

Evaluation of the Antimicrobial Potential of Punica Granatum Leaves Hydro-methanolic Extract against Selected Pathogens

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Abstract: For a long time, extracts of *Punica granatum* have been used in alternative and complementary medicine. The object of the present study was to evaluate the activity of *Punica granatum* leaves hydro-methanolic extract against some Gram-positive, Gram-negative bacteria and *Candida albicans* using agar diffusion assay. The leaves yielded 34.7% of raw semisolid extract after maceration in hydro-methanol (50:50 v/v) and evaporation. After lyophilization, the dry powder was reconstituted in Muller-Hinton broth to get a concentration of 128 mg/mL; from which further 5 lower concentrations have been prepared (64, 32, 16, 8, and 4 mg/mL in Muller-Hinton Broth). The extract exhibited concentration-dependent activity against some Gram-positive bacteria, namely, *Listeria monocytogenes* (20.67 ± 0.82), *Clostridium perfringens* (17.33 ± 0.52), *Staphylococcus aureus* (10.5 ± 0.55), *Bacillus cereus* (8.83 ± 0.41), and *Enterococcus faecalis* (6.33 ± 0.52); and against three Gram-negative bacteria, namely, *Shigella flexneri* (13.33 ± 0.8), *Vibrio parahaemolyticus* (12.17 ± 0.42) and *Proteus vulgaris* (8.5 ± 0.55); as well as against *Candida albicans* (15.8 ± 0.98); (the values are in mm after 128 mg/mL extract). However, the extract was without any visible activity against *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella aerogenes*. The minimum inhibitory concentrations (MICs) against susceptible organisms were 1.06 mg/mL (*Clostridium perfringens*), 1.11 mg/mL (*Vibrio parahaemolyticus*), 2.08 mg/mL (*Staphylococcus aureus*), 2.16 mg/mL (*Bacillus cereus*), 2.76 mg/mL (*Candida albicans*), 3.07 mg/mL (*Listeria monocytogenes*), 3.80 (*Shigella flexneri*), 12.92 mg/mL (*Proteus vulgaris*) and 15.55 (*Enterococcus faecalis*).

These data may indicate that *Punica granatum* leaves extract is active against some pathogenic bacterial strains and thus may be useful in treatment of disease conditions caused by these bacteria at least as a complementary medicine.

Keywords: Antimicrobial; Punica granatum; Complementary medicine

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Introduction

Infectious diseases account for approximately one half of all deaths in developing countries. In addition, in advanced ones, clinical problems due to drug resistant microorganisms and the emergence of microbe-related public health concerns are evident despite the progress made in understanding microbes and their pharmacological control [1]. For this reason, there is always urgent needs to discover and develop newer antimicrobial remedies with various properties, structures and novel mechanisms of action from various sources to sustain upper therapeutic hand over infectious agents.

Despite emphasis being put in research of synthetic chemical drugs, a special interest in medicinal plants has been focused, in part due to many synthetic drugs are potentially toxic or causing side effects to the patient [2]. In addition, plant materials are cheaper and more available contributors to the improvement of human health in terms of cure and prevention of diseases [3].

Along years, medicinal plants are considered as well-established natural sources for the treatment of various diseases with or without scientific bases. About 20,000 plant species used for medicinal purposes are documented and reported by World Health Organisation [4]. Particularly in Eastern countries, traditional therapy is more culturally acceptable and is able to meet psychological needs in a way western medicine does not.

Along this context, the interested personnel focused a spot of light to the use of natural resources for discovering antimicrobial principles. Previous studies on medicinal plants reviewed by [5] have indicated lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) as broad-spectrum antimicrobials; and bearberry (*Arctostaphylos uvaursi*) and cranberry juice (*Vaccinium macrocarpon*) as antimicrobials to treat urinary tract infections. *Punica granatum*, (*Punicaceae*) is a widely distributed tree all over the world including Middle East. The tree has glossy, leathery leaves and bears red flowers at the branch tips. Different extracts from *Punica granatum* different parts have been proven to have pharmacological actions including analgesic [6], antiparasitic [7], antioxidant [8], anti-ulcerative [9], anticancer [10], hepatoprotective [11], vasculoprotective [12], nephroprotective [13].

