



## Pomegranates (*Punica granatum L.*) peel extracts: quantitative assessment and GC-MS analysis for the identification of bioactive phytochemicals

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### Abstract:

Pomegranates (*Punica granatum L.*) is a deciduous tree from the *Punicaceae* family. Pomegranates peels are produced as a waste product during fruit processing and rich in components like antioxidants, organic acids, flavonoids and phenols. These bioactive compounds possess various medicinal properties. In this study, we used a variety of solvents to extract various compounds from the peel of pomegranates (*Punica granatum L.*). Alkaloids, flavonoids, carbohydrates, proteins, and polyphenols were identified by phytochemical screening in ethanolic, aqueous, and petroleum ether extracts which showed a significant antioxidant activity with highest value of antioxidants ( $0.014 \pm 0.01$  mg/ml; IC<sub>50</sub>) observed in ethanolic extract. These extracts were analyzed by GC-MS, which revealed the presence of different secondary metabolites and phytochemical substances such as 5-(Hydroxymethyl)-2-(dimethoxy methyl) furan, 4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6 and Dimethyl phthalate etc.

**Key Words:** *Punica granatum*, Gas Chromatography-Mass Spectrometry, secondary metabolites, antioxidant, bioactive compound

### Introduction

*Punica granatum L.* belongs to *Punicaceae* family is majorly cultivated in Afghanistan, Iran, Turkey, USA, and Far East countries (Ferrentino et al. 2018; Facciola, 1990). The global pomegranate yield is approximately 1.5 million metric tons per year (Holland et al., 2009). The pomegranate fruit can be divided into peels, juice and seeds and usually, consumed fresh or processed into juice. From ancient times, pomegranate is used in ethnobotanical medicines as an antioxidant, anti-inflammatory, and antihyperglycemic agent, as well as to treat cancer and other ailments (Tamborlin et al., 2020; Karwasra et al., 2019; Orgil et al., 2016). Recent research have also demonstrated its ability to treat cardiovascular disorders and diabetes (Jurenka, 2008). The production and consumption of pomegranates have consistently been on the rise due to its delectable flavor and nutritional value. Nevertheless, fruit processing generates a substantial quantity of byproducts, including peels and seeds, which, if not managed well, can result in environmental contamination issues. The pomegranate peel constitutes around 26-30% of the overall weight and is rich in bioactive compounds (Mo et al., 2022).

The fruit processing waste can serve as a valuable source of phenols, antioxidants, organic acids and flavonoids. Pomegranate peels, specifically the husk, membranes, rind and pericarp contain high levels of polyphenols such as ellagitannin and punicalagins, as well as gallic acid, anthocyanidins, flavanones and flavones (Singh et al., 2019; Thangavelu et al., 2017).

The primary phenolic compounds found in pomegranate peel include tannins, flavonoids, and phenolic acids. It also contain dietary fiber and other bioactive compounds, including alkaloids, minerals, and vitamins (El-Hadary and Ramadan, 2019; Singh et al., 2018). These components work as antioxidants by enhancing oxidative indicators and eliminating or counteracting reactive oxygen species, so enhancing their many roles such as reducing inflammation, preventing cancer, fighting against microorganisms, and providing cardiovascular protection (Liu et al., 2019; Mastrogiovanni et al., 2019; Du et al., 2018; Deng et al., 2017). Some researches revealed that pomegranate peel extracts can be used in treatment of leukemia disease (Asmaa et al., 2015; Dahlawi et al., 2013). Various studies on pomegranate revealed its medicinal and agricultural benefits. Pomegranates have antibacterial, antifungal, insecticidal, and molluscicidal properties against diseases that affect both plants and humans (Rosas-burgos et al., 2016). Because pomegranate syrup and peeling alter the structure of proteins, decrease fluid leakage, destroy bacteria, and sting toxins, they are used to cure diarrhoea and dysentery. Pomegranate extracts are used to destroy germs and drive out parasites. Tannins and alkaloids are responsible for this lethal action. Additionally, pomegranates are utilised as a cooling agent for the body's heat, a diuretic, and a tonic (Alhuqail et al., 2018; Hoffmann, 1996).

For pharmaceutical uses, these bioactive substances need to be extracted from the raw material. A variety of extraction methods and solvents are available for the extraction procedure. The unique properties of the sample and intended chemicals should be taken into account when selecting the solvent (Rostagno et al., 2010).

It is anticipated that by gaining a more profound comprehension of the bioactive components found in pomegranate peel, we can enhance the utilization of waste sources for physiological purposes. This study compares the chemical profiles and biological activities of extracts obtained using various solvents, highlighting the significance of solvent choice on extract composition and bioactivity.

#### **Materials and methods:**

- i. **Collection and processing of fruit peel:** The pomegranate peels (healthy and contamination -free) were gathered from the vicinity/markets of Jaitpur (New Delhi, India) as well as from the kitchen garden. In order to eliminate any potential contaminants, the material was given an initial washing process utilizing stream water, followed by a subsequent shade drying step.
- ii. **Preparation of fruit peel extract:** Phytochemicals were extracted from coarsely ground powder of dried fruit peels by the Soxhlet Process (500g powder in 2L solvent) using petroleum ether, ethanol, and distilled water as a solvent. Dried crude extracts were produced after solvent evaporation and stored in refrigerators. The dried crude extract underwent additional examination using GC-MS and phytochemical techniques.
- iii. **Quantitative Estimation of Phytochemicals:**

Following phytochemical analysis were performed on the selected sample extracts:

  - **Test for Total Phenolic Content (TPC)**

The TPC was determined by the Folin-Ciocalteu technique. A standard calibration curve was created by measuring the absorbance at 765nm. The TPC was quantified as milligrams of gallic acid equivalent (GAE) per gram of peel extract, as stated in literature (Rajpal, 2002).
  - **Test for Flavonoids**

The flavonoid compound was assessed through aluminium-chloride colorimetric technique, as described by El Far and Taie in 2009. This approach relies on the generation of acid stable complex of flavonoids and aluminium. A blank was made by substituting  $AlCl_3$  with distilled water. The absorbance was taken at 415 nm wavelength following a 40-minute of incubation period. A calibration curve was generated at a wavelength of 415nm using standardized quercetin concentrations, enabling

the quantification of flavonoid content in test samples. The flavonoid amounts were calculated using this curve and expressed as milligrams of quercetin equivalent per gram of sample.

- **Test for Total Bitter**

Bitter content was assessed through Gravimetry method reported by Bhagwat et al., (2017). The proportion of bitter residue was determined by calculating the weight of the residue and describing it as a percentage of the weight of the air-dried sample.

