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Partial Identification of Antifungal Compounds from *Punica granatum* Peel Extracts

Ira Glazer,[†,#,∥] Segula Masaphy,[†,⊗,∥] Prosper Marciano,[†,⊗] Igal Bar-Ilan,[†,⊗] Doron Holland,[§] Zohar Kerem,[#] and Rachel Amir*[,†,⊗]

[†]Migal Galilee Technology Center, P.O. Box 831, Kiryat Shmona 11016, Israel

[§]Institute of Plant Sciences, Agricultural Research Organization, Newe Ya'ar Research Center, Ramat Yishay 30095, Israel

[#]The Otto Warburg Minerva Center for Agricultural Biotechnology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

[⊗]Tel-Hai Academic College, Upper Galilee 10120, Israel

ABSTRACT: Aqueous extracts of pomegranate peels were assayed in vitro for their antifungal activity against six rot fungi that cause fruit and vegetable decay during storage. The growth rates of *Alternaria alternata*, *Stemphylium botryosum*, and *Fusarium* spp. were significantly inhibited by the extracts. The growth rates were negatively correlated with the levels of total polyphenolic compounds in the extract and particularly with punicalagins, the major ellagitannins in pomegranate peels. Ellagitannins were also found to be the main compounds in the bioactive fractions using bioautograms, and punicalagins were identified as the main bioactive compounds using chromatographic separation. These results suggest that ellagitannins, and more specifically punicalagins, which are the dominant compounds in pomegranate peels, may be used as a control agent of storage diseases and to reduce the use of synthetic fungicides.

KEYWORDS: *Punica granatum L., pomegranate, antifungal activity, hydrolyzable tannins, polyphenol, punicalagin*

INTRODUCTION

Considerable postharvest losses of fruits and vegetables occur by decay caused by fungal pathogens, which, in addition to causing rot, may produce mycotoxins.[1,2] Synthetic fungicides are the primary means of controlling postharvest fungal diseases; however, the global trend has shifted toward the reduction of fungicide application due to their carcinogenicity, high and acute residual toxicity, long degradation periods, and other side effects on humans.[3,4] This trend necessitates the development of alternative strategies that are based on natural products (particularly of plant origin), which are nontoxic and biodegradable.[5] The rapid rise in demand for organically produced fruits and vegetables also has increased the demand for such natural pesticides.[6]

Pomegranates (*Punica granatum* L.), a native shrub of occidental Asia and Mediterranean countries, have a high content of health-promoting compounds. Several studies have reported the efficacy of extracts from different tree parts, such as bark, leaves, fruit, and fruit peel to inhibit the growth of Gram-positive and Gram-negative bacteria, which are food-borne pathogens, spoilage bacteria, and human pathogens.[7−14] Pomegranate extracts also exhibit antifungal activities. First, it was reported that some extracts have anti-*Candida* sp. and anti-*Saccharomyces cervisiae* activity,[13,15] and more recently it was found that powder and extracts from the fruit's peel have the ability to inhibit the growth of *Penicillium* spp., two strains of the mycotoxigenic aspergilli, two stains of *Colletotricum*, *Rhizopus stolonifer*, *Botrytis cinerea*, and *Rhizoctonia solani*.[16−21] However, the chemical natures of the bioactive compounds that are responsible for antifungal activities were not elucidated in these studies. Therefore, the goal of the present study was to investigate the chemical nature of the bioactive compounds having the antifungal activity. Such knowledge about the peel's biological activity is valuable for developing new uses for the agricultural waste of pomegranate juice (PJ) industries and to produce natural fungicides that may replace the synthetic forms.

MATERIALS AND METHODS

Materials. The materials were purchased from Sigma-Aldrich unless specified differently. Punicalagin standard was purchased from CFM Oskar Tropitzsch (Germany).

Plant Material. Three different pomegranate accessions were chosen for this study; accessions PG102-3 and PG203-4, which have high and low levels of polyphenols in their peels, respectively,[16] and the 'Wonderful' accession, which is the major accession grown in Israel. The first two accessions were chosen from a collection in the Newe Ya'ar research center, ARO (registered in the Israel Gene Bank for Agriculture Crops (IBG, Web site: http://igb.agri.gov.il).[23] The fruits from the 'Wonderful' accessions were obtained from a commercial orchard grown in Upper Galilee. Following their harvest, the fruits were immediately transported by a ventilated car to the laboratory, where they were processed as described below.

