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Original Article

Pharmacognostical and Physicochemical Analysis of Stem Bark of *Punica granatum* L.

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ABSTRACT

Background: *Punica granatum* L. (*Lythraceae*), or pomegranate, is a vital medicinal plant in traditional systems like Ayurveda and Unani. Its stem bark is particularly valued for its healing powers and richness in tannins, traditionally used for astringent, anthelmintic, anti-diarrheal, and anti-inflammatory effects. **Objectives:** The primary aim is to develop detailed pharmacognostical and physicochemical criteria for the stem bark to support precise identification, verification, and standardization for future therapeutic applications. **Materials and Methods:** Testing included organoleptic, macroscopic, and microscopic (powder and transverse section) examinations. Physicochemical parameters such as ash values, loss on drying, extractive values, fluorescence analysis, and elemental analysis (CHNSO) were determined. **Results:** The bark appeared as single or double quills (7-15 cm long) with a greenish-brown outer and yellowish-brown inner surface. Microscopic analysis revealed phellem, phellogen, and phelloderm layers with stone cells and phloem parenchyma. Physicochemical results included total ash (3.11%), loss on drying (4.30%), and water extractive value (10.26% w/w). Elemental analysis confirmed the presence of C, H, N, S, and O. **Conclusion:** The data provides essential standard markers for identifying and controlling the quality of Punica granatum L. stem bark, contributing to the scientific validation of its use in herbal medicine.

Keywords: *Punica granatum* L., Physicochemical parameters, Powder microscopy, Fluorescence analysis, Elemental analysis.

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INTRODUCTION

India is a country with one of the most ancient, rich and brightest traditions of medicinal herbs utilization. Plants have played an important role in the treatment of several ailments in the history of human beings- a practice that has been maintained to date. The organization of the World Health Organization (WHO) has reported that 75-80% of the global population makes use of plant-based drugs as the main source of medical treatment (Dipak *et al*., 2012). To treat different illnesses, herbal medicine, also known as phytomedicine, botanical medicine, or herbalism, incorporates different parts of plants, such as leaves, seeds, roots, bark, berries, or flowers (Ettish *et al*., 2022). Although these remedies are natural, they may include highly active bioactive elements, which require the same kind of effectiveness as pharmaceutical medications. It does not mean that natural is a safe condition, and the use of this substance can be harmful to health when used incorrectly (Alkhatib *et al*., 2022). A large portion of the Indian population (up to 90% of it) and parts of Africa rely on herbal remedies as their health care tradition (Wahab *et al*., 2012). What makes herbal remedies so attractive is the low cost, reduced side effects than synthetic medicines, developing resistance to conventional medicines, particularly antibiotics, and the high cost of care in the modern set-up. Also, a herbal treatment is in line with the increasing trend of customized and comprehensive medical services. The world has almost 21,000 known medicinal plant species, according to the World Health Organization, which is considered the world health authority (Kumudhaveni *et al*., 2024).

Continuous pursuits of replacing conventional medicines with natural ones, with the majority of chronic diseases having no confirmed cure, have only contributed to the growing popularity of natural medicine. There is, however, a major drawback of herbal remedies since they do not have all the scientific information about the safety and effectiveness of these treatments related to allopathic medicine. Based on these points, the pharmacognostic and physicochemical analysis of *Punica granatum* L. stem bark is the principal subject of investigation in this paper. This study aims to make the identifying markers undisguised and present a systematic guide on the way of conducting further research on the potential medical applications of this plant (Jeevitha *et al*., 2021).

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Pomegranate/anar or *Punica granatum* L. is a small, shrubby tree that is the *Lythraceae* family and is cultivated worldwide. The genus *Punica L.* has just two species: *Punica protopunica*, which is not edible and is endemic to the Socotra Islands in the Arabian Sea, and Punica granatum, which is an edible species native to the region from Iran to the Himalayas and widely cultivated in the Mediterranean (Dipak *et al.*, 2012).

Punica granatum L. has been used for a long time in traditional medicine systems because of its therapeutic value. Herbal remedies have been employed to treat a wide range of illnesses with the use of the whole plant, including the fruit, peel, seeds, root, and bark, among them, helminthiasis, acidosis, diarrhoea, ulcers, microbiological infections, skin ailments, healing wounds, fever, bleeding, and respiratory issues (Rahimi *et al.*, 2012). *Punica granatum* L. plays an important role in naturopathy, and it is known as a pharmacy on its own. It is mainly appreciated in terms of antiparasitism (Jacob *et al.*, 2019). Yet, it also functions as a blood tonic to enhance the blood quality, and apathies (canker sores) diagnosis, diarrhoea, and ulcer treatment are commonly treated on the basis of the soothing or healing properties (Marrone *et al.*, 2024). Indeed, the therapeutic effect of the plant is explained by its high phytochemical content, namely flavonoids, alkaloids, polyphenols and tannins. Soft seeds, thickly colored peel and arils are some of the distinctive features of the plant. The arils, the nutritious part, specifically, contain a lot of vitamin C, vitamin K, and folate (Marrone *et al.*, 2024). Pomegranate is famous for several pharmacological effects, such as antimicrobial, anticancer, anti-inflammatory, and immunomodulatory activities. The taxonomy of *Punica granatum* L. is given in Table 1 (Kumari *et al.*, 2021).

