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EVALUATION OF PHYTOCHEMICALS AND *IN VITRO* ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY *OF PUNICA GRANATUM L.* PEEL EXTRACTS

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ABSTRACT

Fruit wastes rich in bioactive compounds are increasingly recognized for their potential in various product formulations. The significant global demand for pomegranates and the considerable waste produced during their processing highlights the importance to evaluate the properties of pomegranate peel. The study focuses on a comprehensive phytochemical analysis of pomegranate peel, and its antibacterial and antioxidant activity. Pomegranate peel water extract (POWE) and ethanol extract (POEE) were analyzed using High Resolution Liquid Chromatography Mass Spectrometry Quadrupole Time of Flight (HRLCMS-QTOF) in both positive and negative ion modes. HRLCMS-QTOF identified 98 distinct compounds in POEE and 91 in POWE, with phenolic and polyphenolic compounds being prominent, along with significant amounts of lipid derivatives, organic acid derivatives, and organoheterocyclic compounds. The total polyphenol content was higher in POEE (390.05 ± 2.1 mg GAE/g) compared to POWE (330.25 ±1.4 mg GAE/g), and both extracts demonstrated good antioxidant activity. Both POWE and POEE exhibited similar antibacterial activities with MIC and MBC values ranging between 10 and 40 mg/mL, against tested organisms. Overall, the results indicate that pomegranate peel extracts have significant potential as natural antioxidant and antibacterial agent, which may find applications in formulation of industrial products and functional foods.

Keywords: HRLCMS-QTOF, antioxidant, antibacterial, phytochemicals, polyphenols, pomegranate peels

INTRODUCTION

The worldwide food sector produces an enormous quantity of discarded materials and by-products during food processing. A significant portion of agricultural produce is also damaged during transportation and storage (**Rodrigues *et al.*, 2022**). The fruit and vegetable processing sectors, in particular, contribute to approximately 45% of total by-products (**FAO Report 2015**). The problem of agricultural wastes, in general, is not well comprehended by consumers or industrial sectors. Improper disposal of these wastes has led to increase in greenhouse gases, pollution and related environmental issues (**Rodrigues *et al.*, 2022; FAO Report 2015**). Addressing this problem, pomegranate waste can stand out to be significant contributor for extraction of bioactives from its peels.

Pomegranate (*Punica granatum* L.), part of the Punicaceae family, has a long history of cultivation in Iran, India, China, and the Mediterranean since 3000 B.C. Pomegranate cultivation now extends to diverse regions, including northern and tropical parts of Africa, both Americas, and the Caucasus area. With its production of approximately 3.8 million tons in 2017, the popularity of pomegranates is growing globally due to their flavor and nutritional benefits (**Mo *et al.*, 2022**). In India alone, pomegranate production reached 3 million metric tons in 2021-22 (**APEDA Report 2023**). The fruits are an attractive industrial choice for preparation of juices, jellies, jams, wine, and other flavored food products. The high proportion of peel in pomegranate, coupled with their worldwide popularity and processing, results in significant contributions to both household and industrial wastes (**Marra *et al.*, 2022**). Pomegranate peels are rich in bioactive compounds suitable for various industrial applications, and are thereby attracting attention for sustainable improvisations to waste management protocols (**Mo *et al.*, 2022; Marra *et al.*, 2022**). Hence, although currently a problem, effective repurposing of pomegranate peels could provide India with around 1.5 million metric tonnes of raw material, in times of need (**APEDA Report 2023**).

Ain *et al.*, (2023) have described several uses of pomegranate peels in the pharmaceutical, cosmetic, packaging, and food sectors. The biological properties of pomegranate peels are extensively discussed in a comprehensive review by **Siddiqui *et al.*, (2024)**. The medicinal uses of pomegranate peels are also documented in complementary and traditional practices of India, China, and Iran, among others (**Thangavelu *et al.*, 2017; Liu *et al.*, 2017; Ge *et al.*, 2021**). Research indicates that pomegranate peels possess greater amounts of health-promoting substances compared to the fruit's edible portions. The diverse range of physiological effects of these compounds is due to their individual and synergistic activities (**Singh *et al.*, 2023**). The studies determining the biological effects of the pomegranate peels have typically reported 18 to 510 mg/g dry weight of phenolic compounds in their samples (**Mo *et al.*, 2022**). This concentration is also influenced by the type of solvents used, the extraction processes, and geographical characteristics of the region of cultivation of pomegranate (**Sweidan *et al.*, 2023**). Pomegranate peel extracts include ellagic acid, tannins catechins, flavonoids, gallic acids and anthocyanins; all of which contribute to its medicinal potential. (**Singh *et al.*, 2023**) These phenolic compounds are major secondary metabolites produced through the shikimic acid, pentose phosphate, and phenylpropanoid pathways, resulting in the formation of various water-soluble substances (**Mo *et al.*, 2022**). Among these compounds, tannins (193 to 420 mg/g) and flavonoids (84 to 134 mg/g) are abundant. Punicalagin is one of the promising compounds which exhibit radical scavenging, metal chelating and antibacterial properties. It is an ellagitannin which undergoes endo-esterification to yield ellagic acid. In turn, ellagic acid forms complex ellagitannins through polymerization with sugar ligands. Other constituents in peel extract are phenolic acids such as gallic, caffeic, chlorogenic, butyric, ferulic, and cinnamic acids. The dietary fibre, ranging between 33% and 62%, comprises of lignin, cellulose, uronic acid, xylose and arabinose. In addition to polyphenols and dietary fibres, pomegranate peels contain alkaloids (pseudo grenadine, N-methyl grenadine, and iso-grenadine), vitamins, steroids, and various minerals (potassium, phosphorus, sodium, calcium and magnesium). Besides, they are also rich in enzymes, amino acids, lipids, organo-heterocylclic compounds and their derivatives (**Mo *et al.*, 2022; Man *et al.*, 2022**). In the current study, the phytochemical composition of ethanol and water extract of pomegranate peel is evaluated using High-Resolution Liquid Chromatography Mass Spectrometry with Quadrupole Time-of-Flight (HRLCMS-QTOF) in the negative and positive modes along with antibacterial and antioxidant activity of peel extracts.

MATERIALS AND METHODS

Materials

Pomegranate fruit were procured from the local market of Kalyan, Dist: Thane, Maharashtra, India. All the chemicals (viz, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, methanol) were procured from Sigma Aldrich, Mumbai, India. Food grade ethanol was obtained from Manosol, Mumbai. Muller and Hinton Broth, Nutrient agar, Potato Dextrose agar, De Man, Rogosa and Sharpe (MRS) were procured from Hi Media, India.

Methods

Preparation of pomegranate peel extracts

The pomegranate peels, including the albedo, were separated from the arils and used for preparation of extract. The peels were washed thoroughly with tap water followed by sterile distilled water and then sun-dried for three days. The peels were then finely powdered using a pre-sterilized mixer grinder jar and stored in airtight containers. To extract the active components, 10g of the powdered peel was soaked in 90 mL of 96% ethanol (food grade) for 48h and filtered using Whatman filter paper No.1. The pomegranate peels ethanol extract (POEE) filtrate was concentrated under vacuum at ~40°C using a Trident Labortek Rotary Evaporator. In addition to alcohol extract, pomegranate peel water extract (POWE) was prepared by boiling the peels in the rotary evaporator at 80°C. The efficiency of the extraction process was evaluated by calculating extraction recovery percentage using Eq. 1. The dried extracts obtained were subjected to UV radiation for 2 h and examined for sterility on sterile nutrient agar and sterile potato dextrose agar plates. The sterile extracts were stored in labelled sterile containers at 4°C until further use. The extraction recovery is calculated using Eq. 1(**Venkataramanamma, D., *et al*.,2016**)

$$ER\ (\%) = \frac{A}{B} x\ 100 \quad \ldots \text{Eq. 1}$$

Where ER is the Extraction Recovery in '%', A is the weight of recovered dry extract in 'g', and B is Initial dry weight of peel powder in 'g'.

Determination of total polyphenol content

The total polyphenol content of POEE and POWE was determined using the Folin-Ciocalteu method. The diluted fruit peel extracts (0.5mL) were mixed with 1:10 diluted Folin Ciocalteau reagent (2.5 mL) and 7.5% sodium carbonate (2.5 mL). The mixture was incubated at room temperature for 30mins. The intensity of blue color formed on reaction was quantified using a spectrophotometer at 760nm. The total phenolic content was expressed in Gallic Acid Equivalents (GAE; mg/g) obtained from standard gallic acid curve (0.01 to 0.05 mg/mL) and calculated using Eq. 2 (**Waterhouse 2002**). All analyses were performed in triplicates.

$$T = C\ X\ \frac{V}{M} \quad \ldots \text{Eq. 2}$$

Where T is the total phenolic content in 'mg/g' of the extract, C is the concentration of gallic acid established from the calibration curve in 'mg/mL', V is the volume of the extract solution in 'mL', and M is the weight of the extract in 'g'.