Water Extraction of Pomegranate Peels. Pomegranate arils were removed, and the fruit peels were cut into 0.3−0.5 cm^2 slices and stored at −20 °C until used. To prepare the peel water extracts, 3-fold double-distilled (DDW) water (v/w) was added to the peel pieces, which were shaken at 25 °C for 1 h. The samples were centrifuged at 12000 rpm for 20 min, and the supernatant was filtered through 0.45

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μm filters (Millipore). Two hundred microliter aliquots were freeze-dried and weighed.

To obtain different levels of the bioactive compounds in the water extracts of peels, the pomegranates peels were extracted with DDW at a ratio of 1:3 (w/v) for durations of 1, 5, 15, 30, and 60 min.

For thin layer chromatography (TLC) experiments, 10 g of pomegranate peels was frozen in liquid nitrogen and homogenized using a Retsch mill (model MM301) to prepare peel homogenates. The homogenates were centrifuged (14000*g* for 30 min 4 °C), and the supernatant was used fresh or kept frozen at −70 °C for further analyses.

For peel extract fractionation, 400 g of peels collected from the 'Wonderful' accession was homogenized in 800 mL of DDW and kept at 50 °C for 30 min in the presence of 12 mL of butylated hydroxytoluene (BHT) dissolved in acetone (1:1). The homogenate was centrifuged for 20 min at 10000*g*. The supernatant was collected and stored at −70 °C for further analyses.

Mycelial Growth Inhibition Tests. For determination of the pomegranate peels' antifungal activity we have used fresh pathogenic fungal isolates, obtained from the Fruit Storage and Research Laboratory, Kiryat Shmona. Six mold fungi were chosen for this study: *Alternaria alternata*, *Penicillium digitatum*, *Penicillium expansum*, *Botrytis cinerea*, *Stemphylium botryosum*, and *Fusarium* spp. The fungi were isolated from decayed persimmon, citrus, apple, strawberry, pear, and avocado, respectively, and their identities were ratified by the Plant Protection and Inspection Services of the Ministry of Agriculture and Rural Development, prior to being used for the different experiments. To keep their virulence after isolation, the cultures were transferred to fresh medium up to two times throughout the experiments. The toxicity of the peel extracts against these mold fungi was initially tested using the poisoned plate technique. The peel extracts were added to the culture medium (potato dextrose agar, PDA Difco) after autoclaving, when the medium temperature reached about 40−45 °C, and were mixed thoroughly with final pomegranate water extract concentration adjusted to 25 and 50% (v/v) in the medium. PDA plates without peel extracts served as controls. Mycelial growth inhibition tests were performed by placing 5 mm mycelial agar disks cut from the margin of expanding fungal colonies, in the center of the PDA tested plates. The plates were incubated in the dark at 25 °C. When the fungal growth in the control plates had completely covered the Petri dishes, the colony diameter of all the treatments was measured for each fungus and the growth rate was expressed as centimeters per day. All treatments were done in five replications and the experiments repeated three times.

Total Polyphenol Compound Determination. Total polyphenols were determined using a colorimetric method modified for small volumes.[22,24] Half a milliliter of the water extract sample/standard was mixed with 2.5 mL of diluted Folin−Ciocalteu reagent (1:10 in water). After a short vortex, 20% Na_2CO_3 was added, and the tubes were mixed for 5 min at 50 °C. Quercetin was used as a standard. The absorbance of the cooled samples was measured at 760 nm.

Bioautography. The bioautogram test was performed as previously described.[6,35] Two types of TLC plates were used: silica gel G60/F254 (20 × 20 cm, 0.2 mm thickness, aluminum sheets, Merck, Germany); and Polygram SIL G/UV254 (20 × 20 cm, 0.2 mm thickness, precoated plastic sheets, Macherey-Nagel, Germany). Five hundred microliters of the peel homogenates (4 mg/mL) was applied on TLC plates, and the samples were separated in a glass TLC chamber with the mobile phase. Four types of mobile phases were tested: solvent 1, chloroform/methanol/water (60:35:5 v/v/v); solvent 2, methanol/chloroform/water (60:35:5 v/v/v); solvent 3, methanol/ethyl acetate/water (60:35:5 v/v/v); solvent 4, methanol/water + 0.1% formic acid (95:5 v/v/v). When the mobile phase reached 1 cm from the top of the plate, the plates were dried for 1 h at room temperature. To verify the separations of the compounds, the spots were visualized by UV irradiation of 254 and 366 nm, and the plates were sprayed with H_2SO_4 and fan-dried. When a good separation was obtained, the TLC plates were run in duplicates. One set was used as the reference chromatogram, whereas on the second plates, 5 mL of PDA containing a spore suspension (10^6/mL) or hyphae collected from *A. alternata*, *S. botryosum*, and *Fusarium* spp. was poured, forming a thin layer of solid media with spores or hyphae on top of the TLC plate. The plates were incubated at 25 °C for 2 days for fungal growth. The inhibition zone was visually determined, and the R_f determined by spraying the plates with an aqueous solution (2.5 mg/mL) of thiazolyl blue [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide, BDH, Poole, U.K.], followed by incubation for a further 4 h at 37 °C.[25] The inhibition zones appeared colorless against a purple background. From the reference TLC plates, zones corresponding to the R_f of the inhibition zones were scraped from the plate and eluted with methanol. In addition, zones from two other R_f that did not show any inhibition were eluted and used as negative control. The eluted samples were centrifuged at 5000 rpm for 15 min, and the supernatant was analyzed by LC-MS.