Percentage of bitters (w/w) is calculated as:

$$\frac{(A - B) \times 100}{W}$$

A = Weight of dish + Residue

B = Weight of empty dish

W = Weight of material taken

- **Test for Tannin**

Method described by Atanassova, and Christova-Bagdassarian (2009) was used to estimate the tannin content in pomegranate peel. The sample diluted with distilled water was titrated with N/10 KMnO<sub>4</sub> in the presence of indigo carmine solution until a golden yellow colour was achieved. A blank titration without the sample was also conducted as a control. The amount of indigo carmine solution needed to neutralise the tannin is represented by the difference between the two titrations. Tannin (as gallotannic acid) weighs 0.004157 gm per millilitre of 0.1N KMnO<sub>4</sub>.

- **Test for Total Alkaloids**

Alkaloids were estimated using the spectrophotometric method of Dragendorff's reagent (Sreevidya and Mehrotra, 2003). Absorbance at 435 nm was measured against a nitric acid-thiourea blank. A calibration curve (20-100µg of caffeine) enabled alkaloid estimation. Alkaloids were expressed as mg of caffeine equivalent per gram of extract.

- **Test for saponin**

Method described by Rajpal (2002) was used to estimate the saponin content in pomegranate peel. The saponin percentage was determined and expressed as a weight-to-weight ratio in relation to the air dried sample.

- **Test for protein**

The protein content in pomegranate peel was estimated by following the Kjeldahl procedure. The difference in volume between the two titrations constitute the acid required to neutralize the ammonia present in the sample. Nitrogen is represented by 0.007004 grams per milliliter of 0.5N sulphuric acid (Rajpal, 2002).

- **Test for Carbohydrate**

Hedge and Hofreiter's (1962) anthrone method was performed to calculate the total carbohydrate content. Anthrone has a maximum absorption at 630 nm, and the technique relies on its synthesis from hydroxyl methyl furfural in an acidic solution to give a green colour. The standard curve was prepared and concentration of unknown amount in sample was measured on it.

- **Test for Reducing Sugar**

The reducing sugar was estimated through a method suggested by Marques et al. (2016). 125 ml of extract was measured and topped up in a 250 ml standard flask; ten milliliters of Fehling's solution I and II, boiling, followed by a titration which was repeated until concurrent results were obtained (Marques et al., 2016).

- **Test for Antioxidants**

The DPPH assay was used to measure the ability of the ethanolic and aqueous extracts to quench free radicals. The absorbance was taken at 517 nm following incubation. A matching volume of methanol and DPPH excluding extract or reference ascorbic acid was made as a control sample. As a control, methanol was used (Bag and Devi, 2015).

- iv. **Gas chromatography-Mass spectrometry (GC-MS) analysis**

Samples were analyzed by GC-MS using a GC-2010 gas chromatograph and a TQ8030 mass spectrometer. The sample (1.0  $\mu$ l) was injected into the GC-MS on a glass capillary. Acquisition mode Q3 Scan with ion source temperature of 250° C and an interface temperature of 300° C were used. At a constant flow rate of 1 ml/min and pressure of 57.5 kPa, helium gas was utilized as carrier gas. Libraries were used to check the mass spectrum of the unknown molecule against a database of known compounds.

## Results

### • Quantitative Estimation of Phytochemical constituents

In the present study, variable amount of phytochemicals were obtained for each solvent after quantitative estimation of different extracts of pomegranate peel. Petroleum ether extract of peel showed presence of alkaloid ( $2\pm 0.57$  mg of caffeine equivalent/g) and protein ( $3.92\pm 0.75$ ) in higher amount as compared to other extracts. Ethanolic extract of pomegranate peel showed higher amount of polyphenol ( $22.05\pm 1.72$  GAE/g), bitter ( $12.81\pm 3.07$  %), flavonoid ( $3.06\pm 0.63$  mg quercetin equivalent/g), carbohydrate ( $19.46\pm 1.21$ %) while aqueous extract showed tannin ( $16.68\pm 2.81$ %) and saponins ( $22.36\pm 2.17$ ), in more quantity in comparison with other extracts (Table 1, Fig. 1). The antioxidant potential was measured in terms of IC<sub>50</sub>. The maximum antioxidant activity was reported for ethanolic extract ( $0.014\pm 0.01$  mg/ml) followed by aqueous extract ( $0.023\pm 0.01$ ) whereas the petroleum ether fraction showed minimum ( $0.22\pm 0.12$ ) activity. The presence of reducing sugar was not observed in any of the extracts. Results revealed that ethanolic extraction was the most efficient method for extracting bioactive compounds from pomegranate peel in comparison to aqueous and petroleum ether extracts.

### • Gas chromatography-Mass spectrometry (GC-MS) analysis

GC-MS analysis revealed the presence of various biologically active chemical compounds in pomegranate peel extracts (Fig. 2). The GC-MS chromatogram of the Petroleum ether extract of peel demonstrated one peak representing the presence of one phytochemical constituent (Table: 2) and the ethanol extract of pomegranate peel showed eight peaks representing the presence of eight phytochemical components (Table: 3). In both the extract, the Diethyl phthalate was the major phytochemical constituent. The GC-MS chromatogram of the aqueous extract of peel showed five peaks indicating the presence of five phytochemical constituents. The major phytochemical constituent was 5-(Hydroxymethyl)-2-(dimethoxy methyl) furan (Table: 4).

## Discussion

Despite being shown to have more antioxidant properties than the fruit's edible portions, the pomegranate peel is still regarded as a byproduct in the food and beverage sectors (Fischer et al., 2011). When compared to other fruit parts, the peel has found to be rich in phenolic components (Akhtar et al., 2015). It is the primary source of bioactive substances such as pro-anthocyanidins, ellagitannins, and flavonoids. Ellagitannins are the primary class of phenolic compounds; the main chemicals found inside include punicalagins and ellagic acid (Khalil et al., 2018; Fischer et al., 2011).

