Effect of pomegranate Extract and luteolin on Gram Negative and Positive Bacteria

Entessar. H. Ali
Bio-chemical Technology Division.
Applied Sciences Department. University of Technology

Abstract

The antibacterial effect of rind of pomegranate extract and Luteoline on the growth was evaluated in vitro serial dilution method was used to the growth of various Gram-negative and Gram-positive bacteria. The bactericidal activity of this extract was analyzed by serial dilution in tubes. In this study it was found that Gram positive bacteria are susceptible to very low pomegranate and luteoline concentrations. On the other hand, Gram negative bacteria were more resistant, with minimal bactericidal concentration of this extract ranging between 5 - 10 mg ml^{-1} pomegranate and 2,4 mg ml^{-1} of luteoline , for Gram-positive bacteria and were ranging between 10 - 20 mg ml^{-1} pomegranate and 4,8 mg ml^{-1} of luteoline for Gram-negative bacteria. The results of this study suggest that crude extraction of pomegranate and luteoline is effective in controlling of many diseases as a natural resource to prevent many bacterial infections.

Introduction

Pomegranate has strong antioxidant and anti-inflammatory properties and have anti-cancer activity in several human cancers [1]. In addition, pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a high antioxidant activity [2] . Antimicrobial drug resistance in human bacterial pathogens is a worldwide issue and as a consequence, effective treatment and control of such organisms remain an important challenge. Bacterial resistance has appeared for every major class of antibiotic [3].

The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate (flavonols, flavanols and anthocyanins), condensed in the fruit. Pomegranate polyphenols include flavonoidstannins (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 [4]. HTs are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity . Constituents Pomegranate pericarp (Peel, rind) Phenolic punicalagins;

gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavonols; flavones, flavonones; anthocyanidins [5].

Flavonoids are ubiquitously occurring and widely consumed secondary metabolites of plants and have profound pharmacological properties [6]. They are reported to have anti-viral [7], anti-parasitic [8] and anti-cancer [9] activities. Luteolin (3«,4«,5,7-tetrahydroxy¯avone), an important member of the favonian family, is present in various fruits and vegetables and has contributed to the antioxidant activity of artichoke leaf extract on reactive oxygen species in human leucocytes [10]. Luteolin is also reported to have anti-inflammatory properties and mediates its action by inhibiting of nitric oxide production [11]. Luteolin has anti-allergic properties [12], and a recent report [13] establishes luteolin as a potent inhibitor of human mast cell activation through the inhibition of protein kinase C activation and Ca#+ in¯ux. Luteolin exerts growth inhibitory effects on NK}Ly ascites-tumour-cell cultures in .i.o [14]. The flavonoids luteolin and quercetin are also reported to arrest cell cycle in the G" phase of human melanoma cells [15]. We have previously established that luteolin and quercetin inhibit DNA topoisomerase II of Leishmania and that they can induce cell-cycle arrest leading to apoptosis of Leishmania donovani promastigotes[12].

For the purpose of communicable diseases with bacterial infection and resistant bacteria to manufactured antibiotics. The objectives of this work was: to study the activity of pomegranate extract and luteolin against several Gram-positive and Gram-negative bacteria.

Materials and methods

Extraction of pomegranate *rind*

pomegranate rind samples were collected from Iraqi gardens. For the purpose of extraction, the dried rind of pomegranate was cut into small pieces, and extracted at room temperature with 50 ml of 70% ethanol using ultrasonic bath (Decon FS 300, England) for 90 minutes. Then, the alcoholic extract was evaporated at 50°C until dryness (16). Luteoline take from sigma as standard material.

Antibacterial assay

Six bacterial strains were used: ***Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, streptococcus pneumonia, Streptococcus faecalis*** and ***Bacillus subtillis***.These bacteria were kindly supplied by the Biotechnology department; college of science, university of Baghdad, Baghdad, Iraq .The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5x 10^7 cell ml^{-1}) tubes. It was further diluted to obtain a final of 5 x 10^6 cell ml^{-1}. All bacteria strains were sub- culture in nutrient broth (8). The broth was inoculated by the 0.2 ml/10ml broth either with all bacteria strains, then added 1 ml of (25, 50 and 100 mg) pomegranate and

(2.5,5 and 10) luteoline. The tubes were incubated at 37 C^0 for 24 h. The growth of control bacterial growth due to pomegranate and luteoline was measured by turbidity at 600 nm wavelength. The mean values of inhibition were calculated from triple reading in each test. The minimum bactericidal concentration (MBC) of pomegranate and luteoline was determined by the ten-fold dilution method against bacterial strains in ***in vitro***.

Statistical data analysis: Data were statistically analyzed using SPSS statistical software (version 11.5). The values are given as mean ± standard error.

Results

This study confirms the antibacterial effect of pomegranate extract and luteoline on various Gram-negative and Gram-positive bacteria. In particular,crude of pomegranate and luteoline is a very potent inhibitor of growth of bacteria such as clinical isolates of ***Pseudomonas aeruginosa***, ***Proteus vulgaris***, ***Klebsiella pneumonia, streptococcus pneumonia, Streptococcus faecalis*** and ***Bacillus subtillis.*** All bacterial pathogens failed to grow in higher concentrations of pomegranate extract and luteoline (Table 1,2 and Figure 1,2,3,4).

