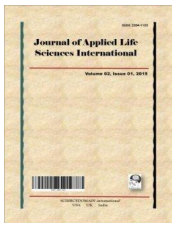

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Effect of Agriculture Waste: Pomegranate (*Punica granatum* L.) Fruits Peel on Some Important Phytopathogenic Fungi and Control of Tomato Damping-off

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Authors' contributions

This work was carried out in collaboration between both authors. Authors TGMM and AAK performed the research work. Author TGMM designed the study and performed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Considerable amount of solid wastes in the form of peels and seeds are generated by the fruit processing industries, and these wastes if not disposed correctly are seen to cause serious environmental problems. The aim of this research was to investigate the chemical constituents of the methanolic extract of pomegranate peel and evaluation of the antifungal activity against economically important phytopathogenic fungi as well as its effect on the linear growth, and the efficiency of pomegranate powder and its extract against damping-off disease caused by *Fusarium oxysporum*. The results showed that, *In vitro*, methanolic extract of pomegranate peel caused inhibitory effect to the linear growth of six economically important fungal phytopathogens, isolated from different hosts including: *Botrytis cinerea, Colletotrichum dematium, Fusarium oxysporum, Fusarium solani, Phoma* spp, and *Rhizoctonia solani.* Also, Pomegranate peel extract (PPE)

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effectively decreased linear growth and spore germination of *F. oxysporum* at 4000 ppm. Under greenhouse conditions, application of pomegranate peel powder as seed treatment or soil treatment deceased pre and post emergence damping off caused by *Fusarium oxysporum,* compared with untreated infected control. Treating tomato seedlings or soil with peel extract before sowing provided a good protection against damping-off. While soil treatment was more effective than seedling treatment. The major components of methanolic extract of pomegranate peel were identified by Gas chromatography/ mass spectrometry analysis.

Keywords: Pomegranate peel; phytopathogenic fungi; tomato; damping-off; GC-MS analysis.

1. INTRODUCTION

The production of tomato is of worldwide agricultural importance. Tomato (*Lycopersicon esculentum* Mill) is one of the most economic vegetable crops cultivated at different locaties in Egypt and all over the world for either local consumption or exportation purposes [1-4]. Also, is an important sourcing of nutrients such as vitamins A, C, E and as well as lycopene- natural antioxidant, which is not found in the other solanaceous crops. It has niacin 0.712 mg, calcium 31 mg and water 94.28 g per 100 g weight, constitutes an important part of the house hold diet and national economy [5-8]. It is subjected to attack by many soil borne fungal diseases specially damping-off and wilt diseases causing considerable losses either in the nurseries or in the field [9].

For many years, synthetic fungicides are currently used as primary means for the control of plant disease. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicide among fungal pathogens, and high development cost of new chemicals. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance [10]. Thus, there has been a growing interest on the research of the possible use of plant secondary metabolites for pest and disease control in agriculture [11,12]. The plants have long been recognized to provide a potential source of different class of chemical compounds, known as phytochemicals, such as terpenoids, alkaloids, phenolics, glucosides, etc., which are effective products for pest control. In order to increase our knowledge of the use of natural substances (plant extracts) to control plant diseases, we tested the antifungal effect of peel extracts of *Punica granatum L.*

The pomegranate (*Punica granatum L.*) is one of the oldest known edible fruit; it has been widely consumed in various cultures for thousands of years [13]. The pomegranate is belongs to the family Punicaceae. It is native from the area of Iran to the Himalayas in northern India, and has been cultivated and naturalized over the entire Mediterranean region since ancient times. Actually, the pomegranate is widely cultivated throughout Iran, India, Mediterranean countries, the drier parts of Southeast Asia, Malaysia, the East Indies; tropical Africa; in tropical and subtropical regions of the world like Turkey, California, Egypt, Italy, India, China, Chile and Spain to some extent, in the United States (drier parts of California and Arizona), China, Japan, and Russia [14-17]. According to Food and Agriculture Organization (FAO), pomegranate production is about 1.5 million tons all over the world [18]. It consists of edible part, seeds, and peel. The pomegranate peels constituents 5% to 15% of its total weight [19]. During the industrial processing of pomegranate, large volume of wastes is produced, which have a wide range of nutritional values. Therefore, in the recent years, scientists have focused on the industrial by-products of pomegranate that have a high potential of antioxidant and antifungal properties [20].

