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Phytochemical Composition and Microscopic Characteristics of the Fruit Bark of *Punica granatum* L.

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ABSTRACT

Background and Objective: The outer layer of the *Punica granatum* L. fruit exhibits a high degree of durability. The majority of the plant's components have been utilized in traditional medicine for the treatment of various ailments. The medicinal properties of *Punica granatum* L. are anti-inflammatory, antioxidant and antibacterial activities. The present investigation was undertaken to establish the foundational pharmacognostic criteria that should be employed in the evaluation of the botanical specimens. **Materials and Methods:** In this study, an analysis was conducted on the phytochemical composition and microscopic characteristics of the fruit bark of *Punica granatum* L. Quantitative phytochemical information was assessed by conducting tests on three distinct extracts derived from the peel of *Punica granatum* L. fruit. **Results:** The periderm of the fruit exhibits a diverse array of microscopic characteristics, encompassing fibrous elements, sclerotic structures, stone cells, prismatic crystals, both simple and compound starch granules, oil droplets and fully developed parenchymal cells. The bark of *Punica granatum* fruits contains various bio-active compounds such as saponins, quinones, terpenoids, phenols, tannins, alkaloids, betacyanin, coumarins, phlobatannins, oil and fats. However, it is notable that glycosides and anthocyanin are not present in significant amounts. **Conclusion:** Pomegranate has a diverse array of potential therapeutic properties due to the presence of numerous chemical constituents. This research may contribute to the production of standardized products derived from this plant.

KEYWORDS

Punica granatum, pharmacognosy, therapeutic properties, chemical constituents, bioactive compounds

INTRODUCTION

Punica granatum L. (pomegranate) belongs to family Punicaceae. It is a small tree that grows up to 20 to 30 feet tall in semi-arid to sub-tropical climates[1]. The spiny branches are stiff and sharp. The leaves are narrow, lance-shaped, shiny and leathery[2]. Flowers can appear alone or in groups of two or three at the end of each branch[3].

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Pomegranate is good for health, according to recent *in vitro* and *in vivo* studies[4,5], owing to its polyphenol content and antioxidant activity. *Punica granatum* fruits, seeds, peels and leaves contain a variety of beneficial compounds. Suman and Bhatnagar[6] describe how these ingredients have been used to treat diseases. Pomegranate peel is loaded with minerals, polysaccharides, flavonoids, ellagitannins, flavonoids and other phenolic compounds. Among other things, its juice contains polyphenols, tannins, anthocyanins, vitamin C, vitamin E and lipoic acid. The fruits, peels and seeds of *Punica granatum* can aid in disease treatment by altering biological processes[7]. Pomegranate juice and peel contain punicalagin, which are very powerful antioxidants[8].

Punica granatum extract and powder are frequently made from its bark due to the high amount of punicalagin and antioxidants[9]. The primary cause of various diseases is long-term tenderness. The diseases due to long-lasting inflammation include type 2 diabetes cancer, overweight and Alzheimer's disease. Tirado-Gallegos *et al.*[10] revealed that the antioxidant effects of *Punica granatum* are mainly responsible for its anti-inflammatory effects. According to laboratory studies, *Punica granatum* extract may slow the growth of cancer cells and even cause them to die through a process known as apoptosis[11]. Pantuck *et al.*[12] discovered that the presence of prostate-specific antigen (PSA) in the blood is an indicator of prostate cancer. Breast cancer is one of the most common types of cancer in women.

Levitsky and Dembitsky[13] discovered that a *Punica granatu*m extract could prevent breast cancer cells from dividing and even kill some of them. Pomegranate plant compounds can help reduce inflammation, so it stands to reason that they could be used to treat arthritis. Laboratory studies showed that *Punica granatum* extract can inhibit enzymes that are known to irritate osteoarthritic joints[14]. Furthermore, a single intraperitoneal injection of pomegranate extract into fish naturally infected with lymphocytic disease virus increased their innate immune response and reduced the number of fish that died from lymphocytic infection[15,16]. *Punica granatum* family plants are also used to treat lung diseases. Extracts are effective against the 13 herpes virus and have high activity against the influenza virus[15,17].