High-Resolution Liquid Chromatography Mass Spectrometry with Quadrupole Time-of-Flight (HRLCMS-QTOF) of extracts

High-Resolution Liquid Chromatography Mass Spectrometry with QuadrupoleTime-of-Flight (HRLCMS-QTOF) was carried out to identify biochemical components in the extract. The untargeted analysis was conducted using a G6550A LC TOF/Q-TOF system (Agilent Technologies, Infinity System LC, Santa Clara, CA, USA), which was coupled with a mass spectrometer equipped with an electrospray ionization (ESI) source (**Galeano G *et al*.,2018**, **Araujo N *et al*., 2020**). Chromatographic separation was performed on ZORBAX Eclipse plus C18 column (150 mm x 2.1 mm, 5 microns particle size, Agilent Technologies, CA, USA) maintained at 40°C. The mobile phases used were 0.1% formic acid in water (Solvent A) and acetonitrile containing methanol (Solvent B), with a flow rate of 0.300 mL/min. The gradient elution protocol was as follows: 0–25 min, 95% A-5% B; 25–30 min, 100% B; 31–35 min, 95% A-5% B. The extracts were diluted in acetonitrile containing methanol and 3 µL of the sample was injected into the column using an autosampler at a pressure of 1200 bar. Mass spectrometry was operated in both negative and positive ESI modes with the following parameters: capillary voltage (VCap), 3500 V; fragmentor, 175 V; skimmer, 65 V; octopole (OCT 1 RF Vpp), 750 V; nebulizer pressure, 35 psi; drying gas temperature, 250°C; and sheath gas temperature, 300°C. Mass spectra were recorded by scanning the mass range from m/z 120 to 1200 in both MS and MS/MS modes, with tandem MS/MS spectra obtained using auto MS/MS acquisition mode. Data processing was carried out using Agilent MassHunter Qualitative Analysis software version B.06.0, which provided a list of potential molecular formulas. The MS data, MS/MS fragmentation profiles, and molecular formulas proposed by MassHunter were compared with literature data and databases such as ChemSpider and MassBank to annotate the phytochemicals analyzed from the extracts.

Determination of total antioxidant activity

The total antioxidant activity of POEE and POWE was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. It is a simple, rapid and inexpensive method used widely to measure free radical scavenging activity of food samples (**Kedare and Singh 2011**). Different dilutions of the extract (0.2 mL each) were mixed with 1.8 mL of 0.5 mM DPPH. The reaction mixture was incubated for 5 min at room temperature under dark conditions. The change in color intensity of the extract was measured using a spectrophotometer at 515 nm and the total antioxidant activity was calculated using Eq. 3 (**Brand-Williams *et al*., 1995**).

$$Antioxidant\ activity\ (\%) = \frac{C-T}{C} X\ 100 \quad \ldots \text{Eq. 3}$$

Where C is the absorbance of the Control sample and T is the absorbance of the Test sample.

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of POEE and POWE was determined using the broth dilution method using sterile Mueller Hinton broth as growth medium and sterile physiological saline as diluent. The test organisms used in the study were *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2036, *Proteus vulgaris* NCIM 2027, *Shigella flexneri* NCIM 5265, *Bacillus cereus* NCIM 2155, *Salmonella enterica* NCIM 5255, *Listeria monocytogenes* NCIM 5260 (Procured from NCL, Pune). *Lactobacillus casei var shirota* was also used in the study and was isolated from commercial drink Yakult on sterile MRS media. The bacterial strains were grown on Mueller Hinton medium for 18 to 24h at 37°C, and the inoculums were adjusted to 0.5 Mac Farland turbidity standards. Various dilutions of peel extracts (ranging from 10 mg/mL to 50 mg/mL) for bacteria were prepared from a stock concentration (500 mg/mL in 10% DMSO). For *Lactobacillus casei var shirota*, the extracts concentration used were in the range of 100-800mg/mL. To each of the tubes 0.1 mL bacterial inoculum was added. Growth, sterility, DMSO and color controls were maintained throughout the experiment. The tubes were incubated at 37°C for 24h, and bacterial growth was determined by observing turbidity. All tests were performed in triplicates. The MIC was determined as the lowest concentration of extract that prevented visible growth of the inoculated test organisms in the broth culture (**Wiegand L *et al*., 2008**).

Determination of Minimum Bactericidal Concentration

To determine the Minimum Bactericidal Concentration (MBC) of POEE and POWE, 0.1 mL volume was removed from MIC tubes showing no growth and spread onto sterile Mueller and Hinton agar plates for bacterial cultures and sterile MRS media for *Lactobacillus casei var shirota*. The plates were incubated at 37°C for 24h. The MBC was identified as the lowest concentration of the extract that inhibited bacterial growth on solid medium. All samples were tested in triplicates (**Andrews J *et al*., 2001**).

RESULTS AND DISCUSSION

Analysis of qualitative characteristics and total polyphenol content of extracts

Fresh pomegranate peels contain approximately 70% moisture, which decreases naturally over time (**Khoualdia *et al*., 2020**). In this study, the peels sample had a moderate moisture content of 51%. While this level of moisture can enhance the extractability of phenols and polyphenols, it also risks increasing oxidase enzyme activity, which can lead to their degradation (**Obasa *et al*., 2024**). Hence, to manage moisture content, the peels were sundried before solvent extraction process, which yielded 27% POEE and 50% POWE. Higher recovery in water extract indicates presence of ionic compounds in higher proportion compared to covalent compounds in pomegranate peels. The pH levels of POEE and POWE were 4.20 and 3.77 respectively. The lower pH of POWE was likely due to the higher concentration of ionic compounds in it.

Despite better extraction recovery in POWE, results indicated higher levels of total polyphenol content in POEE (390.05 ± 2.1 mg GAE/g of extract) compared to POWE (330.25 ±1.4 mg GAE/g of extract). Based on this observation, it is anticipated to contain significant amounts of phenol and polyphenol derivatives in pomegranate peels which were confirmed further in the study, with HRLCMS-QTOF analysis. Although phenols and polyphenols are generally ionic, they can form covalent bonds with inorganic compounds (derivatives) reducing their water solubility. Hence, we used a highly polar solvent (water) along with comparatively less polar solvent (ethanol) to maximise recovery of total polyphenols, and compare their phytochemical constituents and bioactive potential. These factors also explain the variations in efficacy and choice of solvents reported in literature for extraction of phenols and polyphenols from food, with no standardised protocol developed so far (**Mojzer *et al*., 2016**).

Identification of phytochemicals in extracts with HRLCMS-QTOF

During chromatography, the analytes formed during ionization is charged through deprotonation in the negative ion mode, and protonation in the positive ion mode. This makes it easier to detect and verify small molecular weight compounds with single known functional groups based on their polarity. In the present phytochemical analysis, presence of large molecular weight compounds, multiple functional groups, was suspected. Hence, the analysis was conducted in both modes. In total, the HRLCMS-QTOF analysis (+ve and –ve mode) of pomegranate peels identified 98 distinct compounds in the ethanol extract and 91 in the water extract as represented the chromatograms in Figure 1 and 2 for POEE and Figure 3 and 4 for POWE . Tables 1 and 2 represent 27 and 71 compounds identified in the POEE on analysis in the negative and positive mode respectively. Similarly, Tables 3 and 4 represent 26 and 65 compounds identified in the POWE on analysis in the negative and positive mode respectively.

Table 1 Phytochemical profile of pomegranate peel ethanol extract analysed by HRLCMS-QTOF in the negative ion mode

