The effect of pomegranate extracts on bacteria

Shawqi Muhammad Hassan [1], Abdulqadr Khdir Hamad [2], Ahmed Farhan Shallal[3]

Shawqi Muhammad Hassan
MSc in Biomedical science (Medical Microbiology) at the University of Wolverhampton-Wolverhampton- United Kingdome
Position: Assist lecturer at the university of Raparin-
Phone number: 0009647516896949
Email:Shawqi.bio@raparinuni.org

Abdulqadr Khdir Hamad
MSc in Medical biology at the university of Gaziantep- Gaziantep- Turkey
Position: Assist lecturer at the university of Raparin
Phone number: 009647503490660
Email: abdulqadir.bio@raparinuni.org

Ahmed Ferhan Shallal
PhD in immunology at the University of Al-Mustansiriya - Baghdad- Iraq
Position: lecturer at the university of Raparin
Phone number: 009647500855427
Email: ahmed.farhan@raparinuni.org

Abstract

Background and Objective: Natural plants have been evaluated as sources of antimicrobial agents against wide range of microorganisms. The aim of this study is to investigate antibacterial potency of pomegranate peels, seed and whole fruit extracts against these Gram positive bacteria (*Staphylococcus aureus,*

Streptococcus pneumonia, and *Bacillus sp),* and Gram negative bacteria *(Escherichia coli, Klebsiella sp, Shigella sp,* and *Pseudomonas sp*).

Methods: The fresh fruits extracts (peels, seeds and whole fruit) were separately prepared with methanol and distil water (D.W) by standard procedure. The samples were stored in refrigerator at 4°C until use. The bacterial samples were taken from scientific research center in Koya-Erbil-Kurdistan region-Iraq. The antibacterial activity of different pomegranate extracts was determined by using disc diffusion method.

Results: The results of present study showed that different pomegranate extracts with methanol and water have effect on the bacterial growth. The study demonstrated that there was more significant effect on growth of gram positive bacteria (*Staphylococcus aureus, Bacillus sp* and *Streptococcus pneumonia)* than gram negative bacteria (*Escherichia coli, Klebsiella sp, Shigella sp,* and *Pseudomonas sp)* based on the inhibition zone diameter.

Conclusion: This study indicates that different pomegranate extracts peels, seed and whole fruit may serve as natural alternatives due to their potential chemical agents against variety of bacterial infections. Performing more studies require to isolate the active antibacterial agents in pomegranate and know their mechanisms of action so that they could use in therapeutic purposes.

Key words: *Punica granatum*, Gram positive bacteria, Gram negative bacteria, Pomegranate Peel, Seed, Whole fruits.

پوختە

باکگراوەند و ئامانج:

رووەکە سروشتیەکان وەک سەرچا وەیەکی دژە تە ن خە ملێنراون دژی ژمارەیەکی زۆر لە میکرۆبەکان.لە ئێستادا هەنار روەکێکی دەرمانیە بە هۆی چالاکی بەهیزی دژی بەکتریاکان.ئامانجی ئەم توێژینەوە لێکۆلینەوەیە لەکاریگەری پێکهاتە کانی توێکڵ ودەنک و هەموو بەشەکانی هەنار(توێکڵ ودەنک و توێکڵی سپی ناوەوەی هەنار) دژی بەکتریاگرام نێگەتیف گرام (Staphylococcus aureus, Streptococcus pneumonia, and Bacillus sp پۆزەتیف(. Escherichia coli, Klebsiella sp, Shigella sp, and) Pseudomonas sp)

ڕێگاکانی کارکردن:

پێکهاتە کانی میوەی هەنار (توێکڵ و دەنک هەموو میوەکە) بە جیا ئامادەکران لە گەڵ میسانۆل وئاو بەڕێگای ستاندەرد.پێکهاتە ئامادەکراوەکان لەسەلاجە لە ٤پلەی سیلیزی دا پارێزران تا کاتی بەکارهێنان. نمونەکانی بەکتریا وەرگیران لە سەنتەری توێژینەوەلە شاری کۆیە. کاریگەری پێکهاتە جیاوازەکانی هەنار دژی بە کتریاکان دیاری کران بە ریگای دێسک دیفیوژن لە ڕێگەی پێوانی تیرەی زۆنە ڕوونەکە(زۆنەڕوونەکەئە وە پیشان دەدات بەکتریا گەشەی نەکردووە) بە دەوری دێسکەکاندا.