Table 1. Antibacterial properties of pomegranate extract

Strains	Optical Density 600 nm			
	Concentration of pomegranate mg ml^{-1}			
	5	10	20	Control
Pseudomonas aeruginosa	1.59±0.34	1.41±0.11	1.21±0.20	1.59±0.20
Proteus vulgaris	1.45±0.25	1.40±0.30	1.15±0.22	1.58±0.28
Klebsiella pneumonia	1.36±0.24	1.33±0.19	1.23±0.22	1.43±0.19
Streptococcus pneumonia	1.30±0.12	1.29±0.20	0.85±0.12	1.32±0.10
Streptococcus faecalis	1.40±0.14	1.39±0.08	1.34±0.14	1.39±0.11
Bacillus subtillis	0.45±0.06	0.30±0.07	0.31±0.17	1.49±0.36

Pseudomonas aeruginosa:

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 5 mg ml^{-1} (1.59 ± 0.34) and above as compared with control 1.59 ± 0.20 (Table 1). There was also an obvious decrease in the number of viable cells of ***Pseudomonas aeruginosa*** especially at the higher concentration (20 mg ml^{-1}) was 6.05 x10^8 CFU ml^{-1} as compared with control 7.95 x 10^8 CFU ml^{-1} (Figure 1). The MBC of pomegranate was 10 mg ml^{-1}.

Proteus vulgaris:

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 10mg ml^{-1} (1.40±0.30) and above as compared with control 1.58±0.19 (Table 1). There was also an obvious decrease in the number of viable cells of ***Proteus vulgaris*** especially at the higher concentration (10 and 20 mg ml^{-1}) was 7 x10^8 and 5.75 x10^8 CFU ml^{-1}, respectively as compared with control 7.9x10^8 CFU ml^{-1} (Figure 1). The MBC of pomegranate was 5 mg ml^{-1}.

***Klebsiella pneumonia*:**

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 5mg ml $^{-1}$ (1.23±0.19) and above as compared with control 1.32±0.10 (Table 1). There was also an obvious decrease in the number of viable cells of ***Klebsiella pneumonia*** especially at the higher concentrations (10 and 20 mg ml^{-1}) was 6.65 x10^{8} and 6.15 x10^{8} CFU ml $^{-1}$, respectively as compared with control 7.15 x10^{8} CFU ml $^{-1}$ (Figure 1). The MBC of pomegranate was 5 mg ml^{-1}.

Streptococcus pneumonia:

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 20 mg ml $^{-1}$ (0.85±0.12) and above as compared with control 1.39±0.11 (Table 1). There was also an obvious decrease in the number of viable cells of ***Streptococcus pneumonia*** especially at the higher concentration (10 and 20 mg ml^{-1}) was 6.45 x10^{8} and 4.25 x10^{8} CFU ml $^{-1}$, respectively as compared with control 6.6x10^{8} CFU ml $^{-1}$ (Figure 2). The MBC of pomegranate was 5 mg ml^{-1}.

Streptococcus faecalis:

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 10 mg (1.39±0.02) and above as compared with control 1.49±0.36 (Table 1). There was also an obvious decrease in the number of viable cells of ***Streptococcus faecalis*** especially at the higher concentration (10 and 20 mg ml^{-1}) was 6.95 x10^{8} and 6.70 x10^{8} CFU ml $^{-1}$, respectively as compared with control 6.95x10^{8} CFU ml $^{-1}$(Figure 2). The MBC of pomegranate was 10 mg ml^{-1}.

Bacillus subtillis:

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 10mg ml $^{-1}$ (0.30±0.07) and above as compared with control 1.49±0.36 (Table 1). There was also an obvious decrease in the number of viable cells of ***Bacillus subtillis*** especially at the higher concentration (20 mg ml^{-1}) was 1.55 x10^{8} CFU ml $^{-1}$ as compared with control 7.45 x10^{8} CFU ml $^{-1}$ (Figure 2). The MBC of pomegranate was 5 mg ml^{-1}.

Table 2. Antibacterial properties of luteoline

Strains	Optical Density 600 nm			
	Concentration of luteoline mg ml-1			
	0.2	0.4	0.8	Control
Pseudomonas aeruginosa	0.606±0.34	0.575±0.11	0.530±0.20	0.650±0.20
Proteus vulgaris	0.510±0.25	0.496±0.30	0474.±0.22	0.563±0.28
Klebsiella pneumonia	0.363±0.24	0.329±0.19	0.316±0.22	0.468±0.19
Streptococcus pneumonia	0.415±0.12	0.406±0.20	0.306±0.12	0.487±0.10
Streptococcus faecalis	0.404±0.14	0.390±0.08	0.304±0.14	0.449±0.11
Bacillus subtillis	0.451±0.06	0.301±0.07	0.311±0.17	0.490±0.36

Pseudomonas aeruginosa:

The results indicated that luteoline exhibited antibacterial activity at concentrations of 0.2 mg ml $^{-1}$ (0.606 ± 0.34) and above as compared with control 0.650 ± 0.20 (Table 2). There was also an obvious decrease in the number of viable cells of ***Pseudomonas aeruginosa*** especially at the higher concentration (0.8 mg ml^{-1}) was 2.650 x10^{8} CFU ml $^{-1}$ as compared with control 3.250 x 10^{8} CFU ml $^{-1}$ (Figure 3). The MBC of luteoline was 0.2 mg ml^{1}.