Table 1: Taxonomic classification of *Punica granatum* L.

Sl. No.	Kingdom	Plantae
1	Sub Kingdom	Tracheobionta
2	Super division	Spermatophyta
3	Division	Magnoliophyta
4	Class	Magnoliopsida
5	Subclass	Rosidae
6	Order	Myrtales
7	Family	Lythraceae
8	Subfamily	Punicoideae
9	Genus	*Punica* L.
10	Species	*Punica granatum* L.
11	Common Name	Pomegranate/Anar

Taxonomy of the plant

Geographical Distribution of Punica granatum L.

Punica granatum L. is a plant native to the regions of Central Asia, particularly Iran, Afghanistan, India, Pakistan, China, and Japan. Besides its indigenous locations, it has been practised for and very ancient in numerous other parts of the world (Rahimi *et al.*, 2012). *Punica granatum* L. is a well-known plant in Mediterranean countries such as Tunisia, Turkey, Egypt, Spain, and Morocco (Shaygannia *et al.*, 2016). Moreover, it has also been dispersed to other locations such as California in the U.S and Russia, where it is grown, since it is flexible to various climates (Kumari *et al.*, 2021). Experts cultivate *Punica granatum* L. in the vast majority of countries of the world today Iran, Spain, Italy, Afghanistan, America, India, China, Russia, Uzbekistan, Morocco and Greece (Jacob *et al.*, 2019). Even Iran is a major producer of *Punica granatum* L. in the world. The provinces of Markazi, Yazd, Fars, Khorasan, and Kerman make up the largest production rate in Iran (Shaygannia *et al.*, 2016).

Morphology of *Punica granatum* L.

Punica granatum L. is a small tree or a shrub, which is decisive. It is characterised by its prickly branches and red, juicy fruits (Dathan *et al.*, 2024). The *Punica granatum* L. is typically between 6 to 10 meters in height, although the height might differ depending on the growing environment. It has many prickly branches with a smooth and dark grey bark (Maphetu *et al.*, 2022). Giving it a clear contrast to the green-toned leaves and the bright colours of red or orange flowers that it produces before fruit-setting. The leaves are simple, glossy and smooth in colour and seem on the sides of a lateral shoot, oppositely or sub-oppositely (Bapodara *et al.*, 2011). Being carried on short petioles, they can be ovate or oblong. The plant yields 1-5 flowers to each shoot, out of which one of them is terminal (Kumar *et al.*, 2012). These are sessile (do not have a peduncle), whorled-shaped, actinomorphic, and bisexual flowers that are small. The 5- to 8-lobed calyx is tubular, thick, and covered with 5-7 imbricate and wrinkled and bright orange-red petals (Wang *et al.*, 2018). The calyx tube gives rise to numerous free stamens with dorsifixed anthers, and the pollen grains are aperturate. The ovary is poor and multi-locular. The fruit is a huge, round berry of up to 5 and 12 centimetres in diameter, and it varies in colour between brownish-yellow to red. It has a crown of the undying tubular calyx (Jacob *et al.*, 2019). The fruit has two components, one being the hard outer layer known as the pericarp, and the other being the internal spongy mesocarp (albedo). The mesocarp is abnormally partitioned with chambers each having an array of seeds that are soft or hard and differ in colour, ranging from red to white. A juicy aril covers every seed entirely, and it is the edible part of the fruit (Kumari *et al.*, 2021).

Leaves: The leaves of new branches are oppositely located and have an integration-like pattern within the spurs (Shaygannia *et al*., 2016).

Flowers: The plant is 1-5 flowered, 1 of which is normally terminal and the other lateral, being sessile or supported by short peduncles. The flowers are bisexual, colourless, and usually red, but sometimes, they may be yellow or white (Hussain *et al*., 2021).

Fruit: *Punica granatum* L. has fruit of a special kind called a balausta, or tough, leathery rind of a berry. It is typically light red to greenish-yellow in colour when mature, although in some cultivars or wild varieties, it may appear dark purple. It ranges from 6 to 25 cm in diameter and can weigh anywhere from under 200 g to over 820 g (Rahimi *et al*., 2012).

Seed: Seed production is abundant; the seeds are triangular, lack albumin, and are embedded within the aril (Shaygannia *et al*., 2016).