ئە نجام:

ئە نجامی توێژینەوەکە پیشانی دا پێکهاتە جیاوازەکانی هە نار لە گەڵ میسانۆل وئاو کاریگەریان هەیە لە سەر گەشەی بە کتریاکان. توێژینەوەکە دەری خست پێکهاتەکانی هەنار کاریگەریان لە سە ربەکتریا گرام پۆزەتیفەکان زیاترە بەبەراورد لە گەڵ بەکتریا گرام نێگەتیفە کان.

دەرئەنجام:

توێژیەنەوەکە ئەوە پیشان دەدات پێکهاتە جیاوازەکانی هەنار دەکرێ وەک دەرمانی سروشتی بە کار بهێنرێن دژی نە خۆشیە میکرۆبیەکان بە هۆی بوونی مادەی کیمیای بە هێز لە پێکهاتەکانیدا دا.ڕاسپاردەکانی توێژینەوەکە ئە نجام دانی توێژینەوەی زیاترە بۆجیاکردنەوەو زانینی میکانیزمی ماددە دژە بەکتریاکانی ناو پێکهاتە جیاوازەکانی هەنار بۆئەوەی لە بواری دەرمان بە کاربهێنرێن.

الخلاصة

الخلفية والهدف:

تم تقييم النباتات الطبيعية مؤخرا كمصادر لعوامل مضادة للميكروبات ضد مجموعة واسعة من الكائنات الدقيقة. وقد برز الرمان (بونيكا غراناتوم) كمصنع علاجي بسبب النشاط المضاد للميكروبات. الهدف من هذه الدراسة هو دراسة الفاعلية المضادة للجراثيم قشور الرمان والبذور ومقتطفات الفاكهة الكاملة ضد مجموعة من هذه البكتيريا الموجبة للغرام (المكورات العنقودية الذهبية، والبكتريا العقدية، وبكتيريا باسيلوس سب)، و مجموعة من البكتيريا السالبة للجرام (إشيريشيا كولي، كليبسيلا، شيغيلا، بسيودوموناس س).

طرائق العمل:

تم تحضير مستخلصات الفاكهة الطازجة (قشور، بذور وثمرة كاملة) بشكل منفصل مع الميثانول والماء المقطر (D.W) بواسطة الإجراء المعياري. تم تخزين العينات في الثلاجة عند 4 درجات مئوية حتى الاستخدام. أخذت

العينات البكتيرية من مركز البحوث العلمية في كويا-أربيل- إقليم كوردستان - العراق. تم تحديد النشاط المضاد للبكتيريا لمستخلصات الرمان المختلفة باستخدام طريقة نشر الاقراص.

النتائج:

أظهرت نتائج الدراسة الحالية أن مقتطفات الرمان المختلفة مع الميثانول والماء لها تأثير على نمو البكتيريا. وأظهرت الدراسة وجود تأثير معنوي أكبر على نمو البكتيريا الموجبة للجرام (بكتريا ستافيلوكوكوس أوريوس و باسيلوس سب و ستريبتوكوكوس بنيومونيا) من البكتيريا السالبة للجرام (إشيريشيا كولي و كليبسيلا سب و شيغيلا سب و بسيودوموناس سب) على أساس قطر منطقة التثبيط.

