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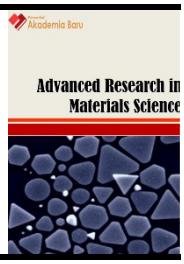

Punica Granatum Linn.: A phytochemical and pharmacological review

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ABSTRACT

The pharmacochemistry of the pomegranate (*Punica granatum Linn*.) was mentioned in the literatures. The natural chemicals and the biological attributes of the plant were determined. The interested properties include the wound healing effect, anti-diarrhoeal, anti-parasitic and antioxidant capacities of the seed and rind, or fruit peel extracts. Biotechnological research was also focused on the potentially natural dye colourant from the pomegranate. The pomegranate peel, producible as flour, could as well, be a promising antibacterial agent. Per the publications, therapeutic applications of pomegranate were reviewed. Manufacturers utilize this plant to produce supplements and cosmetics. Advances in pharmacological studies include the clinical trial to investigate the efficiency of a *Punica*-contained traditional mouthwash, to control gingivitis among the diabetic patients. From the phytochemical screening, the phenolic, alkaloid and terpenoid constituents were detected. The separation of alkaloids was performed by utilising the bulk liquid membrane technique, using rotating discs' contactor. Latest discovery includes the identification of punigratane, a novel pyrrolidine alkaloid with putative efflux inhibition activity. It is anticipated that *P. granatum* fruit peels and seeds could offer potential benefits and medicinal uses for human. The bio-waste of the rind is also appreciated and could possess significant values in the agricultural, biotechnological, chemical and food industries.

1. Introduction

Punica granatum L. is usually known as the pomegranate (Fig. 1). It belongs to the *Punica* genus in Punicaceae family. It is originally planted in area between Iran and northern India such as Afghanistan, Iran, Libya and Tunisia. In the Punicaceae family, *Punica spinosa* and *Punica florida* are synonyms with *P. granatum*. This means there is only one genus in its family. There are only two species in Punicaceae [1], another one is *Punica protopunica*. Its tree commonly grows 12 to 16 feet

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as shrub or small trees. Its flower is big, red and funnel-formed. Its fruit is grenade-shaped (Fig. 1), with bright red, leathery rind, and with calyx. The fruit contains many seeds (arils). The juice is contained in the seeds. The peel encloses membranous, white tissue (endocarp) which in turn, encloses the seeds.

P. granatum is popularly consumed as fresh juice, beverages and food products due to its sweet and delicious taste. It is also extracted to be utilised in herbal medicines or dietary supplement for improving human health. Thus, there are many supplements that are available in the market nowadays in which *P. granatum* extract is used as its active ingredients. It is claimed that these products can be used to treat diseases. The examples of its pharmacological activities include antioxidant, anti-tumors, anti-bacterial and anti-hepatoxicity. Moreover, the plant has been used for treatment of cardiovascular disease, diabetes, dental condition and protection from ultraviolet radiation [2].

Fig. 1. The Indian pomegranate, showing its distinctive ruby red, jewel-like seeds, is also available in local market.

Different parts of *P. granatum* such as the leaves, fruits, flowers and peels have different amounts and types of phytochemicals or biologically active compounds, in them. These differences have caused each part of them to give various effects. There are many phytochemicals that may involve in its biological attributes such as alkaloid, phenolic, gallocatechins, delphinidin, cyanidin, gallic acid and sitosterols. These phytochemicals are believed to be the source of the medicinal uses for *P. granatum* [3].

P. granatum is widely used as traditioal medicine in many parts of the world such as America, Asia, Africa and Europe [4]. It was used to treat tape worm infestation, acidosis, microbial infection, diarrhea, hemorrhage, helminth infection and respiratory pathologies [5]. The literature on *P. granatum* is enriching since 19th century and the journals on it extensively increased starting 2000 onwards. The popularity increases as people start to realize the great advantages it can bring and its potential in the health field [6]. Biomaterial and technological research were also focused on the potentially natural dye colourant from pomegranate [7]. The research statement in most *Punica* studies is that it can give medicinal uses to human. Many investigations were performed on the juice and fruit. However, the public may still be uninformed that the peel and seed parts can also provide medicinal values [8]. These plant parts are even considered as biowaste products by the food industry. Thus, studies should be conducted to reveal *Punica*'s biological characteristics and the constituents that contribute to these benefits. Meanwhile, the objective of this paper is to present

the recent medicinal uses and phytochemicals of *P. granatum* peels and seeds via literature search. The significance of this review would be anticipated. The pharmacological activities that *P. granatum* could offer were studied, since this plant is used for centuries in complementary practices.

