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Studies on Antibacterial and Antifungal Activity of Pomegranate (*Punica granatum* L.)

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Abstract: Recently, natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of microorganisms. This study described the antibacterial and antifungal activities of pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi. The peel extract has shown highest antimicrobial activity compared to other extracts. Among the selected bacterial and fungal cultures, the highest antibacterial activity was recorded against *Staphylococcus aureus* and among fungi high activity against *Aspergillus niger* was recorded.

Key words: *Punica granatum* · Antimicrobial activity · Pomegranate juice · Rind · Phenolics

INTRODUCTION

In ancient Greek mythology, pomegranates are known as the "fruit of the dead", the substances available in Hades for its residents. Hades himself, the master, benefitted amorously when six pomegranate seeds from his realm sealed for him the betrothal of the beautiful daughter of Zeus and Demeter, fair Persephone.

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].

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 pomegranate is an ancient fruit that has not changed much throughout the history of man. It was found in the Indus Valley so early that there is a word in Sanskrit for pomegranate. The pomegranate is also significant in Jewish, Christian and Muslim traditions [2]. The pomegranate is native of Iran and Afghanistan, known in ancient Egypt [3]. Pomegranate belongs to punicaceae family. It is one of the important horticulture fruit to the Mediterranean climate. The edible part of fruit contains considerable saccharides, polyphenol and important minerals.

The physical and chemical properties of pomegranate have been evaluated in Turkey [4], Italy [5] etc. On an average basis the pomegranate has following components as shown in Table A:

The different types of phytochemicals that have been identified from various parts of the pomegranate tree and from pomegranate fruits and seeds and are listed in Table B. The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate in the fruit. Pomegranate polyphenols include flavonoids (flavonols, 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 [6]. HTs are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity [7].

The objectives of the present study were-1) to evaluate the antibacterial and antifungal activity of different parts of pomegranate fruit on selected bacterial and fungal cultures and 2) to find out the most significant part of the fruit with highest antimicrobial activity.

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Table A: Pomegranate Chemical Composition

Food Value	Percentage	Minerals & Vitamins	Concentration in mg
Moisture	78.0%	Calcium	10 mg
Protein	1.6%	Phosphorus	70 mg
Fat	0.1%	Iron	0.3 mg
Minerals	0.7%	Vitamin C	16.0 mg
Fibre	5.1%	Vitamin B complex	Trace amounts
Carbohydrates	14.5%	-	-
	100%	Caloric value	65

Values Per 100gms of edible portions. Bakhru H.K, 2009

Table B: Phytochemicals of Pomegranate

Plant Component	Constituents
Pomegranate Juice	anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid; caffeic acid; catechin, EGCG, quercetin, rutin; numerous minerals, particularly iron; aminoacids.
Pomegranate seed oil	95-percent punicic acid; other constituents, including ellagicacid; other fatty acids; sterols.
Pomegranate pericarp (Peel, rind)	Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavonols; flavones, flavonones; anthocyanidins.
Pomegranate leaves	Tannins (punicalin and punicafolin); and flavones glycosides, including luteolin and apgenin
Pomegranate flower	Gallic acid, ursolic acid; triterpenoids, including maslinic and Asiatic acid; other unidentified constituents
Pomegranate roots and bark	Ellagitannins, including punicalin and punicalagin; numerous piperidine alkaloids.

Julie Jurenka, MT (ASCP), Alternative Medicine Review, 2008

MATERIALS AND METHODS

Pomegranate Fruits: The pomegranate fruits were purchased and collected from a well known market in Hyderabad city. Different parts of the fruits were used in the study- rind, juice, seeds (white and red) and whole fruit extracted with methanol and aqueous extract.

Methods of Extraction: The fresh fruits were cleaned, freeze-dried and grounded into fine powder using an electric blender. The powder was dried in an oven at 40°C for 24 h, then the fine powder was sieved through 24-mesh. The fine powdered sample (10g) was extracted with 250 ml 80% methanol in water at room temperature (≈25°C) for 24 h in a shaking water bath. The extract was filtered by a Millipore filter with a 0.45µm nylon membrane under vacuum at 25°C. The samples were stored at 4°C until use. For aqueous extract the fine powdered sample (10g) was extracted with 100ml of distilled water.