The object of the present study was to investigate the antimicrobial activity of the hydromethanolic extract of *Punica granatum* leaves, growing in our environment, using *in vitro* techniques, against twelve strains of Gram-positive, Gram-negative bacteria as well as *Candida* that are pathogenic and hazardous to both human and animal health if remained uncontrolled. The MIC(s) of the extract against susceptible microorganisms, if any, will be determined.

Material and methods

The plant material & extraction procedure

The green leaves of *Punica granatum* areal parts (Figure 1) were collected from our local environment and identified. Plant leaves were separated and refluxed in running tap water and then with bi-distilled water, shade dried at room temperature and coarsely crushed using a pestle and mortar. Extract was prepared by macerating a weighed amount of the crushed leaves (100 g) in a known volume (1 Litre) of water/organic solvent (bi-distilled water: absolute methanol, 50:50, v/v). Maceration continued for 72 hours in refrigerator with intermittent shaking. The hydro-methanolic extract was then strained through muslin mesh, filtered through Whatman paper #1. The obtained filtrate was then concentrated using a shaking water bath at 56 °C in wide-mouth containers and the residue obtained (yield) was then lyophilized (LyoQuest-85, Telstar, Madrid, Spain), weighed and re-constituted by dissolving in

measured amount of Muller-Hinton broth(Oxoid,Hampshire, UK). A stock solution of 128 mg/mL was prepared and filter-sterilized using sterile syringe filters (EconoFltr PTFE 25 mm 0.45 μ m, Agilent Tech., CA, USA). The stock solution was then serially diluted to get 64, 32, 16, 8, 4, 2 & 1 mg/mL, which were used for the antibacterial activity testing. The method of extraction was modified after^[14].



Figure 1 *Punica granatum* leaves and flowers(the leaves were used for extraction)

Culture media and Microbial strains

Maximum Recovery Diluent (MRD, LAB 103, LAB M Ltd., Lancashire, UK) with typical formula (g/L) of Peptone 1.0 and sodium chloride 8.5 was used for diluting the selected reference bacterial strains to obtain the dilution whose turbidity is equivalent to 0.5 McFarland for each bacterial strain.

Muller-Hinton Broth (Oxoid CM0405, Oxoid Ltd., Hampshire, UK) with typical formula (g/L) of Beef dehydrated infusion 300, Casein hydrolysate 17.5 and Starch 1.5 with pH adjusted at 7.3 \pm 0.1 was used for serial dilution of *Punica granatum* leaves extract.

Muller-Hinton Agar (Biolife Italiana, Milano, Italy) with typical formula (g/L) of Beef extract 2, Acid digest of casein 17.5, Starch 1.5 and Agar 17 was used for antimicrobial assay. The following 12 microbial strains have been used in the present study after preparation of their cultures as described above:

Antimicrobial activity using agar diffusion assay (ADA)

Reference Bacterial Strain	Supplier	NCTC	ATCC
<i>Staphylococcus aureus</i>	Sigma Trade, Cairo, Egypt	10788	6538
<i>Listeria monocytogenes</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	7973	35152
<i>Bacillus cereus</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	7464	10876
<i>Enterococcus faecalis</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	12697	29212
<i>Clostridium perfringenes</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	8237	13124
<i>Escherichia coli</i>	Sigma Trade, Cairo, Egypt	12241	25922
<i>Salmonella typhimurium</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	12023	14028
<i>Shigella flexneri</i>	KWIK-STIK [®] , Microbiologics, Inc., Minnesota USA	--	9199
<i>Klebsiella aerogenes</i>	Sigma Trade, Cairo, Egypt	9528	
<i>Proteus vulgaris</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	4175	13315
<i>Vibrio parahaemolyticus</i>	Selectrol [®] , TCS Biosciences Ltd, Buckingham, UK	10885	--
<i>Candida albicans</i>	LAB M Ltd., Lancashire, UK	--	90028