2. Pharmacological uses of *Punica Granatum* peels and seeds

P. granatum is used for centuries in medicinal purposes. Many studies were done to explore the utilisation of this plant species. The results are mostly positively related to its alternative medicinal uses. From the studies, researchers found many potential therapeutics action of *Punica* species [9]. Pomegranate juice is obtained by extracting the seeds [10]. The nutritional benefits of the pomegranate were listed [11]. The co-product of this procedure is the fruit peel, which could be converted into flour. The polyphenolic profile and antibacterial property of the peel flour was also assessed [12]. The antimicrobial and antiviral effects of pomegranate were lately debated [13].
P. granatum is also known as the ellagic acid-rich fruit. The pharmacological use of the fruit is due to the ellagic acid and ellagitannins content. They are agents, which induce vasorelaxation, oxygen free radical scavenging, hypolipidemic, anti-inflammatory and anti-carcinogenic activities in various animal preparations [14]. The bio-waste of the rind could also possess significant values in agricultural, biotechnological and chemical industries [3, 15-17].

2.1. Antioxidant activity of Punica Granatum peels and seeds

The most famous property of *Punica granatum* is its antioxidant capability. All parts of the *P. granatum* such as juice, seeds and peels show high antioxidant characteristics. The peels which are not used and considered as biowaste materials, showed the highest antioxidant activity [18-19] as compared to the other parts of the plants. It is followed by the flower, leaf and seeds [20]. Punicalagins (Fig. 2) are found to be the most important compounds that contribute to the pomegranate antioxidant property. The compounds are mostly present in the peel, husk and inner yellow membranes surrounding the juice arils of pomegranate fruits. They are present in the pomegranate juice too, but at lower concentration [21]. *P. granatum* which is rich in antioxidant, can help to reduce the risk of getting chronic diseases, caused by oxidative stress. Punicalagin was also included in a novel therapeutic system which was developed for the topical treatment of antiviral infections [22].

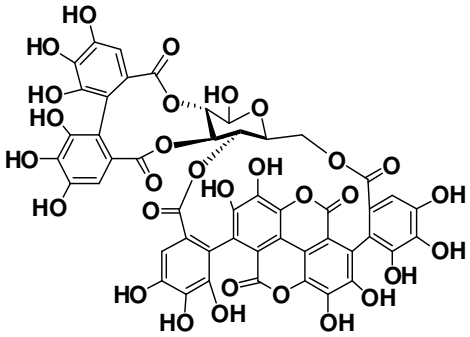

Fig. 2. The chemical structure of punicalagin, a phenolic substance in *P. granatum* peels and seeds.

Peels have higher radical scavenging activity compared to seeds. Higher radical scavenging activity means higher antioxidant ability of the compound [23]. The peels have very high antioxidant

level and even higher compared to vitamin C with same concentration [15]. Free radical also can cause autoxidation such as autoxidation of unsaturated lipids in foods. Antioxidants can intercept the free radical chain of oxidation and donate hydrogen from phenolic hydroxyl group thus no further oxidation of the lipid. The methanol extract of *P. granatum* peel exhibit antilipid peroxidation activity which can longer shelf life of foods. Increase concentration of pomegranate peel will increase antilipid peroxidation activity [23].

Antioxidant activity also helps in preventing the central nervous system (CNS) impairment such as Alzheimer disease. The brain is vulnerable to oxidative stress caused by free radicals. *P. granatum* seeds can help to protect the brain from neuronal damage due to aging process. It is known that plants with flavonoids, saponins and tannins help in treating and preventing CNS disorder. *P. granatum* seeds contain all the three, thus enable them to reduce the CNS disorder by anxiolytic effect [24].

