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HERBICIDAL AND FUNGICIDAL EFFECTS OF POMEGRANATE PEELS AND LEAVE CRUDE JUICES

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Abstract:

The present study aimed to through some light on the herbicidal and fungicidal activities of pomegranate peels and leave crude juices. Therefore, leave and peels of pomegranate plants were manually separated and mechanically pressed to obtain their crude juices. Pomegranate juices were tested in vitro for herbicidal activities against five weed seeds (Avena fatua L., Polypogen monspeliensis L., Imperata cylindrical, Capsella bursa pastoris L. and Amaranthus retroflexus L.). Fungicidal activities were conducted against six fungi (Fusarium solani, Fusarium moniliforme, Fusarium oxysporum, Rhizoctonia solani, Pythium splendes and Pythium ultimum). Pomegranate crude juices reduced to variable degrees the various weed seed germination. Both juices were found to be effective in reduction of mycelia growth for all tested fungi. Pomegranate peels crude juice induced pronounced effect on the germination inhibition and fungi mycelia growth for the aforementioned weed seeds and fungi under study than that of leave crude juice. These results coincided with the data of total phenolic and flavonoid contents of pomegranate crude juices since their values were higher in peels crude juice than in leave crude juice. The results of tested parameters led to suggest that the pomegranate peels crude juice can be used as a safe agent, non-toxic and environmental friendly to possess herbicidal and fungicidal activities.

Keywords*: Pomegranate peels and leave crude juices, Polyphenols, Flavonoids, Herbicidal and fungicidal activities*

Running tittle*: Herbicidal and fungicidal activities of pomegranate crude juices*

INTRODUCTION

Plants have been used a valuable source of natural products for maintain health. Different plant parts such as herbs and spices have been used for many years for the prevention of microbial infection. Pomegranate is one of the important and oldest edible fruits of topical and subtropical regions. This plant is a symbol of life longevity, health, feminity, immortality and spirituality (Mahdihassan, 1984). In Cuban traditional medicine, the pomegranate fruits have been used to treat acidosis, dysentery, microbial infections, diarrhea, helminthiasis, haemorrhage and respiratory pathologies (Fuentes and Exposito, 1995).

The edible parts of pomegranate fruits can be consumed fresh as well as for the preparation of fresh juice, jelly, jam and beverage. The importance of pomegranate fruits is stem from containing high levels of phenolic compounds such as ellagic, flavonoids, gallic acid, coumarin, caffeic acid, … etc. which possess antioxidant activity (Farag *et al*., 2015). In this context, Gaber *et al*. (2015) studied the effects of dimethyl sulfoxide, ethanol and methanol extracts of *Punica granatum* peels and seeds against five gram-positive and gramnegative bacteria. Their results indicated that the studied extracts may be used as a potential source of antimicrobial and mutagen agents.

Naseer *et al*. (2014) mentioned that pomegranate peel extracts induced remarkable antifungal activity compared to other extracts. GC/MS analysis showed the presence of seven phenolic acids, i.e., gallic, p-coumarin, caffeic acid, ferulic acid, protocatechuic acid, cinnamic acid and vanillic acid in the peel extract.

El-Khateeb *et al*. (2013) described that the extract of pomegranate leave caused remarkable reduction on the fungal growth (94.4%) of *B. fabae* while *F. oxysporm* and *F. solani* were the most resistant fungi against the methanolic extract.

Several scientists evaluated the microbial effect of pomegranate different parts extracted by various solvents of different polarities. For instance, Moorthy *et al*. (2013) studied the anti- microbial activity of ethanolic pericarp extract of *P. granatum* against 19 bacteria and 2 fungi. The inhibitory effect could be due to the presence of some of the secondary metabolites like phenolic compounds, flavonoids, terpeniods, phytosterols, glycosides and tannins present in the ethanolic pericarp extract of *P. granatum*.

Orak *et al*. (2011) examined the antimicrobial activity of peel extracts against three bacterial strains, i.e., *Staphylococus aureus*, *Escherichia coli* and *Salmonella enteritidis*. The antifungal activity was tested against two fungal strains, which were named *Aspergillus parasiticus* NRRL 2999 and *Aspergillus parasiticus* NRRL 465. All extracts possessed remarkable antibacterial and antifungal activities against all tested bacteria and fungal strains.