In searching for compounds from plants that are active against plant pathogenic fungi, we have found that the methanol extract of pomegranate peel is highly active against most of the plant diseases tested. So, the present study was aimed to identify the phytocompounds by Gas Chromatography-Mass Spectrometry **(**GC-MS) analysis and study the antifungal activity of methanolic extracts of pomegranate peel against economically important phytopathogenic fungi as well as its effect on the linear growth, spore germination of *Fusarium oxysporum*, to control tomato damping-off, Also, the use of wastes by the food industry becomes viable, since it is a natural alternative to synthetic pesticides and avoid waste disposal into the environment, bringing benefits to both industry and consumers.

2. MATERIALS AND METHODS

2.1 Preparation of Methanolic Extract of Pomegranate Peel

Fresh fruits of Pomegranate (*Punica granatum* L.) were manually peeled. The collected peels were then rinsed with distilled water and dried in an oven by hot air (50ºC) for 48h, and were powdered to get 60-mesh size using a mixing grinder [21]. The powdered peels (2 g) of pomegranate were macerated with 20 milliliter of 80% methanol at room temperature. The macerated material was strained through Whatman No1 filter paper. The extract was concentrated at 40ºC by using Rotary Evaporator (Heidolph, LABOROTA A 4000-Germany) and then dried in an oven at 50ºC for 48 h. Finally, by using the dried extract, different concentrations of methanolic extract i.e. 31.3, 62.5,125, 250, 500, 1000, 2000 and 4000 ppm were prepared.

2.2 Phytopathogenic Fungi Used

Six authentic phytopathogenic fungi isolated from different hosts, were used in this study; *Botrytis cinerea* (pea)*, Colletotrichum dematium* from (soybean)*, Fusarium oxysporum, Fusarium solani, Phoma* spp. and *Rhizoctonia solani* from (tomato). They were kindly provided by Seed Pathology Research Department, Plant Pathology Research Institute, Agricultural Research Center, (ARC), Giza, Egypt, were regularly subcultured and maintained on potato dextrose agar (PDA) medium in a refrigerator at 5±1ºC, throughout the course of the study.

2.3 Effect of Pomegranate Peel Extract (PPE) on the Linear Growth of Some Fungi

The antifungal activity of methanolic extract of pomegranate peel was studied *in vitro* by a poisoned food technique [22]. The Erlenmeyer flasks containing media were sterilized in an autoclave at a pressure of 15 Ib/sq inch and temperature 121ºC for 15 min. The methanolic extract was added to sterilized media, cooled to 30-35ºC, and shaken thoroughly. To avoid bacterial contamination, streptomycin was added to the media before pouring into petri dishes (9 cm diameter). The final concentrations of methanolic extracts were 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 ppm. The media were then poured into a set of three petri dishes (three replicates) under aseptic conditions in a laminar flow chamber with filter (Labconco Corporation, Kansas City, Missouri 6432). After partial solidification of the media in the plate, a disc (4 mm diameter) of the six fungal species, i.e. *B. cinerea, C. dematium, F. oxysporum, F. solani, Phoma* sp and *R. solani* was cut from 1-week-old culture with the help of a cork borer and inoculated to the center of the poured plates of treatments and control sets. The plates were sealed with Parafilm and then incubated (NÜVE San. Mlztirvetic A.S Ankara, Turkey) at 25±2ºC until the fungal growth in the control dishes was completed [23]. Percentage of inhibition was calculated due to the treatments against control, using the following formula [24].

$$\text{Percentage inhibition} = \left(\frac{C - T}{C}\right) x100$$

where C is the average of three replicates of hyphal extension (mm) of control, and T is the average of three replicates of hyphal extension (mm) of plates treated with tested material. EC_{50} values were determined by the linear regression (LPd line Computer Program) of the probit of the tested fungus percentage inhibition vs. logs the concentrations (ppm) of the studied materials. The EC_{50} notation used to indicate the effective concentration (ppm) that causes 50% growth inhibition. In essence, the lower the value of EC_{50} is the higher the efficacy of the tested materials in the test under consideration.

2.4 Effect of PPE on Spore Germination of *F. oxysporum*

Different concentrations of PPE (250, 500, 1000, 2000 and 4000 ppm) were tested. One drop from spore suspension of *F. oxysporum* was added to the after mentioned concentration was pipetted by sterilized pipette on glass slides. Other glass slides, with drop from spore suspension, were prepared using distilled water as control treatment. All slides were incubated at 25±2ºC for 18 h. Three replicates were used for each concentration. Percentage of spore germination was recorded after microscopic examination and percentage of inhibition in germination was calculated as mentioned before.

