Antimicrobial Effect of Pomegranate Peel Extract on Some Pathogenic Microorganisms

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ABSTRACT

A different concentration of pomegranate peel extract were prepared (0.1, 0.2, 0.3, 0.4 and 0.5mg/ml) and tested their effect on the growth of some pathogenic microorganisms including (Staphylococcus aureus, Escherichia coli, Proteus vulgaricus, Candida tropicalis and Candida albicans). The results showed a significant sensitivity of Staphylococcus aureus, Proteus vulgaricus, Candida tropicalis and Candida albicans to the concentrations starting from 0.3mg/ml except E. coli which exhibited significant sensitivity to the concentration 0.2mg/ml. The yeast isolates showed a significant sensitivity to the pomegranate peel extract start from 0.4mg/ml concentration.

Key words: punica granatum, peel extract, pathogenic microorganism

تأثير التضاد المايكروبي لمستخلص قشر الرمان على بعض الأحياء المجهرية الممرضة

الخلاصة

تراكيز مختلفة من مستخلص قشر الرمان تم تحضيرها (٠,١ ، ٠,٢، ٠,٣، ٠,٤ و ٠,٥ ملغم/مل) لغرض فحص تأثيرها المثبط على نمو بعض الأنواع من الأحياء المجهرية الممرضة (Staphylococcus aureus, Escherichia coli, Proteus vulgaricus, Candida tropicalis and Candida albicans) . بينت النتائج المختبرية حساسية أنواع من البكتريا المستخدمة إلى مستخلص قشر الرمان ابتداءاً من التركيز ٠,٣ ملغم/مل إلى التركيز ٠,٥ ملغم/مل باستثناء بكتريا Escherichia coli التي اظهرت حساسية ابتداءاً من التركيز ٠,٢ملغم/مل إلى التركيز ٠,٥ملغم/مل. في حين اظهرت نوعي الخمائر المستخدمة في الدراسة حساسيتها ضد مستخلص قشر الرمان ابتداءاً من التركيز ٠,٤ملغم/مل وإلى التركيز ٠,٥ملغم/مل.

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INTRODUCTION

Fruits are one of the oldest forms of food known to man. There are many references to fruits in ancient literature. Vedas state that fruits form the base of the Food of Gods. According to Qur'an, the fruits like grapes, date, fig, olive and pomegranate are gifts and heavenly fruits of God. The people in ancient times regarded fruits to be endowed with magic or divine properties. The Babylonians regarded the seeds as an agent of resurrection, the Persians as conferring invincibility on the battlefield and for ancient Chinese alchemical adepts, the bright red juice was mythopoetically regarded as a "soul concentrate", homologous to human blood and capable of conferring on a person longevity or even immortality [1]

Pomegranate peels are exploited in traditional medicine because of their strong astringency, making them a popular remedy throughout the world. Different types of phytochemicals that have been identified from various parts of the pomegranate tree and from pomegranate fruits and seeds.[2] The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate in the fruit. Pomegranate polyphenols include flavonoids (flavanols and anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins). Hydrolyzable tannins (HTs) are found in the peels (rind, husk, or pericarp), membranes and piths of the fruit [3]. HTs are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity [4].

In the form of an aqueous decoction (i.e., boiling the hulls in water for 10-40 minutes), it was used for dysentary and diarrhea, and also for stomatitis it can be drunk, used as a mouthwash, douche or enema. In recent years, there has been an increasing interest in determining antioxidant properties of red fruits, due to their rich dietary sources of antioxidant phenolics and anthocyanins [5,6] Furthermore, this species appear to have interesting antiviral activity. Pomegranate extracts have been shown to be effective against the herpes virus [7] and hydroalcoholic extracts of whole fruits have exhibited high activity against the influenza virus [8].

In the present study, we investigated the antimicrobial activities of water extract from pomegranate peel against selected microorganisms.