Sensitivities of the above mentioned microbial strains to *Punica granatum* leaves extract have been measured in terms of zone of inhibition using agar diffusion assay (ADA) [15]. The Mueller-Hinton agar was prepared (31 g of agar powder were mixed with 1080 mL of bi-distilled water in a suitable flask and then autoclaved. The amount of agar solution was divided in 4 smaller flasks, 270 ml each. Evaporation occurs during autoclaving and 250 out of 270 mL remain in every flask. Flasks were kept on water bath at 56 °C to maintain their liquid state. Each flask was then inoculated with 0.5 ml of the microbial inoculum to match a 0.5 McFarland turbidity standard, which is equivalent to 1.5×10^8 cells per mL. After inoculation, the infected agar was spread into sterile Petri dishes (15 ml of agar solution into 10 mm diameter Petri dish to form a uniform layer of about 2.7 mm deep). The plates were left to cool and solidify and then wells (7 mm diameter) have been cut out from the agar plates using a sterilized stainless-steel pore maker. Each well was then filled with 0.1 ml of *Punica granatum* leaves extract at a particular concentration (1–128 mg/mL in Muller-Hinton Broth). The wells of a set of plates were filled only with Muller-Hinton Broth, the solvent of the plant extract, and kept as negative control. All the prepared plate sets were then incubated at 37 °C for 24 h either under anaerobic (*Clostridium*) or aerobic (other bacteria) condition and the diameter of any resultant zone of inhibition was measured. Zone sizes were measured from the edge of the well to the end of the clear zone. For each combination of a particular extract concentration and a bacterial strain, the experiment was performed twice as triplicates (6 observations).

Minimum inhibitory concentration (MIC)

MIC of the *Punica granatum* leaves extract was calculated from Agar diffusion assay results; where, the value of MIC was determined as the zero intercept of a linear regression of the squared size of the inhibition zones (x^2), plotted against the natural logarithm of the antibiotic concentration ($\ln C$) as follows:

$$\ln(\text{MIC}) = \ln(C) - x^2/4Dt$$

Where, D is the diffusion coefficient, presumed to be independent of concentration, and t the time of antibiotic diffusion [16]. $\ln(C)$ was calculated from the linear equation by setting (Y) value as zero; then MIC was calculated as (C) at Y-intercept.

Statistical analysis

All values were expressed as mean \pm standard deviation of the mean of the performed triplicates. Data of concentrations down to 4 mg/mL were only included in calculation. Linear regression was prepared using Excel software, Microsoft office 365.

Results

The yield % from the extracted 100 grams of the chopped *Punica granatum* leaves was 34.7%. This was calculated according to the equation of (Yield % = $100 \times$ Extracted residue / Original plant).

Punica granatum leaves extract showed significant inhibitory effects against most Gram-positive microbial strains, namely, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens* and *Enterococcus faecalis*; and against only three of Gram-negative ones which are *Shigella flexneri*, *Proteus vulgaris* and *Vibrio parahaemolyticus*. The extract also showed a good inhibitory activity against the mycotic strain *Candida albicans*.

Meanwhile, the extract was without effect against most of Gram-negative strains, namely, *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella aerogenes*.

Inhibition zones and the corresponding *Punica granatum* leaves extract concentrations are tabulated in Tables 1 and 2. MICs are depicted from Figure 2 and shown in Table 3.