Researches allover the world have focused their work on the increase of food production to cope with the wide expansion of world's population. Weeds and fungi are the major problems in world agriculture and the use of synthetic herbicides successfully reduced labor cost for weed management and increased crop productivity. However, the intensive application of synthetic herbicides resulted in contaminate soil and ground water beside increase the risk of toxic residues in agricultural products. In addition, synthetic herbicides caused deleterious effects on the environment and human health. Therefore, there is a great need for natural botanical products to induce herbicidal activity and counteract the deleterious effects caused by synthetic herbicides. Consequently, in the present study, leave and peels crude juices of pomegranate were tested for herbicidal and fungicidal activities.

Materials and methods

1. Plant samples

Ripe pomegranate fruits were collected in October, 2014 from pomegranate trees in El- Menia governorate, Egypt. Samples of ripe pomegranate fruits were handpicked from different trees of Wonderful cultivar. The plant was authenticated by Dr.Abdalatif, A. M., Associate Prof. of Horticulture Department, Faculty of Agriculture, Cairo University. The English, scientific and family names of the plant under study are: Pomegranate, *Punica granatum* L. and *Lythraceae*, respectively.

2. Preparations of pomegranate leave and peels crude juices

Leave and peels of ripe pomegranate fruits were manually separated, cleaned from dust followed by seed removal then mechanically pressed by a Carver hydraulic laboratory press (Carver model C S/N 37000- 156; Fred S. Carver nc, Menomonee Falls, WI, USA). The resultant crude juices were concentrated using freeze- dryer (Labconco Corporation, Kansas City, M.O. USA) and kept in brown bottles at -5ºC until use.

3. Total polyphenolic content (TPP)

The total phenolic compounds in the pomegranate peels and leave crude juices were determined by the Folin- Ciocalteau method (El-falleh *et al*., 2012). TPP contents in the crude juices were calculated and expressed as gallic acid equivalent per g dry weight (mg GAE/g DW) by reference to regression equation of standard curve (Y=0.018x – 0.039, R2=0.986).

4. Flavonoid content

The colorimetric aluminum chloride method (El-falleh *et al*., 2012) was used for the determination of the total flavonoid content of the pomegranate crude juices. The concentrations of flavonoids in the pomegranate crude juice samples were calculated from the regression equation of calibration plot (Y=0.010x-0.143, R2=0.989) and expressed as mg quercetin equivalent /g of dry weight sample.

5. Herbicidal activity

a. **Weed seeds** Five weed seeds, i.e., *Avena fatua*, *Imperata cylindrical, Polypogen monspeliensis,* L., *Capsella bursa pastoris L. and Amaranthus retroflexus L.* were used in the present study to envisage the effect of pomegranate crude juices on its germination phenomenon. Table (1) represents English, family, genus and species names of various weed seeds.

b. **Direct contact of pomegranate peels and leave crude juices on weed seed germination** Empty and undeveloped weed seeds under study were discarded by floating on tap water. The weed seeds were surface-sterilized with 5% sodium hypochlorite for 20 min. then rinsed with abundant distilled water and embedded in distilled water for a period of 36 h. Two layers of filter papers were stacked in all petri plates (9.0 cm diameter) and moistened by 3 ml distilled water. The diluted pomegranate peels and leave crude juices were applied at the concentration range of 150-2400 ppm then poured (1.5 ml of each concentration) on the filter paper and allowed to diffuse by covering the plates. Each treatment was performed in triplicates. The weed seeds (20 seeds) under study were placed onto filter paper and kept in each plate maintaining at equidistance. Plates were incubated at 25 ± 1˚C with relative humidity of 55-65% and photoperiod of 16/8 h (light / dark). The germination ability was regularly examined every day. After seven days, the percentage of germination inhibition (Paudel and Gupta, 2008) was calculated by the following equation: Inhibition of seed germination (%) = [(C-T) / C] X 100 Where,
C: represents number of weed seed germination on the control plate.
T: represents number of weed seed germination on the treated plate.

6. Fungicidal activity

a. Fungal strains

Six fungal strains were obtained from the Tropical Product London, England. These strains were checked for purity and identity by the Regional Centre for Mycology and Biotechnology, Al-Azhar University. The fungal strains were: *Fusarium oxysporum* Schlecht. (C 597), *Fusarium solani* Mart. (MTCC 9667), *Rhizoctonia solani* Kuhn. (MTCC 4633), *Fusarium moniliforme* Sheld (MS 31), *Pythium ultimum* Var. (ATCC 2134) and Pythium *splendens* Braun. (ATCC 2232)