Conventionally, herbal treatments have made use of the fruit peel, bark of young shoots and roots, leaves, and flowers of *Punica granatum* L. The entire fruit of *Punica granatum* L. has strong astringent qualities, especially the tannin-rich sections. Also, the pomegranate peel extract is applied as a gargle to treat the inflammation of the pancreas. Since alkaloid is concentrated in them, fresh or dried root barks and plant ethanol extracts are applied to get rid of intestinal parasites. The plant also possesses anti-inflammatory and antibacterial properties, which make it the desired plant in herbal remedies. These effects occur because it produces polyphenols, tannins, flavonoids, and alkaloids in their great amounts, and these exist in various parts of the plant. These advantages not only serve purposes inside the body (e.g., digestive and cardiovascular health) but also can be used topically (e.g., on a wound or skin disease) (Kumudhaveni *et al*., 2024).

MATERIALS AND METHODS

Plant material collection

Fresh *Punica granatum* L. stem bark was collected on 22nd February 2024, at Krishi Anusandhan Kendra, Nagina, Bijnor and Uttar Pradesh, India.

Identification and Authentication

Recognition and verification of the plant specimens was done by Dr Vinay Ranjan, Scientist-E, Head of Office, Botanical Survey of India, Ministry of Environment, Forest and Climate Change, Central Regional Centre, Prayagraj (Allahabad), Uttar Pradesh, India. A voucher specimen with the following order of reference number could be provided under the herbarium BSI/CRC/P/2024&25/128.

Drying and Pulverisation

The sound stem bark of the *Punica granatum* L. was carefully washed, leavened into small pieces and dried under shade at room temperature. After drying it completely, the material was crushed to fine powder using a mechanical grinder and stored in an airtight glass container to be later subject to further investigation (Semwal *et al*., 2013).

Pharmacognostic evaluation

Macroscopic evaluation

Macroscopical analysis of the *Punica granatum* L. stem bark entails the measurement of attributes like dimensions, form, feel, smell, colour, flavour, and other unusual features like fracture, feel, and outer and inner surfaces. The samples were put on a white paper area, and the macroscopical analysis of the stem bark was completed by observing them with the naked eye. Then, organoleptic characteristics were in the form as shape, size, colour, taste, fracture, and odour, were considered (Ts *et al*., 2023).

Microscopic evaluation

Punica granatum L. stem bark dried in a test tube using the required quantity of distilled water and boiled some a few minutes. Once the bark had gone soft, it was carefully cut into thin cross-section slices (Prabhu *et al*., 2010). The next generation is the treatment with the phloroglucinol 0.1% w/v solution, followed by the concentrated hydrochloric acid. The stained slices were observed under the microscope, and the various cell layers were identified, and the characteristic structure of the cell parts was established. The last step involved photomicrography to obtain the images (Kumar *et al*., 2014).

Powder Microscopical studies

The reaction of the finely powdered dried stem bark of *Punica granatum* L. under a microscope was done to determine its cellular structures. Phloroglucinol staining was done in order to detect lignin. A little piece of powdered stem bark was placed on a microscope slide, and 1-2 drops of the newly made 0.1% phloroglucinol solution were added to it. A strong hydrochloric acid drop is then put in. The mixture was covered with a cover slip, and it was inspected using a microscope (Sharma *et al*., 2024). The pink colour or fuchsia hue of the walls of the cell reflected the lignin. In particular, xylem and inter-fascicular fibres, because of the reaction of phloroglucinol with cinnamaldehyde terminal groups in lignin. The preparations of the slides were stained to avoid deterioration of the specimens and to help analyse them under a microscope in glycerol. Photomicrography was employed to capture images of the unusual structures and cell structures to document the findings (Kumar *et al*., 2014).

Physicochemical evaluation

Physicochemical analysis of plants is necessary to assess their physical and chemical properties, the analysis of which is necessary to provide quality assurance, paid to verify, and understand their potential as medicines (Ram *et al*., 2025). It also assists in detecting adulteration and improper treatment of the medicines of plant origin (Jeevitha *et al*., 2021). Physicochemical properties of powdered stem bark of *Punica granatum* L. were determined: Total ash residue, water-soluble ash residue and acid-insoluble ash residue. Value extracts were determined to determine the percentage of soluble elements in the alcohol and water. The moisture content was measured through the loss-on-drying approach in order to estimate the amount of water left in the sample (Rotich *et al*., 2024).