الاستنتاجات:

تشير هذه الدراسة إلى أن مستخلصات الرمان المختلفة من مستخلص البذور ومستخلص الفاكهة ومستخلص الثمرةالكاملة, يمكن أن تكون بدائل طبيعية بسبب ماتحتويه من عوامل كيميائية فعالة ضد مجموعة متنوعة من الالتهابات البكتيرية. تتطلب إجراء المزيد من الدراسات لعزل العوامل المضادة للبكتيريا النشطة في الرمان ومعرفة آليات عملها بحيث يمكن استخدامها ألاغراض علاجية.

1. Introduction

Higher and aromatics plants have traditionally been used in folk therapy, showing inhibition against bacteria and fungi (Bupesh *et al*., 2007). The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic agents. Many investigations have demonstrated the antimicrobial activity of the components of some higher plants that which appears to have the same effects of antibiotics (Hamad *et al*., 2017).

Medicinal plants are always a good source to find new treatment for human health problems. Recently, a wide range of these plants have been screened for antimicrobial property. *Punica granatum,* commonly known as pomegranate, has been highlighted in some studies as they have that property (Sadeghian *et al*., 2011). Certain natural products of plant origin were evaluated as a source of antimicrobial agents against a variety of phytopathogenic fungi (Mohamad and Khalil, 2014).

Pomegranate *(P.granatum Linn*) is one of the oldest known fruits. This fruit is mentioned in the Bible and Quran and is often associated with fertility. According to Qur'an, the fruits like grapes, date, fig, olive and pomegranate are gifts and heavenly fruits of God (Bharani and Namasivayam, 2016).The chemical analysis of different

parts of pomegranate has been studied by many researchers, they found fruit and peel of pomegranate were rich source of polyphenolic compounds. Pomegranate polyphenols include flavonoids (flavonols, flavanols and anthocyanins), condensed tannins (proanthocyanidins), hydrolysable tannins (ellagitannins and gallotannins). Hydrolyzable tannins (HTs) are found in the peels membranes, and piths of the fruit HTs are predominant polyphenols (Hama *et al*., 2014).

P.granatum described as nature's power fruit, has been widely used by traditional remedy in America, Asia, Africa and Europe for the different treatment types of diseases. The fruits of *Pgranatum* have been used to cure acidosis, dysentery, diarrhea, helminthiasis, hemorrhage, respiratory pathologies, and act as antiviral activity (Bupesh *et al* .,2007). In addition, several studies showed the role of different pomegranate extracts in prevention of cancer, cardiovascular disease, diabetes, dental conditions, bacterial infections, male infertility, Alzheimer's disease, arthritis, and obesity (Albarri *et al*., 2017)

Pomegranate is reported in many studies that have antimicrobial activity against a wide range of microorganisms including, Gram positive and Gram-negative bacteria (Hama *et al*., 2014).

Over the years, there have been many studies undertaken in various areas of the world on the bactericidal effects of pomegranates on a number of highly pathogenic and drug-resistant strains (Howell and D'Souza, 2013). The flower, seed oil, seed extract, and peel extract of pomegranate have a potent antioxidant activity (Singh *et al*., 2002). Furthermore, the water and methanol extracts of pomegranate peel possesses antimicrobial activity against microorganisms, *such as Bacillus subtilis, Staphylococcus aureus,Yersinia enterocolitica,* and *Listeria monocytogenes* (Nuamsetti *et al*., 2012). However, few studies have stated antibacterial activity of pomegranate peels, seeds and whole fruits. The aim of this study is to investigate antibacterial impact of methanol and aqueous extracts of pomegranate peels, seed and whole fruit against a range of different bacteria such as *Staphylococcus aureus,*

Streptococcus pneumonia, Bacillus sp, Escherichia coli, Klebsiella sp, Shigella sp, and *Pseudomonas sp* by disk diffusion method.

2. Materials and Methods

2.1. Preparation of Pomegranate powder

The fresh fruits (peels, seeds and whole fruit) were separated manually and rinsed with distilled water (D.W). The collected peels, seeds, and whole fruits separately were dried in oven at 50 °C for 48 hours and grounded into fine powder using an electric blender.