LC-MS Scan Analysis. Chromatographic separations of the compounds isolated from the inhibition zone obtained by TLC were carried out on a reversed phase ODS Hypersil column (2.1 × 100 mm, particle size = 5 μm, Thermo catalog no. 30105-102130), using acetonitrile (Merck 30) and water 50:50 as the mobile phases at a flow rate of 0.4 mL/min in an isocratic mode. Electron spray ionization (ESI) probes in negative and positive modes were used as follows: ESI−, capillary voltage (kV) = 3.2, cone (V) = 150, source temperature (°C) = 120, desolvation temperature (°C) = 350, desolvation gas flow (L/h) = 492, multiplier (V) = 650, mass range of m/z 100−1300; ESI+, capillary voltage (kV) = 0.5, cone (V) = 50, source temperature (°C) = 120, desolvation temperature (°C) = 350, desolvation gas flow (L/h) = 492, multiplier (V) = 650, mass range of m/z 100−1300.

LC-MS Analysis of Hydrolyzable Tannins. The water extracts of the peels (described above) that were extracted for 1 and 5 min in water were diluted with DDW 1:10 (v/v), whereas extracts extracted for 15, 30, and 60 min were diluted to 1:50 (v/v) before LC-MS analysis was carried out. These dilutions were further diluted with acetonitrile (1:1 v/v) to achieve final concentrations of 1:20 and 1:100, respectively. A Waters 2790 HPLC system equipped with a Micromass triple-quadrupole Quatro-Ultima mass spectrometer in series, controlled by Micromass MassLynx ver. 4.0 software, was used. The chromatographic separations were as previously described for punicalagins, punicalins, and gallagic acid,[22] and electron spray ionization in the negative mode was used. High selectivity identification was achieved using the multiple reaction monitoring (MRM) method according to their previously described mother and daughter ions. The mother ion was fragmented by argon using several collision energies, and the daughter ion areas of the standard solutions were compared to those received from the pomegranate samples, as described.[22]

Peel Extract Fractionation. For separation and purification of the active compounds, HPLC fractionation was used. Five hundred milliliters of the peel's crude extract from the 'Wonderful' accession were filtered through Watman filter paper (1 qualitative, circle, 90 mm). The filtrate was subsequently fractionated by flash chromatography (CombiFlash Retrieve, Teledyne Isco, Lincoln, NE; equipped with a RediSep preparative solid phase C-18 extraction column, Teledyne Isco). Stepwise elution was performed using 200 mL of water to wash compounds that were not absorbed by the resin, followed by 400 mL of graded solutions of methanol in water (v/v): 5, 10, and 30% and final elution with acetone, all at a flow rate of 10 mL/min. All fractions were freeze-dried and kept at −80 °C for later analyses.

The 5% methanol elution fraction of the CombiFlash preparative solid phase extraction column was loaded on semipreparative column (Synergy 4u Hydro -RP-80A 250 × 10 mm) using HPLC (HP 1100 connected to photo diode array detector). A gradient of acetonitrile in water was employed to isolate punicalagin: 4% acetonitrile in water for 9 min, 15% acetonitrile in water for 46 min, 90% acetonitrile in water for 0.5 min, and 90% acetonitrile in water for 2.5 min, at a flow rate of 5 mL/min. The two anomers of punicalagin (α and β) were collected in accordance with their absorbance, and the purity of the isolate was confirmed using LC-MS, as above.

Spore Germination Inhibition Tests. To find the active fraction and compounds after HPLC fractionation, a spore germination inhibition assay was adapted for low assay volumes. *S. botryosum* spores were collected from 1-month-old PDA fungal culture cultured at 22 °C, into sterile 5% Tween 80 in water. The suspension was filtered through sterile gauze to eliminate mycelial units. Spore stock suspension was brought to a concentration of 50000 spores/mL. All of the fractions from the CombiFlash RetrievE column were freeze-dried and brought to a concentration of 10 mg dry matter/mL before use. Five microliters of spore suspension was added to 5 μL of either sterile distilled water (as a control) or fractions of pomegranate peel extracts. The tubes were capped and incubated for 4 h at 25 °C for spore germination. Samples were loaded onto microscope slides, and both germinated and ungerminated spores were counted under a microscope in five observation fields. Germination was expressed as percent germinated spores from total spores observed in the sample (mean ± SD).