Total Ash valueApproximately 5 g of powdered stem bark of *Punica granatum* L. was carefully weighed and added to silica crucibles one at a time; it had been weighed and fired earlier (Ram *et al*., 2025). The powder was evenly spread as a thin layer at the crucible's bottom and placed in a muffle furnace after that; the temperature was steadily raised until it reached a dull red-hot, guaranteeing that all of the carbon had been removed. The crucible was weighed once again after cooling. Until the weight remained consistent, this process was repeated. The proportion of total ash was calculated using the air-dried powder (Shirode *et al*., 2024).

$$\% \text{ Ash content} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Weight of crucible with powdered Drug} - \text{Weight of empty crucible}} \times 100$$

Loss on dryingWeigh out 2 to 5 g of powdered stem bark of *Punica granatum* L. into a Petri dish that has been previously weighed and record the weight accurately. Dry the sample at 105°C for 5 to 6 hr, and then let it cool in a silica gel-filled desiccator. Determine the percentage of loss on drying after the material's weight is stable (Shirode *et al*., 2024).

$$\text{LOD \%} = \frac{\text{Wt. of Petri dish with powdered drug} - \text{After drying Wt. of Petri dish with sample}}{\text{Weight of powdered drug}} \times 100$$

Water-soluble ash

The ash residue generated through the total ash value approach is used to estimate the water-soluble ash residue of powdered stem bark of *Punica granatum* L. The total ash residue was boiled for 5 min in 25 mL of distilled water (Ram *et al*., 2025). The insoluble residue was collected on ash-free filter paper and then processed with hot water. The insoluble residue was moved to a silica crucible that had been previously weighed, fired for 15 min at an approximate temperature of no more than 450°C in a muffle furnace, allowed to cool in a desiccator, and then weighed to ascertain the amount of water-soluble ash residue. Until the weight remained consistent, the procedure was repeated. The water-soluble ash residue was determined by deducting the weight of the insoluble matter from the overall weight of the ash residue. The weight of the air-dried sample was subsequently utilised to determine the proportion of water-soluble ash residue (Shirode *et al*., 2024).

$$\% \text{ Water soluble ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Weight of Powdered Crude Drug}} \times 100$$

Determination of Acid-insoluble Ash Value

Punica granatum L. stem bark powder is heated to a high temperature (550°C) in a muffle furnace. Ash, the residue left over after burning up organic materials, is produced by this process. The ash was transferred into a beaker filled with 15 mL of 10% hydrochloric acid. The crucible was rinsed twice with 5 mL of the same acid. The insoluble stuff was collected in a sintered crucible or on ashless filter paper after the beaker was brought to a boil for 5 min. Warm water was used to wash the beaker, and the rinse water was passed through the filter paper. To make sure the residue stayed at the tip of the filter paper cone, this cleaning process was carried out three times. The funnel and filter paper were then dried in an oven at 105°C. The crucible was dried, and the final weight was recorded. After being folded into a little bundle, the filter paper containing the insoluble residue was put back into the crucible. For 2 hr, the crucible was slowly lit at 500°C in a furnace until its weight remained constant. The crucible and its residue were weighed once the residue had cooled to room temperature in a desiccator (Shirode *et al*., 2024).

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Weight of Powdered Crude Drug}} \times 100$$

Determination of Alcohol Soluble Extractive Value

The alcohol-soluble extractive value of *Punica granatum* L. procedure determines the percentage of bark active ingredients that are soluble in alcohol, typically ethanol. 5 g of coarsely ground, air-dried *Punica granatum* L. stem bark were added to a 250 mL conical flask containing 100 mL of 90% ethanol. A stopper was used to close the flask. The sample should be macerated (soaked) for 24 hr, standing for the last 18 hr after being shaken constantly throughout the first 6 hr. To obtain the liquid extract from the plant material, quickly filter the macerated mixture. Transfer 20 mL of the filtrate, weighing it in advance, into an evaporating dish. After the solution was dried by evaporation, the residue was dried for approximately 3 min at 105°C in an oven until the extract was thoroughly dry. After recording the residue's constant weight, the extractive value was computed using extrapolation (Ram *et al*., 2025).

Alcohol soluble extractive value = Weight of residue / Weight of the Powdered drug ×100

Determination of Water Soluble Extractive Value

The water-soluble extractive value of *Punica granatum* L. stem bark is evaluated by weighing 5.0 g of the air-dried, coarsely ground sample precisely. The powder should be transferred to a conical flask-250 mL. Pour in 100 mL of purified water. Tightly cork the flask. After giving the mixture a good shake, leave it for

6 hr. For the next 18 hr, give it another good shake. To prevent solvent evaporation, filter the mixture as soon as possible after a day. Fill a tared (pre-weighed) porcelain plate with the filtrate (25 mL). In a water bath, evaporate until completely dry. The residue is typically dried to constant weight at 105°C. (Rotich *et al*., 2024).