Sr. No	Name of compound	Class	Formula	Mass (DB)	m/z	RT	Diff (DB, ppm)	Diff (DB, mDa)
Amino acid derivatives								
1	Glucobrassicin	Alkylglucosinolates	$C_{16}H_{20}N_2O_9S_2$	448.06	447.05	7.32	8.21	3.68
2	O-Carbamoyl-deacetylcephalosporin C	Peptides	$C_{15}H_{20}N_4O_8S$	416.1	415.1	9.822	-5.67	-2.36
Lipid and lipid like molecules								
3	alpha-L-Rhamnosyl-(1->3)-alpha-D-galactosyl-diphosphoundecaprenol	Prenol lipids	$C_{67}H_{112}O_{16}P_2$	1234.7	616.36	21.596	3.38	4.17
4	Methyl (3x,10R)-dihydroxy-11-dodecene-6,8-diynoate 10-glucoside	Fatty acyl glycosides	$C_{19}H_{26}O_9$	398.16	443.16	7.238	-7.21	-2.87
Organic acid derivatives								
5	Alginic acid	Carbohydrate conjugates	$C_{12}H_{20}O_{12}P_2$	418.04	463.04	6.399	-8.01	-3.35
6	Carboxyifosfamide	Cyclophosphamides	$C_7H_{15}Cl_2N_2O_4P$	292.01	291.01	5.941	-6.07	-1.77
7	Chondroitin 6-sulfate	Glycosaminoglycans	$C_{28}H_{42}N_2O_{28}S_2$	918.14	963.12	1.836	14.5	13.31
Organoheterocyclic compounds								
8	3'-N-Acetyl-4'-O-(14-methylpentadecanoyl)fusarochromanone	Benzopyrans	$C_{33}H_{52}N_2O_6$	572.38	617.38	21.275	8.8	5.04
9	Halosulfuron-methyl	Pyrazoles	$C_{13}H_{15}ClN_6O_7S$	434.04	479.04	2.028	0.41	0.18
Polyphenolic compounds								
10	Isoterchebin	Ellagitannins	$C_{41}H_{30}O_{27}$	954.1	953.08	7.516	11.73	11.19
11	Punicacortein B	Ellagitannins	$C_{27}H_{22}O_{18}$	634.08	633.06	6.342	11.51	7.3
12	Punicacortein D	Ellagitannins	$C_{48}H_{28}O_{30}$	1084.1	1083	4.238	8.75	9.48
13	Punicalin	Ellagitannins	$C_{34}H_{22}O_{22}$	782.06	781.04	2.979	10.85	8.49
14	Sanguiin H3	Ellagitannins	$C_{68}H_{48}O_{44}$	1568.2	783.06	5.654	11.99	18.8
15	Sanguiin H7	Ellagitannins	$C_{34}H_{26}O_{23}$	802.09	799.05	5.664	2527.1	2021.9
16	1-O-Galloylpedunculagin	Gallotannins	$C_{41}H_{28}O_{26}$	936.09	935.07	5.905	10.47	9.8
17	2-O-Galloylpunicalin	Gallotannins	$C_{41}H_{26}O_{26}$	934.07	933.05	5.967	11.99	11.2
18	Castacrenin E	Ellagitannins	$C_{47}H_{28}O_{29}$	1056.1	1101.1	3.334	11	11.62
19	Granatin A	Ellagitannins	$C_{34}H_{24}O_{22}$	784.08	783.06	4.24	11.65	9.14
20	Heterophylliin A	Ellagitannins	$C_{34}H_{26}O_{22}$	786.09	785.07	6.104	12.94	10.17
21	Heterophylliin F	Ellagitannins	$C_{68}H_{50}O_{44}$	1570.2	784.07	6.493	11.27	17.7
22	Sanguiin H11	Ellagitannins	$C_{41}H_{28}O_{27}$	952.08	951.06	4.562	10.61	10.1
23	Vescalin	Ellagitannins	$C_{27}H_{20}O_{18}$	632.07	631.05	4.661	6.27	3.96
24	Kurigalin	Gallotannins	$C_{27}H_{24}O_{18}$	636.1	633.06	6.46	3186	2020.2
25	Catechin	Flavonoids	$C_{15}H_{14}O_6$	290.08	290.08	5.64	7.09	-11.04
Phenolic compounds								
26	TS-TM-calix(4)arene	Benzenoids (cyclic oligomer)	$C_{32}H_{32}O_{16}S_4$	800.06	799.05	5.675	-10.98	-8.78
Others								
27	Heparin	Polyanionic compounds	$C_{26}H_{41}NO_{34}S_4$	1039	1083	5.345	950.8	986.98

Table 2 Phytochemical profile of pomegranate peel ethanol extract analysed by HRLCMS-QTOF in the positive ion mode

Sr. No	Name of compound	Class	Formula	Mass (DB)	m/z	RT	Diff (DB, ppm)	Diff (DB, mDa)
Aminoacid derivatives								
1	N(alpha)-Benzyloxycarbonyl-L-leucine	L-leucine derivative	$C_{14}H_{19}NO_4$	265.13	266.14	8.231	6.85	1.82
2	Prolyl-Arginine	Proline derivative	$C_{11}H_{21}N_5O_3$	271.16	294.15	3.766	3.38	0.92
3	N-(1-Deoxy-1-fructosyl)phenylalanine	Phenylalanine derivative	$C_{15}H_{21}NO_7$	327.13	328.14	4.796	7.11	2.33
4	N-(1-Deoxy-1-fructosyl)proline	Proline derivative	$C_{11}H_{19}NO_7$	277.12	278.12	1.823	7.93	2.2
5	Valyl-Tyrosine	Tyrosine derivative	$C_{14}H_{20}N_2O_4$	280.14	303.13	6.787	-0.93	-0.26
Aromatic polycyclic compounds								
6	3alpha,4,7,7alpha-Tetrahydro-4-hydroxy-1H-isoindole-1,3(2H)-dione	Isoindolones	$C_8H_9NO_3$	167.06	190.05	6.441	-6.12	-1.02
Carbohydrate derivatives								
7	Linamarin	Carbohydrate conjugates	$C_{10}H_{17}NO_6$	247.11	248.11	1.501	6.44	1.59
8	Lotaustralin	Carbohydrate conjugates	$C_{11}H_{19}NO_6$	261.12	262.13	2.427	6.77	1.77
9	2-O-Benzoyl-D-glucose	Monosaccharide derivative	$C_{13}H_{16}O_7$	284.09	307.08	5.473	-0.18	-0.05
10	Methyl (R)-9-hydroxy-10-undecene-5,7-diynoate glucoside	O-acyl carbohydrate	$C_{18}H_{24}O_8$	368.15	391.14	10.979	-1.39	-0.51
Lipid and lipid like molecules								
11	3-Methylbutyl 2-furanbutanoate	Long chain fatty acids	$C_{13}H_{20}O_3$	224.14	247.13	8.728	-2.62	-0.59
12	3-Nonanon-1-yl acetate	Fatty acyl derivative	$C_{11}H_{20}O_3$	200.14	223.13	13.931	-5.47	-1.1
13	7,8-Diaminononanoate	Long chain fatty acids	$C_9H_{20}N_2O_2$	188.15	189.16	5.467	7.03	1.32
14	(9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid	Lineolic acid derivative	$C_{18}H_{26}O_3$	290.19	291.19	15.284	7.46	2.17
15	Osmaronin	Fatty acyl glycosides	$C_{11}H_{17}NO_6$	259.11	260.11	1.766	7.44	1.93