2.2. Preparation of Pomegranate extracts

A. Methanol extract: The powder (10 g) of peels, seeds, and whole fruits were separately placed in beaker and extracted with 100 ml of 99% methanol at room temperature for 24hours.The clear extracts were filtered and sterilized. The samples were stored in refrigerator at 4°C until use.

B. Aqueous extract: The powder (10 g) of peels, seeds, and whole fruits were separately placed in beaker and extracted with 100 ml of D.W at room temperature for 24hours.The clear extracts were filtered and sterilized. The samples were stored in refrigerator at 4°C until use.

2.3. Bacteria and cultures

The test microorganisms *(Staphylococcus aureus, Streptococcus pneumonia, Bacillus sp, Klebsiella sp, Eschericia coli, Shigella sp,* and *Pseudomonas sp*) were taken from Scientific Research Center in Koya- Erbil-Kurdistan Region-Iraq. The tested bacteria were sub-cultured and incubated at 37 ºC for 24 Hours. The organisms were inoculated on Muller Hinton Agar. Sterile paper disks (5 mm diameter) were soaked with (20 µg /ml) of peels, seeds and whole fruits extracts separately. Then the discs were placed on the surface of inoculated agar plates of microorganisms and incubated at 37 ºC for 24 hours (Sadeghian *et al*., 2011)

2.4. Determination of antibacterial activity

The antimicrobial activity of pomegranate extracts were determined by using disc diffusion method. The antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) around disc of pomegranate extract (Dahham *et al.,2011*).

2.5. Statistical analysis

All tests were performed in triplicate. The results were expressed as mean and standard deviation. Statistical significance was calculated by ANOVA test (LSD). The mean difference were considered significant at the 0.05 level (Nuamsetti *et al.,* 2012).

3. Results

The results for methanolic pomegranate extracts on tested bacterial cultures have shown some variation based on diameter of inhibition zone (DIZ) values as showed in table1. The diameter of inhibition zone (DIZ) mean values of methanolic extracts for *Staphylococcus aureus* was between 5.9mm and 20.1 mm. The maximum inhibitory effect was recorded by peel extract with mean value of 20.1 mm DIZ mean value which was more than the mean value of control ciprofloxacin (CIP) with 14.9 mm. The lowest activity is shown by seed extract with a DIZ mean value 5.9 mm. The DIZ mean values of extracts for *Bacillus sp* were between 4.1mm and 19mm. The maximum inhibitory effect was recorded by peel extract. However, the seed extract, whole fruit extract and CIP had less inhibitory effect.

For *Streptococcus pneumonia,* the DIZ mean values were between 10.1mm and 15 mm for the peel and whole fruit extracts. However, the seed extract was not affected on *S.pneumonia*. The DIZ mean values of different extracts for *Klebsiella* were between 9.9mm and 14mm, the maximum inhibitory effect was noted by peel extract. However, the seed extract and CIP presented less inhibitory effect. For *Shigella*, the DIZ mean values of extracts were between 7.4mm and 20.1mm. The whole fruit methanolic extract was more active than peel, ciprofloxacin and seed respectively.

The DIZ mean values of different extracts for *Eschericia coli* were between 7.4mm and 15mm. The maximum antibacterial effect was reported by peel extract against *E.coli.* While the methanolic seed and whole fruit extract were less active. The DIZ

mean values of different extracts for *Pseudomonas sp* were between 10mm and 18mm. The maximum inhibitory effect for *Pseudomonas sp* was noted by peel extract with a DIZ mean value of 18 mm. However, the seed extract showed minimum antibacterial activity and DIZ mean value was 10 mm. The current study for methanol pomegranate extracts, showed the highest antibacterial activity by peel with DIZ mean value 20.1 mm against *S. aureus*. While, the lowest effect was observed on *Bacillus sp* by seed with a DIZ mean value of 4.1 mm.

