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**Pomegranate (Romman) Plant Antimicrobial Activity of the Extracts of
(*Punica grantum* L.)**

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ABSTRACT

Many plants were found to synthesize substances that are useful to the maintenance of health in humans and animals. Romman, pomegranate (*Punica grantum*) has been reported to have some antimicrobial activity. The present study was aiming to investigate the antifungal and antibacterial properties of the Romman fruit peel and flower extracts. The radial growth and the dry weight methods were used for fungi, while, the plate count and the inhibition zone methods were used for bacteria. Both fruit peel and the flower were found to contain high amounts of tannins. The study showed that the extracts were effectively inhibited mycelial growth and dry weight of both *Aspergillus niger* and *Penicillium italicum*. However, the effect was only significant at the higher concentration. On the other hand, the extracts of the fruit peel were highly effective against bacteria. It inhibited growth of both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria, although, the effect was more pronounced on the Gram-negative bacteria.

Keyword: Antimicrobial Activity, Romman, fungi, bacteria.

INTRODUCTION

The World Health Organization (WHO) estimates that 4 billions people (80% of the world population) recently use herbal medicine for some aspects of primary health care. It also stated that plant-derived pharmaceutical medicine was used in modern medicine in ways that correlated directly with their traditional uses as plant medicine by native cultures (Internet, 2008a). However, substances derived from the plants, remain the basis for a large proportion of commercial medications used today for treatment of heart diseases, high blood pressure, pain, asthma, and other problems (Internet, 2008b). Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trials. During the 20th century much of the

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pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. About 25% of the prescribed drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound (Internet, 2008c). Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization (Internet, 2010). The Romman tree or Pomegran-ate (*Punica grantum*) is a member of the family Lythraceae , it is generally described as a perennial tree or shrub. The tree has green foliage and inconspicuous orange flowers, with an abundance of conspicuous red fruits or seeds. The Pomegranate tree has a moderate life span relative to most other plant species and a slow growth rate (Malouf, *et al.*, 2006). Pomegranate contains the highest percentage of ellagitannins of any commonly consumed juice. Ellagitannins are metabolized into urolithins by gut flora, and have been shown to inhibit cancer cell growth in mice (Internet, 2010). Elsaeim (2002) reported that pomegranate is very effective against worms and good against decentary. Pomegranates have also been reported to have antimicrobial activity against a range of Gram- positive and Gram- negative bacteria (Internet, 2008c).

The present study was therefore, conducted to investigate the antimicrobial activities of the extracts of pomegranate against two fungi (*Aspergillus niger* and *Penicillium italicum*) and two bacteria (*E. coli* and *Staphylococcus aureus*).

MATERIALS AND METHODS

Samples of pomegranate (*Punica grantum*) fruits and flowers were obtained from Wad Medani local market. The aqueous extracts of the fruit rind and the flowers of Romman were added to the different media used, to give different concentrations (0.00, 25.0, 50.0, 75.0 and 100mg/ml). The two bacteria, *E. coli* and *Staphylococcus aureus* and the two fungi, *Aspergillus niger* and *Penicillium italicum* were obtained from the Microbiological Laboratory in the Department of Food Science and Technology, Faculty

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of Engineering and Technology, University of Gezira, Wad Medani, Sudan. The media used for growth and maintenance of bacteria were, nutrient agar and nutrient broth. For fungi both Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were used. The effect of pomegranate extracts on bacterial growth was made by the Cup-Plate agar diffusion (Inhibition zone) method, as described by Barry *et al* (1970) and Cruickshank *et al.* (1975) and by the Dilution Plate method as described by Zainal *et al.* (1988). The nutrient broth medium was used for both bacterial tests. The numbers of bacterial colonies obtained from the dilution plate method were calculated as a log number of colonies per 1 ml. The logs of the numbers were plotted against time. The statistical analysis methods used were the ANOVA and the Duncan's multiple range tests.

The Effect of the pomegranate extracts on fungal growth, on the other hand, was made by the radial growth and the mycelial dry weight methods, using PDA and PDB media, respectively. The methods were used as described by Abdel-Rahim *et al.* (2010). For radial growth, the diameter of growth was measured every 48 hours, by taking the average of two crossed diameters for each disc in the Petri dish. The results were then calculated as a percentage from the diameter of the growth of the control treatment. For the mycelial dry weight, the inoculated broth media in the flasks were incubated at room temperature (28 – 30⁰C) for 8 days. After incubation mycelia were collected by filtering the culture through a Whatman No. 1 filter paper and the fresh weight was recorded. The mycelial mats were then dried at 80⁰C for 24 hours, before being reweighed.