16	12-(2,3-Dihydroxycyclopentyl)-2-dodecanone	Fatty acyl derivative	$C_{17}H_{32}O_3$	284.24	307.22	18.179	-0.68	-0.19
17	16-Hydroxy-10-oxohexadecanoic acid	Long chain fatty acids	$C_{16}H_{30}O_4$	286.21	309.2	16.72	-2.23	-0.64
18	16-Oxo-palmitate	Long chain fatty acids	$C_{16}H_{30}O_3$	270.22	293.21	15.362	-2.36	-0.64
19	18-hydroxy-9Z-octadecenoic acid	Long chain fatty acids	$C_{18}H_{34}O_3$	298.25	321.24	19.22	-0.77	-0.23
20	(10S)-Juvenile hormone III diol	Prenol lipids	$C_{16}H_{28}O_4$	284.2	307.19	14.916	-0.01	0
21	GA / Gibberellic acid	Prenol lipids	$C_{19}H_{22}O_6$	346.14	369.13	12.735	-0.48	-0.16
22	Juvenile hormone III	Prenol lipids	$C_{16}H_{26}O_3$	266.19	289.18	14.838	-1.49	-0.4
Nucleoside/ Nucleotide derivatives								
23	5-Methylcytidine	Pyrimidine nucleosides	$C_{10}H_{15}N_3O_5$	257.1	258.11	1.48	2	0.51
24	1-Methylhistidine	Histidine derivative	$C_7H_{11}N_3O_2$	169.09	192.08	6.22	-6.1	-1.03
Organic acid derivatives								
25	Lomustine	Carboxylic acid derivatives	$C_9H_{16}ClN_3O_2$	233.09	256.08	2.722	11.2	2.61
26	AK-toxin I	Carboxylic acid derivatives	$C_{23}H_{27}NO_6$	413.18	414.19	7.477	5.1	2.11
27	D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose	Carboxylic acid derivatives	$C_{10}H_{19}NO_7$	265.12	266.12	1.502	6.33	1.68
28	2E-Decenedioic acid	Organic acid	$C_{10}H_{16}O_4$	200.1	223.1	14.611	-4.7	-0.94
Organoheterocyclic compounds								
29	Cinchoninone	Alkaloid derivative (Cinchona alkaloids)	$C_{19}H_{20}N_2O$	292.16	293.17	1.865	-9.84	-2.87
30	Meteloidine	Alkaloid	$C_{13}H_{21}NO_4$	255.15	256.15	3.441	7.07	1.8
31	Parsonsine	Alkaloid	$C_{22}H_{33}NO_8$	439.22	440.23	9.434	5.26	2.31
32	Isoamericanol A	Benzodioxanes	$C_{18}H_{18}O_6$	330.11	353.1	14.915	-0.72	-0.24
33	Melicopicine	Benzoquinolines	$C_{18}H_{19}NO_5$	329.13	352.12	15.141	4.48	1.48
34	Ketotifen	Cycloheptathiophenes	$C_{19}H_{19}NOS$	309.12	310.13	4.784	-1.69	-0.52
35	5(6)-Pentyl-1,4-dioxan-2-one	Dioxanes	$C_9H_{16}O_3$	172.11	195.1	13.664	-5.77	-0.99
36	2-Heptylfuran	Furans	$C_{11}H_{18}O$	166.14	189.13	7.369	-4.87	-0.81
37	15-Hydroxymarasmen-3-one	Furofurans	$C_{15}H_{20}O_4$	264.14	265.14	6.277	3.26	0.86
38	Dihydrozeatin	Imidazopyrimidines	$C_{10}H_{15}N_5O$	221.13	222.13	5.423	11.48	2.54
39	Levoglucosan	Oxepanes	$C_6H_{10}O_5$	162.05	185.04	5.345	1.74	0.28
40	3',4'-Dihydrodiol	Phenylhydantoins	$C_{15}H_{14}N_2O_4$	286.1	309.08	11.397	-1.24	-0.36
41	Meperidine (pethidine)	Phenylpiperidines	$C_{15}H_{21}NO_2$	247.16	248.16	9.563	6.65	1.64
42	Allixin	Pyrans	$C_{12}H_{18}O_4$	226.12	249.11	7.237	2.67	0.6
43	Isoniazid	Pyridines and derivatives	$C_6H_7N_3O$	137.06	160.05	6.207	-10.95	-1.5
44	7,8-Dihydro-7,8-dihydroxykynurenate	Quinolines and derivatives	$C_{10}H_9NO_5$	223.05	224.05	7.62	5.91	1.32
45	3-Methyl-3H-imidazo[4,5-f]quinoxalin-2-amine	Quinoxalines	$C_{10}H_9N_5$	199.09	222.07	8.265	4.48	0.89
Phenolic compounds								
46	Noroxycodone	Benzenoids (Phenanthrene derivative)	$C_{17}H_{19}NO_4$	301.13	324.12	8.904	-0.54	-0.16
47	3-Methyl-4-phenyl-3-buten-2-one	Benzenoids (phenols)	$C_{11}H_{12}O$	160.09	161.1	16.083	6.17	0.99
48	3-tert-Butyl-5-methylcatechol	Benzenoids (phenols)	$C_{11}H_{16}O_2$	180.12	181.12	13.914	6.67	1.2
49	Butylparaben	Benzenoids (phenols)	$C_{11}H_{14}O_3$	194.09	195.1	8.811	6.68	1.3
50	Halocins	Benzenoids (phenols)	$C_{21}H_{23}NO$	305.18	306.19	4.534	-12.03	-3.67
51	Isobutyl N-methylanthranilate	Benzenoids (phenols)	$C_{12}H_{17}NO_2$	207.13	208.13	6.803	10.03	2.08
52	Uralenneoside	Benzenoids (phenols)	$C_{12}H_{14}O_8$	286.07	309.06	9.318	-0.97	-0.28
53	Methyl 3-(2,3-dihydroxy-3-methylbutyl)-4-hydroxybenzoate	Benzenoids (p-hydroxybenzoic acid derivative)	$C_{13}H_{18}O_5$	254.12	255.12	15.727	9.7	2.46
54	Metoprolol	Benzenoids (tyrosol derivative)	$C_{15}H_{25}NO_3$	267.18	290.17	9.076	-1.21	-0.32
55	3,4-Dimethoxy-1,2-benzenedicarboxylic acid	p-methoxybenzoic acid derivative	$C_{10}H_{10}O_6$	226.05	249.04	8.07	-2.71	-0.61
Polyphenolic compounds								
56	Quercetin	Flavonoid	$C_{15}H_{10}O_7$	302.04	303.05	8.455	8.74	2.64
57	Disperse Blue 1	Polyketide (anthraquinone dye)	$C_{14}H_{12}N_4O_2$	268.1	291.08	6.771	3.65	0.98
58	Dothistromin	Polyketide (anthraquinone)	$C_{18}H_{12}O_9$	372.05	373.05	15.188	6.51	2.42
59	trans-Grandmarin isovalerate	Pyranocoumarins	$C_{20}H_{24}O_7$	376.15	377.16	14.594	4.75	1.79
60	Licoagrodione	Stilbenes	$C_{20}H_{20}O_6$	356.13	357.13	10.031	5.72	2.04
61	4-Hydroxy-3,5,4'-trimethoxystilbene	Stilbenes	$C_{17}H_{18}O_4$	286.12	309.11	12.348	-0.28	-0.08
62	Caffeic acid	Cinnamic acid derivatives	$C_9H_8O_4$	180.04	181.04	15.69	4.35	-
Others								
63	(R)-Reticuline	Alkaloid precursor	$C_{19}H_{23}NO_4$	329.16	352.15	10.393	-1.33	-0.44
64	Tsibulin 2	Carbonyl compounds	$C_{11}H_{18}O_2$	182.13	205.12	14.839	-5.34	-0.97
65	U 0521	Carbonyl compounds	$C_{10}H_{12}O_3$	180.08	181.08	14.682	5.87	1.06
66	6alpha,9-Difluoro-11beta-hydroxypregn-4-ene-3,20-dione	Corticosteroid hormone	C21H28F_2O_3	366.2	367.21	12.676	5.58	2.04
67	2-Formaminobenzoylacetate	Ethyl ester	$C_{10}H_9NO_4$	207.05	208.06	8.149	4.14	0.86
68	Bis(4-isothiocyanatobutyl) disulfide	Isothiocyanates	$C_{10}H_{16}N_2S_4$	292.02	293.03	6.991	-2.45	-0.72
69	2alpha,3alpha-(Difluoromethylene)-5alpha-androstan-17beta-ol acetate	Steroid hormone	$C_{22}H_{32}F_2O_2$	366.24	367.25	13.992	-4.05	-1.48
70	N-Guanylhistamine	Substituted amines	$C_6H_{11}N_5$	153.1	176.09	2.143	-1.54	-0.24
71	(+/-)-3-[(2-methyl-3-furyl)thio]-2-butanone	Thioethers	$C_9H_{12}O_2S$	184.06	185.06	1.141	-6.77	-1.25

Legend: - Not determined

Table 3 Phytochemical profile of pomegranate peel water extract analysed by HRLCMS-QTOF in the negative ion mode

Sr. No	Name of compound	Class	Formula	Mass (DB)	m/z	RT	Diff (DB, ppm)	Diff (DB, mDa)
Amino acid derivatives								
1	4-Hydroxyglucobrassicin	Alkylglucosinolates	$C_{16}H_{20}N_2O_{10}S_2$	464.06	463.05	7.351	1.02	0.47
Carbohydrate derivatives								
2	3,4-Hexahydroxydiphenoylarabinose	Complex carbohydrates	$C_{19}H_{16}O_{13}$	452.06	497.05	6.061	10.47	4.73
Organic acid derivatives								
3	Chondroitin 6-sulfate	Glycosaminoglycans	$C_{28}H_{42}N_2O_{28}S_2$	918.14	963.13	2.466	9.26	8.5
Organoheterocyclic compounds								
4	Diltiazem	Benzothiazepines	$C_{22}H_{26}N_2O_4S$	414.16	459.16	7.323	-2.42	-1
5	Glyceryl lactopalmitate	Phenylpyrazoles	$C_{20}H_{16}N_6O_2S$	404.11	449.1	7.515	-2.63	-1.06
6	Halosulfuron-methyl	Pyrazole	$C_{13}H_{15}ClN_6O_7S$	434.04	479.04	3.626	-7.47	-3.24
Polyphenolic compounds								
7	1-O-Caffeoyl-(b-D-glucose 6-O-sulfate)	Cinnamic acid derivatives	$C_{15}H_{18}O_{12}S$	422.05	481.06	2.13	14.01	5.91
8	2-O-Galloylpunicalin	Gallotannins	$C_{41}H_{26}O_{26}$	934.07	933.06	5.284	5.32	4.97
9	Castacrenin E	Ellagitannins	$C_{47}H_{28}O_{29}$	1056.1	1101.1	4.612	7.47	7.89
10	Emblicanin B	Ellagitannins	$C_{34}H_{20}O_{22}$	780.04	777.02	4.778	2584.9	2011.1
11	Granatin A	Ellagitannins	$C_{34}H_{24}O_{22}$	784.08	783.06	6.876	6.66	5.22
12	Isoterchebin	Ellagitannins	$C_{41}H_{30}O_{27}$	954.1	953.08	8.037	10.46	9.98
13	Punicacortein D	Ellagitannins	$C_{48}H_{28}O_{30}$	1084.1	1083.1	5.137	5.67	6.15
14	Punicalin	Ellagitannins	$C_{34}H_{22}O_{22}$	782.06	781.05	7.524	0.95	0.74
15	Sanguiin H9	Ellagitannins	$C_{54}H_{42}O_{36}$	1266.1	632.06	5.808	7.1	8.99
16	Sanguisorbic acid dilactone	Ellagitannins	$C_{21}H_{10}O_{13}$	470.01	469	7.852	10.16	4.77
17	Vescalin	Ellagitannins	$C_{27}H_{20}O_{18}$	632.07	631.05	7.202	0.46	0.29
18	Punicalagin	Ellagitannins	$C_{48}H_{28}O_{30}$	1084.06	1084.06	5.186	-	-4.26
Phenolic compounds								
19	Difenacoum	Benzenoids (phenylnaphthalenes)	$C_{31}H_{24}O_3$	444.17	443.16	8.187	6.79	3.02
20	TS-TM-calix(4)arene	Benzenoids (cyclic oligomer)	$C_{32}H_{32}O_{16}S_4$	800.06	799.06	6.485	-10.45	-8.36
21	Flufenoxuron	Benzoylurea	$C_{21}H_{11}ClF_6N_2O_3$	488.04	533.03	6.003	13.66	6.67
22	Monomethyl phthalate	Benzoic acid esters	$C_9H_8O_4$	180.04	-	22.035	6.08	-
Others								
23	Ceftibuten	Beta lactams	$C_{15}H_{14}N_4O_6S_2$	410.04	469.06	6.397	-14.7	-6.03
24	Epithienamycin F	Beta lactams	$C_{13}H_{18}N_2O_8S_2$	394.05	453.06	6.305	-0.03	-0.01
25	Oxdemetonmethyl	Organothiophosphates	$C_6H_{15}O_4PS_2$	246.01	291.01	6.934	5.1	1.26
26	5-Hydroxythiophene-2-carbonyl-CoA	Oxidoreductase enzyme	$C_{26}H_{38}N_7O_{18}P_3S_2$	893.09	892.08	5.131	6.84	6.11

