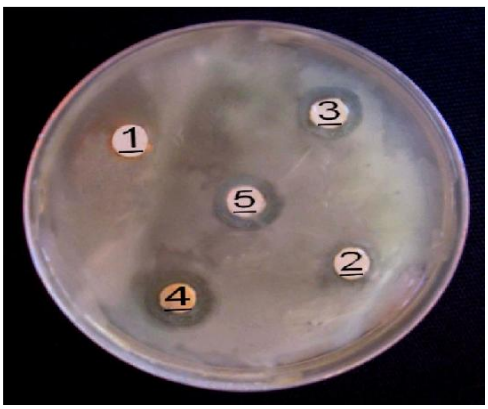
Staphylococcus aureus



Streptococcus sp.



Pseudomonas aeruginosa



Burkholderia pseudomallei

Figure 1: Inhibition Zones Induced by Different Concentrations of Phenolic Extract:(1=6.25mg/ml,2=12.5 mg/m,3=25 mg/ml,4=50 mg/ml and 5=100 mg/ml) on Microorganis-ms Used in This Study.

DISCUSSION

Present study exhibited importance medicinal of the *Punica granatum* peels through antimicrobial activity of the phenolic extraction. Microbs showed a variable susceptibility for different concentrations of phenolic extract. Some of simplest bioactive phytochemical consist of single substituted phenolic ring which are in the highest oxidation state. The common plants contain phenols,which is effective against bacteria[10]. Hydroxylated phenols shown to be toxic to microorganisms. The site´s and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms,with evidence that increased hydroxylation results in increased toxicity[11]. The mechanisms thought to be responsible for phenolic to microorganisms include enzyme inhibition by the oxidized compound,possibly reaction with sulfhydryal groups or through more nonspecific interaction with the proteins[12].

In this study we observed that the studied gram positive pathogenic bacteria were high susceptible more than gram negative pathogenic bacteria. In spite of presence some exceptional,it may be due to cell membrane of gram positive pathogenic bacteria which composed from peptidoglycan,mucopolysaccharids and phospholipids. This will provide suitable medium for possibility to interaction and acts as bactericidal or bacteriostatic agents and give rise to affect as destructive whether on membrane or on building unit of protein structure or nucleic acid synthesis inside the bacterial cell.

Comparative with gram negative bacteria the cell membrane of these bacteria composed from two membrans,outer and inner membrane and separated by the periplasmic space. The outer membrane composed of three materials, mucopolysaccharids,Lipoproteins and phospholipids ,while inner membrane composed from peptidoglycan and glycopeptides. The cell membrane of gram negative bacteria contain 90-95% lipids. These contains were not provided suitable medium to reaction with extracts.

تأثير الفعالية المضادة للميكروبات لمستخلص الفينول من قشور الرمان

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الخلاصة

تم دراسة الفعالية للميكروبات للمستخلص الفينولي لقشور الرمان وقد اختيرت بعض أنواع البكتريا المرضية الموجبة والسالبة لصبغة غرام. بينت الدراسة إن هذه الميكروبات لها حساسية مختلفة تجاه المستخلص وحسب نوع الميكروب والتركيز المستخدم ، وقد لوحظ أن التأثير الأكبر للمستخلص كان على جرثومة Streptococcus sp أما الجرثومتين Burkholderia pseudomallei و Staphylococcus aureus لم تتأثر بالمستخلص إلا عند استخدام التراكيز العالية جداً.

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