2.5 Greenhouse Experiments for Evaluation the Effect of Tomato Seed Treatment and Soil Application with Pomegranate Peel Powder (PPP) on the Incidence of Pre and Post-emergence Damping-off Caused by *F. oxysporum*

2.5.1 Preparation of fungal inoculum

Corn meal-sand medium (3:1 w/w) in 500 ml glass bottles was autoclaved at 121ºC for 30 minutes. The sterilized bottles were then inoculated with discs (5 mm in diam.) of 8 days old culture of *F. oxysporum* and incubated at 25±2ºC for 15 days [25]. Fungal inoculum of *F. oxysporum* was mixed thoroughly with the potted sterilized soil at the rate of 4% inoculum level (w/w). The infested soil was adequately watered for one week to enhance growth and distribution of the fungal inoculum.

2.6 Effect of Tomato Seed Treatment and Soil Application with Pomegranate Peel Powder (PPP) on the Incidence of Pre and Post-emergence Damping-off Caused by *F. oxysporum*

2.6.1 Seed treatment

Tomato seeds (Beto,cv) were treated with Arabic gum (1%) as sticker and then coated with pomegranate peel powder at the rate of 10 g/kg seeds. Another group of the seeds was treated with the commercial fungicide Flowsan 42.7% FS at the rate of 3 g/kg seeds. Untreated seeds was used as a control treatment, then the seeds were sown in pots (30-cm-diam.) containing soil infested with 4% inoculum level of *F. oxysporum*. Six seeds were sown in each pot and four replicates were used for each treatment.

2.6.2 Soil treatment

Pomegranate peel powder was added to pots (30-cm-diam) containing soil infested with 4% inoculum level of *F. oxysporum* at the rate of 100 g/pot, and then the pots were transplanted with untreated tomato seedlings at the rate of 6 seedlings per pot. Four replicates were transplanted in each pot. The growing seedlings in all treatments were examined periodically. Pre and post emergence damping-off was recorded 15 and 30 days after transplanting.

2.7 Effect of Tomato Seedling Treatment and Soil Treatment with Pomegranate Peel Powder (PPP) on Disease Incidence

2.7.1 Seedling treatment

Four-weeks old tomato seedlings were dipped in PPP (4000 ppm) for 1h. Other seedlings were dipped in the commercial fungicide Rizolex T 50% WP at the rate of 3 g/L. Untreated seedlings were used as control treatment. The seedlings were transplanted in pots containing soil infested with 4% inoculum level of *F. oxysporum*. Six seedlings were transplanted in each pot and four replicates were used for each treatment.

2.7.2 Soil application

Pots (30 cm-diameter) containing soil infested with 4% inoculum level of *F. oxysporum* were treated with pomegranate peel extract (10 ml/pot), Transplanting was made with un-inoculated 4-week-old tomato seedlings. Six trans plants were sown in each pot and four replicates were used. The plants in all treatments were examined periodically and the damping-off incidence 60 days after sowing was recorded.

2.8 Preliminary Phytochemical Analysis

The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of active chemical compounds [26]. The preliminary Qualitative phytochemical screening for the presence of various phytochemical compounds was performed using the methanolic extract for the identification of the phenolic compounds, tannins, alkaloids, flavonoids, carbohydrates, reducing sugar and saponins using standard procedures [27-30].

2.9 Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Gas Chromatography-mass Spectrometry analysis of methanolic extract of pomegranate peel: The GC-MS analysis was performed with an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column DB-5 (30 m x 320 µm x 0.25 µm film thickness). Helium was used as carrier gas at approximately 1.0 ml/min pulsed splitless

mode. The solvent delay was 3 min, and the injection volume was 1 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 ev scanning from m/z 50 to 500. The ion source temperature was 230ºC and the quadruple temperature 150ºC. The electron multiplier voltage (EM voltage) was maintained 1050 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60ºC then elevated to 280ºC at rate of 8ºC /min, and 10 min hold at 280ºC. The Detector and injector temperature were set at 280ºC and 250ºC, respectively.

2.10 Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and Willey (Chem Station data system) and by comparison with series of n-alkenes (C_4–C_{28}) and calculation of kovats indices.

2.11 Statistical Analysis

Completely randomized design in factorial arrangement with three replicates [31]. The least significant difference (L.S.D) between means was checked [32].