Pomegranate Juice Processing: For the preparation of pomegranate juice concentrate and pomegranate fruit extract, pomegranates were handpicked, washed, chilled and stored in tanks. The fruit was then crushed, squeezed to yield the juice and pomegranate fruit extract. Pomegranate fruit extract includes not only juice, but also the inner and outer peels and the seeds of the pomegranate. Flavonoids constitute 40% (anthocyanins, catechins and phenols) of total polyphenols in pomegranate juice [8-10]. Both juices were filtered, pasteurized, concentrated and stored at 4°C until use.

Microorganisms and Culture: Seven bacterial strains and five fungal strains were procured from MTCC (Microbial Type Culture Collection Centre and Gene Bank) Chandigarh, India. The strains used are *Bacillus coagulans* MTCC 3164, *Bacillus cereus* MTCC 1307, *Bacillus subtilis* MTCC 6910, *Escherichia coli* MTCC 732, *Klebsiella pneumonia* MTCC 7028, *Staphylococcus aureus* MTCC 7405 and *Pseudomonas aeruginosa* MTCC 4302. The fungal strains used are *Aspergillus niger* MTCC 2196, *Mucor indicus* MTCC 3318, *Penicillium citrinum* MTCC 7124, *Rhizopus oryzae*MTCC 1987 and *Trichoderma reesei* MTCC 3929. The bacterial strains were cultured on nutrient agar medium at 37°C and fungal strains on potato dextrose agar medium at 28°C.

Determination of Antibacterial Activity: An agar-well diffusion method was employed for determination of antibacterial activities [11]. The stored pomegranate extract samples were dissolved in phosphate buffered saline (PBS, P^H 7.0-7.2). All bacteria were suspended in sterile water and diluted to ≈10^6 CFU/mL. The suspension (100µL) was spread onto the surface of nutrient agar medium. Wells (4.6mm in diameter) were cut from the agar with a sterile borer and 60µL extract solutions were

delivered into them. Negative controls were prepared using PBS solution. Penicillin G and gentamycin were used as positive reference standards to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 37°C for 24 h. antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicates.

Determination of Antifungal Activity: Fungal conidial suspension was prepared by the method of S. Guleria *et al*. [12]. Conidia were isolated from the 10 days old culture of the selected fungal cultures (mentioned above) by flooding culture plates with 5 mL of sterile distilled water and conidia were dislodged by using L-shaped glass rod. Conidial suspension was filtered through sterile double layered muslin cloth to remove bits of mycelia. Spore suspension was then prepared in liquid potato dextrose medium (potato 200g, dextrose 20g and water to make total volume of 1 L) to obtain a concentration of 3x 10^5 conidia/mL. For determination of antifungal activity agar-well diffusion method was followed in which the spore suspension was inoculated with molten potato dextrose agar at 45°C and allowed to set. Wells (4.6mm in diameter) were cut in a similar way as for the antibacterial activity with a sterile borer and 60µL extract solutions were delivered into them. The plates were incubated at 28°C for 3 days after which diameter of zones of inhibition (DIZ) were measured. Amphotericin B and fluconazole were used as positive reference standards.

RESULTS

Antibacterial Activities: Four out of seven bacteria used (*B. coagulans, B. cereus B. subtilis* and *S. aureus*) are Gram-positive and three (*E. coli, K. pneumoniae* and *P. aeruginosa*) are Gram-negative. There was significant variation in the antibacterial activities (DIZ values) of different extracts.