Table 1 Inhibition zones of different concentrations of *Punica granatum* leaves extract on selected Gram-positive bacterial strains and *Candida* (Mean \pm SD; 6 observations)

Pg extract conc.(mg/mL)	<i>Staph. aureus</i>	<i>Listeria mono.</i>	<i>Bacillus cereus</i>	<i>Enterococcus faecalis</i>	<i>Clostridium perfringens</i>	<i>Candida albicans</i>
128	10.5 \pm 0.55	20.67 \pm 0.82	8.83 \pm 0.41	6.33 \pm 0.52	17.33 \pm 0.52	15.8 \pm 0.98
64	8.67 \pm 0.52	18.3 \pm 0.52	7.83 \pm 0.41	5.17 \pm 0.42	16.17 \pm 0.41	13.17 \pm 0.75
32	7.67 \pm 0.52	15.3 \pm 0.52	6.83 \pm 0.41	2.83 \pm 0.42	15.17 \pm 0.41	11.17 \pm 0.75
16	6.83 \pm 0.41	13.3 \pm 0.52	5.83 \pm 0.41	0.17 \pm 0.42	12.17 \pm 0.41	10.33 \pm 0.52
8	5.67 \pm 0.52	10.5 \pm 0.55	5.00 \pm 0.63	0.0 \pm 0.0	11.5 \pm 0.55	8.67 \pm 0.52
4	4.67 \pm 0.52	6.17 \pm 0.41	3.67 \pm 0.52	0.0 \pm 0.0	9.80 \pm 0.41	4.83 \pm 0.75

Table 2 Inhibition zones of different concentrations of *Punica granatum* leaves extract on selected Gram-negative bacterial strains (Mean \pm SD; 6 observations)

Pg extract conc.(mg/mL)	<i>E. coli</i>	<i>S. typhi.</i>	<i>Shigella flexneri</i>	<i>Klebsiella aerogenes</i>	<i>Proteus vulgaris</i>	<i>Vibrio para.</i>
128	0.0 \pm 0.0	0.0 \pm 0.0	13.33 \pm 0.82	0.0 \pm 0.0	8.5 \pm 0.55	12.17 \pm 0.42
64	0.0 \pm 0.0	0.0 \pm 0.0	12.17 \pm 0.42	0.0 \pm 0.0	6.33 \pm 0.52	10.83 \pm 0.42
32	0.0 \pm 0.0	0.0 \pm 0.0	11.33 \pm 0.52	0.0 \pm 0.0	5.33 \pm 0.52	9.83 \pm 0.42
16	0.0 \pm 0.0	0.0 \pm 0.0	8.17 \pm 0.42	0.0 \pm 0.0	2.33 \pm 0.52	9.00 \pm 0.63
8	0.0 \pm 0.0	0.0 \pm 0.0	5.17 \pm 0.42	0.0 \pm 0.0	0.0 \pm 0.0	7.83 \pm 0.42
4	0.0 \pm 0.0	0.0 \pm 0.0	3.17 \pm 0.42	0.0 \pm 0.0	0.0 \pm 0.0	6.83 \pm 0.42

Table 3 MICs of *Punica granatum* leaves extract against the studied susceptible bacterial strains.

Micro-organism	MIC of Pg extract (mg/mL)	Micro-organism	MIC of Pg extract (mg/mL)
<i>Staphylococcus aureus</i>	2.08	<i>Shigella flexneri</i>	3.80
<i>Listeria monocytogenes</i>	3.07	<i>Proteus vulgaris</i>	12.92
<i>Bacillus cereus</i>	2.16	<i>Vibriopara haemolyticus</i>	1.11
<i>Enterococcus faecalis</i>	15.55	<i>Candida albicans</i>	2.76
<i>Clostridium perfringens</i>	1.06		

Discussion

Arising from their microorganism eliminating features and disease treatment, antimicrobials are considered indispensable drugs. Among the available great variety of antimicrobial agents, a particular antimicrobial agent should be ideally selected to employ against a disease-causing particular microorganism, to eliminate it effectively without harming the host organism. However, uncontrolled and/or unsupervised use of antimicrobials, either by patients themselves or from prescriptions written without culture analysis, resulted in

emergence of resistant bacterial strains. Increased rate of resistance as well as some other problems associated with safety necessitated more demand and interest in antimicrobials from natural sources including plant extracts^[17].