Water soluble extractive value = Weight of residue / Weight of the Powdered drug ×100

Fluorescence analysis

Fluorescence analysis of materials generated from plants that emit light when subjected to visible or Ultraviolet (UV) light. It is an essential method for recognising, assessing, and describing illicit substances (Kumudhaveni *et al*., 2024). The presence and kinds of phytochemical elements may be determined by monitoring the fluorescence patterns under various wavelengths and following treatment with different reagents (Huang *et al*., 2014). This is to make sure that herbal remedies are standardised and of high quality. The powder of *Punica granatum* L. stem bark was prepared using a normal procedure. 1 g of finely ground powder of *Punica granatum* L. stem bark was put in different test tubes. A different reagent was added to each of the test tubes. The combinations were then left unattended after shaking them a bit for over 30 min (Nirawane *et al*., 2018). The colour of the test tubes was first viewed under the normal light, and the test tubes were subsequently put under UV light. Colour changes were observed at the wavelengths of 254 and 365 nm. The presence of specific groups of phytochemicals, such as terpenoids, phenols, flavonoids, and alkaloids, may be determined based on the patterns of fluorescence (Wazir *et al*., 2021).

Elemental analysis

The elemental analysis determines the type and the content of elements- the usual elements in a sample are as follows: Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S) and Oxygen (O). This study is essential in determining the composition of organic substances and understanding their composition as well as the purity. The exact purpose of such an elemental analysis is the determination of the proportions of oxygen, sulfur, nitrogen, hydrogen, and carbon (Shamala *et al*., 2022). The spectra of each of the following elements were obtained in the case of stem bark powder *Punica granatum* L.: C, H, N, S, and O.

Ethical Statement

In the current research work, there were no experiments performed on human subjects or animals. In the study, the plant used was *Punica granatum* L. stem bark, which was obtained from Krishi Anusandhan Kendra, Nagina, Bijnor, Uttar Pradesh, India, as per the guidelines for collecting medicinal plants. The plant was taxonomically identified and authenticated by a qualified authority at the Botanical Survey of India, Central Regional Centre, Prayagraj, and a voucher specimen was deposited for future reference. As there are no human or animal subjects used in the research, obtaining the necessary ethical committee approval was unnecessary for the above study. All procedures were carried out in accordance with institutional and national guidelines for the use of plant resources.

Statistical Analysis

All quantitative determinations such as ash values, extractive values, and loss on drying were carried out in triplicate. The results obtained from three independent measurements were recorded and expressed as mean±Standard Deviation (SD). Constant weight was ensured by repeated heating, cooling, and weighing until no significant change in weight was observed. Any abnormal variation between readings was rechecked and the experiment was repeated. Descriptive statistical analysis was used since the study focused on standardization and quality evaluation rather than comparative or inferential analysis. This procedure ensured accuracy, reproducibility, and reliability of the reported physicochemical data.

RESULTS

Macroscopic Evaluation

The macroscopic nature of *Punica granatum* L. stem bark depicts that the bark resembles one or two coiled quills, most of the time with a width of 0.3 to 0.7 cm and a thickness of 0.3 cm, and a length of 7 to 15 cm (Figure 1). The exterior is greenish-brown, with longitudinal creases and sporadic greyish lichen spots. The fracture is short and granular, and the inner surface is yellowish-brown with tiny striations. The bark tastes harsh and astringent, and it has a little smell.

Microscopic Evaluation

The following characteristics were found when the stem bark of *Punica granatum* L. was examined under a microscope:

In the transverse section of the stem bark of *Punica granatum* L., cork is arranged in both radial and tangential rows, Phellogen and a broad parenchymatous phelloderm with sparse clusters of lignified sclereids. Phloem covers most of the bark, with medullary rays interspersed throughout (Figure 2).

The cork

Two to three rows of alternating layers of thin-walled, tangentially elongated, radially orientated suberized cells make up the cork cell. These cells are packed with amorphous tannin masses that contain dark brown pigments. The cork cells seem polygonal with straight anticlinal walls when viewed from the surface (Figure 2).

Phelloderm

The phelloderm is made up of several rows of tangentially elongated parenchyma cells that contain simple or compound (two- to four-component) starch granules. These granules range in shape from oval to spherical, and the bigger ones have a

Figure 1: Macroscopy of *Punica granatum* L. stem bark.

centred hilum. There are also clusters of calcium oxalate crystals and prisms. Small groups of sclereids, usually two to three per group, are also found in the phelloderm. These sclereids are oval to rounded, with narrow lumens and thick, striated, lignified walls (Figure 2).

Phloem

With pyramid-shaped medullary rays that taper in a cone-like manner toward the outside, the phloem zone is composed of phloem parenchyma and alternating tangential bands of sieve areas. While the sieve regions are made up of sieve tubes and partner cells, the phloem parenchyma contains starch granules and small clusters of calcium oxalate arranged in tangential rows (Figure 2).