For *B. cereus*, the DIZ values of 5 methanolic extracts were between 10 and 25 mm. The maximum inhibitory effect was recorded by rind extract; however, the red seed extract had very less inhibitory effect. The effect of aqueous extracts was less on *B. cereus* compare to methanolic extracts except for juice where more antibacterial effect was observed with a DIZ value of 26mm which is highest for this bacterium. For *B. coagulans*, the inhibitory effects were recorded only by 2 methanolic extracts of the pomegranate- rind extract and juice, the DIZ values were 22 and 10mm, respectively. The maximum inhibitory effect was shown by rind extract and 3 out of 5 extracts did not show any inhibitory effect on the bacterium. Whereas the inhibitory effect of aqueous extracts was more on *B. coagulans* with a highest activity of rind extract with a DIZ value of 23mm. The white seed aqueous extract has also inhibited the growth of the bacterium. In the case of *B. subtilis*, the DIZ values were between 9 and 18mm, all the 5 extracts were inhibitory for this bacterium, the high inhibitory effect was exhibited by rind extract followed by juice, red seed and white seed, the lowest effect was exhibited by whole fruit. Comparatively the effect of aqueous extracts was less on *B. subtilis* with the DIZ values between 8.0 and 19 mm. More inhibitory effect was observed with rind extract and juice with DIZ values of 16 and 15 mm, respectively. (The results of methanolic extracts on bacteria are presented in Table 1 & Fig. 1).

Out of seven bacterial cultures tested, the highest antibacterial activity was recorded on the fourth Gram-positive culture - *S. aureus* by the methanolic extracts of pomegranate, wherein the DIZ values were between 10 and 25 mm. The rind extract has shown maximum DIZ value of 25 mm, followed by juice with 23 mm, red seed with 19 mm and whole fruit with 16 mm. The lowest activity was shown by white seed with a DIZ value of 10 mm. The antibacterial effect of aqueous extracts was comparatively less, but high antibacterial activity was recorded by juice with a highest DIZ value of 26 mm. The lowest antibacterial effect was exhibited by white seed with a DIZ value of 10 mm.

Out of three Gram-negative bacteria tested, for methanolic extracts, the highest antibacterial activity was observed for *K. pneumoniae,* followed by *P. aeruginosa* and the less effect was observed on *E. coli*. The DIZ values for *K. pneumoniae* were between 9.0 m and 25 mm, with more antibacterial effect by juice and less effect by white seed. For P. aeruginosa the DIZ values recorded were between 9.0 and 22 mm. The high effect was noted for rind extract followed by juice and less antibacterial effect was noted for whole fruit and seeds. The effect on *E. coli* was comparatively less than the other two bacterial cultures. Rind extract exhibited more antibacterial effect with a zone of 20 mm and least effect was observed for white seed with 8.0 mm.

Table 1: Effect of Different Methanolic Pomegranate Extracts on The Bacterial Cultures

			Diameter of Inhibition Zone in mm (DIZ)[a]				
				Seed			
S.No	Bacterial Culture	MTCC. No	Rind	Red	White	Whole Fruit	Juice
1.	*B. cereus*	1307	25	8.0	10.0	10.0	18
2.	*B. coagulans*	3164	22	4.6*	4.6*	4.6*	10
3.	*B. subtilis*	6910	18	13.0	10.0	9.0	17
4.	*E. coli*	732	20	11.0	8.0	15.0	17
5.	*K. pneumoniae*	7028	20	18.0	9.0	12.0	25
6.	*P.aeruginosa*	4302	22	10.0	10.0	9.0	21
7.	*S. aureus*	7405	25	19.0	10.0	16.0	23

[a]The zone diameter of wells cut in nutrient agar medium is 4.6mm and the diameter of inhibition zone (DIZ) of negative control for each bacterium is also 4.6mm. If the DIZ value is 4.6mm (*), that means the extract has no inhibitory activity against that bacterium

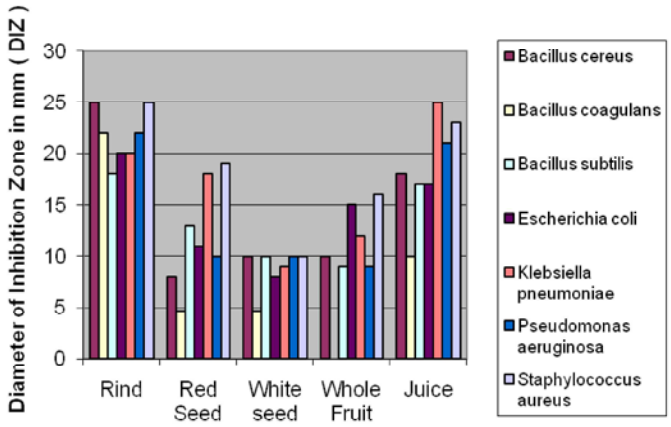

Fig. 1: Effect of different methanolic pomegranate extracts on the bacterial cultures