For centuries, medicinal plants have been used as remedies for various human and animal disease conditions depending on the basis that they contain bioactive principles of therapeutic value^[18]. Antimicrobials of plant origin are not associated with side effects, cheap, available worldwide and have good therapeutic potential to heal many infectious diseases^[1]. For these reasons, antimicrobial assays are continuing to screen plants for their antimicrobial activity with the hope of discovery of very effective, yet safer, pharmacological antimicrobial tools.

In the present study, we investigated the possible antimicrobial activity of *Punica granatum* leaves extract against twelve microbial pathogens that are potentially hazardous in both human and veterinary fields if they are not controlled. Agar gel diffusion assay was applied for fulfilling this purpose.

One of the most important tasks in the clinical microbiology laboratory as well as clinical pharmacology laboratory is the performance of antimicrobial susceptibility tests on significant bacterial isolates and strains. Susceptibility testing is thus useful to predict the possible outcome of treating a patient's infection with a particular antimicrobial agent^[19].

Disk Diffusion Test (Bauer-Kirby Procedure) is one of the simplest and most reliable susceptibility testing methods^{[20],[15]}. This method has been widely studied and well standardized over several years. The test is performed by applying a standardized inoculum of up to 1.5×10^8 cfu/mL to the surface of a large (150-mm diameter) Mueller-Hinton agar plates. Up to 12 commercially prepared, fixed-concentration filter-paper antibiotic disks are placed on the inoculated agar surface. Plates are incubated for 16–18 hours in ambient air at 35 °C before the results are determined. The diameters of the zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimetre by viewing the plate with reflected light when it is held a few inches above a black, non-reflecting background. The diameter of the zone of inhibition is related to the susceptibility of the isolate and to the rate of diffusion of the drug through the agar medium. The zone diameter correlates inversely with the approximate MIC for that antibiotic, i.e., linear regression analysis of zone diameters plotted against natural log of MIC values demonstrate a consistent relationship. However, in practice, the results of a disk diffusion test are interpreted by comparing the measured zone diameter with the interpretive criteria published by the National Committee on Clinical Laboratory Standards, NCCLS,^[21] and included in the disk's FDA-approved product insert. The results of the disk diffusion test are “qualitative” (Susceptible, Intermediate, or Resistant) is derived from the test in addition to an MIC, which is the endpoint of the test or the lowest concentration of antibiotic that prevents visible growth of microbes. In general, the results of a susceptibility test are interpreted as drugs with the lowest MICs for a given bacterial isolate are the best for treatment of an infection due to that isolate.

The antimicrobial potential of *Punica granatum* leaves extract was evaluated using the same pattern with some modification, where antibiotic discs were replaced by making pores into the infected agar and filling such pores with a particular extract concentration. In the present study, the extract exhibited positive result against *Staphylococcus aureus* whose infections are of a great threat to both humans and animals; it spreads pneumonia at slow rates^[22]. An inhibition zone of about 10 mm was recorded by extract concentration of 128 mg/mL and MIC of 2.08 mg/mL. This result is inconsistent with that of^[23] who found that *Punica granatum* methanolic extract showed good effects on the growth of *Staphylococcus aureus* (from 10 to 25 mm inhibition zone according to the part used)

with the highest inhibition by the fruit rind. The data is parallel to those of [24] who found that sun dried fruit extract, among different types of pomegranate extracts, inhibit *Staphylococcus aureus* (up to 27 mm) and *Pseudomonas aeruginosa* (up to 25 mm) but not *Escherichia coli*. The data is also consistent with that of [25] who stated that 80% methanolic extract of peels was a potent inhibitor for *S. aureus* and *Listeria monocytogenes*.