2.2. Antibacterial effect of Punica Granatum peels and seeds

For the antibacterial effect, the peels of *P. granatum* is better compared to the seeds. Numerous studies were done that showed pomegranate peels have high antibacterial and antimicrobial properties. It can fight against a wide range of bacteria include gram positive and gram negative bacteria [25-26]. *Escherachia coli, Pseudomonas aeruginosa, Staphyllococus aureus*, *Bacillus cereus, Bacillus coagulans, Bacillus subtilis and Klebsiella pneumonia* can be inhibited by the *P. granatum* peels [25-27]. Other parts of the plant such as the seeds and juice showed antibacterial effect but not as great as the peels. The sequence is as follows; peels > juice > whole fruits > seeds [25]. Different extraction solvent will also effect the capability of the peel extract to inhibit growth of the bacteria. The ethanol extract showed the highest antibacteria activity. It is even greater compared to tetracycline. This is known by the zone of inhibition in agar good diffusion method [27]. The *P. granatum* peels also effective against some common oral pathogens such as *S. epidermidis, S. aureus, L. acidophilus, S. mutans, S. sanguinis* and *S. salivarius* but not effective against *A. viscosus* and *C. albicans* [28].

Another concern on bacteria potential harm to human is that they can evolve to be resistant to the antibacterial drugs. Bacterial Multidrug Resistance (MDR) is an emerging crisis that will cause the treatment of bacterial infection to be ineffective. One of the most contributing factor to MDR is the increasing efflux activity of the bacterial membrane that pump the drugs out of the cells. Thus, the use of efflux pump inhibitor is becoming popular in preventing drug resistance in bacteria. *P. granatum* peels were recently discovered to can inhibit efflux pump of the bacteria. It is used as adjuvant with antimicrobial to increase the accumulation of the drug in the bacterial cell. Punigratane (Fig. 3) is the new alkaloidic compound that give the efflux pump inhibitor effect in the peel of *P. granatum*. It is added as adjuvant with norfloxacin to fight against *Klebsiella pneumoniae* which has a high efflux pump activity. From the study, the accumulation of norfloxacin in the bacterial cell is increased, thus increasing the drug effectiveness in killing the bacteria [17].

3. Phytochemistry of *Punica Granatum* peels and seeds

Phytochemicals are mostly from the second metabolite of the plants. They are used for protection and other non-nutritional purposes. Researchers discovered that the phytochemicals provide benefits to humans in medicinal field. Extensive studies were performed on *P. granatum* to explore these phytochemicals. From the phytochemical screening, the phenolics, terpenoids and alkaloid constituents were detected from the peels [29-30]. Meanwhile, the composition of amino acids of

pomegranate from China were analyzed. The total glutamate- , aspartate- , pyruvate- and serine-related amino acids were higher, when compared among six cultivars from two regions [31]. It is interesting that contradicting results were announced when local researchers embarked on study of pigments in *Punica* and commented about the absence of glycosides [32].

3.1. Alkaloids in Punica species

The peel of *P. granatum* contains alkaloid which was screened by using Wagner's test [33]. The separation of *Punica* alkaloids was performed by utilising the bulk liquid membrane technique, using rotating discs contactor [34]. Punigratane (Fig. 3) was identified from the peel. It is a pyrrolidine-like structure and possess the efflux inhibition activity. Punigratane contains a heterocyclic ring, having a nitrogen atom, which is like pyrrolidine [17].

3.2. Lipid in Punica species

Lipid is an array of esters of fatty acids. In plant, it is the primary metabolite which is essential for the plant's life. Fatty acids mostly accumulate in the seeds. In *P. granatum*, there are high concentration of fatty acids, such as palmitic acid and stearic acid. While the carotenoids could be obtained via green ultrasound-assisted extraction of *P. granatum* wastes or peels, by using vegetable oils as solvents [35].

Nevertheless, the major constituent of fatty acid in the seeds is punicic acid (Fig. 4) which stands for up to 80% of fatty acid in the seeds oil. Punicic acid is exclusively named for *P. granatum*. It is a polyunsaturated fatty acid which contains eighteen carbons with three conjugated double bonds. The seeds also contain sterols such as stigmasterol and sitosterol [36]. The sterol is a group of steroid which has the same structure as cholesterol. It is known due to its capability of lowering cholesterol level in the body.

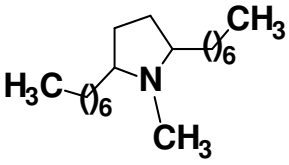

Fig. 3. The chemical structure of punigratane or 2,5-diheptyl-*N*-methylpyrrolidine.