The results for aqueous pomegranate extracts on the three Gram-negative cultures has shown some variation. High antibacterial effect was recorded on *E. coli*, followed by *P. aeruginosa* and less effect was recorded for *K. pneumoniae,* but the effect of red seed was highest on *K. pneumoniae* with a DIZ value of 12 mm which is more than recorded for the other two bacteria. For *E. coli*, the DIZ values were recorded between 8.0 and 20 mm, with a high effect by rind extract and juice extract which gave a same DIZ value of 20 mm. The least effect was recorded for white seed with 8.0 mm (The results for aqueous extracts were presented in Table 2 and Fig. 2). In the case of *K. pneumoniae* high antibacterial effect was observed for rind extract, juice and red seed extract with DIZ values of 18, 20 and 12 mm. The less effect was observed for whole fruit and white seed extract with zones of 10 and 7.0 mm, respectively.

Antifungal Activities: In the present study the antifungal effect of different pomegranate extracts was studied on five different fungi and following results were obtained:

For methanolic extracts of pomegranate, high antifungal activity was recorded on *A. niger* followed by *P. citrinum, R. oryzae, T. reesei* and least activity was recorded on *M. indicus*. For *A. niger* the DIZ values were between 8.0 and 23 mm. A high inhibitory effect was recorded by rind extract with a zone of 23 mm and then juice with a zone of 20mm, the least antifungal effect was recorded by white seed with a zone of 8.0 mm. The next highest antifungal activity was recorded against *P. citrinum* with a range of DIZ values between 8.0 and 22 mm. For this fungus high antifungal effect was exhibited by rind extract (22 mm), followed by juice (18 mm), red seed (8.0 mm) and whole fruit (8.0 mm). The least activity was recorded by white seed with a zone of 6.0 mm. For *R. oryzae*, rind extract has shown more antifungal effect with a DIZ value of 19 mm and juice with a zone of 13 mm whereas the effect of red and white seed extract was moderate with DIZ values of 6.0 and 7.0 mm. A very less effect was recorded for whole fruit with a zone of 4.8 mm. The antifungal effect recorded for *T. reesei* was quite different from other fungi. For this fungus rind extract

Table 2: Effect of Different Aqueous Pomegranate Extracts on the Bacterial Cultures

			Diameter of Inhibition Zone in mm (DIZ)[a]				
				Seed			
S.No	Bacterial Culture	MTCC. No	Rind	Red	White	Whole Fruit	Juice
1.	*B. cereus*	1307	25	7.0	10.0	10.0	26
2.	*B. coagulans*	3164	23	4.6*	8.0	4.6*	19
3.	*B. subtilis*	6910	16	9.0	10.0	8.0	15
4.	*E. coli*	732	20	9.0	8.0	10.0	20
5.	*K. pneumoniae*	7028	18	12.0	7.0	10.0	20
6.	*P.aeruginosa*	4302	20	9.0	7.0	9.0	19
7.	*S. aureus*	7405	25	12.0	10.0	12.0	26

[a]The zone diameter of wells cut in nutrient agar medium is 4.6mm and the diameter of inhibition zone (DIZ) of negative control for each bacterium is also 4.6mm. If the DIZ value is 4.6mm (*), that means the extract has no inhibitory activity against that bacterium

Table 3: Effect of Different Methanolic Pomegranate Extracts on the Fungal Cultures