Medullary rays

The upper portion of medullary rays is made up of tangentially curved to square cells, which are mostly uniseriate and sometimes biseriate. The cells grow radially elongated as the phloem reaches its end, and tiny clusters of calcium oxalate and starch granules are present (Figure 2).

Vessels

The term "vessels" describes a particular kind of cell that makes up a significant portion of the xylem, the plant tissue that conducts water. The vessels of *Punica granatum* L. stem bark are wider in diameter than tracheids. Vessel diameters normally fall between 1 micrometre (µm) and 100 µm. Additionally, vessel lengths might differ, ranging from 100 µm to 10 cm (Figure 2).

Powder Microscopical studies

Punica granatum L. powdered stem bark has a yellowish-brown hue, a slight smell, and an astringent, bitter flavour. The following features of the powdered stem bark are revealed by microscopic analysis:

Under a microscope, the surface of cork cells shows a characteristic brick-wall or honeycomb pattern of densely packed, polygonal, dead cells with thick walls (Figure 3A).

Cork cell fragments are dark, oval to rounded sclereids with thick polygonal shapes and straight anticlinal walls; some of the cells have a lignified appearance (Figure 3B).

Phelloderm is the inner layer of the plant's protective periderm. It is made up of living, thin-walled parenchyma cells that are formed by the cork cambium (phellogen) towards the inside of the stem (Figures 3C and 3D). These cells are used for storage and occasionally for photosynthesis. In essence, it is the "secondary cortex" and a vital part of the bark of woody plants that stores food and provides support.

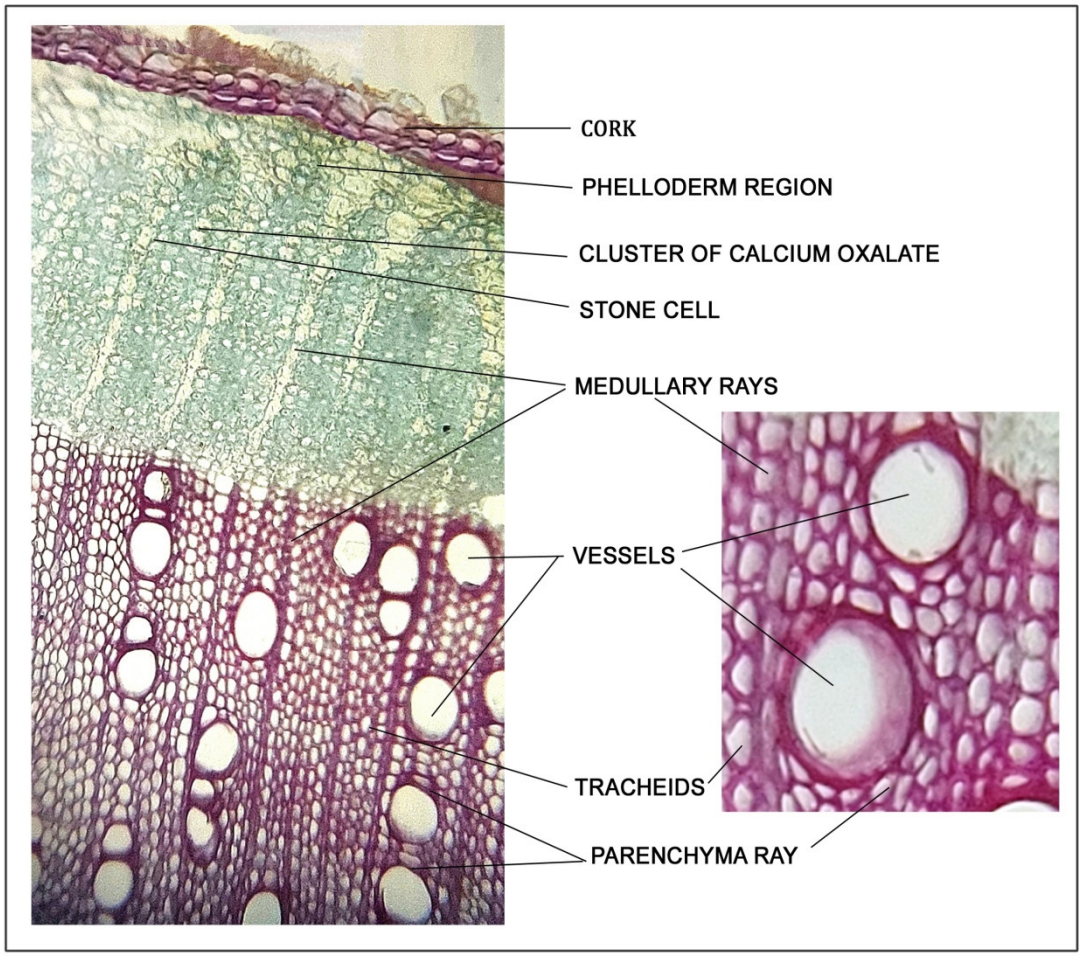

Figure 2: A transverse section of *Punica granatum* L. shows microscopic characteristics of the stem bark, such as cork cells, tannin-containing phelloderm cells, calcium oxalate crystal clusters, stone cells, medullary rays, tracheids, parenchyma rays, and vessels.