RESULTS AND DISCUSSION

Pomegranate Peel Extracts Inhibit the Growth of Three Postharvest Mold Fungi. PJ and peel extracts were previously reported to be able to inhibit the growth of some pathogenic fungi, as well as yeasts.[16−21,24−26] The inhibition ability was suggested to be attributed to the high levels of polypolyphenols, which are mostly water-soluble.[1,22,28] Therefore, we have examined the effect of aqueous peel extracts on the growth rates of six rot filamentous fungi, *A. alternata*, *P. digitatum*, *P. expansum*, *S. botryosum*, *B. cinerea*, and *Fusarium* spp. Two strains, *P. expansum* and *B. cinerea*, that cause pomegranate decay during storage;[33] Ruth Ben Arie, personal communications), served as a negative control. The four other fungi were selected because they cause severe storage rot diseases in certain fruits and vegetables. We consider it important to select strains that cause severe disease after harvest, instead of using defined stains, which in our experience may lose some of their natural traits including pathogenic virulence during their storage in strain banks.

The growth rates of the fungi were measured on media containing PDA with 25 and 50% (v/v) of the 'Wonderful' aqueous peel extracts (equivalent to 860 and 1720 mg of the water extract dry weight, respectively) in 100 mL of PDA medium. This water extract contains organic acids[30] and other organic compounds including primary and secondary metabolites in addition to phenols and sugars. It was found that, whereas the growth rates of *P. expansum* and *B. cinerea* were enhanced as expected, no effect was observed on the growth rate of *P. digitatum*, whereas the growth rates of *A. alternata*, *S. botryosum*, and *Fusarium* spp. were inhibited by the presence of the peel water extracts (Figure 1).

Fungal Growth Rate Is Negatively Correlated with the Level of Total Phenolic Compounds and with the Level of Punicalagins in the Peel Extracts. The major class of pomegranate phytochemicals is the polyphenols. Therefore, we studied whether inhibition of fungal growth correlates with the levels of polyphenols in the peels. To this end, two pomegranate accessions (PG102-3 and PG203-4) that have relatively high or low contents of total phenols, respectively,[22] were selected. The phenols were extracted from the peels by incubation for different time intervals (Figure 2, right panel) and then added to the medium. In both pomegranate accessions, negative correlations between the levels of total phenols in the media and the growth rates of *A. alternata*, *Fusarium* spp., or *S. botryosum* were found (Figure 2, left panel; Table 1).

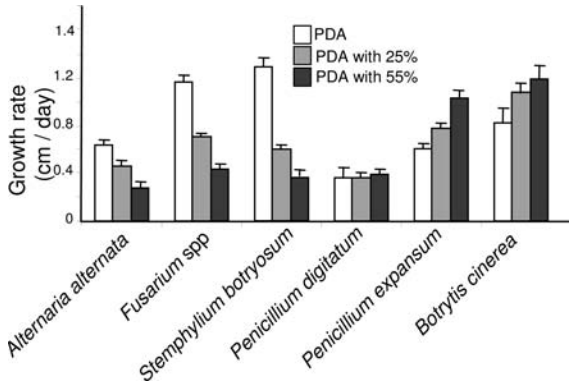

Figure 1. Inhibition of mold fungi by pomegranate peel extract. Growth rate (cm/day) of six rot mold fungi grown on media without and with pomegranate peel extracts. The data show the results of one representative experiment of the three experiments carried out, indicating the mean growth rate ± SD of five replicates.

Hydrolyzable tannins (HTs) are recognized as the major water-soluble phenolic compounds in extracts of pomegranate peels.[22,28] This group of compounds consists of gallotannins (gallic acid esters of glucose) and ellagitannins (ellagic acid esters of glucose). Punicalagins and punicalins (that have two anomers, α and β) as well as gallagic acid, which are the major HT compounds in the peels,[31] belong to ellagitannins. Because it is well-known that ellagitannins exhibit antibacterial activities[32−34] and that peel extracts have the ability to inhibit the growth of some rot fungi,[16−21] we next examined the levels of ellagitannins, punicalagins, punicalins, and gallagic acid, as well as the levels of ellagic acid in the crude extracts, using LC-MS. The levels of these compounds increased with extraction time (Figure 2, right panel). A significant negative correlation was found between the growth rates of the three rot fungi and the level of punicalagins in both pomegranate accessions (Table 1). Gallagic acid levels were negatively correlated with the growth rate of *Fusarium* and *Alternaria*, whereas punicalin levels were negatively correlated with the *Alternaria* growth rate (Table 1). Ellagic acid, however, did not correlate with the growth rate of these three fungi. The results suggest that ellagitannins contribute to most of the antifungal activity of the peel extracts.