The results of this study for aqueous pomegranate extracts, showed some variation based on diameter of inhibition zone (DIZ) mean values as shown in table2. High antibacterial effect was recorded by peel on *S.aureus* with a 13mm DIZ mean value, and minimum effect was noted for *Pseudomonas sp* with a 10.2 mm DIZ mean value. The seed extract of Pomegranate with 7.8mm DIZ mean value was recorded the highest influence on *Klebsiella*, and the lowest effect showed on *Bacillus sp* with 2mm DIZ mean value. The seed extract was not affected on *S.pneumonia*. Whole fruit was more active than seed, but less than peel. Whole fruit was recorded the highest antibacterial activity with 9.7 mm DIZ mean value on both *E.coli* and *Pseudomonas sp,* and the lowest activity was observed with a DIZ mean value of 4mm on *Kliebsella sp.*

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	Diameter of inhibition zone in mm(DIZ)

Name of Bacteria	Peel (20 µg /ml)	Seed (20µg/ml)	Whole fruit (20µg/ml)	Ciprofloxacin 20 µg
Staphylococcus aureus	20.1±0.26*	5.9±0.15 *	13±0.14 *	14.9±0.3 *
Bacillus sp	19±0.14 *	4.1±0.17 *	12 .4±0.45 *	11.1±0.32 *
Streptococcus pneumonia	15±0.5 *	0.00	10.1±0.26 *	19.8±0.26 *
Klebsiella sp	14±0.3 *	9.9±0.35 *	12.9±0.15 *	12.9±0.15 *
Shigella sp	15 ±0.15 *	7.4±0.45 *	20.1±0.2 *	14.1±0.15 *
Escherichia coli	15±0.15 *	7.4±0.45 *	13±0.11 *	20±0.15 *
Pseudomonas sp	18±0.2 *	10±0.15 *	12.2±0.25 *	19.7±0.62 *

Table (1). Effect of different methanol pomegranate extracts on the bacterial growth, expressed as mean ± standard deviation.

Means and standard deviation for n = 3. For each bacterium, the experimental values within each row that have significantly different ($p < 0.05$) according to ANOVA test (LSD). ***** Mean difference is significant at the 0.05 level.

Table (2). Effect of different aqueous pomegranate extracts on the bacterial growth, expressed as mean ± standard deviation.

	Diameter of inhibition zone in mm(DIZ)			
Name of Bacteria	Peel (20 µg /ml)	Seed (20µg/ml)	Whole fruit (20µg/ml)	Ciprofloxacin 20 µg
Staphylococcus aureus	13±0.2 *	4±0.2 *	5.9±0.25 *	15±0.2 *
Bacillus sp	12±0.25 *	2±0.2 *	8±0.15 *	11±0.3 *
Streptococcus pneumonia	11±0.25 *	0.0	8±0.15 *	20±0.3 *
Klebsiella sp	11.93±0.3 *	7.8±0.17 *	4±0.2 *	13±0.2 *
Shigella sp	11.93±0.3 *	4.86±0.15 *	9.3±0.4 *	13.4±0.5 *
Escherichia coli	11.4±0.4 *	4.8±0.15 *	9.7±0.36 *	19.5±0.5 *
Pseudomonas sp	10.2±0.2 *	4.8±0.15 *	9.7±0.36 *	19.3±0.6 *

Means and standard deviation for n = 3. For each bacterium, the experimental values within each row that have significantly different ($p < 0.05$) according to ANOVA test (LSD). ***** Mean difference is significant at the 0.05 level.