MATERIALS AND METHODS

Methods of Extraction

Pomegranate fruit (*Punica granatum*) were collected from the local markets. Peels were removed and dried in an oven by hot air (50°C) for 48hr. Dried peels were powdered to get 60-mesh size using a mixing grinder. For obtaining water extracts of pomegranate peels, 20 g of dried ground samples were with 200 ml of boiling water and heated on stirrer hot plate for 30min. The mixture was filtered through over Whatman No.1 filter paper to remove of peel debris. The filtrate was concentrated to 20 ml and then autoclaved at 121ºC and 15 lb pressure for 15 min. The extract was cooled and immediately assayed for antimicrobial activity [9].

Test for tannins

One ml of aqueous extract (1 ml) was mixed with 10 ml of distilled water and filtered. Ferric chloride reagent (3 drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic tannins[10].

Test for flavonoids

5ml of aqueous extract was mixed with mixture containing 10ml of 1:1solution (50% ethanol plus 10ml of 50% NaOH). A yellow precipitate in the solution confirmed the presence of flavonoids[10].

Test for phenolics

3ml of aquous extract put in test tube then 2ml of 1% Ferric chloride was added. Blue green precipitate in the solution confirmed the presence of flavonoids[11].

Microbial cultures

Local identified isolates of (*Staphylococcus aureus, Escherichia coli, Proteus vulgaricus, Candida tropicalis* and *Candida albicans*) which were supplied from the Central Health laboratories in Baghdad.

The bacterial isolates on nutrient agar plates and incubated ant 37°C for 24hr, while the yeast isolates activated on Potato Dextrose Agar plates and incubated at 28°C for 24hr. The bacteria and yeast isolates stored at 4°C for regular sub-culturing. Ten ml of cell suspension was prepared from each culture using nutrient broth and Sabouraud Dextrose Broth (Difco) in 100ml flasks.

They were grown (bacteria species) on nutrient agar (Hi-media) in Petri dishes and incubated at 37°C for 24hr, while yeast species were grown on potato dextrose agar (Hi-media) in Petri dishes and incubated at 28°C for 24hr, and both (bacteria and yeast) stored at 4°C for regular sub-culturing. 10 ml of cell suspension was prepared for each culture using Sabouraud Dextrose Broth and nutrient broth (Difco) in 100ml flasks.

Determination the antifungal activity of extract

The antimicrobial activity of pomegranate peel crude extract against some species of microorganisms was tested after the adjusting of cell number to give 1×10^7 cell/ml that equal to 0.2 OD when read its turbidity at 600nm wavelength [12]. 100µl of each cell suspensions of microorganisms isolates was inoculate in tubes containing 5ml of Sabouraud Dextrose Broth (for yeast isolates) and nutrient broth (for bacteria isolates) and different concentrations of the crude extract (0.1, 0.2, 0.3, 0.4 and 0.5mg/ml). The cultures and control tubes were incubated at 28ºC (for yeast isolates) and 37 ºC (for bacteria isolates) for 24h. Then the antimicrobial activity and the MIC (Minimum Inhibitory Concentration which inhibits cells growth at low concentration)[13] of pomegranate peel extract were recorded by measuring the turbidity at 600nm against blank. A triple reading has been done for each test.

Statistical Analysis

The results were expressed as (mean±SD). t-test was used to compare the growth of each yeast and bacteria species between control group and each of test tubes that contain the different concentrations of pomegranate peel extract. The threshold of significance was chosen as ($P < 0.05$) [14].

Result and Discussion

The aqueous extract of pomegranate peel was given positive tests for gallic tannins, flavonoids and phenolics.

Table (1).

Parameter	Result
Tannins	+
Flavonoids	+
Phenolics	+

The antimicrobial activity of five different prepared concentration of pomegranate peel extract (0.1, 0.2, 0.3, 0.4 and 0.5mg/ml) against microorganisms including (Staphylococcus aureus, Escherichia coli, Proteus vulgaricus,Candida tropicalis and Candida albicans) was recorded after 24hr of incubation. The result was analyzed statistically and compared with the control of each of selected microorganisms to find out the significant differences in the microbial growth after treatment with pomegranate peel extract.