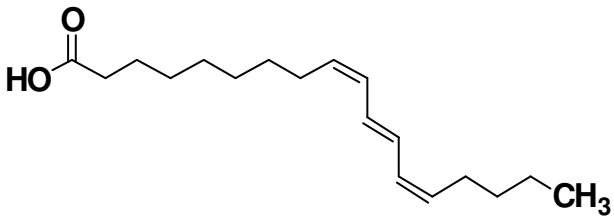

Fig. 4. The chemical structure of punicic acid.

3.3. Phenolics in Punica Species

Phenolic is a large group of compounds which is characterised by containing at least one aromatic ring substituted with at least one hydroxyl group. However, not all compounds containing phenol are classified as phenolic compound. Another thing that must be considered is their biosynthetic pathway. There are two routes for plant phenols biosynthesis, which are shikimate pathway and acetate pathway. Phenols can be divided into two large groups which are flavonoids and non-flavonoids (Table 1). Flavonoids have 6 subgroups which are flavones, anthocyanidins, flavonones, isoflavones, flavonols and flavanols. Meanwhile, the non-flavonoids are subdivided into phenolic acid, lignans and stilbenes. Both peels and seeds of *P. granatum* contain phenolic compounds, where peels having higher amount of the phenolics.

The compound that is responsible for the antioxidant property in the peel of *P. granatum* is punicalagin (Table 1, Fig. 2). It is the most abundant in the peel part when compared to the other part of the plant. Punicalagin (Table 1, Fig. 2) is an ellagitannin which is a hydrolyzable tannin. The structure of punicalagin is characterized by the esterified hexahydroxydiphenic acid with sugar (Fig. 2). Apart from that, catechin and quercetin are also responsible to the antioxidant activity of the peels. Both are flavonoid, in which their structure is characterized by the presence of two aromatic rings and one heterocyclic ring with oxygen [21].

Table 1
The phenolic substances in both peels and seeds of *P. granatum* [4].

Phenolics	Peels	Seeds
Flavonoids	Anthocyanin, quercetin, catechins, rutin	Anthocyanin
Non-Flavonoids	Ellagic acid, gallic acid, protocatechuic acid, caffeic acid, punicalagins (ellagitannin)	Punicalagins (the anomeric isomers of punicalagin molecule)

In terms of extraction and separation methodologies, the coloured aqueous extract of *P. granatum* could be obtained by boiling the peels, arils and seeds [37]. One holistic approach to gain the whole pomegranate polyphenols was proposed by using aqueous solutions of cyclodextrins [38]. The purification of phenolics from *P. granatum* juice could be achieved by using ultra- and nanofiltration membranes [39]. These polyphenols can be detected qualitative- and quantitatively by high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometry [40]. Meanwhile, the matrix solid-phase dispersion method was found as an efficient technique for the quantitative extraction of ellagic acid from the pomegranate peels [41].

4. Products of *Punica Granatum*

Due to its high nutrition value, many processed products of *P. granatum* were introduced. Advances in pharmacological studies include the clinical trial to investigate the efficiency of a *Punica*-contained traditional mouthwash, to control gingivitis among the diabetic patients [42]. Meantime, there are still not many products from pomegranate peel. As for pomegranate seeds, products are made in oily form, as they are rich in oils and lipids. As for the whole fruit, *P. granatum* fruit juice was widely used and famous. This fruit juice is very nutritious and is recommended for patients with gastric trouble [43]. It is also suggested that *P. granatum* juice could protect against the effects of a high-fat diet and high cholesterol levels [44]. An example of a supplement that uses pomegranate as its active ingredient is in the form of concentrated fruit juice [45]. Other substances that were included are honey, gamat and fish oil. It is claimed that the product can be used to prevent stroke, tumor, gastric and arthritis.

5. Conclusion

From the review, the phytochemicals from *P. granatum* peels and seeds were obtained via ethanolic extraction. The phytochemicals were analysed by using chromatographic and spectroscopic approaches. It is anticipated that *P. granatum* fruit peels and seeds could offer potential benefits and medicinal uses for human. The bio-waste of the rind is also appreciated and could possess significant values in the agricultural and food industries.

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