b. Reduction growth percentage of pathogenic fungi

The efficiency of pomegranate peels and leave crude juices were tested *in vitro* by linear growth of six tested fungal pathogens. Different concentrations, i.e., 20, 40, 80 and 160 ppm were used from each crude juice by Poisoned Food Technique as proposed by Jay, *et al*. (2005). PDA (20 ml, 4%) was autoclaved and poured in sterilized Petri dishes (9 cm). Each crude juice quantity (1ml) was added to the medium just before solidification. Discs of each fungus (5 mm diameter) were taken from 7-day old cultures and placed in the center of the plates. The control treatment consisted of medium free from of any juices. Three plates were used for each concentration and incubated at 27±1˚C. The linear growth (mm) of each tested fungus was measured taking into account the fungus growth in the control treatment (Deans and Svoboda, 1990). The reduction percentage for each mycelia growth of the pathogenic fungus was calculated by the following equation:
Inhibition (%) = [(C-T) / C] X 100
Where,
C: represents the colony diameter of the mycelium on the control plate (mm).
T: represents the colony diameter of the mycelium on the treated plate (mm).

7. Statistical analysis

The statistical analysis was performed using SPSS statistical software (Landau and Everitt, 2004). One-way analysis of variance (ANOVA) was applied for comparison between the values of the studied parameters. Data were expressed as mean ± standard error. The statistical significance of differences between individual means was determined by student "t" test.

Results and discussion

Several researchers have been studied the constituents and characteristics of internal sap of plant parts through extraction with different solvents of varied polarities (Miguel *et al*., 2004 and Tiwari *et al*., 2011). In the present work, the internal plant sap was obtained by mechanical press without recourse to solvents. Hence, the present work is focused on the whole substances present in the pomegranate crude juices and not in particular components extracted by solvents of different polarities. One has to point out that the pomegranate botanical parts are safe organs and regarded as waste materials since it obtained from annual pruning of pomegranate trees. Therefore, the main target of the present work was to determine quantitatively the total polyphenolic and flavonoid compounds. The herbicidal and fungicidal activities of pomegranate peels and leave crude juices were also evaluated.

1. Total phenolics and flavonoids of pomegranate peels and leave crude juices

Table 2 presents the quantities of total polyphenols and flavonoids of pomegranate leave and peels crude juices. The data demonstrated that the levels of polyphenols and flavonoids varied according to the pomegranate botanical part. Peel crude juice contained higher amounts of total polyphenols and flavonoids, being about 1.22 and 1.43 times as great as that in leave crude juice, respectively. Similar results were obtained by El-falleh *et al*. (2012).

2. Herbicidal activity

Weeds are extensively present in agricultural systems which reduce yield of many basic crops. They are usually controlled by using mechanical methods and synthetic herbicides as well. Meanwhile, mechanical methods are labor intensive and time consuming and using of herbicides not only creates perceived hazardous impacts on agricultural products but also enhances environmental pollution (Batish *et al*., 2007). Plant allelopathic effects have been considered to suppress weed germination in agricultural systems (Dayan *et al*., 2009). The allelopathic potential of various plants and their effectiveness for weeds control have been studied under both laboratory and field conditions. The herbicidal effect of pomegranate peels and leave crude juices on different weed seed germination is presented in Table 3.

The results illustrated that the germination inhibition (%) of weed seeds was generally increased with the increase of pomegranate peels and leave crude juices concentrations. Also, pomegranate peels crude juice induced more profound effect on the germination inhibition for weed seeds under study than that of pomegranate leave crude juice (Table 3). These results coincided with the data of total polyphenolic and flavonoid contents of pomegranate crude juices. In other words, high herbicidal activity of pomegranate peels crude juice may be attributed to its high contents of total phenolic and flavonoid substances. In this respect, Asghari and Tewari, (2007) mentioned that the toxicity of pomegranate allelochemicals such as phenolic compounds might be the basic factor for reducing the weed seeds germination. In addition, Esmaeilli *et al*. (2012) reported that allelochemicals compounds are known as a phenomenon for ecological control of weeds. These allelochemicals inhibit binary grass germinations and seedling. These allelochemicals consisted of ferulic acid, m-coumaric acid, p- coumaric acid, vanillic acid, p-hydroxy benzoic acid and cinnamic acid. These compounds were found in pomegranate crude juice (Farag *et al*., 2015).