Proteus vulgaris:

The results indicated that luteline exhibited antibacterial activity at concentrations of 0.2 mg ml $^{-1}$ (0.510±0.25) and above as compared with control 0.563±0.28 (Table 2). There was also an obvious decrease in the number of viable cells of ***Proteus vulgaris*** especially at the higher concentration (0.4 and 0.8 mg ml^{-1}) was 2.48 x10^{8} and 2.37 x10^{8} CFU ml $^{-1}$, respectively as compared with control 2.815 x10^{8} CFU ml $^{-1}$ (Figure 3). The MBC of luteoline was 0.2 mg ml^{-1}.

***Klebsiella pneumonia*:**

The results indicated that luteoline exhibited antibacterial activity at concentrations of 0.2 mg ml $^{-1}$ (0.363±0.19) and above as compared with control 0.468±0.10 (Table 2). There was also an obvious decrease in the number of viable cells of ***Klebsiella pneumonia*** especially at the higher concentrations (0.4 and 0.8 mg ml^{-1}) was 1.645 x10^{8} and 1.58 x10^{8} CFU ml^{-1}, respectively as compared with control 2.34 x10^{8} CFU ml $^{-1}$ (Figure 1). The MBC of luteoline was 0.2 mg ml^{-1}.

Streptococcus pneumonia:

The results indicated that luteoline exhibited antibacterial activity at concentrations of 0.2 mg ml $^{-1}$ (0.415±0.12) and above as compared with control 0.487±0.11 (Table 2). There was also an obvious decrease in the number of viable cells of ***Streptococcus pneumonia*** especially at the higher concentration (0.4 and 0.8 mg ml^{-1}) was 2.030 x10^{8} and 1.53 x10^{8} CFU ml $^{-1}$, respectively as compared with control 2.435x10^{8} CFU ml $^{-1}$ (Figure 4). The MBC of luteoline was 0.2 mg ml^{-1}.

Streptococcus faecalis:

The results indicated that pomegranate exhibited antibacterial activity at concentrations of 0.4 mg (0.390±0.08) and above as compared with control 0.449±0.11(Table 2). There was also an obvious decrease in the number of viable cells of ***Streptococcus faecalis*** especially at the higher concentration (0.4 and 0.8 mg ml^{-1}) was 1.95 x10^{8} and 1.52 x10^{8} CFU ml $^{-1}$, respectively as compared with control 2.245x10^{8} CFU ml $^{-1}$(Figure 4). The MBC of pomegranate was 5 mg ml^{-1}.

Bacillus subtillis: The results indicated that pomegranate exhibited antibacterial activity at concentrations of 0.2 mg ml -1 (0.451±0.06) and above as compared with control 0.490±0.36 (Table 2). There was also an obvious decrease in the number of viable cells of Bacillus subtillis especially at the higher concentration (0.8 mg ml-^{1}) was 1.555 x10^{8} CFU ml $^{-1}$ as compared with control 2.45 x10^{8} CFU ml $^{-1}$ (Figure 2). The MBC of pomegranate was 0.2 mg ml^{-1}.