Figures (1, 2) and 3 illustrate the mean absorbance of the growth of selected bacteria in controls and the test tubes that are treated with different concentration of pomegranate peel extract. The result indicated a significant effect of pomegranate peel extract on decreasing the bacterial growth at concentration starting from 0.3mg/ml to 0.5mg/ml except E. coli which it's growth has been decreased significantly from 0.2mg/ml.

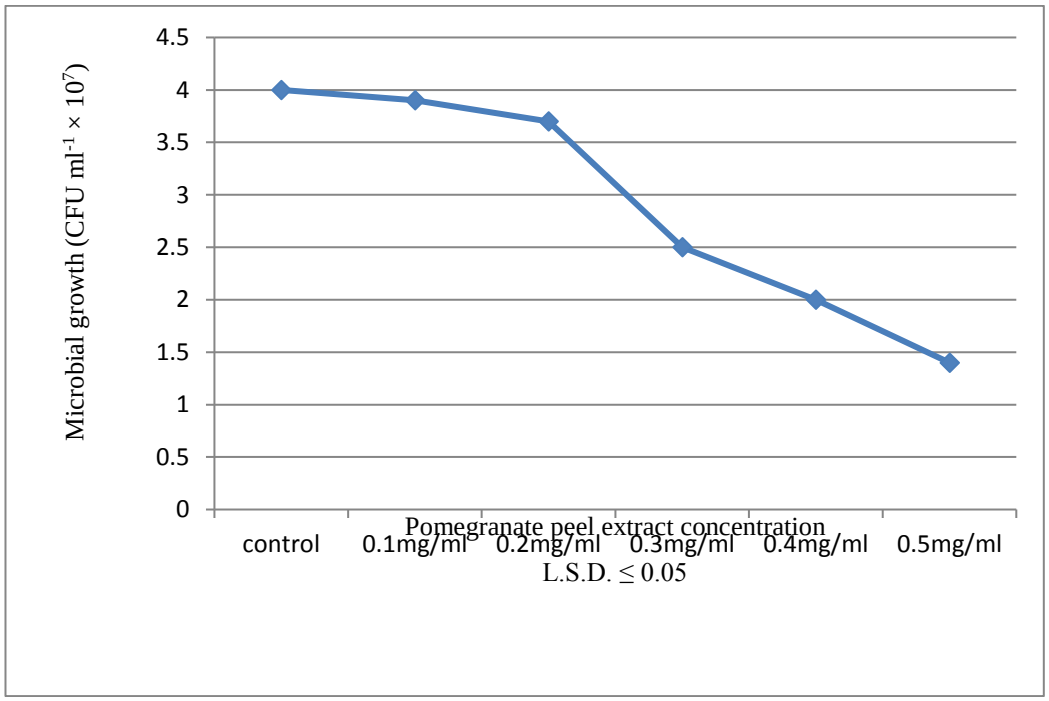
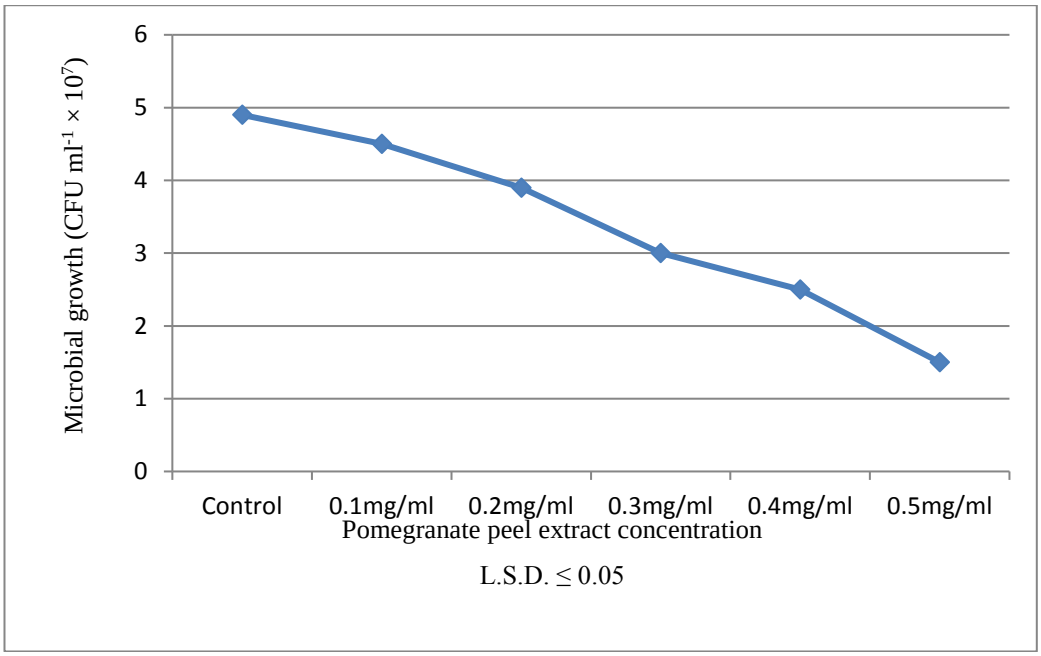