3. Fungicidal activity

a. Reduction percentages of the fungi mycelia growth

A set of experiments was conducted to envisage the antifungal effect of pomegranate juices obtained from peels and leave botanical parts by pressing on some fungi. Figures 1 and 2 show the influence of various concentrations of pomegranate peels and leave crude juices on mycelia growth of fungi under study. The results illustrated that both juices increased the reduction percentages of the fungi mycelia growth. In addition, the pomegranate peels crude juice induced more effect in reduction of fungi mycelial growth than that of leave crude juice. It seems that the degree of fungi mycelial growth reduction was entirely dependent upon the pomegranate crude juice concentration and fungus type. The reduction of fungi growth followed the sequence due to the application of pomegranate juice as follows: *F. solani* > *F. moniliforme* > *F. oxysporum*. In general, the least and most resistant fungi related to pomegranate juices were *F. solaniand F. oxysporum* at all tested juice concentrations, respectively.

The data in Figure 2 show that the influences of various concentrations of pomegranate peels and leave crude juices on *Rhizoctonia solani*, *Pythium splendens* and *Pythium ultimum,* respectively. The results demonstrated that both juices increased reduction percentages of the fungi mycelial growth. Also, the pomegranate peels crude juice was more effective especially on *Rhizoctonia solani* than leave crude juice**.** The arrangement order towards reduction growth phenomenon was: *P. splendens* > *R. solani* > *P. ultimum*.

The outcome of this set of experiments is that the peel crude juice of S pomegranate can be used as natural agent for weed control. In addition, the germination of the above mentioned weed seeds was remarkably decreased and one would expect that the yield of the main crop will increase. Hence, spraying the seeds of the main crop with pomegranate crude peels juice will increase the production of the crop of interest. It is worth noting that pomegranate peels are priceless since it obtained from pomegranate fruits after consumption and induce no hazard effect for the handler. The data of this study can be used to develop bio-herbicides as alternative to hazardous synthetic herbicides from pomegranate peel crude juice and eco-friendly.

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Table (1) English, family, genus and species names of weed seeds.

	English name	Family name	Genus name	Species name
***Avena fatua* L.**	Wild oat	Poaceae	Avena	Fatua
***Polypogen monspeliensis*, (L.) Desf.**	Bristle grass	Poaceae	Polypogen	Monspeliensis
Imperata cylindrical	Halfa grass	Poaceae	*Imperata*	*Cylindrical*
***Capsella bursa-pastoris* (L.) Medik.**	shepherd’spurse	Brassicaceae	Capsella	Bursa-pastoris
***Amaranths retroflexus* Var.**	Red root pigweed	Amaranthaceae	Amaranthus	Retroflexus

Table 2. Total polyphenolic and flavonoid contents of pomegranate peels and leave crude juices.

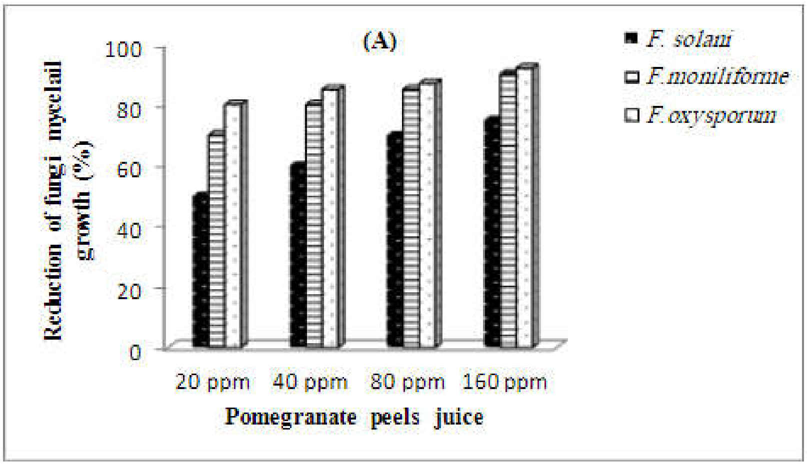
Parameter	Peel crude juice	Leave crude juice
Total polyphenolics (TPP) (GAE mg/g dry weight)	58.63±0.129a	48.02±0.071b
Total flavonoids (TF) (Q E mg/g dry weight)	47.32±0.032a	33.02±0.009b

Values are means of three replicates of each parameter ± standard error. Means within each row followed by the same letter are not significantly different at p > 0.01. GAE and QE refer to gallic acid and quercetin, respectively.

Table 3. Germination inhibition (%) of some weed seeds treated with pomegranate peels and leave crude juices.