The conventional method (maceration, Soxhlet extraction, and steam distillation) and nonconventional methods (pressurized liquid extraction, microwave-assisted extraction, supercritical fluid extraction, ultrasound-assisted extraction, and steam distillation) can be used to extract natural compounds (Chemat et al., 2017; Rostagno and Prado, 2013). In present study, three different solvents (Petroleum ether, Ethanol and Distilled Water) were used for isolation and identification of phytochemicals by Soxhlet method. Ethanolic extract of peel of pomegranate fruit was found to be richer source of various bioactive compounds during the current study. Quantity and type of the extracted phytochemicals varies according to the solvent used for their extraction. The profile of phenolic compounds and biological activity of pomegranate peel extracts depend critically on the type of solvent used (Tamborlin et al., 2020). Water or its hydroalcoholic mixtures are suitable for isolation of phenolic compounds due to their polar nature (Venkataramanamma et al., 2016; Singh et al., 2014). The concluding chemical profile of the extract is determined by the type of extraction solvent utilised during the process. As a result, the extracted substances' bioactivity will be greatly impacted by both their absolute and relative

concentrations (Sumere et al., 2018). When punicalagin was extracted from pomegranate peels using several kinds of solvents, such as ethyl acetate, ethanol, and methanol, significant variations were seen; these variations also had an impact on the antioxidant activity of the extracts (Khalil et al., 2018). According to Kartikaran and Vidya (2019), aqueous extract of the peel of pomegranate fruit has the highest amount of phytochemicals, such as phenols, glycosides, flavonoids, terpenoids, proteins, carbohydrates, and amino acids while pomegranate peel extracts containing ethanol and acetone were the most effective at inhibiting the development of bacteria. Muhammad et al., (2023) dried the pomegranate peel by different method and found that solar dried powder of pomegranate peel possess highest nutritional values in comparison to other powders.

In our study, variable amount of phytochemicals such as alkaloids, proteins, polyphenols, flavonoid, carbohydrate, bitter, tanins, saponins and antioxidants were obtained for each solvent after quantitative estimation of different extracts of pomegranate peel. According to Segura et al., (2005) numerous investigations have revealed that different plant parts of pomegranate contain a variety of chemical components, including flavonoids, phenols, terpenes, alkaloids, acids, sugars, and other substances. Similar to catechin, pomegranate peel extract effectively scavenged DPPH radicals. It is possible that the peel extract also had the potential to donate protons and worked in conjunction with many hydroxyl groups to stabilise free radicals (Nair et al., 2016). Due to their capacity to induce leakage in cell membrane by precipitation of protein, which leads to lysis of the cell and ultimately death of cell, the polyphenolic and tannin components of pomegranate fruit peel extracts has been proposed to have antibacterial action (Elshafie et al., 2021). According to Yassin et al., (2021), methanolic extract of pomegranate fruit peel had the greatest polyphenolic content (277.8 mg of GAE/g) while the aqueous extract of pomegranate had the lowest polyphenolic content (159 mg GAE/g). The hydromethanolic extract analysis of pomegranate fruit peel revealed that its total phenolic content, DPPH, and FRAP antioxidant test were 1577.65 mg/g GAE, 54 µg/ml, and 483.24 mM respectively and phenolic compound viz. punicalagin, ellagic acid, and gallic acid were most abundant in the extract (Ghasemi et al., 2023).

Pomegranate peels have various possible health advantages and are rich in bioactive elements such as quercetin, catechin, antioxidants, vitamins and minerals (Pirzadeh et al., 2020). These compounds plays important role in maintaining health and offer protection against certain chronic diseases like cardiovascular disease, diabetes and some cancers (Gull et al., 2023). Making almost half of the pomegranate's weight, the peel contains flavonoids, tannins, and phenolics with antibacterial qualities. These components have the power to eliminate free radicals and inhibit lipid oxidation in fatty meals (George et al., 2019). Due to higher phenolic content and crude fibre content, pomegranate peel offers several health advantages, including antibacterial, antihypertensive, antioxidant, antilipidemic, and anti-diabetic effects (Ain et al., 2023; Ranjitha et al., 2018). Flavonoids have been shown to have potent antioxidant properties according to a recent study that looked at their health benefits (Derakhshan et al., 2018). Pomegranate peel extract has been found useful in prolonging the shelf life of several kinds of food goods (Akhtar et al., 2015). According to Belal et al. (2020), Polyphenols, flavonoids and tannins content present in pomegranate peel reduce oxidative stress and have a neuroprotective impact by blocking TNF- $\alpha$ , IL-1 $\beta$ , and NO in microglia cells. Presence of ellagic, gallic, and other phenolic components provide wound-healing properties to pomegranate peel (Hayouni et al., 2011).

In our study, GC-MS study of different extracts of pomegranate peel demonstrated the presence of various biologically active chemical compounds. The GC-MS chromatogram of the ethanol extract of pomegranate peel demonstrated maximum peaks among the three extracts. Diethyl phthalate was the major phytochemical constituent in both petroleum ether extract and ethanolic extract while 5-(Hydroxymethyl)-2-(dimethoxy methyl) furan was major phytochemical of the aqueous extract of pomegranate fruit peel. Our results were supported by the study of Hanafy et al. (2021) who observed the presence of 23 components in methanolic extract and 31 components in the ethanolic extract during GC-MS analysis and 5-hydroxymethylfurfural was the main constituent in both methanolic and ethanolic extracts. Similarly Yassin et al. (2021) reported 5-Hydroxymethylfurfural as an important active ingredient in GC-MS study of the methanolic extract and acetonetic extract of pomegranate peel. Ethyl

acetate extract of pomegranate peel revealed the presence of 5-hydroxymethylfurfural and 4-fluorobenzyl alcohol in highest ratio during the GC-MS analysis (Barathikannan et al., 2016). According to the investigation carried out by Arkan and Taiba, (2021) on the salami variety, ethanolic extract of pomegranate peel comprised 28 different chemical components and 2H-Pyran-2-one, hexadecanoic acid, and ethyl ester were found in highest ratio. GC-MS analysis ethyl acetate extract of *P. granatum* peel revealed the presence of 20 chemical compounds, the most prominent of which were Pyrogallol followed by Hydroxymethylfurfural-5 and D-Allose (Sangeetha and Vijayalakshmi, 2011). According to Ashok and Vijayalakshmi's (2011) GC-MS investigation, the ethylene extract of pomegranate peel includes 26 different chemical components, such as glycerin, guanosine, and pyrogallol in higher amount.