Oval to spherical starch granules that are either simple or compound (comprising two to four components), with a central hilum discernible in the bigger granules.

Throughout the sample, there are numerous clusters of calcium oxalate and prismatic crystals that are either isolated or encased in parenchyma cells (Figure 3E).

Xylem fibres are long, dead cells with a hollow lumen in the centre and thick, lignified (hardened) walls. They are pointed at both ends and give the plant mechanical support, but they can also help move nutrients and water (Figure 3F).

Certain wood species include cellular structures called medullary rays, sometimes referred to as vascular rays or pith rays. They can be seen with the unaided eye as radial planar structures that are perpendicular to the growth rings (Figure 3G). They show up as radiating lines from the log's centre in a transverse section.

The stem bark contains specific fibres called lignified fibres, which are distinguished by their thicker walls. Because of their high lignin concentration, which increases their strength, these fibres are essential for giving plants structural support (Figure 3H).

In plants, calcium oxalate crystals take on a variety of shapes, including needles (raphides), prisms, and clusters (druses), mostly within specialised vacuolar cells (idioblasts) for calcium regulation, defiance, and detoxification. These shapes range from simple prisms to complex forms that are essential for taxonomy (Figure 3I).

Physicochemical evaluation

These factors are essential for determining a drug's quality and purity. While the acid-insoluble ash was 0.45% w/w and the water-soluble ash was 3.10% w/w, the overall ash content of the coarsely ground *Punica granatum* L. stem bark was 3.11% w/w, which was a reasonably significant amount. Compared to the extractive value that is soluble in alcohol, 6.46% w/w, the powdered stem bark of *Punica granatum* L. exhibited the highest water-soluble extractive value, 10.26% w/w, suggesting that more constituents are soluble in water than in alcohol.

These extractive values are essential in the evaluation of crude drugs, as they indicate the types of chemical compounds that can be extracted using specific solvents. Additionally, the moisture