Species	Pomegranate juice concentration (ppm)	The inhibition of weed seed germination (%)	
		Leave juice	Peels juice
Avena fatua L	150	44.56±0.005a	50.77±0.03b
	300	65.75±0.09a	71.49±0.007b
	600	66.81±0.005a	77.87±0.05b
	1200	67.87±0.001a	78.94±0.04b
	2400	70.00±0.09a	80.00±0.01b
Imperata cylindrical	150	70.12±0.02a	80.45±0.09b
	300	97.92±0.001a	98.75±0.09b
	600	98.96±0.01	100
	1200	100	100
	2400	100	100
Polypogen monspeliensis L.	150	15.24±0.02a	50.21±0.09b
	300	27.79±0.03a	72.23±0.05b
	600	44.46±0.01a	77.79±0.08b
	1200	55.66±0.04a	83.34±0.06b
	2400	72.23±0.009	100
Capsella bursa pastoris L.	150	40.98±0.01a	70.45±0.04b
	300	66.67±0.03a	88.89±0.07b
	600	77.78±0.05a	94.46±0.08b
	1200	83.35±0.08a	98.35±0.05b
	2400	94.45±0.07	100
Amaranthus retroflexus L.	150	50.23±0.09a	60.34±0.05b
	300	97.92±0.07a	93.75±0.03b
	600	98.96±0.06	100
	1200	100	100
	2400	100	100

Values are means ± SE of measurements. Means within each row followed by different letters are significant different at P < 0.01.



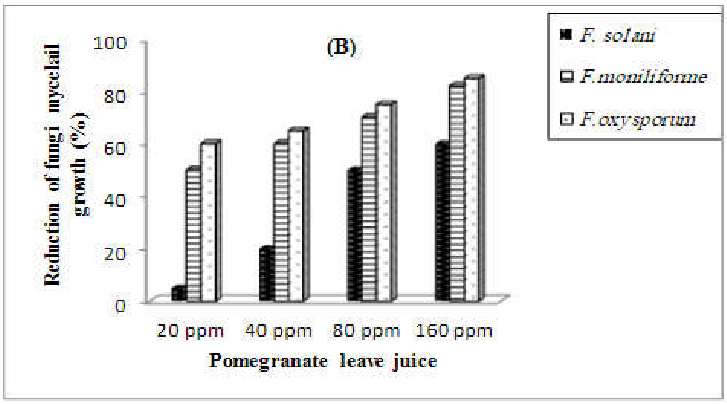
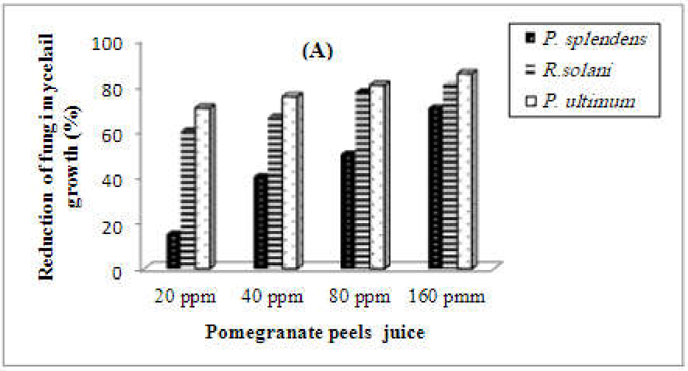


Fig. 1. Reduction of mycelial growth (%) of some *Fusarium* fungi groups using pomegranate peels (A) and leave (B) crude juices.



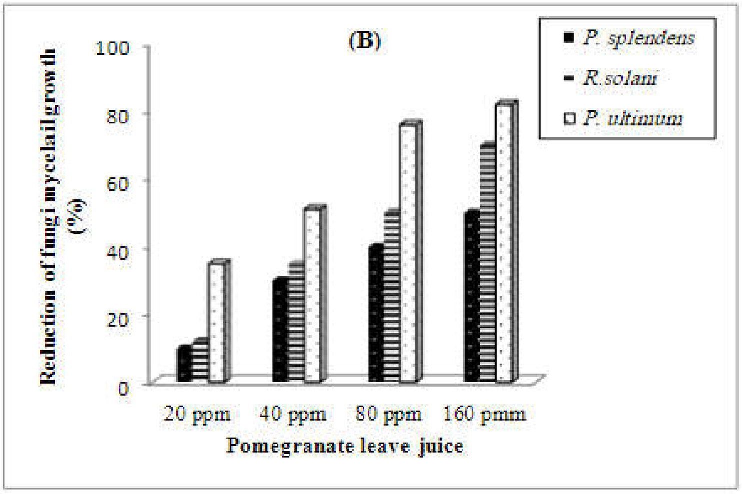


Fig. 2. Reduction of mycelia growth (%) of some fungi using pomegranate peels (A) and leave (A) crude juices.