Ellagitannins Were Also Found in the Inhibition Zones Produced by the Bioautogram Test. To determine if ellagitannins are responsible for the antifungal activity of pomegranate peels, a bioautogram test[6,35] was conducted to identify the bioactive compound/s extracted from pomegranate peel of the 'Wonderful' accession.

Four mixed-solvent systems were used to select the optimal separation by TLC. The best resolution was achieved using methanol/ethyl acetate/water (60:35:5 v/v/v) on two types of TLC plates, that is, silica gel G60/F254 and Polygram SIL G/UV254. The R_f value of the inhibition spot obtained for the silica gel G60/F254 plate was 0.07 for all three fungi tests (spore and hyphae), whereas the R_f value for the Polygram SIL G/UV254 plate was 0.59. The methanol-extracted inhibition spot from the TLC samples was analyzed by LC-MS using the scan procedure. In both inhibition zones, the three ellagitannins, punicalagins (m/z 1084), punicalins (m/z 782), and gallagic acid (m/z 602), which can be formed also from the degradation product of the punicalagins,[36] as well as ellagic acid (m/z 302), were found to be the dominant compounds in the chromatogram (Figure 3). Six other compounds, represented as

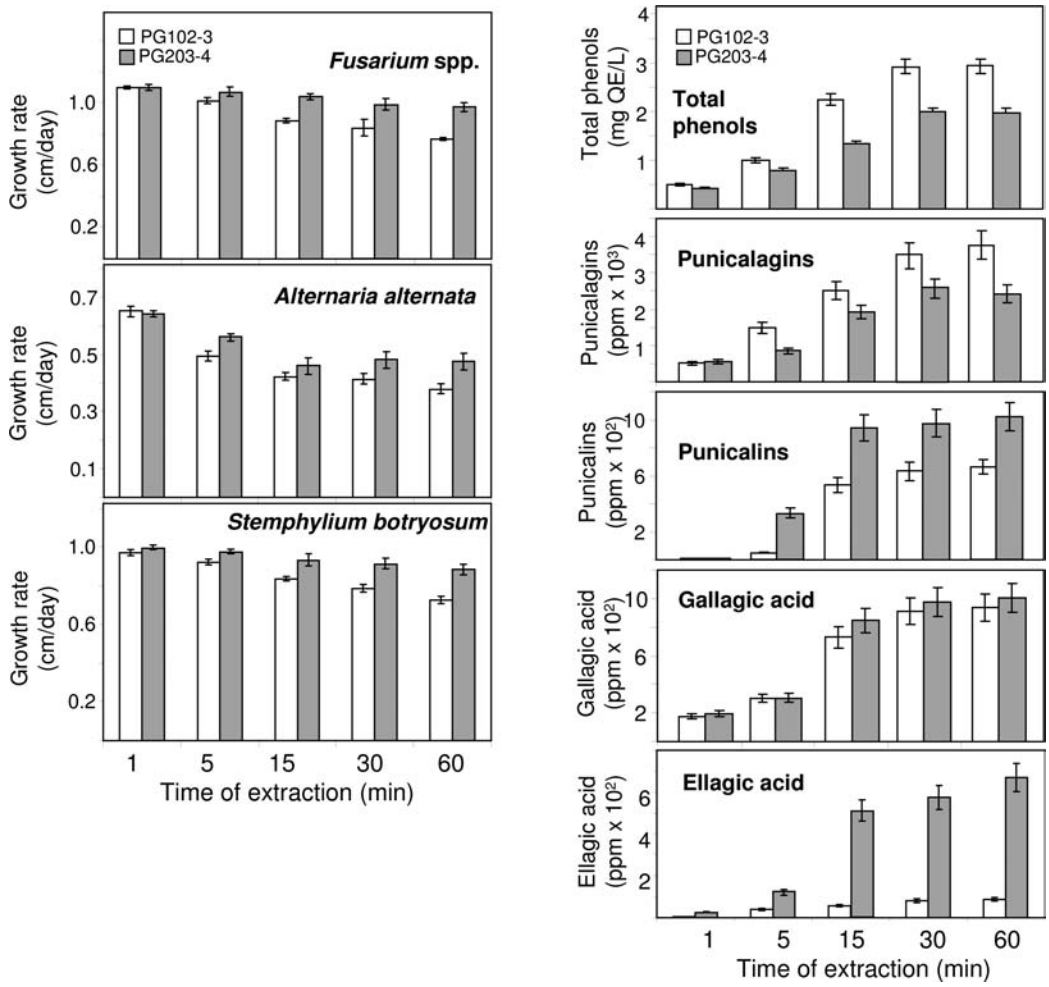

Figure 2. Correlation of fungus growth inhibition with the phenol and ellgitannin contents in the pomegranate peel: (left) growth rate (cm/day) of three mold fungi grown on PDA medium containing 25% aqueous peel extracts; (right) total phenolic content and content of punicalagins, punicalins, gallagic acid, and ellagic acid in the peel extracts of two accessions. Values are the mean ± SD of five replicates from each accession.

Table 1. Correlation Matrix (Pearson Test) Conducted on Data Obtained from the Peel Extracts of Accessions PG102-3 and PG203-4[a]