Diethyl phthalate (DEP) is a synthetic compound that is used extensively in many different industries. It is primarily used in the manufacturing of plasticizer, toothbrushes, car parts, tools, toys, cosmetics, food packaging, insecticides, and medications like aspirin. Furthermore, it plays a number of functions such as neurotoxic and teratogenic agent (NCBI, 2023). Another important compound identified in our study is 4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6, which comes under category of pyrans, which play an important role in agriculture as antimicrobial agent, root growth promoter for plants, soil quality improver, biostimulator of soil, foliar fertilizer, and nematicide (Wei et al., 2010). In addition, it is utilized for the purpose of sterilization, as food additives and smoke flavoring (Li et al., 2017; Gomez et al., 2021). According to Mohammed et al. (2016), 5-hydroxymethylfurfural has antioxidant activity, whereas 4HPyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- possesses laxative, antibacterial, and antioxidant properties. Similarly, Nafea et al. (2011) studies demonstrated HMF's bactericidal efficacy to varying degrees against a wide range of microorganisms. According to earlier reports, the antifungal action of pomegranate extracts is most likely associated with the molecule 4HPyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (Teoh and Mashitah, 2016). Prior research has indicated that the phytochemicals such as Furfural, 5-hydroxymethylfurfural,  $\gamma$ -Sitosterol, 2-furancarboxaldehyde, 5-methyl, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl, Hexadecanoic acid, methyl ester, and Octadecanoic acid found in pomegranate peels that were identified using GC-MS analysis may have antioxidant and antiproliferative properties (Ismail et al., 2021; Zhu et al., 2021; Isbilen et al., 2018; Amaechi et al., 2017).

A recent study demonstrated that due to presence of antioxidant and antimicrobial compounds, pomegranate peel extract, both aqueous and ethanolic, reduced microbial flora number during cold storage, preserved the product's appearance, quality, and stability, and extended the product's shelf life in products such as bighead carp fillets (Zhuang et al., 2019), Ready-to-cook breaded fish sticks' (Panza et al., 2021), chicken nuggets (Bashir et al., 2022) etc.

Research indicates that pomegranate peel byproducts will provide a creative and sustainable solution to prevent food degradation and plant infections. It is a good substitute for synthetic pesticides because of its great effectiveness and broad-spectrum activity against bacteria, fungus, and viral diseases (Belgacem et al., 2021). Citrus fruit post-harvest invasion by green and blue mould can be controlled by using its antifungal qualities. It was noted that pomegranate fruit peel extracts were efficient against *Penicillium digitatum*, and for complete control of green mould in citrus fruits, it was advised to utilise pomegranate peel extract sanitizers to disinfect storage rooms and recirculated water in packing houses (Pangallo et al., 2021). Various researches suggested that the extract from pomegranate peel is a good source of bioactive components, such as minerals, phenolic acids, flavonoids (anthocyanins), and hydrolyzable tannins (gallic acid), that are essential to the fruit's biological activity. Given their well-established chemical properties and ethnobotanical relevance, the macromolecules present in pomegranate peel and peel extract have been proposed as alternatives to synthetic nutraceuticals, food additives, and chemo-preventive agents (Azmat et al., 2024).

In general fruit peels are regarded as a waste material and used as a fertilizer to nourish the soil. Peels of pomegranate contain various phytochemicals and polyphenolic compound due to which it possess various medicinal properties and can be used for treatment of various human as well as plant diseases. Our study showed the presence of various phytochemicals in variable amount in different extracts. It

also showed the abundance of Diethyl phthalate, 4H-Pyran-4-one 2 3-dihydro-3 5-dihydroxy-6and 5-(Hydroxymethyl) -2- (dimethoxy methyl) furan in pomegranate peel, which possess medicinal and industrial benefits.

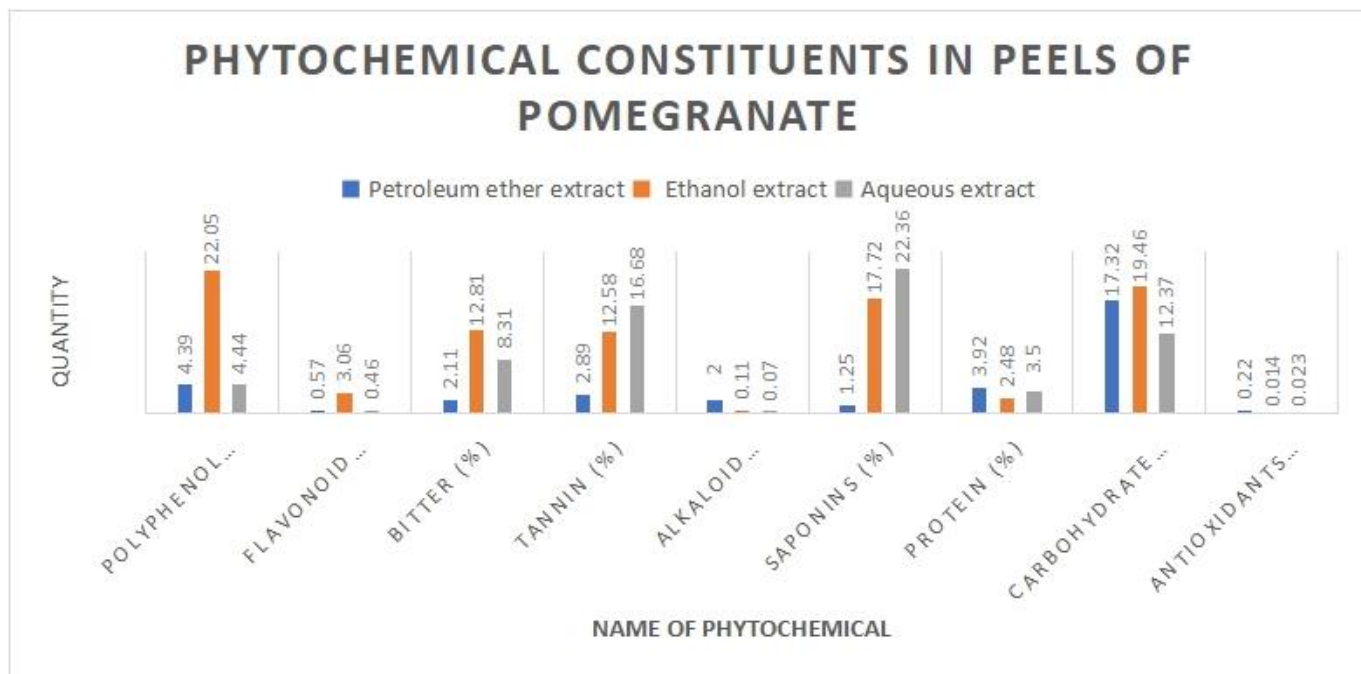


Figure 1: Graph showing comparative quantitative estimation of Phytochemical constituents in various extracts of Pomegranate peel