Humans have always used plants as medicine to treat life-threatening diseases and despite the fact that this is the oldest medical practice, it remains popular to discover new drug candidates[18]. Botanical natural health products are gaining worldwide popularity. Orhan[18] estimates that roughly 80% of the world's population utilises traditional medicine, primarily herbal remedies. Consequently, pharmacognosy continues to be prominent in pharmaceutical sciences and plays an essential role in drug discovery. Pharmacognosy is the study of medicines derived from plants and other natural resources[19]. It is concerned with the study of natural medicines its standardization and authentication.

The majority of pharmacognosy investigation has centered on identifying the controversial plant species and validating conventionally used medicinal plants by using morphological, physicochemical and phytochemical analysis[20].

During the last few decades, the importance of pharmacognosy has grown. As compared to taxonomic identification, the pharmacognostic study includes different parameters for identifying and detecting dry powder adulteration[21]. This is necessary because the dried powder lost its morphological identity and is susceptible to adulteration[22]. Plant identities are verified and standard parameters are established to prevent adulteration in pharmacognostic research.

The presence of phytochemical constituents in different parts of *Punica granatum* makes it pharmacologically valuable. Phytochemical screening is the process of extracting, testing and identifying the substances in plants that can be used as medicine[23]. The phytochemical is a naturally occurring bioactive compound found in plants that, when combined with nutrients and fibres, works as a disease-fighting defence system[24]. Most of the time, the healing effects of plant parts come from their

combination of secondary products. However phytochemical research has shown secondary constituents present in plants are alkaloids, carotenoids, antioxidants, terpenoids and phenolic compounds flavonoids, etc. Screening of phytochemicals can help to find new drugs and may shed light on the traditional uses for more chemical screening. Craig[25] says that plants have about 4,000 phytochemicals that can be used to prevent, lessen the effects of, or treat diseases like strokes, cancer and metabolic syndrome.

The present investigation was undertaken to establish the foundational pharmacognostic criteria that should be employed in the evaluation of the botanical specimens.

MATERIALS AND METHODS

Study area: The present research work was undertaken from June 2023 to December 2023. The samples were collected from different locations in Hazara Division Khyber Pakhtunkhwa, Pakistan.

Drying and powder process: The 3 kg of separated fruit bark was air-dried at room temperature for 12 days. Afterwards, the powder of the bark was produced using an electric grinder for the evaluation of powder drug study.

Preservation: When the bark was finely powdered, it was packed into beakers with methanol at a ratio of 140 g of powder to 550 mL of methanol and left to soak for several days[26]. Daily shaking ensured that the powder was evenly distributed throughout the solvent.

Microscopical characters: Powdered bark was analyzed for the cells and tissues present using a microscope and the established protocol[27]. After heating the mixture for at least 30 minutes, a pinch of powder was added to the test tube and about 70% of chloral hydrate solution was added[28]. After that, used a spirit lamp to melt safranin gel on a slide. Different steps were taken to prepare slides for observation by pouring a solution from a test tube onto safranin gel and then placing a cover slip on top.

Secondary metabolites analysis: Standard protocol was used to analyse secondary metabolites after conducting phytochemical tests on a powdered sample of bark[29,30].

Extract preparation: After soaking 5 g of bark powder in 50 mL each of ethyl and methyl alcohols and distilled water for seven days, the mixture was stirred constantly with a stirrer to ensure proper evaporation[31]. The phytochemical analysis was performed after the extracts had been filtered through Wattman's filter paper for seven days.

Chemicals tests

Tannins: Dark blue and greenish-black color was obtained by adding 1 mL bark extract to 1 mL 5% of ferric chloride[32].

Saponins: The frothing layer on the upper surface of solution was obtained by shaking 1 mL bark extract with 1 mL distilled water[30].

Quinones: Red color of the solution was confirmed by using 1 mL bark extract with 1 mL conc. H_2SO_4[30].

Flavonoids: Yellow shading of solution appeared by analyzing 1 mL bark extract with 1 mL of sodium hydroxide[30].

Alkaloids: The yellow cream-colored formation for alkaloids were obtained by adding 1 mL bark extract in 2 mL conc. HCl and few drops of Mayer's reagent[30].

Glycosides: The formation of pink color for glycosides were obtained by using 1 mL peel extract to 3 mL chloroform and 10% ammonium solution[32].

Cardiac glycosides: Brown ring at the interface for cardiac glycosides was observed by analyzing 1 mL peel extract with 2 mL of glacial acetic acid and a few drops of 5% $FeCl_3$, under-layered with 1 mL of conc. H_2SO_4.

Terpenoids: The formation of reddish-brown color in the inner face of the solution was investigated by using 1 mL peel extract to 2 mL chloroform and conc. sulphuric acid.