Figure (1) antimicrobial activity of pomegranate peel extract against Staphylococcus aureus.



Figure(2) antimicrobial activity of pomegranate peel extract against Escherichia coli.

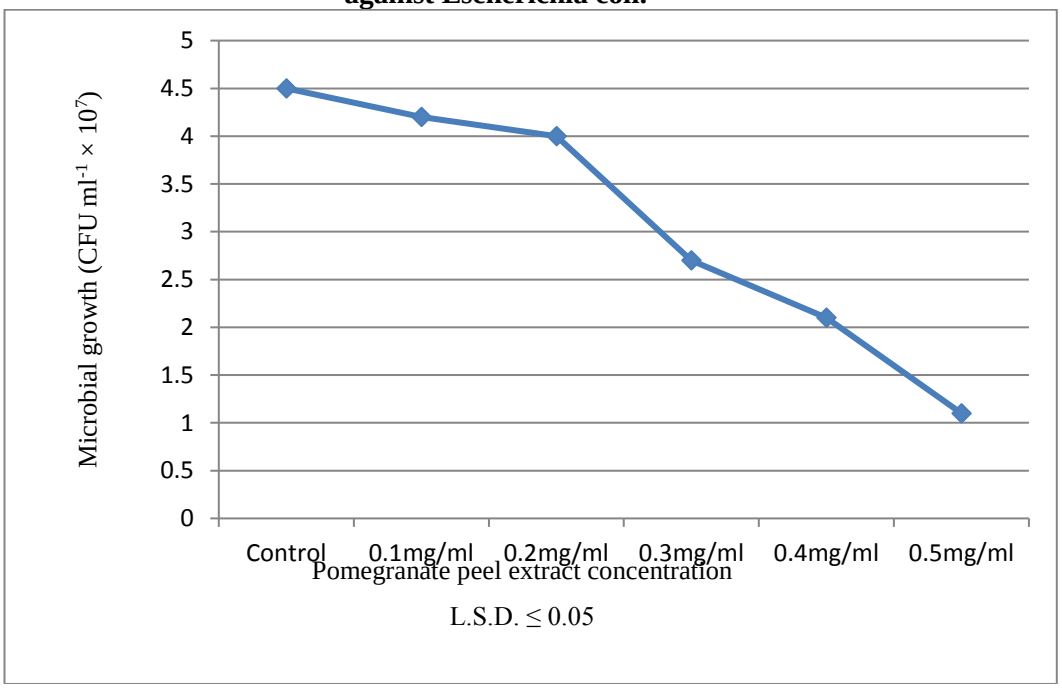
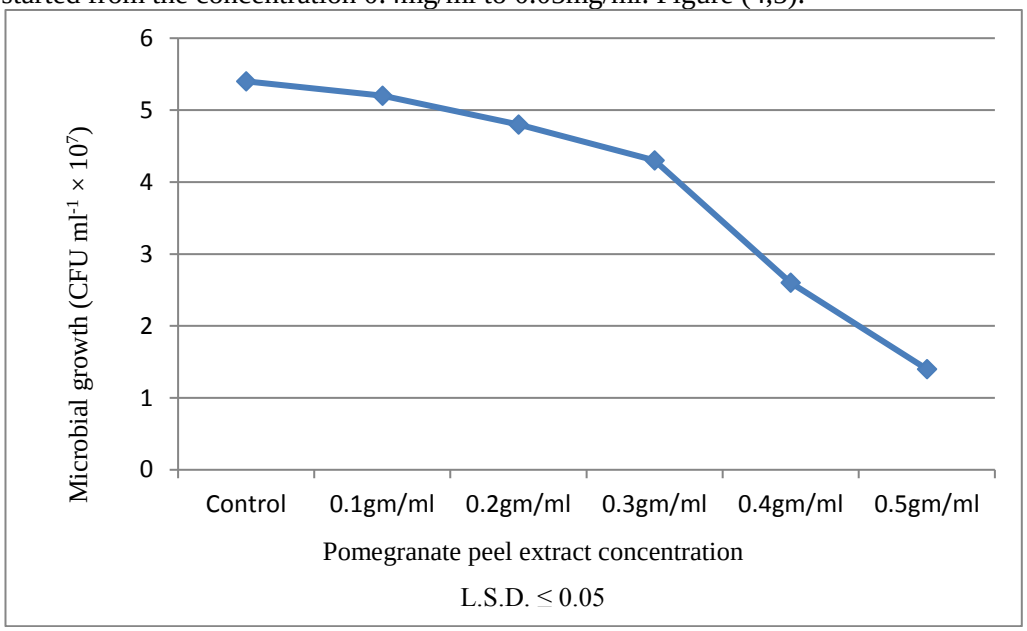
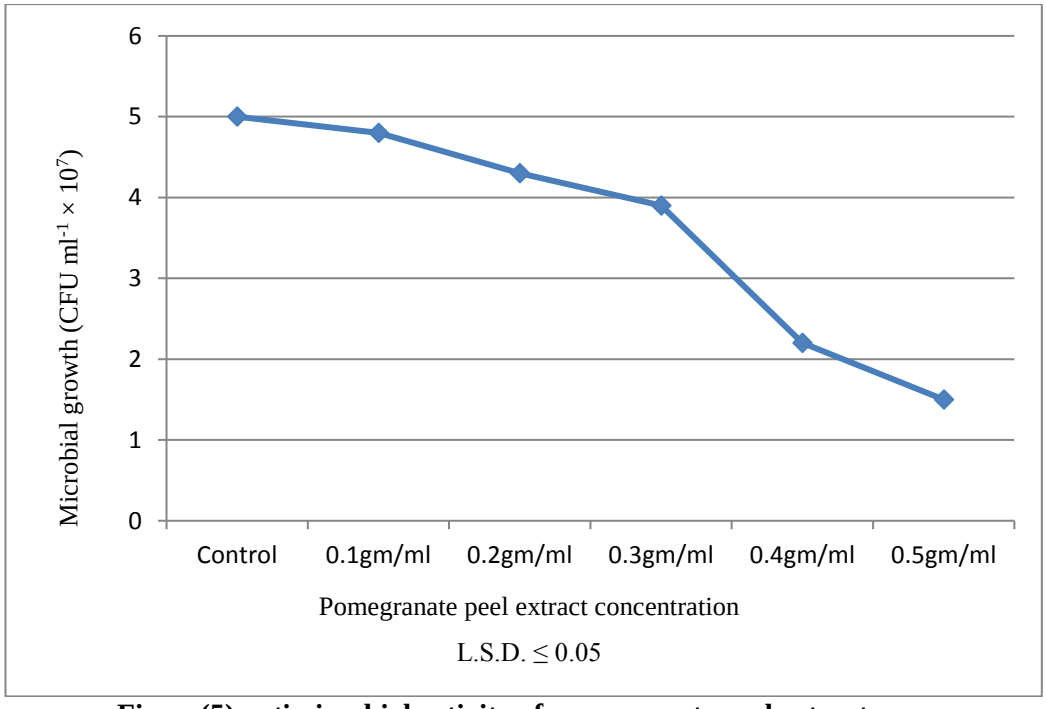