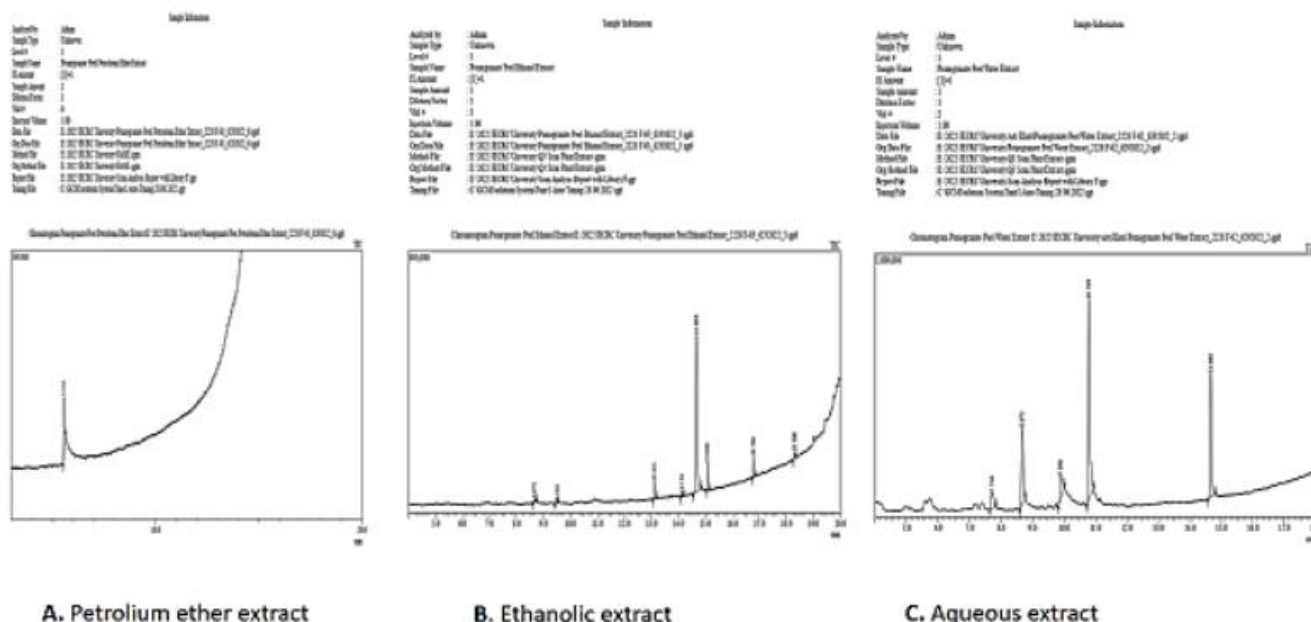


Figure 2: GC-MS chromatogram of Pomegranate peel extracts

Table 1: Results of quantitative estimation of Phytochemical constituents in various extracts of Pomegranate peel

Sample Name	Polyphenol (GAE/g)	Flavonoid mg quercetin equivalent/g	Bitter (%)	Tannin (%)	Alkaloid mg of caffeine equivalent/g	Saponins (%)	Protein (%)	Carbohydrate (%)	Antioxidants (mg/ml) (IC <sub>50</sub> )
Petroleum ether extract	4.39±0.52	0.57±0.13	2.11±1.12	2.89±0.81	<b>2±0.57</b>	1.25±0.47	<b>3.92±0.75</b>	17.32±1.35	0.22±0.12
Ethanol extract	<b>22.05±1.72</b>	<b>3.06±0.63</b>	<b>12.81±3.07</b>	12.58±1.08	0.11±0.10	17.72±2.59	2.48±0.33	<b>19.46±1.21</b>	<b>0.014±0.01</b>
Aqueous extract	4.44±0.91	0.46±0.20	8.31±0.51	<b>16.68±2.81</b>	0.07±0.05	22.36±2.17	3.5±0.26	12.37±1.11	0.023±0.01

**Table 2: Retention time and phytochemical compounds identified in petroleum ether extract of Pomegranate peel through GC-MS technique**

Peak	R.Time	Area	Area%	Name	Molecular Formula	Molecular Weight (g/mol)
1	5.555	288018	100	Diethyl Phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222.24
		288018	100			

**Table 3: Retention time and phytochemical compounds identified in ethanolic extract of Pomegranate peel through GC-MS technique**

Peak	R.Time	Area	Area%	Name	Molecular Formula	Molecular Weight (g/mol)
1	8.671	36614	1.98	4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.12
2	9.514	30724	1.66	Estragole	C <sub>10</sub> H <sub>12</sub> O	148.20
3	13.115	85896	4.65	Cycloheptasiloxane, tetradecamethyl-	C <sub>14</sub> H <sub>42</sub> O <sub>7</sub> Si <sub>7</sub>	519.08
4	14.134	15372	0.83	Kessane	C <sub>15</sub> H <sub>26</sub> O	222.37
5	14.668	1409236	76.34	Diethyl Phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222.24
6	15.09	133133	7.21	Cyclooctasiloxane, hexadecamethyl-	C <sub>16</sub> H <sub>48</sub> O <sub>8</sub> Si <sub>8</sub>	593.23
7	16.79	100588	5.45	Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.39
8	18.306	34484	1.87	Cyclodecasiloxane, eicosamethyl-	C <sub>20</sub> H <sub>60</sub> O <sub>10</sub> Si <sub>10</sub>	741.54
		1846047	100			

**Table 4: Retention time and phytochemical compounds identified in aqueous extract of Pomegranate peel through GC-MS technique**

Peak	R.Time	Area	Area%	Name	Molecular Formula	Molecular Weight (g/mol)
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1	7.716	361505	6.39	Methyl 2-furoate	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11
2	8.675	1155830	20.42	4H-Pyran-4-one 2 3-dihydro-3 5-dihydroxy-6	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.12
3	9.896	501788	8.86	5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11
4	10.789	2252397	39.79	5-(hydroxymethyl)-2-(dimethoxymethyl)-furan	C <sub>8</sub> H <sub>12</sub> O <sub>4</sub>	172.18
5	14.663	1389766	24.55	Diethyl Phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222.24
		5661286	100			

### Conclusion

The current study demonstrated the occurrence of various bioactive components with antioxidant potential in various extracts of pomegranate peels. Our findings demonstrated that the composition, quantity and biological activities of different phytochemicals were influenced by various solvents used. By identifying antioxidant compounds such as 4H-Pyran-4-one 2 3-dihydro-3 5-dihydroxy-6, the potential biological and pharmacological significance of the peel extracts might be ascertained. This indicates that pomegranate peel may be a source of beneficial antioxidant compounds and creates new opportunities for cutting-edge medicinal industry. To fully understand the potential of pomegranate peels, additional research is required.