			Diameter of Inhibition Zone in mm (DIZ)[a]				
				Seed			
S.No	Fungal Culture	MTCC. No	Rind	Red	White	Whole Fruit	Juice
1.	*A. niger*	2196	23	10.0	8.0	10.0	20
2.	*M. indicus*	3318	15	5.0	6.0	5.5	12
3.	*P. citrinum*	7124	22	8.0	6.0	8.0	18
4.	*R. oryzae*	1987	19	6.0	7.0	4.8	13
5.	*T. reesei*	3929	18	9.0	5.0	8.0	10

[a]The zone diameter of wells cut in potato dextrose agar medium is 4.6mm and the diameter of inhibition zone (DIZ) of negative control for each fungus is 0.0mm

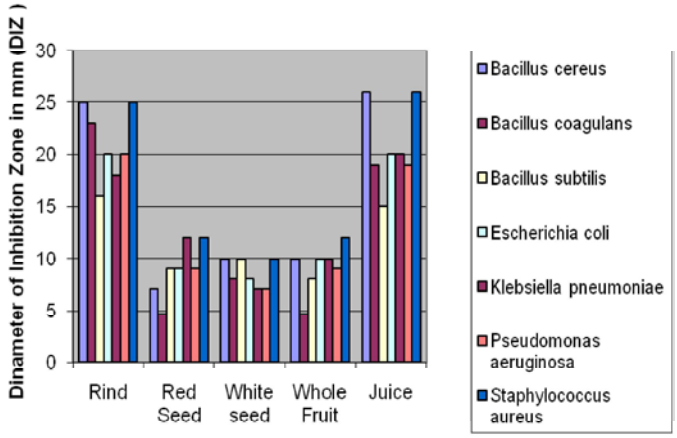

Fig. 2: Effect of different aqueous pomegranate extracts on the bacterial cultures

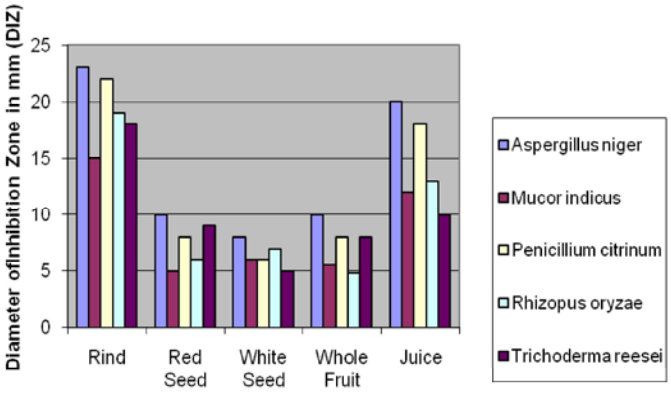

Fig. 3: Effect of different methanolic pomegranate extracts on the bacterial cultures

Table 4: Effect of Different Aqueous Pomegranate Extracts on the Fungal Cultures

			Diameter of Inhibition Zone in mm (DIZ)[a]				
				Seed			
S.No	Fungal Culture	MTCC. No	Rind	Red	White	Whole Fruit	Juice
1.	*A. niger*	2196	22	10.0	9.0	12.0	19
2.	*M. indicus*	3318	15	6.0	5.5	6.0	11
3.	*P. citrinum*	7124	20	8.0	8.0	10.0	18
4.	*R. oryzae*	1987	17	5.0	6.0	8.0	12
5.	*T. reesei*	3929	16	5.0	5.0	9.0	10

[a]The zone diameter of wells cut in potato dextrose agar medium is 4.6mm and the diameter of inhibition zone (DIZ) of negative control for each fungus is 0.0 mm

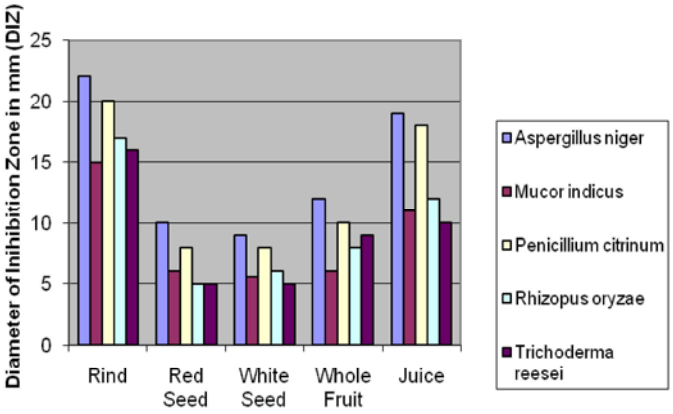

Fig. 4: Effect of different aqueous pomegranate extracts on the bacterial cultures