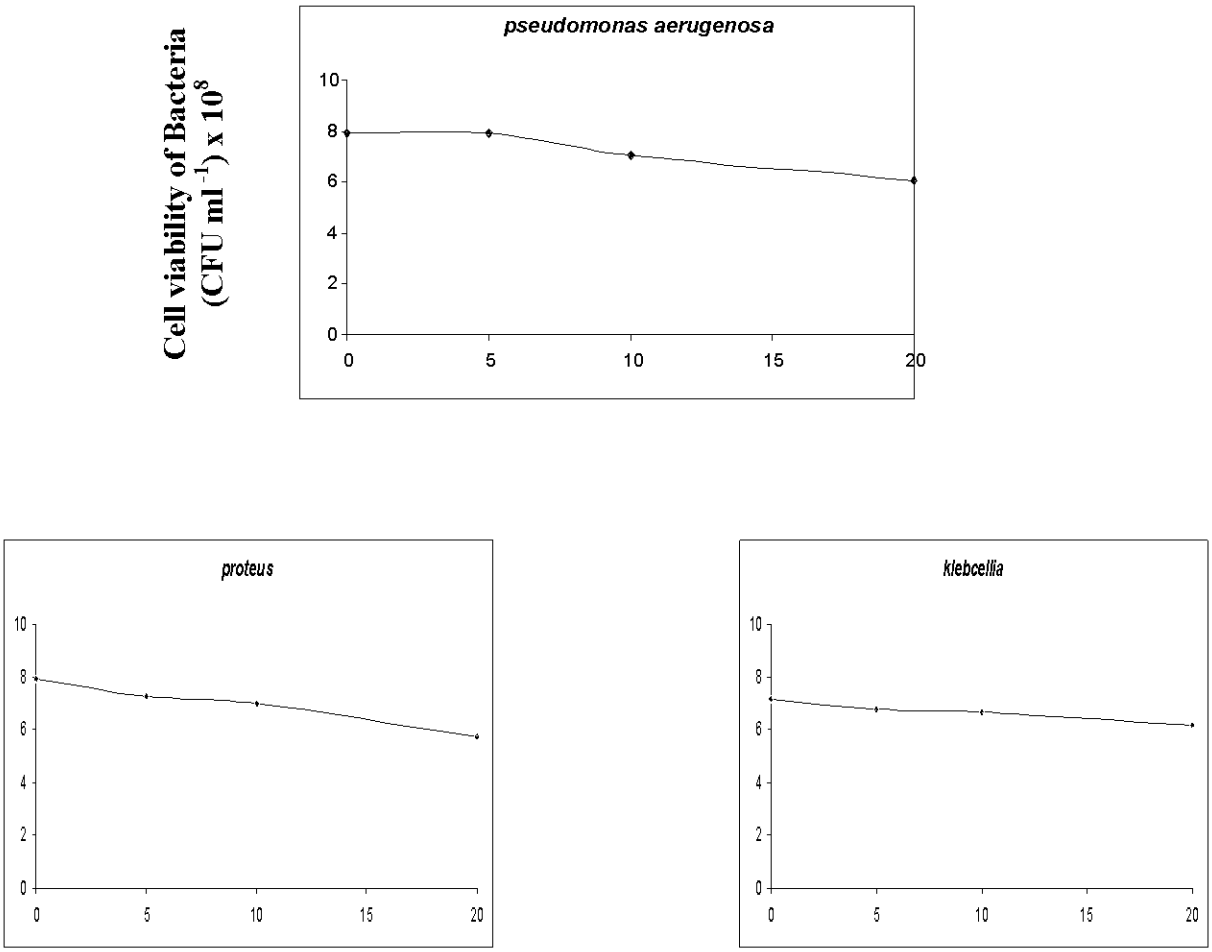
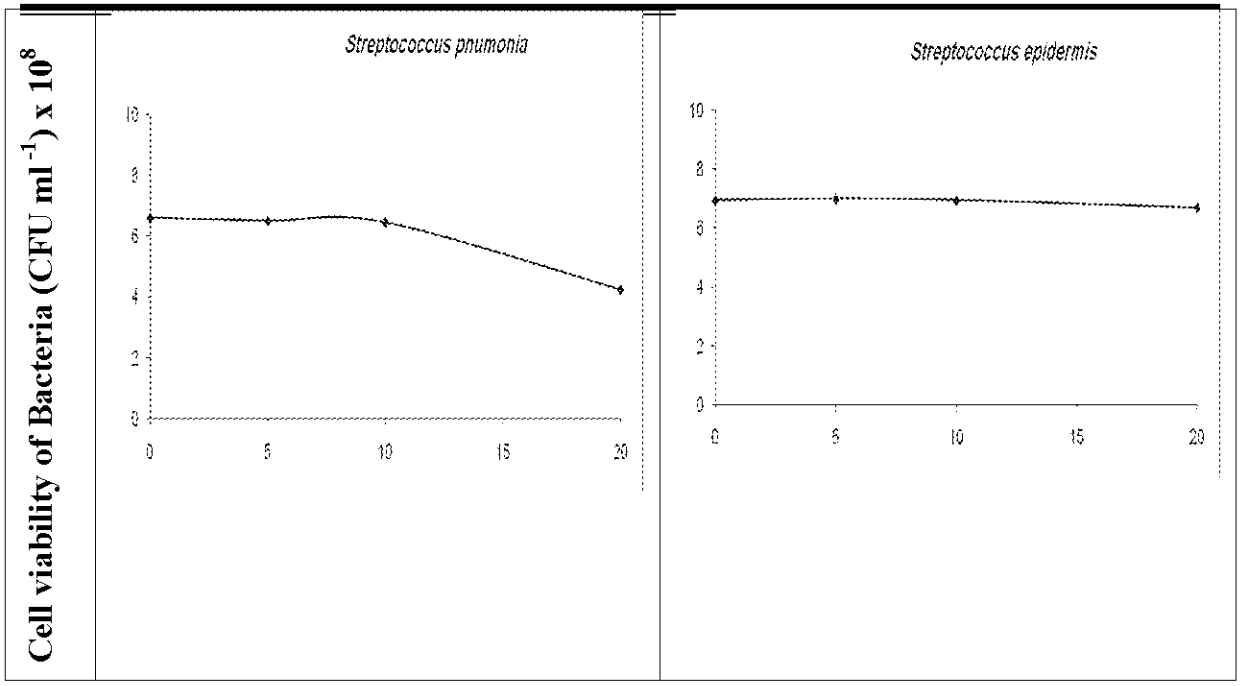
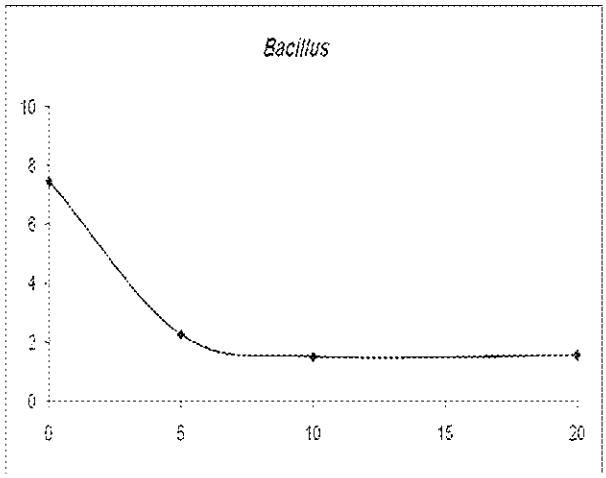