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### References

1. Ain, H.B.U., Tufail, T., Bashir, S., Ijaz, N., Hussain, M., Ikram, A., Farooq, M.A., and Saewan, S.A. (2023). Nutritional importance and industrial uses of pomegranate peel: A critical review. *Food Science & Nutrition*, 11(6), 2589–2598.
2. Akhtar, S., Ismail, T., Fraternal, D., and Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 174, 417–425. <https://doi.org/10.1016/j.foodchem.2014.11.035>
3. Alhuqail, A.A., Elgaaly, G.A., and Ibrahim, M.M. (2018). Identification of bioactive phytochemicals from two Punica species using GC–MS and estimation of antioxidant activity of seed extracts. *SJBS*. 25, 1420–1428.
4. Amaechi N.C., and Okonkwo U. (2017). Identification of compounds in Garcinia kola (Heckel) fruit pulp using gas chromatography-mass spectrometry. *Arch. Curr. Res. Int.* 10, 1–10. doi: 10.9734/ACRI/2017/36190.
5. Arkan Ali Al-Tai and Taiba Falih Al-Mayyahi. (2021). A Chemical Study By Using GC-Mass Spectrometry Of The Peel And Seeds Of *Punica Granatum* L. *Plant. Sys Rev Pharm*, 12(1), 1414-1421.
6. Ashok, K. and Vijayalakshami, K. (2011). The GC-MS analysis of phytochemical constituents in ethanolic extract of *Punica granatum* peel and *Vitis vinifera* seeds. *IJPBS*, 2, 0975-6299. <https://ijpbs.net/abstract.php?article=OTk4>
7. Asmaa, M.J., Ali, A.J., Farid, J.M., and Azman, S. (2015). Growth inhibitory effects of crude pomegranate peel extract on chronic myeloid leukemia, K562 cells. *International Journal of Applied and Basic Medical Research*, 5(2), 100–105. <https://doi.org/10.4103/2229-516X.157154>
8. Azmat, F., Safdar, M., Ahmad, H., Khan, M.R.J., Abid, J., Naseer, M.S., Aggarwal, S., Imran, A., Khalid, U., Zahra, S.M., Islam, F., Cheema, S.A., Shehzadi, U., Ali, R., Kinki, A.B., Ali, Y.A., and Suleria, H.A.R. (2024). Phytochemical profile, nutritional composition of pomegranate peel and peel extract as a potential source of nutraceutical: A comprehensive review. *Food Science & Nutrition*, 00, 1–14. <https://doi.org/10.1002/fsn.33777>
9. Barathikannan, K., Venkatadri, B., Khusro, A., Al-Dhabi, N.A., Agastian, P., Arasu, M.V., Choi, H.S., and Kim, Y.O. (2016). Chemical analysis of *Punica granatum* fruit peel and its in vitro and in

- vivo biological properties. *BMC complementary and alternative medicine*, 16, 264. <https://doi.org/10.1186/s12906-016-1237-3>
10. Bashir, S., Arshad, M.S., Khalid, W., Nayik, G.A., Al Obaid, S., Ansari, M.J., Moreno, A., and Karabagias, I.K. (2022). Effect of antimicrobial and antioxidant rich pomegranate peel based edible coatings on quality and functional properties of chicken nugget. *Molecules*, 27(14), 4500.
  11. Belal, S.K.M., Afifi, O.K., and Afeefy, A.A. (2020). Evaluation of mobile phone radiation-induced structural changes of rat brain with emphasis on the possible protective role of pomegranate peel extract. *International Journal of Radiation Research*, 18(4), 753–764.
  12. Belgacem, I., Li Destri Nicosia, M.G., Pangallo, S., Abdelfattah, A., Benuzzi, M., Agosteo, G.E., and Schena, L. (2021). Pomegranate peel extracts as safe natural treatments to control plant diseases and increase the shelf-life and safety of fresh fruits and vegetables. *Plants*, 10(3), 453.
  13. Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A.S., and Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A Review. *Ultrasonics Sonochemistry*, 34, 540–560. <https://doi.org/10.1016/j.ultsonch.2016.06.035>
  14. Dahlawi, H., Jordan-Mahy, N., Clench, M., McDougall, G.J., and Maitre, C.L. (2013). Polyphenols are responsible for the proapoptotic properties of pomegranate juice on leukemia cell lines. *Food Sciences and Nutrition*, 1(2), 196–208. <https://doi.org/10.1002/fsn3.26>
  15. Deng, Y., Li, Y., Yang, F., Zeng, A., Yang, S., Luo, Y., et al. (2017). The extract from *Punica granatum* (pomegranate) peel induces apoptosis and impairs metastasis in prostate cancer cells. *Biomed Pharmacother.* 93, 976–84. doi: 10.1016/j.biopha.2017.07.008
  16. Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M. S., Conti, G.O., and Sadrabad, E.K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and Chemical Toxicology*, 114, 108–111.
  17. Du, L., Li, J., Zhang, X., Wang, L., Zhang, W. (2018). Pomegranate peel polyphenols inhibits inflammation in LPS-induced RAW264. 7 macrophages via the suppression of MAPKs activation. *J Funct Foods*. 43, 62–9. doi: 10.29219/fnr.v63. 3392
  18. El-Hadary, A.E., & Ramadan, M.F. (2019). Phenolic profiles, antihyperglycemic, antihyperlipidemic, and antioxidant properties of pomegranate (*Punica granatum*) Peel Extract. *Journal of food biochemistry*. 43(4), e12803. <https://doi.org/10.1111/jfbc.12803>
  19. Elshafie, H.S., Caputo, L., De Martino, L., Sakr, S.H., De Feo, V., and Camele, I. (2021). Study of bio-pharmaceutical and antimicrobial properties of pomegranate (*Punica granatum* L.) leathery exocarp extract. *Plants*.10, 153. doi: 10.3390/plants10010153.
  20. Facciola, S.C. (1990). *A Source Book of Edible Plants*. Vista: Kampong Publications. p. 166.
  21. Ferrentino, G., Asaduzzaman, M., Scampicchio, M.M. (2018). Current technologies and new insights for the recovery of high valuable compounds from fruits byproducts. *Crit Rev Food Sci Nutr*. 58, 386–404. doi: 10.1080/10408398. 2016.1180589
  22. Fischer, U.A., Carle, R., and Kammerer, D.R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS(n). *Food Chemistry*, 127(2), 807–821. <https://doi.org/10.1016/j.foodchem.2010.12.156>
  23. George, N.S., Cheung, L., Luthria, D.L., Santin, M., Dawson, H.D., Bhagwat, A.A., and Smith, A.D. (2019). Pomegranate peel extract alters the microbiome in mice and dysbiosis caused by *Citrobacter rodentium* infection. *Food Science & Nutrition*, 7(8), 2565–2576.
  24. Ghasemi, R., Mohajeri, F.A., heydari, A., Yasini, S.A., Tafti, A.D. and Sadrabad, E.K. (2023). Application of Pomegranate Peel Extract, a Waste Agricultural Product, as a Natural Preservative in Tahini. *International Journal of Food Science*. 2023, 8860476, <https://doi.org/10.1155/2023/8860476>
  25. Gomez, J.P., Velez, J.P.A., Pinzon, M.A., Arango, J.A.M., and Muriel, A.P. (2021). Chemical characterization and antiradical properties of pyroligneous acid from a preserved bamboo, *Guadua angustifolia* Kunth. *Brazilian Archives of Biology and Technology*, 64, e21190730.