and juice has shown less antifungal effect than other fungi, with DIZ values of 18 and 10 mm, whereas the effect of red seed (9.0 mm), white seed (5.0mm) and whole fruit (8.0 mm) was more than *R. oryzae* and *P. citrinum*. Among all the fungi tested the least antifungal effect was observed on *M. indicus*. The significant antifungal effect was shown by only two extracts- rind and juice with a DIZ value of 15 and 12 mm, respectively. A meagre antifungal effect was observed for red seed, white seed and whole fruit (The results for methanolic pomegranate extracts are presented in Table 3 and Fig. 3).

The effect of aqueous pomegranate extracts was of similar pattern like methanolic extracts; where in the following order of decreasing antifungal effect was observed for tested fungi:

A. niger > *P. citrinum* > *R. oryzae* > *T. reesei* > *M. indicus*

For *A. niger* the DIZ value were between 9.0 and 22 mm, the effect of rind extract was highest with a zone of 22 mm. The next higher antifungal effect was exhibited by juice (19 mm), whole fruit (12 mm) and white seed extract (9.0 mm). The effect of aqueous extracts on *P. citrinum* indicates that high antifungal effect was due to rind extract (20 mm), followed by juice (18 mm) and whole fruit (10 mm). The effect of red seeds and white seeds was same with a DIZ value of 8.0 mm for both the seeds. In the case of *R. oryzae* a significant antifungal effect was recorded for rind extract, juice and whole fruit with DIZ values of 17, 12 and 8.0 mm and a minimum effect was seen for red and white seeds (5.0 and 6.0 mm). The antifungal effect on *T. reesei* was of similar pattern like *R. oryzae* wherein, more inhibitory effect was observed for rind extract, juice and whole fruit with zones of 16, 10 and 9.0 mm. The effect of red and white seeds was lowest with zones of 5.0 mm each. Among all the five tested fungal strains, the lowest antifungal effect was observed for *M. indicus*. Out of five extracts only two (rind and juice) has shown better antifungal effect with DIZ values of 15 and 11 mm. The remaining three extracts (Red seed, white seed and whole fruit) has shown very less inhibitory effect with DIZ values of 6.0, 5.5and 6.0(Table 4 and Fig. 4).

DISCUSSION

The antimicrobial effects of pomegranate were previously studied. Indeed, it is reported that the bark, leaves, flowers and fruits of pomegranate are widely used as phytotherapeutic agents in Brazil [13].

Ahmad and Beg [14] reported that alcohol extracts of pomegranate fruits showed antibacterial activity when tested against *S. aureus*, *E. coli* and *Shigella dysentriae*. Prashanth *et al*. [15] also reported methanolic extracts of *Punica granatum* fuit rind to be active against all microorganisms tested in their study. These results are in accordance to results obtained in the present study for bacteria wherein antibacterial activity was observed for all the seven bacterial cultures tested. Mathabe *et al*. [13] showed that methanol, ethanol, acetone and water extracts obtained from pomegranate were active and effective against the tested microorganisms (*S. aureus, E. coli, Salmonella typhi, Vibrio cholera, S. dysenteriae, S. sonnei, S. flexneri* and *S. boydii*), showing an inhibition zones of 12-31 mm. Melendez and Capriles [16] have also reported that extracts from pomegranate fruits possess in vitro antibacterial activity against many bacteria tested (*E. coli, Enterobacter cloacae, P. fluorescens, Proteus vulgaris, Alcaligenes faecalis, Serratia marcescens, E. aerogenes, S. aureus, Arthrobacter globiformis, M.luteus, B. cereus, B. subtilis, B. coagulans, Micrococcus roseus, M. phlei, M. rodochrus, M. smegmatis*; showing an inhibition zones of 11-31 mm). Interestingly, they stated that in Puerto Rico, it is very common practice to use these plant extracts as remedies for colds and bacterial infections. Their results provide evidence for the presence of antimicrobial compounds in the crude methanolic extracts of these plants. These findings and the results obtained in our study clearly confirm the effectiveness of pomegranate fruit on inhibition of microbial activity. Ahmet Duman *et al*. [17] also reported the *In vitro* antibacterial activity of extracts obtained from six pomegranate cultivators against the bacteria *B. megaterium, P. aeruginosa, S. aureus, C. xerosis, E. coli, E. faecalis* and *M. luteus*, showing inhibition zones ranging from 13-26 mm.