	growth rate							
	Fusarium	*Stemphylium*	*Alternaria*	total polyphenols	punicalagins	punicalins	gallagic acid	ellagic acid
growth rate *Fusarium*	1	0.94**	0.77**	−0.89**	−0.85**	−0.24	−0.56*	0.23
growth rate *Stemphylium*		1	0.62*	−0.73**	−0.72**	−0.18	−0.33	0.41
growth rate *Alternaria*			1	−0.85**	−0.80**	−0.56*	−0.74**	−0.22
total polyphenols				1	0.97**	0.61*	0.85**	0.15
punicalagins					1	0.66*	0.86**	0.21
punicalins						1	0.93**	0.85**
gallagic acid							1	0.65*
ellagic acid								1

[a]The *r* value of the correlation that is given and significant ($p < 0.05$) is identified by one asterisk (*), whereas $p < 0.01$ is identified by two asterisks (**).

peaks at *m/z* 275, 452, 576, 722, 764, and 934, were also found in both LC-MS chromatograms at low abundances (Figure 3). These 10 compounds were not found in the other eluted TLC strips used as controls, thus strengthening the hypothesis that one or more of the ellagitannins exhibit antifungal activity.

Fractionation Studies Show That Punicalagins Are the Main Compounds Responsible for Inhibition of the Germination of Fungal Spores. Two ellagitannins, punicalagins and punicalins, were previously shown to have antibacterial and anti-*Candida* activities. They were both shown to impede the growth of *Staphylococous aureus*, *Pseudomonas aeruginosa*, *Salmonella*, *Escherichia coli*, *Mycobacterium tuberculosis*, some species of the genus *Vibrio*, and *C. albicans*.[14,37−41] In addition, gallic acid, the major monomer in the composition of HTs, also exhibits antibacterial and antifungal activities by inhibiting corynebacteria, staphylococci, streptococci, *Bacillus subtilis*, *Shigella*, *Salmonella*, *Vibrio cholera*, *E. coli*,[40] *Cryptococcus neoformans*,[43] *C. albicans*,[26] and *Colletotricum truncatum*.[20]