Listeria monocytogenes a Gram-positive, facultative anaerobic, motile bacterium responsible for listeriosis infections in man and animals. In man it is responsible for 10% of gastroenteritis and meningoencephalitis in animals. In the present study, *Punica granatum* leaves extract exhibited average growth inhibition zone of 20.67 mm at concentration of 128 mg/mL with MIC of 3.07 mg/mL. The result may be parallel with that of [25].

A modest activity was expressed by the present extract against *Bacillus cereus* that was 8.83 mm at concentration of 128 mg/mL with MIC of 2.16 mg/mL. *Bacillus cereus* is a Gram-positive, rod-shaped, motile bacterium that is harmful to humans causing foodborne illness. However, some strains are beneficial as probiotics [26]. The present result may be partially incomparable with those of [23] who reported an inhibition zone up to 25 mm for *B. cereus* by pomegranate rind extract.

Punica granatum leaves extract showed 17.33 mm growth inhibition zone with MIC of 1.06 mg/mL against *Clostridium perfringenes*. The bacterium that was formerly known as *C. welchii* is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium. It is one of the most common causes of food poisoning. The anticlostridial data of the *Punica granatum* leaf extract found in the present study may be consistent with that of [27] who found that both sour and sweet pomegranate peel extracts were effective against the growth of *Cl. perfringenes*.

A weak antibacterial activity was exhibited by the extract against *E. fecalis* with about 6 mm inhibition zone at concentration 128 mg/mL and MIC of about 15 mg/mL. This result is inconsistent with that of [27] who found good activities for both sour and sweet peel extracts against *E. fecalis* with inhibition zone of 17 and 15 mm, respectively; however MICs were still high being 31 and 15 mg/mL, respectively.

Among the selected Gram-negative bacteria in the present study, *Punica granatum* leaves extract was only effective against *Vibrio parahaemolyticus*, *Shigella flexneri* and *Proteus vulgaris* with growth inhibition zones of about 12, 13 and 8.5 mm at concentration of 128 mg/mL with MICs of 1.1, 3.8 & 12.9 mg/mL, respectively. *Vibrio parahaemolyticus* is a curved, rod-shaped motile bacterium found in brackish saltwater, which, when ingested, causes gastrointestinal illness. The present result of *Punica granatum* leaves extract against *Vibrio* may be partially parallel with that of [28] who recorded inhibitory activities for un-ripened, ripened, methanolic and acetone fruit extracts against *Vibrio cholerae* with inhibition zones up to 22 mm. *Shigella flexneri* is a Gram-negative bacterium that can cause diarrhea in humans. *S. flexneri* infections can usually be treated with antibiotics, although some strains have become resistant. *Punica granatum* leaves extract was found effective against *S. flexneri* in the present study, with inhibition zone of 13 mm at concentration of 128 mg/mL and MIC of 3.8 mg/mL. The present result against *S. flexneri* may be parallel with that of [28] who recorded inhibitory activities for un-ripened, ripened, methanolic and acetone fruit extracts against *S. flexneri* with inhibition zones ranging between 12 and 25 mm. *Proteus vulgaris* is a rod-shaped bacterium that inhabits the intestinal tracts of humans and animals. It is an opportunistic pathogen and is known to cause urinary tract infections and wound infections. Our result may be parallel with that of [29] who reported inhibition zone of 20 mm against *P. vulgaris* by pomegranate ethanolic extract.

Other studied Gram-negative bacteria, namely, *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella aerogenes* were found resistant to *Punica granatum* leaves extract at all its studied

concentrations. These results are inconsistent with^[30] who recorded inhibition zones for *Salmonella* species including *S. typhi*, *paratyphi*, *enteritidis* and *gallinerum* growths in presence of pomegranate peel ethanolic extract (100 – 500 µg paper discs). Also, the result of *K. pneumoniae* is inconsistent with that of [23] who recorded a wide range of inhibition zone (7 – 25 mm) exhibited by different fruit parts; 7 mm was exhibited by the white pomegranate seeds, while 25 mm was exhibited by pomegranate juice.