Figure 1. Pomugranate effect against cell viability of Gram negative bacteria





Concentration of pomugranate mg ml^{-1}

Figure 2. Pomugranate effect against cell viability of Gram posative bacteria

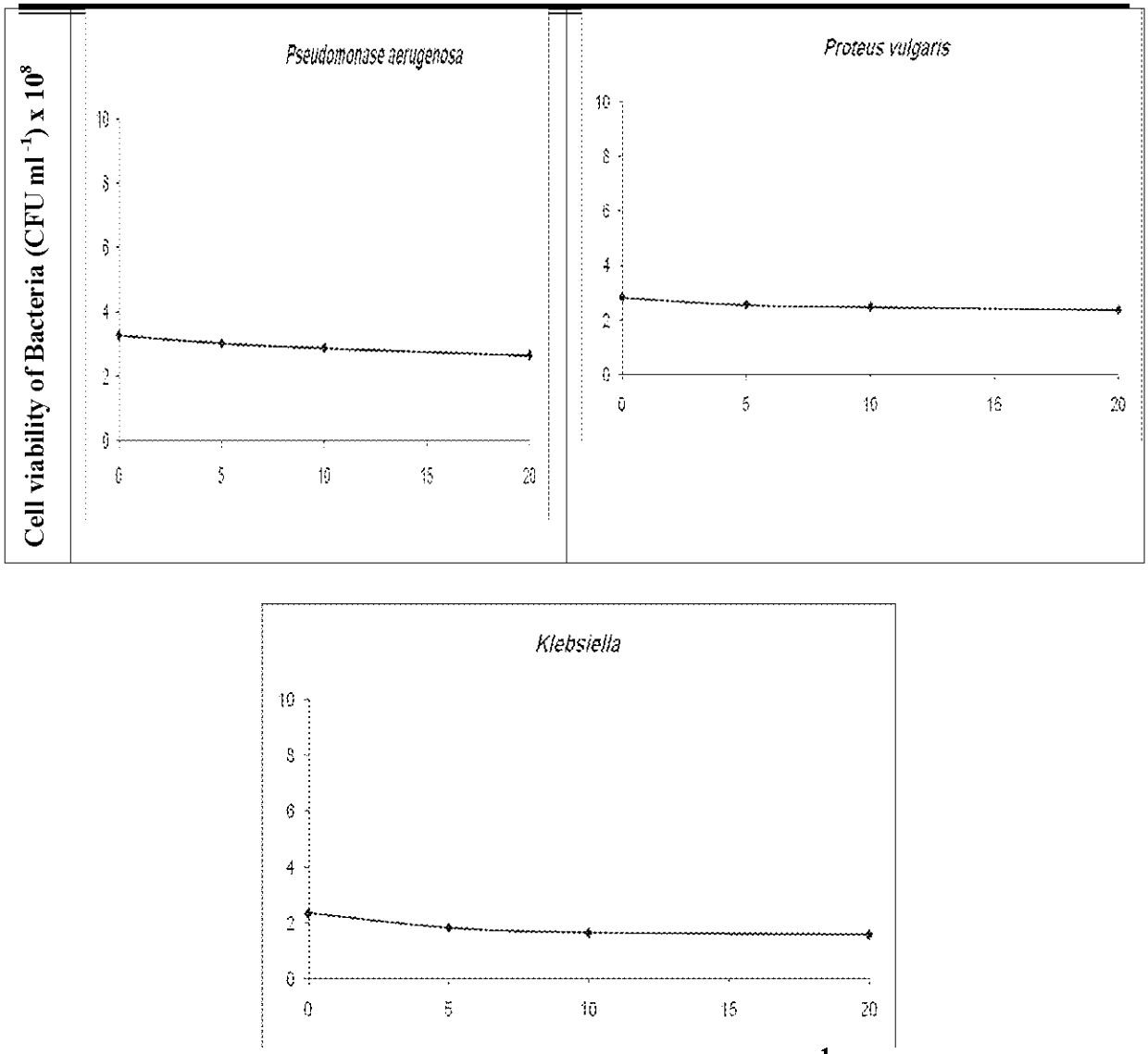


Figure 3. Luteoline effect against cell viability of Gram negative bacteria

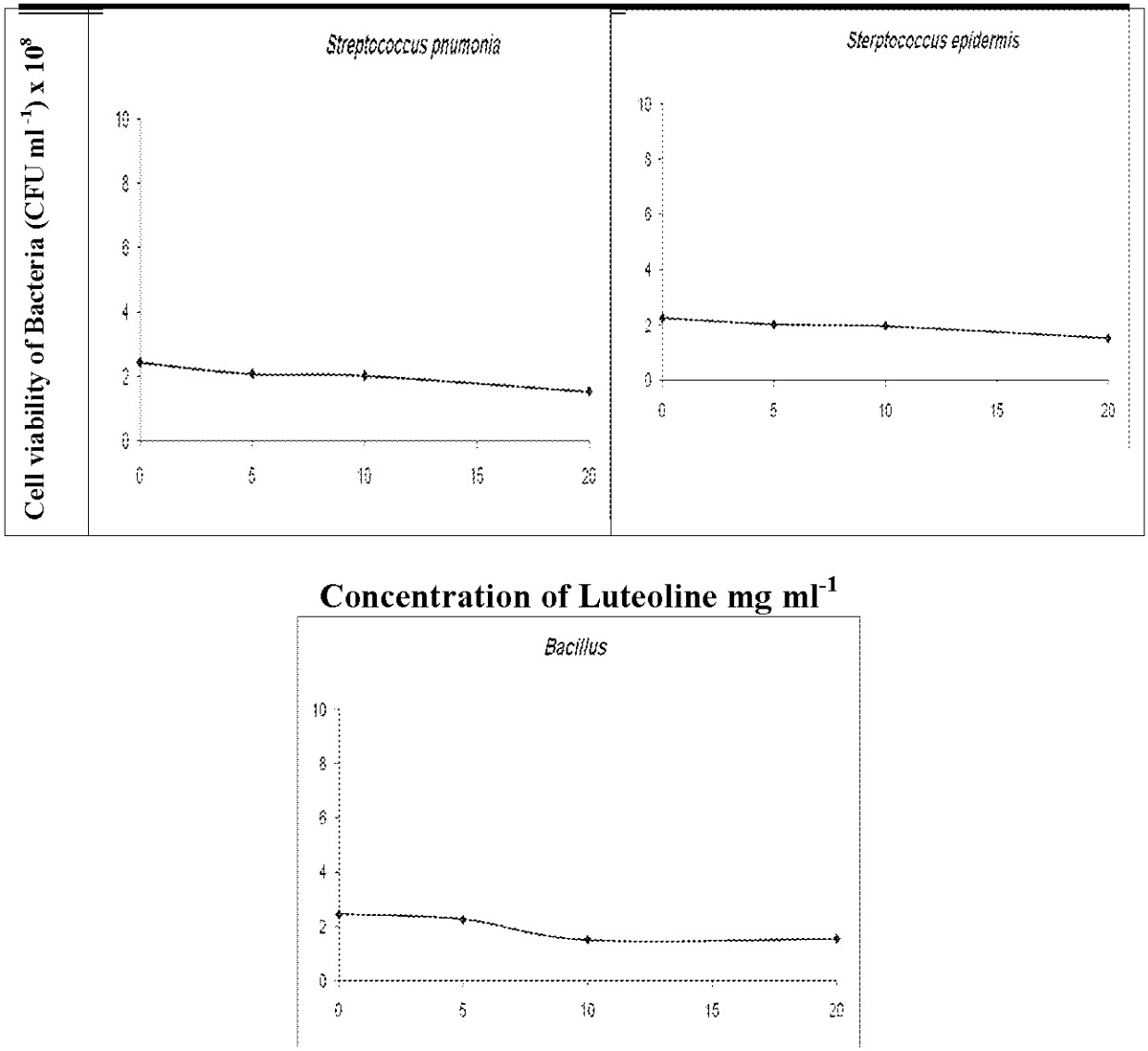


Figure 4. Luteoline effect against cell viability of Gram posative bacteria

Discussion

There has been only limited research on antibacterial activity of pomegranate and luteoline (9). In this Study, we could verify that Gram positive bacteria (***Streptococcus pneumonia, Streptococcus epidermis*** and ***Bacillus subtillis***) are susceptible to very low pomegranate concentrations. On the other hand, Gram negative bacteria ***(Pseudomonas aeruginosa***, ***Proteus vulgaris*** and ***Klebsiella pneumonia***) were more resistant. Previous studies also reported that Gram-negative bacteria were less susceptible to lower minimal inhibitory concentrations (MIC) than Gram-positive strains (10, 11, 12, 13, 14, 15). However, with respect to the magnitude of the MIC, pomegranate show lower activity against ***Pseudomonas aeruginosa*** than ***Proteus vulgaris*** and ***Klebsiella pneumonia*** and was more active against ***Streptococcus pneumonia*** and

Streptococcus epidermis than ***Bacillus subtillis***. The quantitative and qualitative chemical composition could provide an explanation for the observed differences. Crude pomegranate contains a mixture of a large number of biologically active substances (16) that belong chemically to the terpenes, caffeic acid and its esters, flavonoids, free amino acids, aldehydes and ketones. Many studies have shown that fatty acid esters, phenolic compounds and cinnamic acid were the main propolis constituents and some of them were shown to possess antibacterial activity (17, 18). Crude pomegranate was shown more effective than single chemicals, a possible explanation of why pomegranate is more effective than its individual compounds. Of course, mixtures are more likely to contain toxic constituents, and they must be thoroughly investigated and standardized before approved for use on a large-scale basis in the West (19,20). Many researchers had investigated the