26. Gull, H., Ikram, A., Khalil, A.A., Ahmed, Z., and Nemat, A. (2023). Assessing the multitargeted antidiabetic potential of three pomegranate peel-specific metabolites: An in silico and pharmacokinetics study. *Food Science & Nutrition*, 11(11), 7188–7205.
27. Hanafy, S.M., Abd El-Shafea, Y.M., Saleh, W.D. et al. (2021). Chemical profiling, in vitro antimicrobial and antioxidant activities of pomegranate, orange and banana peel-extracts against pathogenic microorganisms. *J Genet Eng Biotechnol.* 19, 80. <https://doi.org/10.1186/s43141-021-00151-0>
28. Hayouni, E.A., Miled, K., Boubaker, S., Bellasfar, Z., Abedrabba, M., Iwaski, H., Oku, H., Matsui, T., Limam, F., and Hamdi, M. (2011). Hydroalcoholic extract based-ointment from *Punica granatum* L. peels with enhanced in vivo healing potential on dermal wounds. *Phytomedicine*, 18(11), 976–984.
29. Hoffmann, D. (1996). *The complete illustrated holistic herbal: a safe practical guide for making and using herbal remedies*, Element book, Great Britain, p132.
30. Holland, D., Hatib, K., Bar-Ya'akov, I. (2009). Pomegranate: Botany, Horticulture, Breeding. In *Horticultural Reviews*; Janick, J., (Ed.); John Wiley & Sons, Inc.: Hoboken, NJ, USA, pp. 127–191.
31. Isbilen, O., Rizaner, N., and Volkan E. (2018). Anti-proliferative and cytotoxic activities of *Allium autumnale* PH Davis (Amaryllidaceae) on human breast cancer cell lines MCF-7 and MDA-MB-231. *BMC Complement. Altern. Med.* 18, 1–13. doi: 10.1186/s12906-018-2105-0
32. Ismail, B.A., Nassar, D.A., Abd El-Wahab, Z.H., and Ali O.A. (2021). Synthesis, characterization, thermal, DFT computational studies and anticancer activity of furfural-type schiff base complexes. *J. Mol. Struct.* 1227, 129393. doi: 10.1016/j.molstruc.2020.129393.
33. Jurenka J.S. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): a review. *Alternative medicine review : a journal of clinical therapeutic*, 13(2), 128–144.
34. Karthikeyan, G., and Vidya, A.K. (2019). Phytochemical analysis, antioxidant and antibacterial activity of pomegranate peel. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 5(1), 218.
35. Karwasra, R., Singh, S., Sharma, D., Sharma, S., Sharma, N., and Khanna, K. (2019). Pomegranate supplementation attenuates inflammation, joint dysfunction via inhibition of NF-kappaB signaling pathway in experimental models of rheumatoid arthritis. *Journal of Food Biochemistry*, 43(8), e12959. <https://doi.org/10.1111/jfbc.12959>
36. Khalil, A.A., Khan, M.R., Shabbir, M.A., and Khalil, R. (2018). In vitro antioxidant activity and punicalagin content quantification of pomegranate peel obtained as agro-waste after juice extraction. *Pakistan Journal of Agricultural Sciences*, 55, 197–201. <https://doi.org/10.21162/PAKJA S/18.5663>
37. Li, R., Narita, R., Nishimura, H., Marumoto, S., Yamamoto, S.P., Ouda, R., et al. (2017). Antiviral Activity of Phenolic Derivatives in Pyroligneous Acid from Hardwood, Softwood, and Bamboo. *ACS Sustain Chem Eng.* 6(1), 119–26.
38. Liu, C., Guo, H., DaSilva, N.A., Li, D., Zhang, K., Wan, Y., et al. (2019). Pomegranate (*Punica granatum*) phenolics ameliorate hydrogen peroxide-induced oxidative stress and cytotoxicity in human keratinocytes. *J Funct Foods.* 54, 559–67. doi: 10.1016/j.jff.2019.02.015
39. Mastrogiovanni, F., Mukhopadhyaya, A., Lacetera, N., Ryan, M.T., Romani, A., Bernini, R., et al. (2019). Anti-Inflammatory effects of pomegranate peel extracts on in vitro human intestinal Caco-2 cells and ex vivo porcine colonic tissue explants. *Nutrients.* 11, 548. doi: 10.3390/nu11030548
40. Mo, Y., Ma, J., Gao, W., Zhang, L., Li, J., Li, J. and Zang, J. (2022). Pomegranate Peel as a Source of Bioactive Compounds: A Mini Review on Their Physiological Functions. *Front. Nutr.* 9:887113. doi: 10.3389/fnut.2022.887113
41. Mohammed, G.J., Al-jassani, M., and Hameed, I.H. (2016). Antibacterial, antifungal activity and chemical analysis of *punica grantanum* (pomegranate peel) using GC-MS and FTIR spectroscopy. *Int J Pharmacognosy Phytochem Res.* 8(3), 480–494.
42. Muhammad, A., Dayisoylu, K.S., Pei, J., Khan, M.R., Salman, M., Ahmad, R., Ullah, H. and Noor, G.R. (2023). Compositional analysis of natural pomegranate peel powder dried by different methods