3. RESULTS

3.1 Antifungal Activity of Methanolic Extract of Pomegranate Peel

The antifungal activity *in vitro* by using the poisoned food technique of the methanolic extract of pomegranate peel was studied against the six phytopathogenic fungal species: *B. cinerea, C. dematium, F. oxysporum, F. solani, Phoma* spp and *R. solani.* The results (Table 1) reveal variation in the antifungal activity of the methanolic extract, that extract displayed high inhibitory effect on the growth of *B. cinerea, F.oxysporum, F. solani*, *Phoma* spp, *C. dematium* and *R. solani*, at concentrations from ranging between 1000 to 4000 ppm.

The *in vitro* antifungal activity of PPE expressed as a half inhibitory concentration (EC_{50}) of mycelial growth with the corresponding 95 % Confidence limits is shown in Table 2. It is clear that the antifungal activity increased with increasing the concentrations of the extract. *F. oxysporum* and *C. dematium* showed greater sensitivity to PPE than *Phoma* spp, *R. solani*, *B. cinerea* and *F. solani.* The EC_{50} of the tested phytopathogenic fungi was 266.40, 272.47, 305.59, 307.88, 318.36 and 787.41 ppm, respectively.

3.2 Effect of Different Concentrations of PPE on the Linear Growth and Spore Germination of *F. oxysporum*

Different concentrations of PPE were used to study their effect on the linear growth and spore germination of *F. oxysporum*. Data in Table 3 show that PPE significantly decreased the linear growth and spore germination of *F. oxysporum* compared to untreated control. Increasing the concentration was associated with an additional decrease with maximum records for linear growth and spore germination inhibition at 4000 ppm, being 76.6 and 82.6%, respectively.

Data present in Table 4 show that application of pomegranate peel powder and the commercial fungicide Flowsan 42% FS significantly decreased damping-off and recorded the highest survivals and the lowest damping-off and the highest efficiency, being 4.2 and 92.3% respectively. Soil treatment with pomegranate peel powder was higher than seed treatment in reducing the efficiency of damping-off being 76.9% and 46.1%, respectively.

3.3 Effect of Tomato Seed Application and soil Treatment with Peel Powder on Pre and Post- emergence Damping off Caused by *F. oxysporum*

Data present in Table 4 show that application of pomegranate peel powder and the commercial fungicide Flowsan 42% FS significantly decreased damping-off and recorded the highest survivals and the lowest damping-off and the highest efficiency, being 4.2 and 92.3% respectively. Soil treatment with pomegranate peel powder was higher than seed treatment in reducing the efficiency of damping-off being 76.9% and 46.1%, respectively.

3.4 Effect of Seedling Treatment and Soil Treatment with Pomegranate Peel Extract before Transplanting on Disease Incidence of Tomato

Data present in Table 5 indicate that the used treatments significantly decreased disease incidence compared to untreated control. The fungicide treatment recorded maximum decrease

(4.2%) followed by soil treatment with PPE (8.3%). Whereas, seedling treatment with the same extract was the lowest effective one, being 20.8%.

Table 1. *In vitro* antifungal activity of methanolic extract of pomegranate peel on mycelial growth of some economically important phytopathogenic fungi

Concentration (ppm)	Tested fungi % inhibition at different concentrations (ppm)					
	B. cineara	***C. dematium***	***F. oxysporum***	***F.solani***	***Phoma spp***	***R. solani***
0.0	0.0	0.0	0.0	0.0	0.0	0.0
31.3	16.0	20.9	7.70	7.7	13.7	21.8
62.5	24.1	29.1	16.7	13.2	22.3	29.3
125	44.1	38.5	30.7	20.9	33.4	37.9
250	49.6	48.7	48.3	30.7	46.2	47.2
500	55.2	58.9	66.3	42.1	59.3	56.6
1000	66.3	68.6	81.1	54.2	71.5	65.6
2000	72.6	77.1	90.0	65.9	81.6	73.8
4000	83.0	84.2	90.0	76.3	89.1	80.9
LSD ≥ 0.05	0.9	1.5	1.4	1.3	1.3	0.9

- *Data are mean of 3 replicates*

Table 2. Half inhibitory concentration (EC_{50}) of the mycelial growth of the six pathogenic fungi

Tested Fungi	EC_{50}	95 % Confidence limit	
		Lower	Upper
B. cinerea	318.36	240.4	419.4
C. dematium	272.47	207.6	353.3
F. oxysporum	266.40	194.2	361.3
F. solani	787.41	340.7	2998.4
Phoma sp	305.59	186.1	491.8
R.solani	307.88	281.3	336.7