EXPERIMENTAL RESULTS

Results of the fruit peel and flower extracts of pomegranate on radial growth on *P. italicum* are shown in Tables (1 and 2), respectively. Although, at the higher concentration (100 µg/4 ml) there is some degree of reduction in radial growth, the reduction was not statistically significantly different from that of the control and the other lower concentrations (Table, 1). Similar effects were also shown by the extract of

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the pomegranate flower against mycelial radial growth of the fungus (Table, 2). The effects of the Pomegranate fruit peel and flower, on mycelial growth (radial growth and of mycelial weights) of the fungus *A. niger* are shown in Tables (3 and 4). The higher concentration (100 %) of the peel extract gave significantly better reduction in mycelial radial growth of this fungus compared to the control and the lower concentration (25%). However, the second higher concentration (75 %) was only significantly better than the control treatment (Table 3). On the other hand, only the higher concentration of the extract of the flower was significantly better (Table 4).

Table (1): Effects of different concentrations of pomegranate fruit peel extract on radial growth (mm) of *P. italicum*.

Concentration %	Incubation(days)				Mean
	2	4	6	8	
0	2	3.15	4.75	5.3	3.800 ^a
25	1.85	3.00	3.97	4.80	3.405 ^a
50	1.80	2.90	3.85	4.36	3.228 ^a
75	1.75	2.70	3.78	4.05	3.070 ^a
100	1.63	1.98	2.45	3.27	2.332 ^a
Mean= 3.164	SE = 0.26	SD = 1.15			

*Figures with the same letter are insignificantly different.

Table (2): Effects of different concentrations of pomegranate Flower extract on radial growth of *P. italicum*

Concentration %	Radial growth (mm)				Mean
	Incubation days				
	2	4	6	8	
0	2.83	3.1	4.75	4.93	3.915 ^{a *}
25	2.7	3.0	4.4	4.58	3.670 ^a
50	2.2	2.7	3.8	3.35	3.262 ^a
75	2.0	2.5	3.7	4.05	3.063 ^a
100	1.4	2.4	3.43	4.0	2.808 ^a
Mean = 3.341	SE = 0.22	SD = 1.00			

*Figures with the same letter are insignificantly different.

Table (3): Effects of different concentrations of pomegranate fruit peel extract on radial growth of *A. niger*.

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Concentration %	Radial growth (mm) Incubation (days)				
	2	4	6	8	mean
0	3.75	4.33	4.39	4.6	4.267 ^a
25	3.65	3.68	4.33	4.51	4.065 ^{ab}
50	3.33	3.5	4.2	4.2	3.807 ^{abc}
75	3.15	3.3	3.45	3.9	3.450 ^{ab}
100	2.9	3.01	3.27	3.84	3.257 ^c

Mean = 3.764 SE = 0.12 SD = 0.52

*Figures with the same letter are insignificantly different.

Table (4): Effects of different concentrations of pomegranate (flower) extract on radial growth of *A. niger*

Concentration %	Radial growth (mm)				Mean
	Incubation (days)				
	2	4	6	8	
0	2.6	3	3.23	3.23	3.015 ^a
25	2.48	2.93	2.75	3.05	2.803 ^{ab}
50	2.23	2.68	2.68	2.78	2.592 ^{ab}
75	2.04	2.63	2.6	2.7	2.493 ^{ab}
100	1.85	2.45	2.48	2.58	2.340 ^b

Mean = 2.648 SE = 0.08 SD = 0.35

*Figures with the same letter are insignificantly different.

The effect of Pomegranate extracts on mycelial dry and fresh weights of both *A. niger* and *P. italicum*, are shown on Fig. (1) and (2), respectively. Results on Fig. (1) showed the effect of the extracts on mycelial weights of *A. niger*. From the results it is clear that the extracts were highly effective in reducing both the fresh and dry weights of the fungus. However, the effect was increasing with increasing concentration. On the other hand, *P. italicum* was found tolerant to the extract. Its growth was even enhanced at the lower concentrations, although, at the higher concentration (100%) there was a reduction.