- and nutritional and sensory evaluation of cookies fortified with pomegranate peel powder. *Front. Nutr.* 10, 1118156. doi: 10.3389/fnut.2023.1118156
43. Nafea, E.A., Moselhy, W.A., and Fawzy, A. (2011). Does the hmf value affect the antibacterial activity of the bee honey? *Egyptian academic journal of biological sciences.* 4(1), 13–19.
  44. Nair, R.K., Haridas, A., and Ezhuthupurakkal, D.R. (2016). Diversity and comparative account on phytochemical and antioxidant properties of two varieties of Musa, Nendran and Kunnan. *South Ind. J Biol Sci.* 2(1), 203–206.
  45. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 6781, Diethyl Phthalate. Retrieved August 2, 2023 from <https://pubchem.ncbi.nlm.nih.gov/compound/Diethyl-Phthalate>.
  46. Orgil, O., Spector, L., Holland, D., Mahajna, J., and Amir, R. (2016). The anti-proliferative and anti-androgenic activity of different pomegranate accessions. *Journal of Functional Foods*, 26(Supplement C), 517–528. <https://doi.org/10.1016/j.jff.2016.08.024>
  47. Pangallo, S., Li Destri Nicosia, M.G., Scibetta, S., Strano, M.C., Cacciola, S.O., Belgacem, I., Agosteo, G.E., and Schena, L. (2021). Preharvest and postharvest applications of a pomegranate peel extract to control citrus fruit decay during storage and shelf life. *Plant Disease*, 105(4), 1013–1018.
  48. Panza, O., Conte, A., and Del Nobile, M.A. (2021). Pomegranate byproducts as natural preservative to prolong the shelf life of breaded cod stick. *Molecules*, 26(8), 2385.
  49. Pirzadeh, M., Caporaso, N., Rauf, A., Shariati, M. A., Yessimbekov, Z., Khan, M.U., Imran, M., and Mubarak, M.S. (2020). Pomegranate as a source of bioactive constituents: A review on their characterization, properties and applications. *Critical Reviews in Food Science and Nutrition*, 61(6), 982–999. <https://doi.org/10.1080/10408398.2020.1749825>
  50. Ranjitha, J., Bhuvaneshwari, G., Terdal, D., and Kavaya, K. (2018). Nutritional composition of fresh pomegranate peel powder. *International Journal of Chemical Studies*, 6(4), 692–696.
  51. Rosas-burgos, E.C., Burgos-hernández, A., Noguera-artiaga, L., Kačániová, M., Hernández-garcía, F., and Cárdenas-lópez, J.L. (2016). Antimicrobial activity of pomegranate peel extracts as affected by cultivar. *J Sci Food Agric.* 97(93), 802–810.
  52. Rostagno, M.A. and Prado, J.M. (2013). *Natural Product Extraction: Principles and Applications*, (pp. 1–500). London: Royal Society of Chemistry. <https://doi.org/10.1039/9781849737579>
  53. Rostagno, M.A., D'Arrigo, M., Martínez, J.A., and Martínez, J.A. (2010). Combinatory and hyphenated sample preparation for the determination of bioactive compounds in foods. *TrAC Trends in Analytical Chemistry*, 29(6), 553–561. <https://doi.org/10.1016/j.trac.2010.02.015>
  54. Sangeetha, J., and Vijayalakshmi, K. (2011). Determination of Bioactive Components of Ethyl Acetate Fraction of *Punica granatum* Rind Extract. *Int J Pharm Sci Drug Res.* 3, 116-122.
  55. Segura, J.J., Morales-Romos, L.H., Verde-Star, J., Guerra, D. (2005). Growth inhibition of *Entamoeba histolytica* and *E. invadens* induced by pomegranate root (*Punica granatum* L.). *Arch. Invest. Med. Mex.*, 21, 235 – 239. <https://europepmc.org/article/med/2131771>
  56. Singh, B., Singh, J. P., Kaur, A., and Singh, N. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (*Punica granatum* L.) peel: A review. *Food chemistry*, 261, 75–86. <https://doi.org/10.1016/j.foodchem.2018.04.039>
  57. Singh, B., Singh, J. P., Kaur, A., and Singh, N. (2019). Antimicrobial potential of pomegranate peel: a review. *Int. J. Food Sci. Technol.* 54(4), 959-964.
  58. Singh, M., Jha, A., Kumar, A., Hettiarachchy, N., Rai, A.K., and Sharma, D. (2014). Influence of the solvents on the extraction of major phenolic compounds (punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril. *Journal of Food Science and Technology*, 51(9), 2070–2077. <https://doi.org/10.1007/s13197-014-1267-0>
  59. Sumere, B.R., De Souza, M.C., Dos Santos, M.P., Bezerra, R.M.N., Da Cunha, D.T., Martinez, J., and Rostagno, M.A. (2018). Combining pressurized liquids with ultrasound to improve the extraction of phenolic compounds from pomegranate peel (*Punica granatum* L.). *Ultrasonics Sonochemistry*, 48, 151–162. <https://doi.org/10.1016/j.ultsonch.2018.05.028>

60. Tamborlin, L., Sumere, B.R., de Souza, M.C., et al. (2020). Characterization of pomegranate peel extracts obtained using different solvents and their effects on cell cycle and apoptosis in leukemia cells. *Food Sci Nutr.* 8, 5483–5496. <https://doi.org/10.1002/fsn3.1831>.
61. Teoh, Y.P. and Mashitah, M.D. (2016). Extraction of 4h-pyran-4-one, 2,3- dihydro -6-methyl-, an alternative antifungal agent, from schizophyllum commune: optimization and kinetic study. *Borneo Sci.* 37(1), 1–22.
62. Thangavelu, A., Elavarasu, S., Sundaram, R., Kumar, T., Rajendran, D., and Prem, F. (2017). Ancient seed for modern cure - pomegranate review of therapeutic applications in periodontics. *J Pharm Bioallied Sci.* 9(5), 11–14.
63. Venkataramanamma, D., Aruna, P., and Singh, R.P. (2016). Standardization of the conditions for extraction of polyphenols from pomegranate peel. *Journal of Food Science and Technology*, 53(5), 2497–2503. <https://doi.org/10.1007/s13197-016-2222-z>
64. Wei, Q., Ma, X., and Dong, J. (2010). Preparation, chemical constituents and antimicrobial activity of pyrolygneous acids from 12 Peña, G. J. et. al. *Brazilian Archives of Biology and Technology. J Anal Appl Pyrolysis.* 87(1), 24–8.
65. Yassin, M.T., Mostafa, A.A., and Al Askar, A.A. (2021). In Vitro Evaluation of Biological Activities and Phytochemical Analysis of Different Solvent Extracts of *Punica granatum* L. (Pomegranate) Peels. *Plants*, 10(12), 2742. <https://doi.org/10.3390/plants10122742>
66. Zhu S., Jiao W., Xu Y., Hou L., Li H., Shao J., Zhang X., Wang R. and Kong D. (2021). Palmitic acid inhibits prostate cancer cell proliferation and metastasis by suppressing the PI3K/Akt pathway. *Life Sci.* 286, 120046. doi: 10.1016/j.lfs.2021.120046
67. Zhuang, S., Li, Y., Jia, S., Hong, H., Liu, Y., and Luo, Y. (2019). Effects of pomegranate peel extract on quality and microbiota composition of bighead carp (*Aristichthys nobilis*) fillets during chilled storage. *Food Microbiology*, 82, 445–454.