Table 3. Effect of different concentrations of PPE on the linear growth and spore germination of *F. oxysporum*

Concentration (ppm)	Linear growth (cm)	Inhibition (%)	Spore germination (%)	Inhibition (%)
0.0	9.0	-	91.4	-
250	6.3	30.0	69.4	24.1
500	5.2	42.2	60.8	33.4
1000	4.1	54.4	56.7	37.9
2000	3.1	65.5	32.2	64.7
4000	2.1	76.7	15.8	82.7
LSD≥ 0.05	1.9	-	14.6	-

Table 4. Effect of tomato seed application and soil treatment with peel powder on pre and post-emergence damping off caused by *F. oxysporum*

Treatments	Damping off (%)		Total (%)	Efficiency (%)
	Pre-emergence	Post-emergence		
Seed treatment	12.5a *	16.7ab	29.2	46.1
Soil treatment	8.3a	4.2b	12.5	76.9
Fungicide (Flowsan)	0.0a	4.2b	4.2	92.3
Control	20.8a	33.3a	54.2	-

**Means within the same column followed by the same letter are not significantly different according to Duncan's multiple range test (P≥0.05)*

3.5 Preliminary Phytochemical Determination of Methanolic Extract of Pomegranate Peel

In general, plants produce phytoalexins as a defensive tool in response to microbial invasion. [33]. The Preliminary qualitative phytochemical analysis of methanolic extract of pomegranate peel extract was carried out for detection of secondary metabolites is presented in Table 6. The results indicate that the alkaloids and saponin were totally absent showing the negative test. Carbohydrates, reducing sugar, sterols, glycosides, phenolic compounds, tannins and flavonoids were found to be present by the qualitative test. The similar findings were also reported by [34-38]. This indicating the methanolic extract of pomegranate peel is good source of secondary metabolites having an important role in metabolism.

Table 5. Effect of seedling treatment and soil treatment with pomegranate peel extract before transplanting on disease incidence of tomato

Treatments	Disease incidence (%)	Efficiency (%)
Seedling treatment	20.8b*	58.3
Soil treatment	8.3b	83.3
Fungicide (Rizolex T)	4.2b	91.6
Control	50.0a	-

**Means within the same column followed by the letters are significantly different according to Duncan's multiple range test (P≥0.05)*

3.6 GC-MS Analysis of Methanolic Extract of Pomegranate Peel

Now a day the study of the organic compounds from plants and their activity has increased. The combination of a best separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds [39]. The most abundant components found in the methanolic extract of pomegranate peel were (1); 5-hydroxymethyl-2 Furancarboxaldehyde followed by (2); 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one.(3);2-methyl-2,5-furandione.(4); dibutyl phthalate and (5); 9-Octadecanoic acid, methyl ester (Table 7)

4. DISCUSSION

Considerable amount of solid wastes in the form of peels and seeds are generated by the fruit processing industries, and these wastes if not disposed correctly are seen to cause serious environmental problems such as water pollution, unpleasant odors, explosions and combustion, asphyxiation, and greenhouse gas emissions. There are several reports highlighting the integral exploitation of bioactive compounds from these wastes and their potential applications as antioxidant, antimicrobial, flavoring, colorant, and texturizer agents [40-42]. Solid wastes, particularly from processes such as peeling and coring, typically have a high nutritional value and may be used as animal feed [43]. Since last decade, efforts have been made to improve methods and ways of reusing fruits and vegetables wastes.

In vitro obtained results indicated that pomegranate peel extract exhibited inhibitory effect on the linear growth of economically important six phytopathogenic fungi isolated from different hosts, with minor variation among them. Furthermore, the tested extract effectively decreases the linear growth and spore germination of *F. oxysporum* (The causal of tomato damping-off and the maximum reduction was recorded at 4000 ppm.