4. Discussion

The change in the inhibition zone of bacterial growth around pomegranate extract discs are given in tables (1 and 2). There were significant variations in the antibacterial activities (Diameter of Inhibition Zone mean values) of methanol and aqueous extracts. Results of present study indicated that methanol and aqueous pomegranate extracts have influence on the growth of bacteria *S.aureus, Bacillus sp, S.pneumonia, E.coli, Klebsiella sp, Shigella sp,* and *Pseudomonas sp*. The current study is agreed with previous study conducted by Duman *et al*., 2009 who showed

that methanol, ethanol, and water extracts obtained from pomegranate were active and effective against the tested microorganisms *S. aureus, E. coli, Salmonella typhi, Vibrio cholera, S. dysenteriae,* and *S. sonnei*. This study has shown that the antimicrobial activity of pomegranate extracts have more effect on the growth of gram positive bacteria (*S.aureus, Bacillus sp,* and *S. pneumonia*) than gram negative bacteria (*E.coli, Klebsiella sp, Shigella sp* and *Pseudomonas sp*). This is may be due to the different structures of the cell walls of Gram-positive and Gram-negative (Hamad *et al*., 2017).

From the present results we observed that methanol peel extracts of pomegranate have high antibacterial activity against *S.aureus* and *E.coli*. It has been reported that methanol extract of pomegranate peels is a potent inhibitor for *S. aureus, Listeria monocytogenes*, *E. coli* and *Yersinia enterocolitica* (Albarri *et al.,* 2017). The presence of some phytocompounds in the pomegranate peel extract may be responsible for the inhibitory effect (Elfalleh *et al*., 2012). Similarly, it has been stated that the antibacterial activity of pomegranate peels may be indicative of presence of some metabolic toxins or broad spectrum antibiotic compounds (Hama *et al*., 2014).

The peels of pomegranate are more active than whole fruit and seed respectively, because peels of *P.granatum* contains many potent compounds such as (anthocyanins, hydrolysable, tannis ellagic, flavones, gallic acid and vitamin C(Yehia *et al*., 2011). Similar results were clearly recorded in our study which have shown that methanolic peel extract of pomegranate *(P.granatum*) were more effect than whole fruit and seed.

Our results support that methanol extracts are more active than water extracts. Similarly, Devatkal *et al*., 2013 showed that aqueous pomegranate extracts have weaker effect than acetone and methanol extracts. Another study is agree with the current study which revealed that acetone and aqueous extracts were estimated against both Gram positive and Gram-negative bacteria. The acetone extract showed

the highest antibacterial activity, followed by methanol and water extract (Devatkal *et al*., 2013). Moreover, it has been reported that methanol extracts were more active than water extracts against *E. coli, S. aureus,* and *B. subtilis* (Nuamsetti *et al*., 2012).

S.aureus were more sensitive to aqueous peel extracts than *Pseudomonas sp* as shown in table1 and table2. Similarly, Sadeghian and his colleagues *et al* (2011) indicated that the growth inhibition activity of aqueous peel extract started at lower concentration for *S. aureus* than for *P*. *aeruginosa*. Therefore, the aqueous peel extract may be more effective on Gram positive bacteria as compare to Gram negative bacteria.

The methanol extracts of *P.granatum* whole fruit is known to be active against all microorganisms tested in their study (Duman *et al*., 2009). Our results also reported that methanol whole fruit extract has antibacterial activity on all tested bacteria. It has reported that antibacterial activity of pomegranate may be related to the presence of hydrolysable tannins and polyphenolics in the pomegranate extracts, especially punicalagin and gallagic acid. The antimicrobial influence of tannins is associated to its molecular structure and toxicity for bacteria. Tannins were able to act on the bacterial cell wall and cell membrane (Kiany *et al*., 2016).

5. Conclusions

In conclusion, our results obviously demonstrated broad spectrum activity of pomegranate extracts against wide range of bacteria. The results also indicated that methanol extracts of pomegranate are more effective against bacteria than the water extracts. The presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major active constituents may be responsible for these activities. The results of the present study for antibacterial activity of pomegranate extracts give chance to pharmacists to use them in the alternative therapy. Further studies are required to isolate the strong antibacterial agents in pomegranate and know their mechanisms of action. This could be used in medicinal purposes. Further studies are recommended to investigate the effect of pomegranate extracts on other species of bacteria.

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