In the present study, *Punica granatum* leaves extract showed good activity against *Candida albicans*. This result is consistent with that of [31] who stated that pomegranate peel extract (among others) exhibited highest inhibition of *Candida albicans* with a mean zone of inhibition of 22 mm. However, our results are inconsistent with the study of^[32] who recorded that no concentration of methanolic extract of *Punica granatum* peel inhibited *C. albicans*.

The antimicrobial activities of *Punica granatum* extract might be attributed to the presence of phenols. The antimicrobial mechanisms of phenolic compounds involve the reaction of phenolics with microbial cell membrane proteins and/or protein sulfhydryl groups that yield bacterial death due to membrane protein precipitation and inhibition of enzymes such as glycosyltransferases^[33].

Conclusion

The findings of the present study have shown clearly that *Punica granatum* leaves extract is active against some pathogenic bacterial and fungal strains and thus may be useful in treating disease conditions caused by these micro-organisms.

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NA

Conflict of interest disclosure

The authors declare that there is no conflict of interest related to the present study.

References

1. Iwu M, Duncan AR, Okunji CO. New antimicrobials of plant origin. *Perspectives on new crops and new uses*. ASHS Press, Alexandria, VA. 1999:457-462
2. Akinpelu D, Onakoya T. Antimicrobial activities of medicinal plants used in folklore remedies in south-western. *African journal of Biotechnology*. 2006, 5
3. Okoko T, Oruambo IF. The effect of hibiscus sabdariffa calyx extract on cisplatin-induced tissue damage in rats. *Biokemistri*. 2008, 20
4. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of ethnopharmacology*. 2006, 106:290-302
5. Rios J, Recio M. Medicinal plants and antimicrobial activity. *Journal of ethnopharmacology*. 2005, 100:80-84
6. Bagri P, Ali M, Aeri V, Sultana S, Bhowmik M. Evaluation of anti-inflammatory and analgesic activity of punica granatum linn. *International Journal of Drug Development and Research*. 2010, 2
7. Al-Megrin WA. Efficacy of pomegranate (*punica granatum*) peel extract against *hymenolepis nana* in infections mice. *Biosciences Biotechnology Research Asia*. 2016, 13:103-108
8. Gheith I, El-Mahmoudy A. Potent anti-oxidant and anti-inflammatory potentials of punica granatum leaf and flower hydromethanolic extracts in vitro. *Bioscience Journal*. 2017, 33:434-446
9. Chauhan I, Agrawal S, Goel RK. Status of inflammatory markers and growth factor in gastric ulcer protective effects of punica granatum l. Peel extract in rat. *National Journal of Physiology, Pharmacy and*