Legend: - Not determined

Table 4 Phytochemical profile of pomegranate peel water extract analysed by HRLCMS-QTOF in the positive ion mode

Sr. No	Name of compound	Class	Formula	Mass (DB)	m/z	RT	Diff (DB, ppm)	Diff (DB, mDa)
Aminoacid derivatives								
1	Lysyl-Threonine	Threonine derivative	$C_{10}H_{21}N_3O_4$	247.15	248.16	9.495	-6.35	-1.57
2	N-(1-Deoxy-1-fructosyl)phenylalanine	Phenylalanine derivative	$C_{15}H_{21}NO_7$	327.13	328.14	4.708	7.68	2.51
3	N-(1-Deoxy-1-fructosyl)proline	Proline derivative	$C_{11}H_{19}NO_7$	277.12	278.12	1.699	7.66	2.12
Carbohydrate derivatives								
4	Actinamine	Aminocyclitols (pseudosugars)	$C_8H_{18}N_2O_4$	206.13	207.13	16.85	-0.04	-0.01
5	Lotaustralin	Carbohydrate conjugates	$C_{11}H_{19}NO_6$	261.12	262.13	2.01	6.86	1.79
6	Sorbose	Complex sugars	$C_6H_{12}O_6$	180.06	203.05	12.544	11.19	2.01
Lipid and lipid like molecules								
7	(x)-1-Nonen-3-yl acetate	Carboxylic acid esters	$C_{11}H_{20}O_2$	184.15	207.14	7.244	-3.83	-0.71
8	Gibberellin A75	Diterpenoids	$C_{19}H_{24}O_8$	380.15	403.14	14.802	1.61	0.61
9	17beta-Methylestra-1,3,5(10)-trien-3-ol	Ergostane steroids	$C_{19}H_{26}O$	270.2	293.19	15.613	-10.61	-2.87
10	Osmaronin	Fatty acyl glycosides	$C_{11}H_{17}NO_6$	259.11	260.11	1.723	6.73	1.74
11	Dihydrozeatin-O-glucoside	Fatty acyl glycosides	$C_{16}H_{25}N_5O_6$	383.18	384.19	5.249	6.21	2.38
12	16-Hydroxy-10-oxohexadecanoic acid	Long chain fatty acids	$C_{16}H_{30}O_4$	286.21	309.2	16.701	1.75	0.5
13	16-Oxo-palmitate	Long chain fatty acids	$C_{16}H_{30}O_3$	270.22	293.21	13.543	0.04	0.01
14	3-Methylbutyl 2-furanbutanoate	Long chain fatty acids	$C_{13}H_{20}O_3$	224.14	247.13	10.923	-1.21	-0.27
15	3-Nonanon-1-yl acetate	Fatty acyl derivative	$C_{11}H_{20}O_3$	200.14	223.13	13.911	-2.9	-0.58
16	2E-Decenedioic acid	Long chain fatty acids	$C_{10}H_{16}O_4$	200.1	223.09	14.578	-3.79	-0.76
17	(9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid	Lineolic acid derivative	$C_{18}H_{26}O_3$	290.19	291.19	16.702	8.34	2.42
18	Geijerone	Monoterpenoids	$C_{12}H_{18}O$	178.14	201.13	15.312	-3.66	-0.65
19	Nigakilactone B	Triterpenoids	$C_{22}H_{32}O_6$	392.22	415.21	16.455	1.03	0.41
Nucleoside derivatives								
20	Succinoadenosine	Adenosine derivative	$C_{14}H_{17}N_5O_8$	383.11	384.11	5.263	5.57	2.13
21	5-Methylcytidine	Cytidine derivative	$C_{10}H_{15}N_3O_5$	257.1	258.11	1.443	2.98	0.77
Organic acid derivatives								
22	Prolyl-Arginine	Amino acid analogue	$C_{11}H_{21}N_5O_3$	271.16	294.15	3.644	5.03	1.36
23	7,8-Diaminononanoate	amino monocarboxylic acid	$C_9H_{20}N_2O_2$	188.15	189.16	5.437	7.66	1.44
24	D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose	Carboxylic acid derivatives	$C_{10}H_{19}NO_7$	265.12	266.12	1.444	6.02	1.6
25	2,4-Hexadienyl isobutyrate	Carboxylic acid derivatives	$C_{10}H_{16}O_2$	168.12	191.1	15.732	-4.18	-0.7

26	Lomustine	Carboxylic acid derivatives	$C_9H_{16}ClN_3O_2$	233.09	256.08	2.439	12.47	2.91
27	Valganciclovir	Carboxylic acids and derivatives	$C_{14}H_{22}N_6O_5$	354.17	377.16	14.58	-7.25	-2.57
28	Propyl levulinate	Ketoacids	$C_8H_{14}O_3$	158.09	181.08	14.629	-5.93	-0.94
Organoheterocyclic compounds								
29	5,6,8,14-Tetradehydro-3,6-dimethoxy-17-methyl-morphinan-4,7-diol	Alkaloid derivative (morphinans)	$C_{19}H_{23}NO_4$	329.16	352.15	10.398	0.13	0.04
30	Meteloidine	Alkaloid	$C_{13}H_{21}NO_4$	255.15	256.15	2.085	8.97	2.29
31	2-Acetyl-1,5,6,7-tetrahydro-6-hydroxy-7-(hydroxymethyl)-4H-azepine-4-one	Azepines	$C_9H_{13}NO_4$	199.08	222.07	8.057	-1.51	-0.3
32	3',4'-Dihydrodiol	Azolidines	$C_{15}H_{14}N_2O_4$	286.1	309.08	11.302	-0.35	-0.1
33	Carbendazim	Benzimidazoles	$C_9H_9N_3O_2$	191.07	192.08	6.141	7.4	1.41
34	3-(3,4-Methylenedioxyphenyl)propenal	Benzodioxoles	$C_{10}H_8O_3$	176.05	177.05	15.369	7.14	1.26
35	Ketotifen	Cycloheptathiophenes	$C_{19}H_{19}NOS$	309.12	310.13	4.695	-0.75	-0.23
36	5(6)-Pentyl-1,4-dioxan-2-one	Dioxanes	$C_9H_{16}O_3$	172.11	195.1	13.411	-4.58	-0.79
37	2-Formaminobenzoylacetate	Ester	$C_{10}H_9NO_4$	207.05	208.06	8.064	6.06	1.25
38	2-Heptylfuran	Furans	$C_{11}H_{18}O$	166.14	189.13	7.282	-4.65	-0.77
39	15-Hydroxymarasmen-3-one	Furofurans	$C_{15}H_{20}O_4$	264.14	265.15	6.209	2.39	0.63
40	Theophylline	Imidazopyrimidines	$C_7H_8N_4O_2$	180.06	181.07	5.876	3.44	0.62
41	Imiquimod	Imidazoquinolines	$C_{14}H_{16}N_4$	240.14	263.13	20.233	0.19	0.04
42	3alpha,4,7,7alpha-Tetrahydro-4-hydroxy-1H-isoindole-1,3(2H)-dione	Isoindolines	$C_8H_9NO_3$	167.06	190.05	6.329	-4.32	-0.72
43	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid	Lactones	$C_{12}H_{20}O_4$	228.14	251.13	17.002	-0.58	-0.13
44	Allixin	Pyrans	$C_{12}H_{18}O_4$	226.12	249.11	8.632	-2.07	-0.47
45	Flutriafol	Triazols	$C_{16}H_{13}F_2N_3O$	301.1	302.11	2.132	6.1	1.84
Phenolic compounds								
46	2-O-Benzoyl-D-glucose	Benzenoids (benzoic acid derivative)	$C_{13}H_{16}O_7$	284.09	307.08	5.434	-0.78	-0.22
47	3-Methyl-4-phenyl-3-buten-2-one	Benzenoids (benzoic acid derivative)	$C_{11}H_{12}O$	160.09	183.08	16.495	-5.92	-0.95
48	3,4-Dimethoxy-1,2-benzenedicarboxylic acid	Benzenoids (benzoic acid derivative)	$C_{10}H_{10}O_6$	226.05	249.04	7.902	-3.2	-0.72
49	Methyl 3-(2,3-dihydroxy-3-methylbutyl)-4-hydroxybenzoate	Benzenoids (benzoic acid derivative)	$C_{13}H_{18}O_5$	254.12	255.12	15.657	8.87	2.25
50	Methyl N-methylanthranilate	Benzenoids (benzoic acid derivatives)	$C_9H_{11}NO_2$	165.08	188.07	5.974	-6.44	-1.06
51	Halocins	Benzenoids (diphenylmethanes)	$C_{21}H_{23}NO$	305.18	306.19	4.398	-11.27	-3.44
52	(±)-threo-1-(4-Hydroxyphenyl)-1,2,3-propanetriol	Benzenoids (phenols)	$C_9H_{12}O_4$	184.07	207.06	13.434	-3.77	-0.69
53	5-Hydroxydopamine	Benzenoids (phenols)	$C_8H_{11}NO_3$	169.07	192.06	2.463	-4.33	-0.73
54	Anastrozole	Benzenoids (phenylpropanes)	$C_{17}H_{19}N_5$	293.16	294.17	13.64	12.31	3.61
55	Colchicine	Tropones	$C_{22}H_{25}NO_6$	399.17	400.18	6.046	-11.26	-4.49
Polyphenolic compounds								
56	cis-Sinapic acid	Cinnamic acid derivative	$C_{11}H_{12}O_5$	224.07	247.06	13.755	-1.36	-0.3
57	Quercetin	Flavonoids	$C_{15}H_{10}O_7$	302.04	303.05	8.635	7.66	2.31
58	Ellagic acid	Phenolic acid derivative	$C_{14}H_6O_8$	302.01	303.01	2.377	8.26	2.5
59	Disperse Blue 1	Polyketide (anthraquinone dye)	$C_{14}H_{12}N_4O_2$	268.1	291.08	6.713	5.44	1.46
60	N'-Hydroxyneosaxitoxin	Saxitoxins	$C_{10}H_{17}N_7O_6$	331.12	332.13	3.401	-0.89	-0.29
61	Saxitoxin	Saxitoxins	$C_{10}H_{17}N_7O_4$	299.13	300.14	6.352	-1.58	-0.47
62	Caffeic acid	Cinnamic acid derivatives	$C_9H_8O_4$	180.04	181.04	15.70	4.55	-
Others								
63	Discadenine	Enzyme	$C_{14}H_{20}N_6O_2$	304.16	305.17	4.247	10.69	3.25
64	Bis(4-isothiocyanatobutyl) disulfide	Isothiocyanates	$C_{10}H_{16}N_2S_4$	292.02	293.03	6.857	-0.74	-0.22
65	(+/-)-3-[(2-methyl-3-furyl) thio]-2-butanone	Thioester	$C_9H_{12}O_2S$	184.06	185.06	1.23	-6.35	-1.17