antibacterial activity of pomegranate and its extracts against Gram-positive and Gram-negative strains and found that pomegranate had antibacterial activity against a wide range of Gram-positive rods but had a limited activity against Gram-negative bacilli (21, 22). In vitro studies have demonstrated that pomegranate extracts are more effective against Gram-positive coccii (***Staphylococcus aureus***, ***Streptococcus*** β-haemolyticus), but are only active against some Gram-negative bacteria, such as ***Escherichia coli*** or ***Pseudomonas aeruginosa*** (12). On the other hand, other studies (7,22), have indicated that the bacteriostatic or bactericidal effects of pomegranate depend on the dose and that Gram-negative aerobic bacteria may also be inhibited at concentrations higher than 5 mg ml^{-1}. Also, the minimum inhibitory concentration of pomegranate against 35 ***S. aureus*** strains and 92 other bacterial strains (23). 19 elements in pomegranate, 3 fractions were obtained and tested against ***Staphylococcus***. Another experiment (24,25) found that the sensitivity of 90% of ***Staphylococci*** to ethanolic extract of pomegranate was lower than in a standard strain of ***S. aureus***. Prepared ethanolic extracts from samples of pomegranate collected in 18 regions of the former USSR(26), were serially diluted in agar, in Petri dishes. The dishes were then inoculated with the bacteria ***Bacillus cereus***, ***Staphylococcus auneus***, ***Escherichia coli*** and ***Pseudomonas aeraginosa***, and the fungus ***Candida albicans***, and incubated at 37 °C or 20-25 °C for 48h. Pomegranate at 125-500 μg ml^{-1} inhibited the growth of ***B.Cereus*** and ***S. aureus***, but not that of the other 2 bacteria, or the fungus, even at concentrations higher than 1000 μg ml^{-1}. These findings confirm that, the antimicrobial properties of pomegranate possibly attributed to its high flavanoids content. The anti-bacterial activity of European pomegranate is due to its flavonoid aglycones (galangin & pinocembrin) and phenolic compounds (pinobanksin, pinobanksin 3-O-acetate, bencyl-p-coumarate, caffeic acid esters, and ferulic and caffeic acids), and in pomegranate from the Canary Islands, lignan furofurans (27). The German variety, rich in phenylethyl-trans-caffeate,