- Pharmacology*. 2018, 8:12-17
10. Bassiri-Jahromi S. Punica granatum (pomegranate) activity in health promotion and cancer prevention. *Oncology reviews*. 2018, 12
 11. Al-sallami AS, Al-Bideri AW, Alsaedi SH. Hepatoprotective effect of pomegranate peel (punica granatum l) against thioacetamide-induced cirrhosis. *THE EGYPTIAN JOURNAL OF EXPERIMENTAL BIOLOGY (Zoology)*. 2018, 14:41-47
 12. Wang D, Özen C, Abu-Reidah IM, Chigurupati S, Patra JK, Horbanczuk JO, Józwick A, Tzvetkov NT, Uhrin P, Atanasov AG. Vasculoprotective effects of pomegranate (punica granatum l.). *Frontiers in pharmacology*. 2018, 9
 13. Mestry SN, Gawali NB, Pai SA, Gursahani MS, Dhodi JB, Munshi R, Juvekar AR. Punica granatum improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. *Journal of Ayurveda and integrative medicine*. 2018,
 14. Harborne J. Phytochemical methods, a guide to modern techniques of plant analysis, jb harborne. 1973,
 15. Bauer A, Kirby W, Sherris JC, turck, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*. 1966, 45:493
 16. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of antimicrobial chemotherapy*. 2008, 61:1295-1301
 17. Friedman M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular nutrition & food research*. 2007, 51:116-134
 18. Nostro A, Germano M, D'angelo V, Marino A, Cannatelli M. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology*. 2000, 30:379-384
 19. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: General principles and contemporary practices. *Clinical Infectious Diseases*. 1998:973-980
 20. Woods GL. Antibacterial susceptibility tests : Dilution and disk diffusion methods. *Manual of clinical microbiology*. 1995:1327-1341
 21. Cockerill FR, Clinical, Institute LS. *Performance standards for antimicrobial disk susceptibility testing: Approved standard. National Committee for Clinical Laboratory Standards*; 2012.
 22. Simor AE, Ofner-Agostini M, Bryce E, McGeer A, Paton S. Laboratory characterization of methicillin-resistant staphylococcus aureus in canadian hospitals: Results of 5 years of national surveillance, 1995–1999. *Journal of Infectious Diseases*. 2002, 186:652-660
 23. Dahham SS, Ali MN, Tabassum H, Khan M. Studies on antibacterial and antifungal activity of pomegranate (punica granatum l.). *Am.–Eurasian J. Agric. Environ. Sci*. 2010, 9:273-281
 24. Opara LU, Al-Ani MR, Al-Shuaibi YS. Physico-chemical properties, vitamin c content, and antimicrobial properties of pomegranate fruit (punica granatum l.). *Food and Bioprocess Technology*. 2009, 2:315-321
 25. Al-Zoreky N. Antimicrobial activity of pomegranate (punica granatum l.) fruit peels. *International journal of food microbiology*. 2009, 134:244-248
 26. Ryan K. *Sherris medical microbiology. 4th ed. New York: McGraw Hill*. 2004,
 27. Naziri Z, Rajaian H, Firouzi R. Antibacterial effects of iranian native sour and sweet pomegranate (punica granatum) peel extracts against various pathogenic bacteria. *Iranian Journal of Veterinary Research*. 2012, 13:282-288
 28. Pradeep B, Manojbabu M, Palaniswamy M. Antibacterial activity of punica granatum l. Against gastro intestinal tract infection causing organisms. *Ethnobotanical leaflets*. 2008, 2008:143
 29. Nimri LF, Meqdam M, Alkofahi A. Antibacterial activity of jordanian medicinal plants. *Pharmaceutical biology*. 1999, 37:196-201
 30. Choi J-G, Kang O-H, Lee Y-S, Chae H-S, Oh Y-C, Brice O-O, Kim M-S, Sohn D-H, Kim H-S, Park H. In vitro and in vivo antibacterial activity of punica granatum peel ethanol extract against salmonella. *Evidence-Based Complementary and Alternative Medicine*. 2011, 2011
 31. Pai MB, Prashant G, Murlikrishna K, Shivakumar K, Chandu G. Antifungal efficacy of punica granatum, acacia nilotica, cuminum cyminum and foeniculum vulgare on candida albicans: An in vitro study. *Indian Journal of Dental Research*. 2010, 21:334
 32. Abdollahzadeh S, Mashouf R, Mortazavi H, Moghaddam M, Roozbahani N, Vahedi M. Antibacterial and antifungal activities of punica granatum peel extracts against oral pathogens. *Journal of Dentistry (Teheran, Iran)*. 2011, 8:1
 33. Naz S, Siddiqi R, Ahmad S, Rasool S, Sayeed S. Antibacterial activity directed isolation of compounds from punica granatum. *Journal of food science*. 2007, 72

Figure 2 Plotting of natural logarithmic concentrations (X-scale) against squares of inhibition zones (Y-scale) to calculate MICs of *Punica granatum* leaves extract against susceptible bacteria.