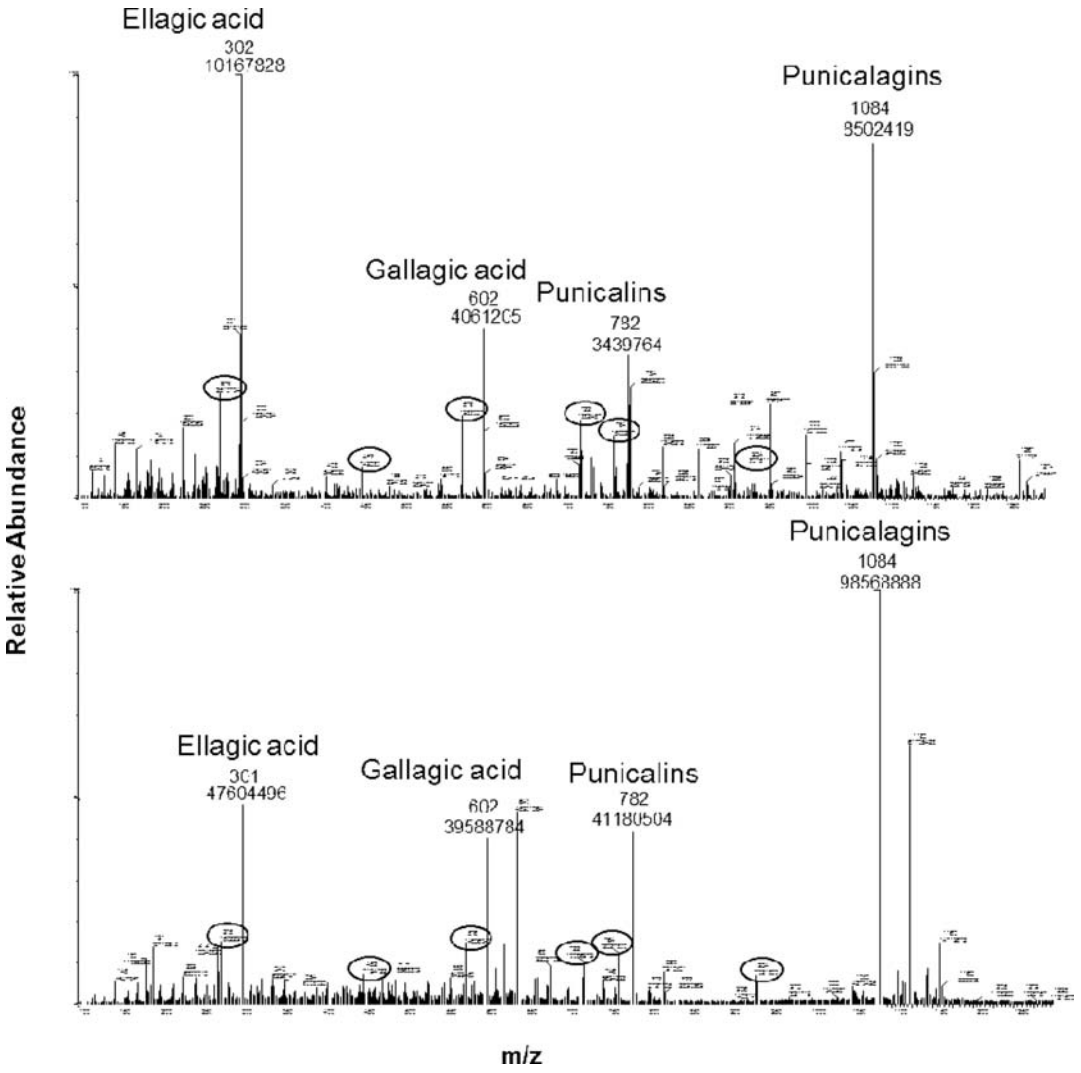

Figure 3. LC-MS analysis of compounds present in the fungal growth inhibition zone following separation by two TLC systems: (top) Polygram SIL G/UV254 TLC plate; (bottom) silica gel G60/F254 of the TLC system. The relative abundance of compounds was obtained from LC-MS analyses of two TLC systems from the elution of the fungal inhibition zones (according to bioautography results). The names of the four hydrolyzable tannins are given above their relevant peaks. Additional compounds found in both TLC systems are circled.

To assess whether punicalagins are the main compounds responsible for the antifungal activity of the peel extracts, we have searched for a method to enrich and separate punicalagins. The CombiFlash preparative solid phase extraction column that absorbs hydrophilic compounds to the resin was selected for this purpose. The crude extract of peel homogenates was loaded on the column, and the flow-through was collected. The column was washed with water (the wash water fraction), and the absorbed compounds were eluted with 5, 10, and 30% methanol in water. The eluted fractions were lyophilized, and each of these fractions was tested for their ability to inhibit spores of *S. botryosum* germination in a concentration of 5 mg/mL. As shown in Figure 4 the crude extract inhibited *S. botryosum* spore germination by 74% compared to control (germination in water). The flow-through fraction gave similar results to the control, suggesting that the inhibitory metabolites were absorbed by the column. Similarly, no inhibitory activity was obtained for the water wash fraction. The eluted 5 and 10% methanol fractions showed 85% inhibition of *S. botryosum* spore germination, whereas 63% inhibition was found for the 30% methanol fraction.

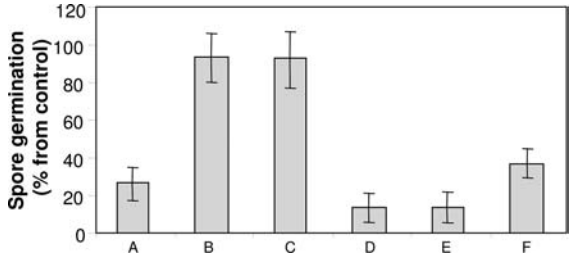

Figure 4. Effect of peel extract fractions on *Stemphylium botryosum* spore germination: (A) crude peel extract; (B) flow through (the fraction (dead volume) collected from the column after the crude extract was loaded on the column); (C) water elution (the fraction eluted with water); (D) 5% methanol elution; (E) 10% methanol elution; (F) 30% methanol elution. The effect of fractions obtained following separation by CombiFlash preparative solid phase extraction column on germination rate is displayed relative to control (germination of spores on water considered as 100%).