bencyl ferulate and galangin, is more effective against ***Staphylococcus aureus*** and ***Escherichia coli***, than the French variety, rich in bencyl caffeate and pinocembrin. The Mediterranean type (Bulgarian, Turkish, Greek and Algerian), composed of flavonoids, esters of caffeic and ferulic acids, diterpenes and hydroxyditerpenes, also show significant bacteriostatic and bactericidal properties (28, 29). In the case of Egyptian pomegranate, anti-microbial activity differs in accordance with its region of origin (7, 22, 30). The same variation in the antibacterial activity was shown in Iraqi pomegranate (9). In Brazilian pomegranate, phenolic compounds have been identified, most notably 3,5-diprenyl-p-coumaric acid, which possesses significant anti-bacterial activity and without seasonal differences against ***Escherichia coli***, ***Pseudomonas aeruginosa***, ***Staphylococcus aureus*** and ***Streptococcus faecalis*** (27,31,32). The inhibition of bacterial viability by pomegranate extract was probably due to the loss of their ability to bind to DNA (33). This fact suggested that pomegranate might act by inhibiting DNA replication and cell reproduction. In conclusion the Iraqi pomegranate extract exhibit significant antibacterial activity. These results confirm that antibacterial properties of pomegranate possibly attributed to its high flavonoids content (9) and volatiles (34). Hence, pomegranate should be viewed more appropriately as a complex natural resource for the control of microorganisms rather than antimicrobial drugs.

References

1. Arora A, Nair NG, Strasburg GM. 1998. Structure activity relationships for antioxidant activities of a series flavonoids in a liposomal system. Free Radic Biol Med 24: 1350–1363.
2. Bors W, Saran M. 1987. Radical scavenging by flavonoid antioxidants.Free Radic Res Commun 2: 289–294.
3. Chang W-C, Hsu F-L. 1992. Inhibition of platelet activation and endothelial cell injury by polyphenolic compounds isolated from Lonicera japonica Thunb. Prostaglandins Leuko Essent Fatty Acids 45: 307–312.
4. Cos P, Calomme M, Sindambiwe JB et al. 2001. Cytotoxicity and lipid peroxidation-inhibiting activity flavonoids. Planta Med 67: 515–519.
5. Hammerschmidt R, Nuckles EM, Kuc J. 1982. Association of enhanced peroxidase-activity with induced systemic resistance of cucumber to Colletotrichum lagenarium.Physiol Plant Pathol 20: 73.
6. Harborne JB. 1994. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In The Flavonoids, Advances in Research. Chapman and Hall: London, 619.
7. Hirano T, Higa S, Arimitsu J et al. 2004. Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4and interleukin-13 production by activated human basophils.Int Arch Allergy Immunol 134: 135–140.
8. Ilarianov L, Rainova L, Nakov N. 1979. On anti-inflammatory and anti-ulcer activity of some flavonoides, isolated from the genus Genista L. Farmatsiia (Sofia) 29: 39.
9. Ajaikumar KB, Asheef M, Babu BH, Padikkala J (2005). The inhibition of gastric mucosal injury by Punica granatum L. (pomegranate) methanolic extract. J. Ethnopharmacol., 96: 171- 176.
10. Adhami VM, Mukhtar H (2007). Anti-oxidants from green tea and pomegranate for chemoprevention of prostate cancer. Mol. Biotechnol., 37: 52-57.
11. Abdel MAE, Dkhil MA, Al-Quraishy S (2011). Studies on the effect of pomegranate (Punica granatum) juice and peel on liver and kidney in adult male rats. Rev,22:50-55.
12. Lambert PA (2005). Bacterial resistance to antibiotics: Modified target sites. Adv. Drug Deliv. Rev.,57:1471-148
13. Wu, X., G. Cao and R.L. Prior, 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. J. Nutrition. 132: 1865.
14. Abd El Hady, F. K. and Hegazi, A. G. (2002).Egyptian propolis: chemical composition, antiviral and antimicrobial activities of east Nile delta propolis. Z. Naturforsch., 57c: 386-394.
15. Cruickshank, R.; Duguid, J. P.; Masion, B. P. and Swain R. H. (1979). Medical microbiology. 12th (Ed.), Churchill Livingstone, Edinburgh, London, New York.