Phenols: The bluish color of phenols was obtained by adding 1 mL peel extract to 2 mL distilled water and a few drops of 10% ferric chloride.

Steroids: One mL of the peel extract was mixed in 2 mL acetic anhydride. The conversion of color into blue or green indicates steroid presence.

Coumarins: The yellow color for the presence of coumarins was investigated by adding 1 mL peel extract to 1 mL 10% NaOH[32].

Anthocyanin and betacyanin: Bluish-green color for anthocyanin and yellow for betacyanin was observed by using 1 mL peel extract and 1 mL sodium hydroxide, heated for 5 min at 100°C[33].

Phlobatannins: A few drops of 2% of HCl were added to 1mL of bark extract. Formation of red color precipitation indicated the presence of phlobatannins[34].

Carbohydrates: One mL of bark extract was added to 2 mL of iodine solution. The appearance of violet or blue color shows the presence of carbohydrates[35].

Altered oil and fats: The plant's powder was rough on the filter paper used in this experiment. The oily residue on filter paper suggests that the plant powder contains oil.

RESULTS

Different types of cells, including simple and compound, starch grains, fibres, scleroid, stone cells, prismatic crystals, oil cells, vascular bundles, parenchyma cells, colouring matter and pollen grains (Table 1) were studied during powder drug study of *Punica granatum* peel (Fig. 1a-p).

Table 1: Microscopical characters of *Punica granatum* peel showing various cells

Serial number	Types of cells
1	Fibers
2	Sclereids
3	Colouring matter
4	Styloid crystals
5	Prismatic crystal
6	Acicular crystals
7	Calcium oxalate crystals
8	Pollen grain
9	Starch grains
10	Oil cells
11	Vascular bundles
12	Stone cells
13	Parenchyma cells