Figure (3) antimicrobial activity of pomegranate peel extract against Proteus vulgaricus.

While the inhibitory action of pomegranate peel extract against yeast growth started from the concentration 0.4mg/ml to 0.05mg/ml. Figure (4,5).



Figure(4) antimicrobial activity of pomegranate peel extract against Candida albicans.



Figure(5) antimicrobial activity of pomegranate peel extract against Candida tropicalis.

In general, the extent of the inhibitory effects of the pomegranate extracts could be attributed to their phenolic and anthocyanin content.[15] The bioactivity of aril extracts on the microorganisms tested has high total flavonols, phenolics, anthocyanins and organic acids.[16] Similarly, [17] confirmed that phenolics were the most important compounds against bacteria, among those gallic acid was identified as the most active compound for inhibition of bacteria tested. Our findings are also support these results. The inhibitory effect of phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation [18]. These results confirmed the antibacterial potential of pomegranate and its use in traditional medicine [19].

The inhibitory action of pomegranate peel extract is attributed also to tannins or polyphenols which are major components of pomegranate peel [3]. In previous work, Endo, et al. [20] reported that some fungi species, including Aspergillus niger, were sensitive to extract of pomegranate fruit peel.

Similarly, Tayel, et al. [21] stated that ellagic acid and punicalagins obtained from pomegranate juice byproducts revealed antifungal activity when assayed against *Aspergillus fumigatus.* Variable activity of pomegranate peel also was observed in an earlier study and some researchers, namely Satish,et al. [22] reported that pomegranate peel had no effect on the growth of *Aspergillus flavus* and Aspergillus parasiticus. Our finding exhibited the existence of antifungal activity of selected Punica granatum against Candida spp. and this finding was also reported by [23] . In this study, the extraction method, that be used may save the antioxidant compounds. Additionaly, differences in the activity of peel extracts among studies could be explained by variations in antioxidant compounds of different pomegranate varieties.

CONCLUSIONS

From this research results we can conclude that peel extracts of Punica granatum have important antmicrobial activities. On the basis of the knowledge of these properties, will be possible to use of pomegranate peel extracts to formulate new products to be used in food industry as natural antioxidant, replacing synthetic antioxidants, and also as natural food preservatives and pharmalogical studies.

REFERENCES

[1]. Lansky, E.P.; Shubert, S. and Neeman I.(2004). Pharmacological and therapeutic properties of pomegranate. p.231-35.

[2]. Lansky, E.; Shubert, S. and Neman, I. (2007) Pharmacological and therapeutic properties of pomegranate. CIHEAM - Options Mediterraneennes. J Ethnopharmacol 109:177-206.

[3]. Reddy, M.K.; Gupta, S.K.; Jacob, M.R.; Khan, S.I. and Ferreira, D.(2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. Planta Med. 73, 461-467.

[4]. Proestos, C.; Chorianopoulos, N.; Nychas, G.J.E. and Komaitis, M. (2005). RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem*. *53*, 1190-1195.

[5]. Ozgen, M.; Serce, S.; Gunduz, K.; Yen, F.; Kafkas, E.; Paydas, S.(2007). Determining total phenolics and antioxidant capacities of selected *Fragaria* genotype. *Asian J. Chem.19*, 5573-5581.