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The present study also investigated the effect of pomegranate extract on two bacteria (*E. coli* and *Staph. aureus*). Both the dilution plate count and the inhibition zone methods were used. The extracts of fruit peel of pomegranate were found highly effective in reducing the number of colonies of both bacteria; *E. coli* and *Staph. aureus* (Table 5 and 6, respectively). However, the effect was much greater against *E. coli*. The numbers of both bacterial colonies were highly significantly reduced even at the low concentrations of the extracts. Similar results were also obtained for the inhibition zone diameter of growth of both bacteria (table 7 and 8). The inhibition zone was far greater than that of the control. However, the effect was more pronounced with *E. coli*.

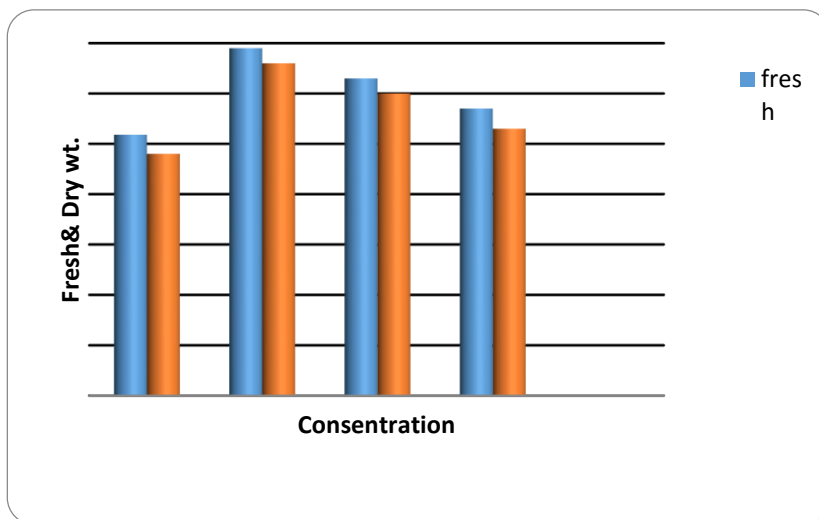


Figure (1): Different concentrations of Pomegranate Peel extract on Fresh and Dry weights of *P. italicum*.

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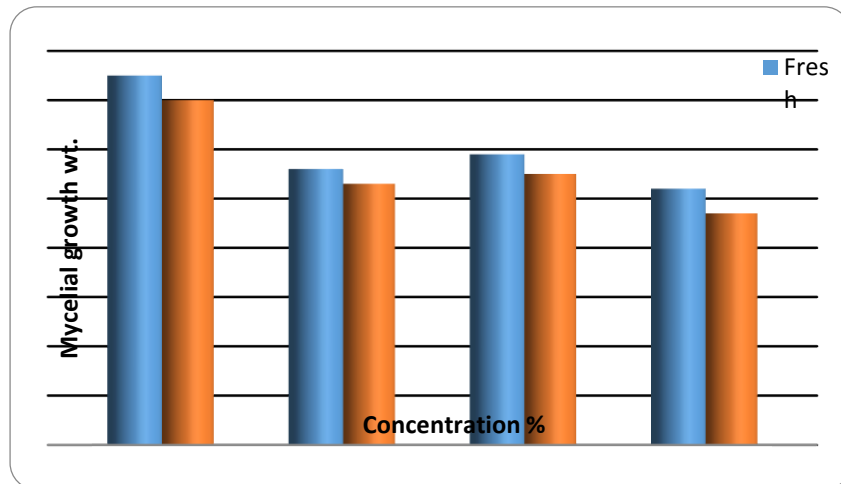


Figure (2): different concentrations of pomegranate Peel extract on Fresh and Dry weights of *A. niger*

Table (5): Effect of different concentrations of Pomegranate fruit peel extract on the number of colonies of *E. Coli*.