Phytochemical screening tests are responsible for the identification of components which are responsible for antimicrobial activity of plant, thus these traditional species can be used as a potential source of antifungal pesticides against plant pathogenic fungi. These compounds can cause antifungal activity of these extracts. In addition, by their free radical scavenging capacities, they can lead to a high antioxidant activity. Also, the presence of some phytocompounds in the pomegranate peel extract may be responsible for the inhibitory effect [20]. The antifungal activity of pomegranate extract against some phytopathogenic fungi has been previously reported, the different pomegranate extracts on linear growth of different fungi; the highest antifungal activity was recorded on *Aspergillus niger* followed by *Penicillum citrinum* and *Rhizopus oryzae*, respectively. Similarly, pomegranate extract reduced linear growth of *Alternaria alternata*, *F. oxysporum*, *Phoma destructive*, *R. solani* and *Sclerotium rolfsii* with different degrees of activity against the tested fungi [44,45].

Under greenhouse conditions, application of pomegranate peel powder as seed treatment or soil application before sowing and transplanting tomato transplants in soil infested with *F. oxysporum* effectively decreased pre and post emergence damping-off compared to untreated infected control. At the same time, seedling treatment or soil treatment with pomegranate peel extract before sowing in soil infested with *F. oxysporum* provided good protection against damping-off incidence. Soil treatment was more effective than seedling treatment. Obtained results are in agreement with those reported by [46]; the antifungal activity against seed-borne pathogens of *Aspergillus* spp as the result of application pomegranate peel extract. Also, pronounced decrease was found in citrus green mould disease (*Penicillium digitatum*) as a result of application of pomegranate peel extract [47]. In another study, soil treatment with pomegranate leaf extract before sowing, effectively reduced damping-off disease of French bean caused by *Rhizoctonia solani*, under greenhouse and field conditions.

The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. The chemical constituents of methanol extract of pomegranate peel using GC-MS. GC-MS analysis showed the existence of various compounds with different chemical structures. All these components have been tested previously and reported to have antimicrobial activity [48,49]. It is possible that these compounds are mainly responsible for the antimicrobial effects observed in this study. An antimicrobial activity of pomegranate extract is probably due to the presence of large scale of antibiotic compounds [50]. In most investigated plants, phenols and tannins are the most important compounds in this field [51]. [52] reported that different parts of pomegranate plant such as peels and leaves contain phenolic compounds that lead to antimicrobial activity of their extracts.

In fact, phenolic materials along with high-molecular weight proteins constitute a complex, thus they can react with the cellular enzymes (oxidoreductase) that exist in cytoplasm and cell wall. Furthermore, these materials can inhibit the access of cellular receptors against microorganisms [52]. The presence of various bioactive compounds confirms the application of the agro-industrial waste as natural fungicide. [52-55].

Table 6. Qualitative phyto-consituents analysis of methanolic extract of pomegranate peel

Test	Phytochemical compound	Reaction
Molish's test	Carbohydrates	+
Benedict's test	Reducing sugar	+
Mayer's test	Alkaloids	-
Salkowski's test	Sterols	+
Borntrager's test	Glycosides	+
Froth's test	Saponins	-
Ferric chloride test	Phenolic compounds	+
Gelatin test	Tannins	+
Lead acetate test	Flavonoids	+

+ Present
- Absent

Table 7. GC-MS of methanolic extract of Egyptian pomegranate peel

Substance	Identified molecules	KI $_{exp}$	KI $_{Lit}$	Nature of compound	Biological activity*
1	$C_6H_6O_3$	1228	1224	Furan aldehyde	Antimicrobial, Preservative
2	$C_6H_8O_4$	1137	1134	Flavonoid	Antimicrobial, Anti-inflammatory, Antiproliferative Antioxidant
3	$C_5H_4O_3$	920	NF	Furan Ketone	Antimicrobial, Preservative
4	$C_{16}H_{22}O_4$	1929	1922	Plasticizer compound	Antimicrobial, antifouling
5	$C_{19}H_{36}O_2$	2089	2085	Fatty acid	Antiadrogenic, Allergenic, Flavor

RI (exp): experimentally obtained Retention; KI (Lit): Literature Kovats index.
*NF: Not found in the Literature; * Dr Duke´s ethnobotanical database*

5. CONCLUSION

Taken together, a methanol extract prepared from the non-edible part of pomegranate fruit: Agro-industrial waste was found to exhibit a great antifungal activity in comparison with commercial fungicide to overcome pesticide pollution and maintaining environment safety. Methanolic extract of pomegranate peel contains the several bioactive compounds have been reported to have antimicrobial activity. Also, this study indicated that the methanolic extract of Egyptian pomegranate has a potential antifungal activity against some economically important phytopathogenic fungi. Further studies are required to formulate the pomegranate peel extract in the suitable formulation as new cost effective botanical fungicide to control pathogenic fungi in agricultural fields.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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