There are many reports of antimicrobial activity of pomegranate [18-21] showing that pomegranate juice is inhibitory to *Staphylococcus epidermidis* and *Klebsiella pneumoniae*. Similar results were recorded in our study for the pomegranate juice which has shown second highest antibacterial activity after rind extract. *Kirilenko et al.* [22] reported that the antibacterial action of pomegranate juice varied with variety and depended on the contents of phenolic compounds, pigments and citric acid. De *et al*. [23] also reported potential antimicrobial activity of pomegranate seeds against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. Pomegranate fruit peel compound punicalagin is reported to have antimicrobial activity against *S. aureus* and *P. aeruginosa* [24]. Negi and Jayaprakasha [25] extracted pomegranate peels with different polar solvents at room temperature and assayed them for antibacterial activity. Acetone, methanol and water extracts were evaluated against both Gram-positive and Gram-negative bacteria. The acetone extract showed the highest antibacterial activity, followed by methanol and water extract. Results obtained in our study also confirm that methanolic extracts have high antibacterial activity followed by water extracts (aqueous extracts).

Regarding fungi the inhibitory effect of *Punica granatum* against mycelial fungi was reported by Qiao Shuhua *et al*. [26]. Their findings indicated that methanolic extracts are more effective than water extracts. The novelty of applying pomegranate peel extract as an alternative, reduced-risk antifungal agent for controlling citrus green mould invasion was investigated by A.A. Tayel *et al*. [27]. Their results indicated that most potent antifungal peel extracts against *Penicillium digitatum* isolates are those extracted with methanol, ethanol and water, respectively. The main active constituent in pomegranate extract are tannins and alkaloids. The main phytochemical constituent in the peel of *P. granatum* are gallotannins, ellagic acid derivatives, catechns & procyanidins and flavonols [2]. Also pomegranate peel extract was reported to include active antifungal compounds such as punicalagin, castagalagin, granatin, catechin, gallocatechin, kaempferol, querectin [28]. The synergistic interactions of these compounds increases the antifungal activity of the pomegranate peel extract.

Punicalagin isolated from the fruit peel of pomegranate was reported to have antimicrobial activity against *Candida albicans* [24]. Fungistatic activity of pomegranate peel varied with test organisms [29] as it inhibited the growth of *Penicillium citrinum* for 8 days, *P. patulum* for 4 days and *P. roquefortii* and *Aspergillus ochraceous* for 3 days. However, it had no effect on the growth of *A. flavus* and *A. parasiticus*. Similar results were obtained in the present study in which it was observed that high antifungal effect was recorded for *A. niger* and *P. citrinum*. Jassim [30] reported antifungal and antiviral compositions comprising pomegranate extract. These compositions prevented the growth of fungus and virus but were not able to affect bacterial viability substantially. Ahmed and Beg [31] reported that a host of plant extracts including ethanolic extracts of pomegranate showed antifungal activity against *Candida albicans*. *In vitro* studies have revealed that the extract of pomegranate inhibited the growth of oral bacteria and candida species [32, 33].

In conclusion, the results obtained in this study clearly demonstrate broad spectrum antimicrobial activity of pomegranate against both bacteria and fungi. More importantly the results indicated that methanolic extracts of pomegranate are more effective against bacteria and fungi than the aqueous extracts. The presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major active constituents may be responsible for these activities.

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