16 .Syrovets, T., Buchele, B., Gedig, E., Slupsky, R. J. and Simmet, T. (2000) Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerase I and IIa. Mol. Pharmacol. 58, 71±81
17. Havsteen, B. (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. 32, 1141±1148
18. Wollenber, E. (1988) Occurrence of ¯avonoid aglycones in medicinal plants.Prog. Clin. Biol. Res. 280, 45±55
19. Vrijsen, R., Everaert, L. and Boeye, A. (1988) Antiviral activity of ¯avones and potentiation by ascorbate. J. Gen. Virol. 69, 1749±1751
20. Mittra, B., Saha, A., Chowdhury, A. R., Pal, C., Mandal, S., Mukhopadhyay, S.,Bandopadhyay, S. and Majumder, H. K. (2000) Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. Mol. Med. 6, 527±541.
21. Yoshida, M., Sakai, T., Hosokawa, N., Marui, N., Matsumoto, K. A., Nishino, H. and Aoike, A. (1990) The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. FEBS Lett. 260, 10±13.
22. Perez-Garcia, F., Adzet, T. and Canigueral, S. (2000) Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. Free Radical Res. 33, 661±665.
23. Kim, H. K., Cheon, B. S., Kim, S. Y. and Kim, H. P. (1999) Effects of naturally occurring ¯avonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure±activity relationships. Biochem. Pharmacol. 58, 759±765.
24. Kimata, M., Inagaki, N. and Nagai, H. (2000) Effects of luteolin and other flavonoids on IgE-mediated allergic reactions. Planta Med. 66, 25±29.
25.Passamonti, S., U. Vrhovsek, A. Vanzo andF. Mattivi, 2003. The stomach as a site for anthocyanins absorption from food, FEBS Letters544(1): 210-213.
26.LD Reynolds and NG Wilson, "Scribes and Scholars" 3rd Ed. Oxford: 1991. pp193–4.
27.S. M. Fiuza. "Phenolic acid derivatives with potential anticancer properties—a structure–activity relationship study. Part 1: Methyl, propyl and octyl esters and gallic acids".doi:10.1016/j.bmc.2004.04.026.
28. Tsao, Makepeasce (July 1951). "A New Synthesis Of Mescaline". Journal of the. American Chemical Society 73 (11): 5495–5496. doi:10.1021/ja01155a562. ISSN 0002-7863
29. phytochemicals.info.Goodarzain,H.and Ekrami,E(2010):wool dyeing with extracted dye from pomegranate (punica Granatum)peel .world Applied science journal,8(11):1387-1389 .
30.Sawant L, Prabhakar B, Pandita N, Quantitative HPLC analysis of Ascorbic Acid and Gallic Acid in *Phyllanthus Emblica*. J. Anal. Bioanal Techniques, 2010; 1: 111.
31.Hafsa D., Pradnya J. P., Development of RP-HPLC method for qualitative analysis of active ingredient (Gallic acid) from Stem Bark of Dendrophthoe falcate Linn., International Journal of Pharmaceutical Sciences and Drug Research, 2011; 3(2): 146-149.
32.Chow, K. C., Macdonald, T. L. and Ross, W. E. (1988) DNA binding by epipodophyllotoxins and N-acyl anthracyclines : implications for mechanism of topoisomerase II inhibition. Mol. Pharmacol. 34, 467±473
33. LePecq, J. B. and Paoletti, C. (1967) A ¯uorescent complex between ethidium bromide and nucleic acids. Physical±chemical characterization. J. Mol. Biol.27, 87±106
34. Kawaii, S., Tomono, Y., Katase, E., Ogawa, K. and Yano, M. (1999) Antiproliferative activity of ¯avonoids on several cancer cell lines. Biosci. Biotechnol. Biochem.63, 896±899.

الخلاصة

تم تقييم الفعالية المضادة للبكتيريا لمستخلص قشور الرمان ومركب اليوتيولين في الزجاج على سلالات مختلفة من البكتيريا السالبة والموجبة لصبغة غرام .وقد استخدمت طريقة التخافيف المتسلسلة في الانابيب لقياس الفعالية المثبطة للنمو البكتيري .اظهرت الدراسة الحالية بان البكتيريا الموجبة لصبغة غرام كانت حساسة لتراكيز المستخلص الخام والمركب النقي الواطئة . من ناحية اخرى اظهرت البكتريا السالبة لصبغة غرام مقاومة اكبرفقد تراوحت قيم التركيز الادنى للتثبيط البكتيري بين 5 , 10 مليغرام/مليليتر لقشور الرمان و2 , 4 مليغرام/مليليتر لليوتيولين للبكتيريا الموجبة لصبغة غرام بينما تراوحت بين 10 , 20 مليغرام/مليليتر لقشور الرمان و4 , 8 مليغرام/مليليتر لليوتيولين للبكتريا السالبة لصبغة غرام . تقترح نتائج هذه الدراسة بان للمستخلص الخام لقشور الرمان والمركب النقي اليوتيولين فعالية للسيطرة على العديد من الامراض من خلال كونه مصدرا طبيعيا لمنع العديد من الاصابات الجرثومية .