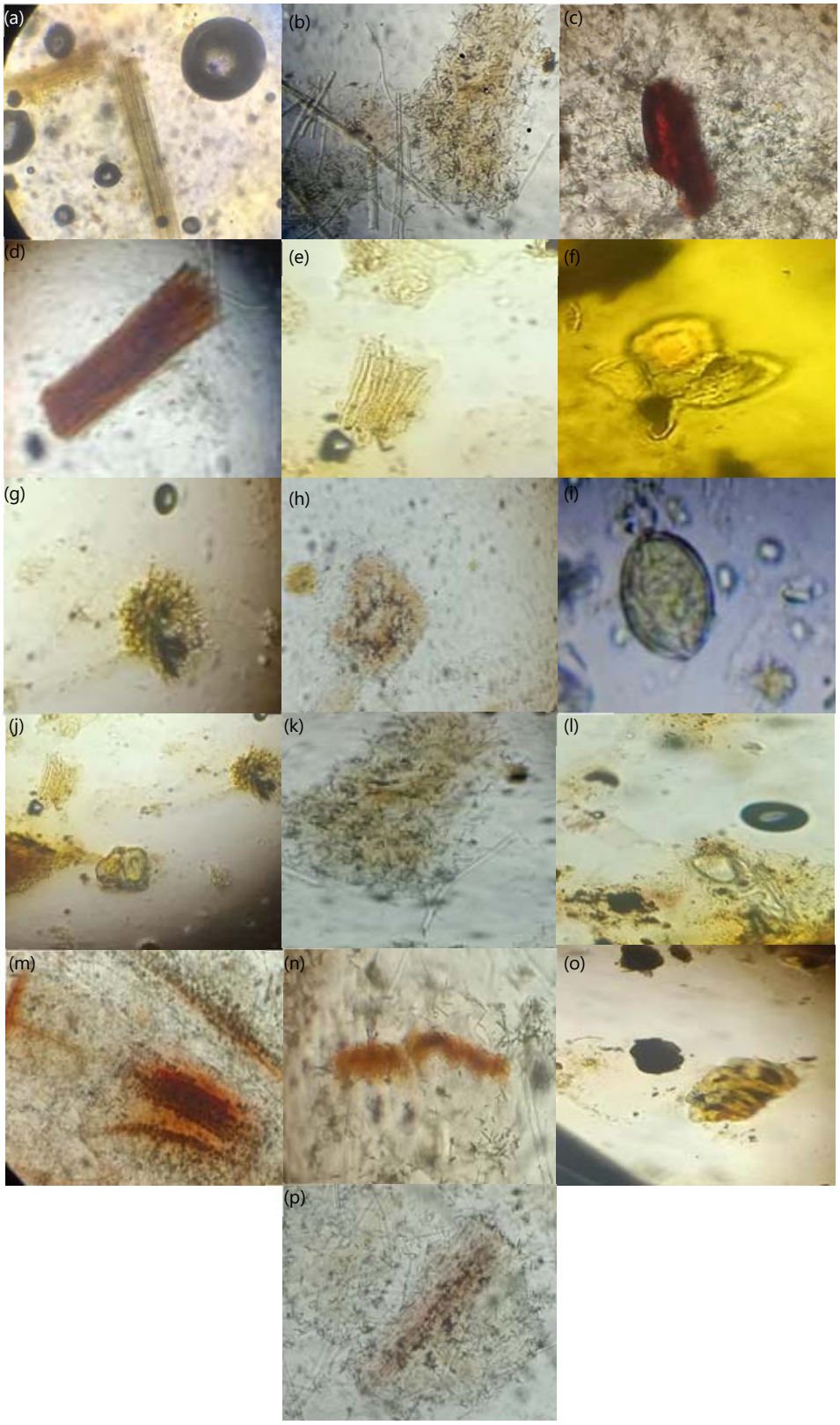

Fig. 1(a-p): Different types of cells and tissues during powder microscopy of *Punica granatum* L., (a) Fibers, (b) Fibers with blunt ends, (c) Scleriod, (d) Colouring matter, (e) Styloid crystals, (f) Prismatic crystal, (g) Acicular crystals, (h) Calcium oxalate crystals, (i) Pollen grain, (j) Starch grains, (k) Starch grains , (l) Oil cells, (m) Vascular bundles, (n) Fragment of tracheid, (o) Stone cells with lumen and (p) Elongated parenchyma cells

Fig. 2: Various phytochemical tests for detection of secondary metabolites

Table 2: Detection of secondary constituents in methanolic, ethanolic and distilled water extraction during phytochemical screening of powdered peel of *Punica granatum* L

Chemical compound	Methanolic extract	Ethanolic extract	Distilled water extract
Carbohydrates	-	+	-
Terpenoids	+	-	+
Saponins	+	+	+
Alkaloids	-	+	+
Flavonoids	-	+	+
Quinones	+	+	+
Glycosides	-	-	-
Cardiac glycosides	+	+	-
Tannins	+	-	+
Coumarins	+	+	-
Phenols	+	-	+
Phlobatannins	-	-	+
Oil and fats	+	+	+
Betacyanin	-	+	+
Anthocyanin	-	-	-

Present (+) sign showing the presence of chemical constituents and Absent (-) sign showing the absence of chemical constituents

Qualitative phytochemical analysis of bark: Phytochemical screening analysis was used to determine the presence of various organic compounds in the bark of *Punica granatum*. The phytochemical investigation of *Punica granatum* fruit peel (Fig. 2) was carried out in three different types of extracts (methyl alcohol extract, ethyl alcohol extract and distil water extract) to observe the quantitative phytochemical which is as follows. Saponins and quinones perform well in all three types of extract. However, the bark extract contains terpenoids, phenols and tannins in methanol and distilled water, but not in ethanol extract. The presence of alkaloids and betacyanin was found in ethanol and distilled water but not in methanol. Coumarins were found in methanol and ethanol but not in distillation water. Phlobatannins were only found in distilled water and were absent in methanol and ethanol extracts. All three extracts contained oil and fats. Glycosides and anthocyanin were not present in any of the three extracts (Table 2).

DISCUSSION

There are many different kinds of cells in the powdered *Punica granatum* bark, as evidenced by organoleptic and microscopical analysis. These include fibres, long fibres with blunt ends, scleroid, colouring matter, styloid crystals, prismatic crystals, acicular crystals, calcium oxalate crystals, pollen grains,

starch grains, starch grains with indistinct hilum and lamina, oil cells and thick everything that could be seen at such a small scale was seen. Similar results were found when comparing the current investigation to previous studies, suggesting that it is valid and should be used for powder drug authentication.