Legend: - Not determined

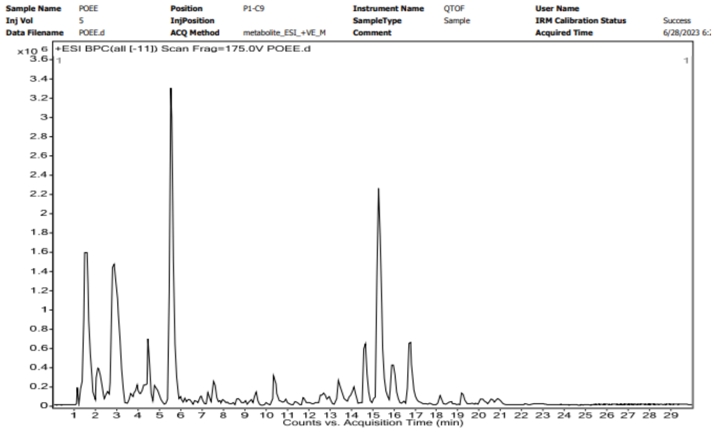

Figure 1 Chromatogram of pomegranate peel ethanol extract in positive ion mode

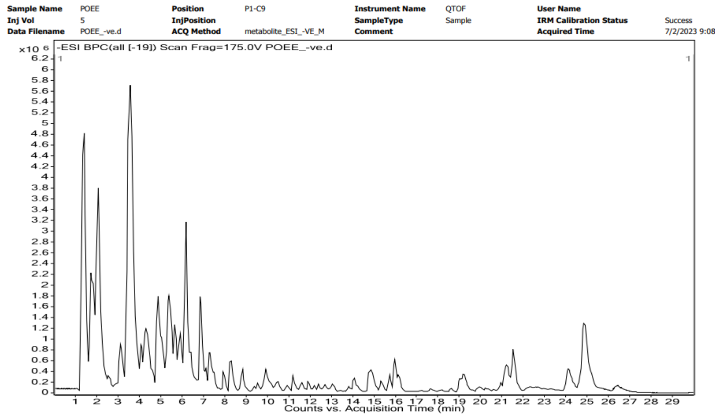

Figure 2 Chromatogram of pomegranate peel ethanol extract in negative ion mode

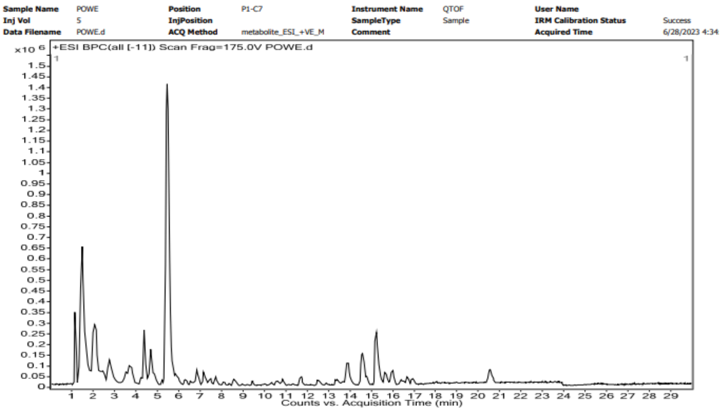

Figure 3 Chromatogram of pomegranate peel water extract in positive ion mode

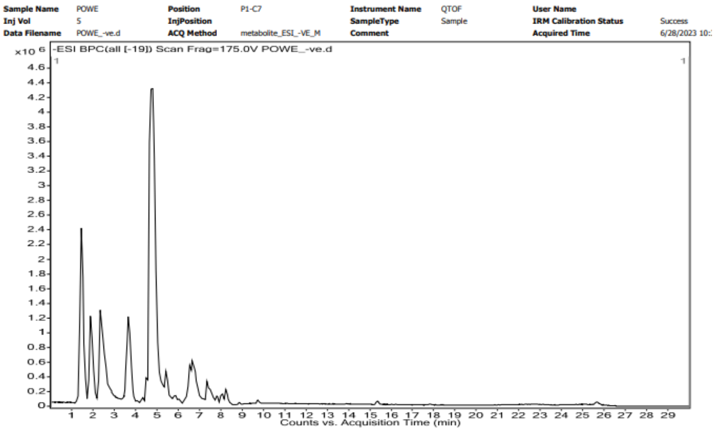

Figure 4 Chromatogram of pomegranate peel water extract in negative ion mode

Overall, 21 phenolic and 31 polyphenolic compounds were identified in the pomegranate peel extracts. The phenolic compounds included 6 phenolic acids, 6 benzoic acid derivatives (3 benzene derivative, 1 benzoic acid ester, 1 p-hydroxybenzoic acid derivative and 1 p-methoxybenzoic acid derivative), 1 cyclic oligomer, 1 diphenylmethane, 1 gallolyl ester, 1 phenanthrene derivative, 1 phenylnaphthalene, 1 tyrosol derivative, 1 phenylpropane, 1 benzoylurea, and 1 tropone. The polyphenolic compounds included 19 tannins, 3 cinnamic acid derivatives, 2 anthraquinone, 2 saxitoxins, 2 stilbenes, 2 flavonoids and 1 pyranocoumarin. Additionally, the HRLCMS-QTOF analysis identified 21 lipid derivatives (belonging to 8 different classes), 12 organic acid derivatives (belonging to 6 different classes), and 31 organoheterocyclic compounds (belonging to 25 different classes).

The present study showed abundance of tannins, organic acids and phenolic acid derivatives in the pomegranate peel extracts. In addition to these compounds, published literature reports abundance of flavonoids in pomegranate peels (**Mo *et al.*, 2022; Marra *et al.*, 2022**). However, only two flavonoids (catechin and quercetin) were detected in this study. The phytochemical constituents of plants can vary based on soil conditions (pH, acidity, fertility, microbiome and moisture), environmental factors (temperature, humidity) during growth or variation in cultivars (**Man *et al.*, 2021**).

Irrespective of the analytical methods used, literature consistently reports abundance of gallic acids, ellagic acids and tannins in pomegranate peels (**Singh *et al.*, 2023**). Although gallic acid was not identified distinctly in our analysis, its presence in pomegranate peels is apparent. Gallic acid is the molecular precursor of both gallotannins and ellagitannins, and hydrolysis of ellagitannins generates ellagic acid (which again is a dimeric derivative of gallic acid). The HRLCMS-QTOF analysis identified 16 ellagitannins (isoterchebin, Punicacortein B and D, Punicalagin, Punicalin, Sanguiin H3, H7, H9 and H11, Castacrenin E, Emblicanin B, Heterophylliin A and F, Sanguisorbic acid dilactone, Vescalin and Granatin A) and 3 gallotannins (1-O-Galloylpedunculagin, 2-O-Galloylpunicalin and Kurigalin), in addition to ellagic acid in this study. Ellagitannins are widely reported group of bioactive polyphenols exhibiting anti-inflammatory, anticancer, antioxidant and antimicrobial properties (**Banc *et al.*, 2023**). Among the ellagitannins, punicalagin and sanguiin H6 are most investigated, and described as promising natural compounds with an array of possibilities in pharmacological interventions (**Gesek *et al.*, 2020; Xu *et al.*, 2021; Kiran *et al.*, 2024**). Sanguiin H11 (of the 4 sanguiins detected in this study) is associated with neuroprotective and anti-inflammatory properties (**Song *et al.*, 2019; Konishi *et al.*, 2000**). Punicalin has shown potential in wound healing, immune-modulation and treatment of cancers (**Kumar *et al.*, 2022a; Colic *et al.*, 2021; Sharma *et al.*, 2022**). In general, phenolic acids and their derivatives show good anti-oxidation potential, anti-inflammatory and antimicrobial properties (**Kumar and Goel 2019**).