Concentration%	Number of colonies (cfu)				Mean
	Incubation (days)				
	2	4	6	8	
0	300	300	310	350	303.333a
25	55	84	87	87	144.000b
50	15	17	20	23	34.750bc
75	9	12	15	17	14.750 bc
100	1	2	3	3	5.750c

SE = 28.24 SD = 123.08

*Figures with the same letter are insignificantly different

Table (6): Effect of different concentration of Pomegranate fruit peel extract on number of colonies of *Staphylococcus aureus*

Concentration%	Number of colonies (cfu)				Mean
	Incubation (days)				
	2	4	6	8	
0	40	56	77	88	65.25
25	31	44	58	59	48.00
50	15	45	47	50	39.25
75	15	24	25	25	22.25
100	10	13	14	14	12.75

SE = 5.05 SD = 22.29

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*Figures with the same letter are insignificantly different.

Table (7) Effect of different concentration of Pomegranate peel extract on inhibition zone of *E. Coli*.

Concentration%	Number of colonies (cfu)				Mean
	Incubation (days)				
	2	4	6	8	
0	0	0	0	0	0.0000b
25	0.05	0.6	0.85	1.09	0.6475a
50	0.73	0.74	0.87	1.25	0.9025 a
75	0.88	0.91	0.91	1.28	0.9950 a
100	0.89	0.91	0.97	1.29	0.0150 a

SD =0.45 SE =0.010

*Figures with the same letter are insignificantly different

Table (8): Effect of different concentration of Pomegranate fruit peel extract on inhibition zone of *Staphylococcus aureus*.

Concentration %	Incubation time (days)				Mean
	2	4	6	8	
0	0	0	0	0	0.0000C
25	0.01	0.03	0.04	0.07	0.0375 C
50	0.08	0.10	0.13	0.13	0.1100 C
75	0.21	0.65	0.67	0.69	05550 b
100	0.64	0.93	1.19	1.26	1.0130 a

SD =0.45 SE =0.010

*Figures with the same letter are insignificantly different.

Discussion

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The results of the study indicated that the extracts of fruit peel and flower of pomegranate plant were highly effective against the two fungi tested. Mycelial radial growth and both fresh and dry weights of mycelia were reduced, although, the reduction was only significantly different at the higher concentration. Extracts of many plant species were reported to have antifungal activities (Abdel-Rahim *et al.*, 1989; Abdel-Rahim *et al.*, 2002; Sulieman *et al.*, 2008). On the other hand, Zainal *et al.*, (1988) reported a pronounced effect of the leaf litter extracts of mesquite (*Prosopis juliflora*) on *A. niger* and *Candida albicans*. Lai and Roy (2004) reported that many herbs and spices can yield medicinal compounds. Spices and herbs have been used for thousands of years by man in traditional medicine.

The present study also investigated the effects of fruit peel extract of pomegranate on two bacteria (*E. coli* and *Staph. aureus*), as indicated in tables 5 and 6, respectively. The results indicated that the pomegranate fruit peel extracts are more effective against bacteria compared that reported for fungi. Moreover, Elsaem (2002) reported that pomegranate is very effective against worms and good against decantary. Pomegranate has also been reported to have antimicrobial activity against a range number of Gram - positive and Gram - negative bacteria (Internet, 2008c). Antibacterial activity of plant extracts are well documented (Alicia, 1981). Vlietinet *et al.* (1995) screened about 100 medicinal plants used by traditional healers to treat infections in Rwanda, for their antibacterial, antifungal and antiviral properties. Their study showed that 45% of the plants were active against *Staph. aureus*, 2%, against *E. coli*, 16% against *Pseud. aerogenosa* and 7% against *Candida albicans*. Moreover, about 27% of the plants tested exhibited antiviral properties. In Sumatra (Indonesia), 114 plant extracts were assayed for their antibacterial activity (Ahmed, 2002). About 82% of the extracts were active against *Staph. aureus* while 32% of them were active against *E. coli*. According to Encarnation *et al.*(1994), the flavones isolated from the medicinal plant *Cullianandra*

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calitornia exhibited an antibacterial activity against the two bacterial isolates tested. The extracts of plant leaves of *Tagetes minuta* were found to exhibit some activity against both Gram - positive and Gram - negative bacteria (Tereschuk *et al.*, 1997).

In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 plants against Gram - positive and Gram - negative bacteria as well as *Candida albicans*. He found a marked effect against the Gram positive *Staph. aureus* followed by *E. coli* and *Candida albicans*, respectively. The use of plants and their extracts as remedies for curing many diseases have stimulated studies for investigating the presence of effective antimicrobial substances in them (Abdel Daim, 2001, Sulieman *et al.*, 2008, Abdel-Rahim *et al.*, 2010).

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