Many common pharmaceuticals derive their active ingredients from plants[34]. *Punica granatum*, a plant in the family Punicaceae, is used in numerous treatments. Saponins, quinones, alkaloids, glycosides, terpenoids, phenols, tannins, volatile oils and fats are just some of the unique substances found in *Punica granatum* bark that give it its medicinal value[35]. Pomegranate peel is rich in a variety of nutrients, including minerals, polysaccharides, flavonoids, ellagitannins and other phenolic compounds[7]. The fruit's bark has been used medicinally for centuries to treat everything from heart disease and cancer to diabetes and Alzheimer's. The antioxidant punicalagins in pomegranates are responsible for the fruit's anti-inflammatory effects[10]. Among female cancers, breast cancer ranks high.

According to Levitsky and Dembitsky[13], pomegranate extract can stop the growth of breast cancer cells and even kill some of them. Those who suffer from osteoarthritis may be interested to learn that pomegranate extract has been shown in lab studies to block the activity of enzymes that cause joint damage. The astringent and ethnomedical benefits of *Punica granatum* peel. Traditional medicine prescribes *Punica granatum* bark for a wide variety of ailments, including but not limited to diarrhea, dysentery, intestinal parasites, wound healing and sore throat. Cattle feed often includes *Punica granatum* peel.

Extracts of *Punica granatum* bark were tested in three different solvents to determine their relative concentrations of the following phytochemicals: Methyl alcohol, ethyl alcohol and distilled water. The saponins and quinones in all three extracts work admirably. Terpenoids, phenols and tannins are found in the bark extract of methanol and distilled water, but not in ethanol. Alkaloids and betacyanin were present in ethanol and distilled water extracts, but not in methanol extract. Methanol and ethanol, but not distillation water, contained coumarins. Extracts made with methanol and ethanol did not contain phlobatannins, but those made with distilled water did. There was oil and fat in all three of the extracts. All three extracts were devoid of glycosides and anthocyanin. Plant extract contains the aforementioned phytochemical constituents found in the bark of *Punica granatum*, which provide essential pharmaceutical properties for human health[13]. This finding was confirmed by a comparison with other published data.

In addition to their use as drugs and supplements, terpenoids, alkaloids and flavonoids have also been studied for their potential to prevent and even reverse the development of various forms of cancer[11]. The three different extracts of pomegranate all contain different phytoconstituents, which are responsible for the fruit's therapeutic effects. The phenolics found in plants are the primary antioxidants or free radical scavengers. This class of compounds includes flavonoids and tannins. The presence of these compounds in the extracts suggests that they are responsible for pomegranate's strong antioxidant properties. Secondary metabolites and other chemical constituents are responsible for medicinal plants' beneficial effects. For instance, saponins have been shown to have cardiode pressant and hypotensive effects. Glycosides are used to treat arrhythmias and congestive heart failure because they are naturally cardioactive. Ishtiaq *et al*.[34] speculated that the presence of saponins in all extracts of pomegranate contributes to its cardioprotective potential.

Punica granatum is believed to be used for the ailment of different diseases. Keeping in mind the importance of this plant it is strongly recommended for detailed phytochemical and medicinal investigation. The plant should be planted at a large scale in every possible habitat.

The research findings will facilitate the confirmation and reproducibility of the herbal products and resulting in the care and effectiveness of natural products. The present investigation will undertake to establish the foundational pharmacognostic criteria that should be employed in the evaluation of the botanical specimens.

CONCLUSION

Punica granatum L. the oldest known medicinal fruit plant, was the subject of the current study, which aimed to provide in-depth information on it. This study provides a wealth of information regarding the pharmacognostic identification of *Punica granatum* fruit bark. Current pharmacological research indicates that this strategy possesses exceptional biological potential. Due to the presence of bioactive compounds, *P. granatum* has been associated with a wide range of pharmacological activities, including antioxidant, antimicrobial, antiviral, anti-diabetic, anti-cancer and anti-Alzheimer's disease effects, among others. The current phytomedicinal value of *Punica granatum* is believed to pique the interest of researchers in using this plant in modern medicine. This study reveals that *Punica granatum* is a promising source of antioxidant and antimicrobial compounds. The results of microscopic studies provide a passport for the correct identification of various cells found in the bark of *Punica granatum*.

SIGNIFICANCE STATEMENT

This study's findings regarding the physicochemical properties of fruit bark and the chemical composition of its constituents will be useful for standardizing herbal drug material. Pomegranate has a diverse array of potential therapeutic properties due to the presence of numerous chemical constituents. This research may contribute to the production of standardized products derived from this plant. Its flavonoids indicate that it possesses antioxidant activity which is valuable in poly herbal formulation.

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