Total antioxidant activity

The radical scavenging activity of POEE and POWE are represented in Table 5. Both POEE and POWE showed significant antioxidant activity at different concentrations. At lower concentrations (200 and 400 µg/mL) POEE and POWE exhibited activity higher than ascorbic acid. However, at higher concentrations (800 µg/mL and 1000 µg/mL), their activity was slightly lower, yet comparable to ascorbic acid. The IC_{50} values further confirm the excellent antioxidant potential of extracts with strongest activity observed for POWE (199.94 µg/mL), followed by POEE (256.84 µg/mL). Ascorbic acid had a significantly higher IC_{50} value (421.68 µg/mL) indicating lower antioxidant activity compared to both extracts.

The antioxidant activity of these extracts can be primarily attributed to polyphenols, particularly ellagitannins. Notable compounds such as sanguiin H-6 and H-11 are reported for their exceptional antioxidant potential (**Banc *et al.*, 2023; Gesek *et al.*, 2020; Song *et al.*, 2019**). Additionally, other polyphenols like ellagic acid, punicalagin (ellagitannins), along with catechin (a flavonoid), are also recognized for their antioxidant properties (Siddiqui *et al.*, 2024). The hydroxyl groups in these polyphenols effectively scavenge reactive oxygen species (**Gesek *et al.*, 2020**). Catechins are known for their varied bioactive potential including oxidative stress mitigation properties which aids cardiovascular health and prevents cancer (**Siddiqui *et al.*, 2024**). Although present in smaller amounts, alkaloids and terpenoids in pomegranate peels also contribute significantly to its antioxidant potential (**Mo *et al.*, 2022**).

Our results suggest that pomegranate peel extracts, particularly POWE, can be used as a potent natural antioxidants with potential applications in various industries. Literature reviews have also described numerous studies showcasing the antioxidant potential of pomegranate peels (**Ain *et al.*, 2023; Singh *et al.*, 2023**). A key application suggested by these reviews is in the preservation of meat and fish products. Pomegranate peel extracts can prevent lipid peroxidation by blocking the radical chain reaction during the oxidation process, thereby preserving quality and extending the shelf life of food. Thus this study, along with above reviewed studies, supports the potential of pomegranate peel extracts to possibly replace synthetic antioxidants, given the high demand for natural antioxidants in global market.

Table 5 Radical scavenging activity of pomegranate peel extracts

Concentration in µg/mL	% Inhibition		
	POEE	**POWE**	**Ascorbic Acid**
200	48.9 ± 1.12	48.86 ± 0.75	28.40 ± 2.29
400	54.54 ± 3.21	59.09 ± 0.48	48.86 ± 1.27
600	63.63 ± 0.95	61.36 ± 1.07	67.04 ± 3.23
800	76.13 ± 2.32	72.72 ± 1.28	87.5 ± 3.25
1000	81.81 ± 0.59	76.13 ± 1.32	92.045 ± 1.97
IC_{50} values	256.84 ± 5.5 µg/mL	199.94 ± 6.7 µg/mL	421.68 ± 15.3 µg/mL

Legend: - POEE- Pomegranate peel ethanol extract, POWE- Pomegranate peel water extract, *Mean value ±SD, n=3

Antibacterial activity

The antibacterial activity of POEE and POWE is represented in Table 6. The MIC and MBC of extracts against test pathogens were in the range of 10 and 40 mg/mL. For *Staphylococcus aureus* NCIM 2079, *Salmonella enterica* NCIM 5255, *Proteus vulgaris* NCIM 2027 and *Pseudomonas aeruginosa* NCIM 2036, the inhibitory as well as bactericidal concentration was 20 mg/mL for both extracts. Other isolates required higher concentration of POEE or POWE for bactericidal effect as compared to inhibitory effect. Despite differences in the recovery % of extracts and total polyphenolic content, there was no notable difference observed in the antibacterial activities of POEE and POWE. This indicates that components responsible for antibacterial activity were extracted in both solvents with equal efficiency.

Literature correlates antibacterial efficacy of pomegranate peels with abundance of phenolic acids and polyphenols. Also, alkaloids and terpenoids are reported as significant contributors to this effect (**Singh *et al.*, 2023**). Polyphenols, in general, promote precipitation of microbial membrane proteins and inhibits glycosyl transferases leading to cell disintegration. Punicalagins bind to the functional domains of PhcA (the bacterial transcriptional regulator), causing impairment of microbial metabolic and regulatory functions (**Siddiqui *et al.*, 2024; Ain *et al.*, 2023**). Flavonoids rupture cell membranes and block vital enzymes (**Kumar *et al.*, 2022b**). The phenolic acids diffuse through cell membranes and cause cytoplasmic acidification. However, increase in hydroxyl groups of phenolic acids like hydroxybenzoic acid reduces its antibacterial potential. Similarly, absence of double bond of the side chain impacts activity of hydroxycinnamic acid (**Banc *et al.*, 2023; Siddiqui *et al.*, 2024**).

Kiran *et al.*, (2024) described antimicrobial activity of punicalagin against multi-drug resistant pathogens. Moreover, with the threat of growing antibiotic resistance and the lack of new antimicrobials, sanguine H6 (ellagitannin) and ellagic acid are believed to be the most promising agents, at present, with no unpleasant side effects (**Gesek *et al.*, 2021**). Collectively, our findings coupled with these reports make pomegranate peel extracts valuable in food preservation and potential therapeutic applications.

Table 6 Antibacterial activity of pomegranate peel extracts

Bacteria	POEE		POWE	
	MIC	MBC	MIC	MBC
***Staphylococcus aureus* NCIM 2079**	20	20	20	20
***Bacillus cereus* NCIM 2155**	30	40	30	40
***Salmonella enterica* NCIM 5255**	20	20	20	20
***Listeria monocytogenes* NCIM 5260**	20	30	20	30
***Escherichia coli* NCIM 2065**	20	30	20	30
***Proteus vulgaris* NCIM 2027**	20	20	20	20
***Shigella flexneri* NCIM 5265**	10	20	10	20
***Pseudomonas aeruginosa* NCIM 2036**	20	20	20	20
Lactobcillus casei var shirota	<800	<800	<800	<800

Legend: POEE- Pomegranate peel ethanol extract; POWE- Pomegranate peel water extract; MIC and MBC are represented as mg/ml

Effect of pomegranate peel extracts on *Lactobacillus casei var shirota*

The MIC and MBC of POWE and POEE is greater than 800mg/mL against *Lactobacillus casei var shirota*. The interaction between pomegranate peel extracts and *L casei var shirota* is a positive sign for evolving functional foods. Punicalagin the most abundant ingredient in the peel extract has anti-inflammatory, anti-atherosclerotic, anticancer activity (**Al-Hindi. R. *et al.*, 2020**). **Ibrahim. A., *et al*., (2020)** have reported prebiotic like effect of pomegranate peel in bioyoghurt containing *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and *Bifidobactium bifidum*. The high antioxidant activity, presence of various polyphenols in pomegranate peel will help in reducing gut inflammation and oxidative stress, thus supporting a healthier gut environment.

CONCLUSION

In conclusion, the HRLCMS-QTOF analysis confirmed the rich phytochemical profile of pomegranate peels with abundance of a variety of phenolic and polyphenolic compounds and a significant amount of lipid derivatives, organic acid derivatives, and organoheterocyclic compounds. The negligible price of pomegranate waste and their radical scavenging potential exceeding ascorbic acid is in itself a motivation for solving problems of waste management, with added economic advantage due to surpassing disposal efforts and formulation of innovative functional foods. Also, given the safety, antioxidant capacity and antimicrobial potential of pomegranate peels, it can be added to variety of food products to improve their quality and shelf life.