[6]. Ozgen, M.; Serce, S. and C. Kaya. (2009). Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *M. rubra* fruits. *Sci. Hortic. 119*, 275-279.

[7]. Jurenka, J. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Altern Med Rev.13*, 128-144.

[8]. Lamar, A.S.; Fonseca, G.; Fuentes, J.L.; Cozzi, R.; Cundari, E.; Fiore, M.; Ricordy, R.; Perticone,P.; Degrassi, F. and Salvia, R.D.(2008). Assessment of the genotoxic risk of *Punica granatum* L. (*Punicaceae*) whole fruit extracts. *J. Ethnopharmacol. 115*, 416-422.

[9]. Al-Zoreky, N.S. (2009). Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels International Journal of Food Microbiology Volume 134, Issue 3, Pages 244-248

[10]. Jaffer, H. J.; Mahmod, M. J.; Jawad, A. M.; Naji, A. and AL-Naib,A.
(1983). Phytochemical and biological screening of some Iraqi plan Fitoterapia Lix 299.

[11]. Ben NC, Ayed N, Metche M (1996). Quantitative determination of the polyphenolic content of pomegranate peel. Z. Lebensm. Unters. Forsch., 203: 374-378.

[12]. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing*. *The 9th International Supplement, M100-S9*, NCCLS: Villanova, PA, USA, 1999.

[13]. Thygesen, L.; Lokke, M.; Marie, M.; Elisabeth, E. and Siren, B. (2003). Vibrational microspectorscopy of food science and technology, (14 ref.)pp.50-57.

[14] Webb, N. and Blackmore, R. (1985). Statistics for biologists. Cambridge university press, pp.26-45.

[15]. Sweetie, R.; Kanatt, R. and Chander, A. (2010). Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products International Journal of Food Science & Technology Volume 45, Issue 2, pages 216–222.

[16]. Erin, M.; McCarrell**,** S.; Gould, W.J.**;** Mark, D.; Fielder, A.; Kelly F.;Waffa, El Sankary and Declan, P.(2008). Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C, School of Life Sciences, Kingston University, Kingston-upon-Thames, UK *BMC Complementary and Alternative Medicine* , 8**:**64doi:10.1186/1472-6882-8-64

[17]. Ahmet, D.; Duman, M.; Ozgen, S.; Dayisoylu, K.; Erbil N. and Durgac, C. (2009). Antimicrobial Activity of Six Pomegranate (*Punica granatum* L.) Varieties and Their Relation to Some of Their Pomological and Phytonutrient Characteristics. Molecules 14: 1808-1817.

[18]. Vasconcelos, L.C.; Sampaio, F.C.; Sampaio, M.C.; Pereira, S.; Higino, JS. and Peixoto, MH.(2006). Minimum inhibitory concentration of adherence of *Punica granatum* Linn (pomegranate) gel against *S. mutans, S. mitis* and *C. albicans*. Braz Dent J. 17:223-7.

[19]. Melendez, P.A. and Capriles, V.A. (2006). Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine, 13*, 272-276.

[20]. Endo, E.H.; Cortez, D.A.; Ueda-Nakamura, T.; Nakamura, C.V. and Dias Filho, B.P.(2010). Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against Candida albicans. Res Microbiol.161(7):534-40.

[21]. Tayel, A.A.; El-Baz, A.F.; Salem, M.F. and El-Hadary, M.H. (2009). Potential applications of pomegranate peel extracts for the control of citrus green mould. J. Plant Diseases and Protection, 116(6): 252-256.

[22]. Satish, S.; Mohana, D.C.; Raghavendra, M.P.j and Raveesha, K.A. (2007). Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. J Agric TechnolJ Agric Technol 3:109-19.

[23]. Pai, M.B.; Prashant, G.M.; Murlikrishna, K.S.; Shivakumar, K.M. and Chandu, G.N.(2010). Antifungal efficacy of Punica granatum, Acacia nilotica, Cuminum cyminum and Foeniculum vulgare on Candida albicans: An in vitro study. Indian J Dent Res;21:334-6.