The 5% methanol fraction was further analyzed by HPLC. Thirty-four peaks were detected, in which the two anomers of punicalagins having retention time of 26 and 35 min were the

most dominant (Figure 5). The punicalagins were the main compounds in this fraction comprising 83% of the total peaks

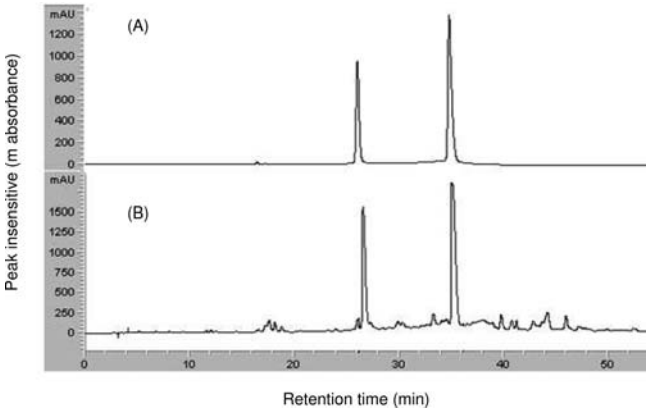

Figure 5. HPLC analysis of the 5% methanol elution fraction of the CombiFlash preparative solid phase extraction column: (A) HPLC analysis of standard of punicalagins; (B) analysis of the 5% methanol elution fraction of the CombiFlash preparative solid phase extraction column.

in the fraction. This fraction was also injected directly to LC-MS. Analysis (Figure 6) confirmed that punicalagins (m/z 1083) are the major compounds found in this fraction (76% according to the abundance of the compounds in the profile) followed by ellagic acid (m/z 301).

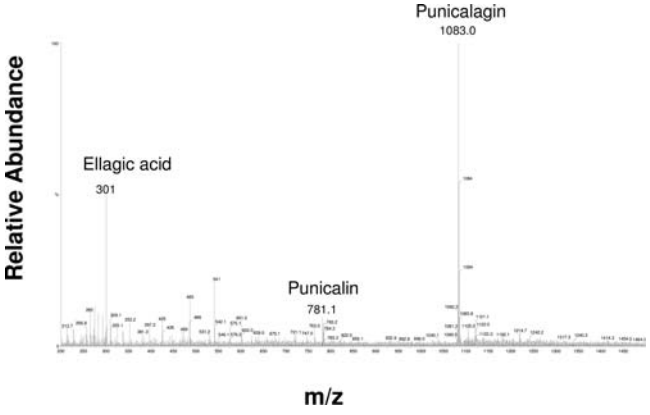

Figure 6. Relative abundance of compounds obtained by further separation by LC-MS of the 5% methanol elution fraction of the CombiFlash preparative solid phase extraction column.

The results show that among ellagitannins, punicalagins, which are the main compounds in the peels,[22,28] are the most bioactive compounds having antifungal activity. This is based on the following findings: (i) punicalagin level correlates significantly with the growth inhibition of the three fungi *A. alternata*, *Fusarium* spp., or *S. botryosum* (Table 1); (ii) punicalagins were the dominant metabolites found in the extraction from the two bioautograms; and (iii) punicalagins were the dominant metabolites in the most active fractions eluted from the CombiFlash column.

Further study is required to clarify the mode of action of these compounds. However, we assume that punicalagins act similarly to other HTs. HTs are well-known to have protein-binding capability, forming irreversible interactions with nucleophilic amino acids in proteins, often leading to the inactivation of the protein and loss of their function.[44] The interactions are nonspecific, occurring through hydrogen bonding and hydrophobic effects and possibly also through the reaction with sulfhydryl groups, as well as by covalent bond formation.[44−46] Probable targets in the fungal cell are the surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes and receptors. HTs can also interact with enzymes secreted by the fungi to degrade substrates and thus can lead to fungal cell starvation.[1,46] In addition, HTs and ellagitannins can destabilize the plasma membrane and thus increase its permeability.[43] The advantage of such a nonspecific action would be that resistant fungus variants cannot emerge easily.

Further studies are required to determine if the peel water extracts, or extracts obtained from the CombiFlash column, can replace the use of synthetic fungicides during storage periods. If successful, it will be an important byproduct of PJ industries, because peels are their main waste.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Laboratory of Plant Science, Migal Galilee Technological Center, P.O. Box 831, Kiryat Shmona 11016, Israel. Phone: 972-4-6953516. Fax: 972-4-6944980. E-mail: Rachel@migal.org.il.

Author Contributions

‖I.G. and S.M. contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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