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REFERENCES

Ain HB U, Tufail T, Bashir S, Ijaz N, Hussain M, Ikram A, Farooq MA, Saewan SA (2023) Nutritional importance and industrial uses of pomegranate peel: A critical review. Food Sci Nutr 11(6):2589–2598. https://doi.org/10.1002/fsn3.3320
Al-Hindi, R. R., & Abd El Ghani, S. (2020). Production of functional fermented milk beverages supplemented with pomegranate peel extract and probiotic lactic acid bacteria. *Journal of Food Quality*, *2020*(1), 4710273. https://doi.org/10.1155/2020/4710273
Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48(Suppl_1), 5-16. https://doi.org/10.1093/jac/48.suppl_1.5
APEDA Report (2023) APEDA facilitates export of first trial shipment of fresh pomegranate to USA via air; Increase in pomegranate exports to USA would result in higher price realisation and increase in farmers' income: Encouraging response from importers in USA. Ministry of Commerce & Industry. Available at https://www.pib.gov.in/PressReleasePage.aspx?PRID=1946623 (accessed July 9, 2024).
Araujo, N. M. P., Arruda, H. S., Dos Santos, F. N., de Morais, D. R., Pereira, G. A., & Pastore, G. M. (2020). LC-MS/MS screening and identification of bioactive compounds in leaves,pulp, and seed from Eugenia calycina Cambess. Food Research International, 137, 109556. https://doi.org/10.1016/j.foodres.2020.109556
Banc R, Rusu ME, Filip L, Popa DS (2023) The impact of ellagitannins and their metabolites through gut microbiome on the gut health and brain wellness within the gut–brain axis. Foods 12(2):270. https://doi.org/10.3390/foods12020270
Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
Colic M, Mihajlović D, Bekić M, Marković M, Dragišić B, Tomić S, Miljuš N, Šavikin K, Škrbić R (2021) Immunomodulatory activity of punicalagin, punicalin, and ellagic acid differs from the effect of pomegranate peel extract. Molecules 27(22):7871. https://doi.org/10.3390/molecules27227871
FAO Report (2015) global initiative on food loss and waste reduction. Food and Agriculture Organization of the United Nations FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy. Available at https://openknowledge.fao.org/server/api/core/bitstreams/57f76ed9-6f19-4872-98b4-6e1c3e796213/content (accessed July 9, 2024)
Galeano Garcia, P., Neves dos Santos, F., Zanotta, S., Eberlin, M. N., & Carazzone, C. (2018). Metabolomics of Solanum lycopersicum infected with Phytophthora infestans leads to early detection of late blight in asymptomatic plants. Molecules, 23(12), 3330. https://doi.org/10.3390/molecules23123330
Ge S, Duo L, Wang J, Yang J, Li Z, Tu Y (2021) A unique understanding of traditional medicine of pomegranate, *Punica granatum* L. and its current research status. J Ethnopharmacol 271:113877. https://doi.org/10.1016/j.jep.2021.113877
Gesek J, Jakimiuk K, Atanasov AG, Tomczyk M (2020) Sanguiins—promising molecules with broad biological potential. Int J Mol Sci 22(23):12972. https://doi.org/10.3390/ijms222312972
Gil-Martín E, Forbes-Hernández T, Romero A, Cianciosi D, Giampieri F, Battino M (2022) Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. Food Chem 378:131918. https://doi.org/10.1016/j.foodchem.2021.131918
Ibrahim, A., Awad, S., & El-Sayed, M. (2020). Impact of pomegranate peel as prebiotic in bio-yoghurt. *British Food Journal*, *122*(9), 2911-2926. https://doi.org/10.1108/BFJ-04-2019-0296
Kedare SB, Singh RP (2011) Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48(4):412–422. https://doi.org/10.1007/s13197-011-0251-1
Khoualdia B, Ben-Ali S, Hannachi A (2020) Pomegranate arils osmotic dehydration: Effect of pre-drying on mass transfer. J Food Sci Technol 57(6):2129–2138. https://doi.org/10.1007/s13197-020-04248-1
Kiran S, Tariq A, Iqbal S, Naseem Z, Siddique W, Jabeen S, Bashir R, Hussain A, Rahman M, Habib FE, Rauf W, Ali A, Sarwar Y, Jander G, Iqbal M (2024) Punicalagin a pomegranate polyphenol sensitizes the activity of antibiotics against three MDR pathogens of the Enterobacteriaceae. BMC Complement Med Ther 24(1):93. https://doi.org/10.1186/s12906-024-04376-7
Konishi K, Urada M, Adachi I, Tanaka T (2000) Inhibitory effect of sanguiin H-11 on chemotaxis of neutrophil. Biol Pharm Bull 23(2):213-218.
Kumar A, Mishra R, Singh VD, Mazumder A, Mazumder R, Kumar A (2022a) Wound healing activity of punicalin and punicalagin isolated from *Punica granatum* L. Rasayan J Chem 15(1):183-189. http://dx.doi.org/10.31788/RJC.2022.1516639
Kumar D, Ladaniya MS, Gurjar M, Kumar S (2022b) Impact of drying methods on natural antioxidants, phenols and flavanones of immature dropped *Citrus sinensis* L. Osbeck fruits. Sci Rep 12(1):1-12. https://doi.org/10.1038/s41598-022-10661-7
Kumar N, Goel N (2019) Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol Rep 24:e00370. https://doi.org/10.1016/j.btre.2019.e00370
Liu J, Tang J (2017) Effects of pomegranate extract in supplementing gonadotropin-releasing hormone therapy on idiopathic central precocious puberty in Chinese girls: a randomized, placebo-controlled, double-blind clinical trial. Food Funct 8(2):695–700. https://doi.org/10.1039/c6fo01616b
Man G, Xu L, Wang Y, Liao X, Xu Z (2022) Profiling phenolic composition in pomegranate peel from nine selected cultivars using UHPLC-QTOF-MS and UPLC-QQQ-MS. Front Nutr 8:807447. https://doi.org/10.3389/fnut.2021.807447
Marra F, Petrovicova B, Canino F, Maffia A, Mallamaci C, Muscolo A (2022) Pomegranate wastes are rich in bioactive compounds with potential benefit on human health. Molecules Basel Switzerland 27(17):5555. https://doi.org/10.3390/molecules27175555
Mo Y, Ma J, Gao W, Zhang L, Li J, Li J, Zang J (2022) Pomegranate peel as a source of bioactive compounds: a mini review on their physiological functions. Front Nutr 9:887113. https://doi.org/10.3389/fnut.2022.887113
Mojzer BE, Hrnčič KM, Škerget M, Knez Ž, & Bren U (2016) Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules (Basel, Switzerland), 21(7):901. https://doi.org/10.3390/molecules21070901
Obasa P, Adenike AB, Agajo J, Tunde OS, Fadipe L (2024) Polyphenol extraction for the enhancement of food lipid quality with an emphasis on the roles of extraction technologies moisture and drying temperature. IntechOpen https://doi.org/10.5772/intechopen.112946

Rodrigues JPB, Liberal Â, Petropoulos SA, Ferreira ICFR, Oliveira MBPP, Fernandes Â, Barros L (2022) Agri-food surplus, waste and loss as sustainable biobased ingredients: A review. Molecules (Basel Switzerland) 27(16):5200. https://doi.org/10.3390/molecules27165200

Sharma K, Kesharwani P, Prajapati SK, Jain A, Jain D, Mody N, Sharma S (2022) An insight into anticancer bioactives from *Punica granatum* (Pomegranate). Anti-Cancer Agents Med Chem 22(4):694–702. https://doi.org/10.2174/1871520621666210726143553

Siddiqui SA, Singh S, Nayik GA (2024) Bioactive compounds from pomegranate peels - Biological properties, structure–function relationships, health benefits and food applications – A comprehensive review. J Funct Foods 116:106132. https://doi.org/10.1016/j.jff.2024.106132

Singh J, Kaur HP, Verma A, Chahal AS, Jajoria K, Rasane P, Kaur S, Kaur J, Gunjal M, Ercisli S, Choudhary R, Bozhuyuk MR, Sakar E, Karatas N, Durul MS (2023) Pomegranate peel phytochemistry, pharmacological properties, methods of extraction, and its application: A comprehensive review. ACS Omega 8(39):35452–35469. https://doi.org/10.1021/acsomega.3c02586

Song JH, Kim SY, Hwang GS, Kim YS, Kim HY, Kang KS (2019) Sanguiin H-11 from Sanguisorbae radix protects HT22 murine hippocampal cells against glutamate-induced death. Bioorg Med Chem Lett 29(2):252-256. https://doi.org/10.1016/j.bmcl.2018.11.042

Sweidan N, Abu Rayyan W, Mahmoud I, Ali L (2023) Phytochemical analysis, antioxidant, and antimicrobial activities of Jordanian Pomegranate peels. PLOS One 18(11):e0295129. https://doi.org/10.1371/journal.pone.0295129

Thangavelu A, Elavarasu S, Sundaram R, Kumar T, Rajendran D, Prem F (2017) Ancient seed for modern cure - pomegranate review of therapeutic applications in periodontics. J Pharm Bioallied Sci 9(Suppl 1):S1-–S14. https://doi.org/10.4103/jpbs.JPBS_101_17

Venkataramanamma, D., Aruna, P., & Singh, R. P. (2016). Standardization of the conditions for extraction of polyphenols from pomegranate peel. Journal of Food Science and Technology - Mysore, 53(5), 2497-2503. https://doi.org/10.1007/s13197-016-2222-z

Waterhouse A (2002) Current protocols in food analytical chemistry. John Wiley & Sons Ltd.; New York, USA: Determination of total phenolics.

Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimum inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, 3(2), 163–175. https://doi.org/10.1038/nprot.2007.521

Xu J, Cao K, Liu X, Zhao L, Feng Z, Liu J (2021) Punicalagin regulates signaling pathways in inflammation-associated chronic diseases. Antioxidants 11(1):29. https://doi.org/10.3390/antiox11010029