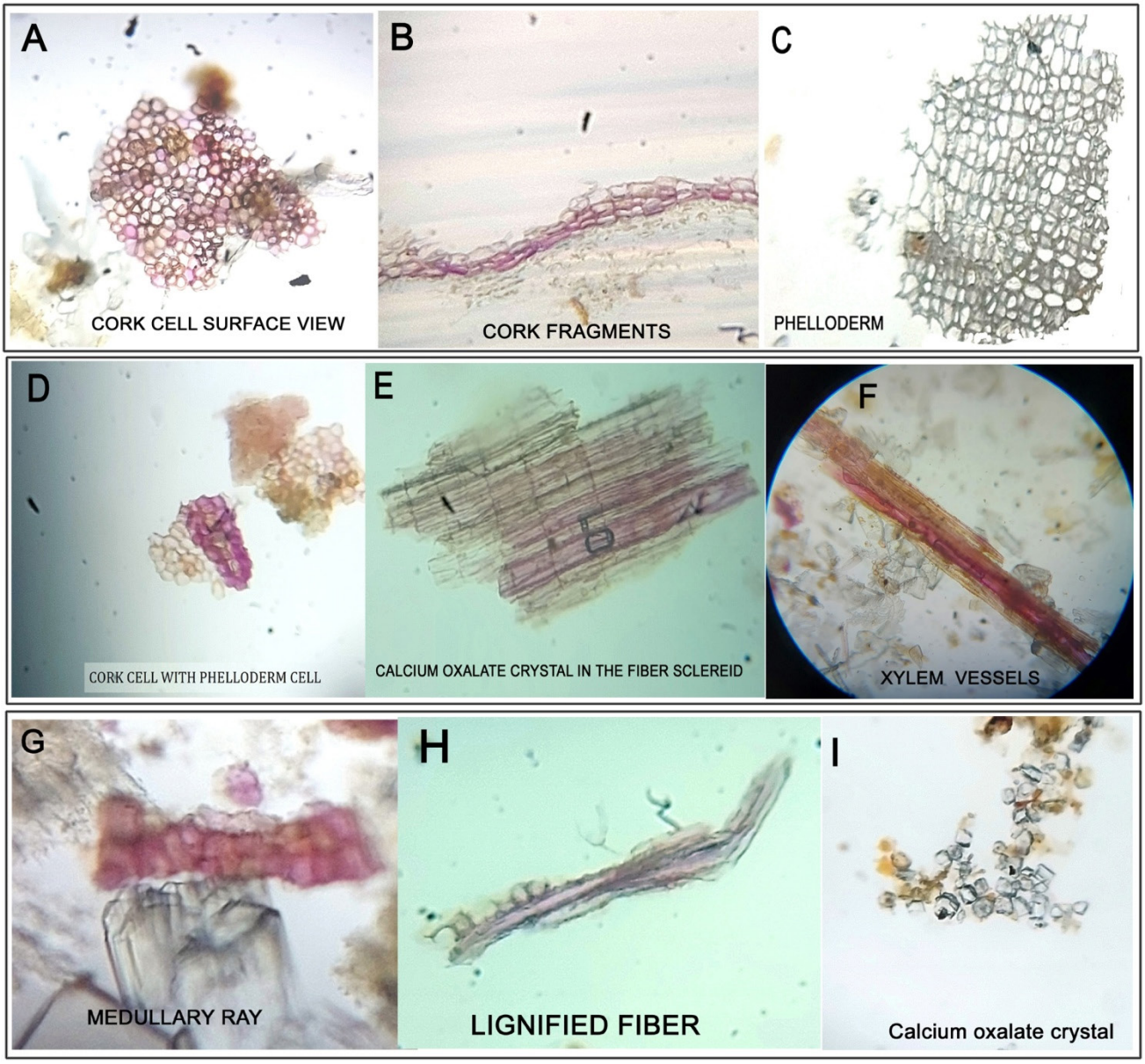

Figure 3: Powdered Microscopical characters of the stem bark of *Punica granatum* L. A. Cork cell surface view. B. Cork cell fragments. C. Phelloderm cell. D. Cork cell with phelloderm cell. E. Calcium oxalate crystal in fibre sclereid. F. Xylem vessels. G. Medullary ray. H. Lignified Fibre. I. A group of Calcium oxalate crystals.

content (loss on drying) of *Punica granatum* L. stem bark was also assessed at 4.30% w/w.

Fluorescence analysis

Fluorescence analysis was carried out on coarsely powdered *Punica granatum* L. stem bark, subjected to visible spectrum light as well as ultraviolet light at specific wavelengths of 254 nm (UV-C) and 366 nm (UV-A), after being treated with different chemical compounds to detect any characteristic colouration. The powdered stem bark of *Punica granatum* L. exhibited different fluorescence when treated with specific reagents and exposed to UV light at both 254 nm and 366 nm, as shown in Table 2. This fluorescence suggests the presence of chromophores within the plant material. Because fluorescence analysis may identify chemical markers unique to a plant, it is a useful method for standardising crude medications. Certain phytoconstituents, when reacted with suitable reagents, produce fluorescent compounds visible under UV or visible light. The qualitative evaluation of crude medications is aided by this conversion to fluorescent derivatives. In the search, the powdered stem bark of *Punica granatum* L. had been subjected to several chemical substances in order to determine its reactivity. Having ammonia, petroleum ether, hydrochloric acid, sulfuric acid, formic acid, toluene and other chemical substances. These fluorescence tests were done in various solvents and reagents, and their findings are outlined in Table 2.

Fluorescence analysis provides an understanding of the possible colouration of the sample drug subjected to ultraviolet and visible

Table 2: Fluorescence analysis of *Punica granatum* L. stems bark.

Test tube no	Powdered stem bark of *Punica granatum* L. + Chemical Reagents	Visible / Daylight	UV 254 nm (short)	UV 365 nm (long)
	Powdered drug	Yellowish brown	Dark olive green	Blackish brown
1	Powdered drug + Chloroform [$CHCl_3$]	Brownish	Lime tree green	Light grey
2	Powdered drug + Ethanol [C_2H_6O]	Brownish	Lime tree green	Grayish brown
3	Powdered drug + Acetic Acid [CH_3COOH]	Brownish	Olive green	Violet
4	Powdered drug + Pet. Ether 60-80^0	Brownish	Lime tree green	Light pink
5	Powdered drug + Ethyl Acetate [$C_4H_8O_{2]}$	Brownish	Lime tree green	Pink
6	Powdered drug + Acetone [CH_3COCH_3]	Brownish	Dark olive green	Lilac pink
7	Powdered drug + Iodine Solution [I_2]	Dark brown	Black	Black
8	Powdered drug + Methanol [CH_3OH]	Brown	Lime tree green	Grayish brown
9	Powdered drug + Toluene [C_7H_8]	Brown	Light lime tree green	Pink
10	Powdered drug + Formic Acid [CH_2O_2]	Brown	Light olive green	Dark brown
11	Powdered drug + Formaldehyde [CH_2O]	Brown	Dark olive green	Dark greyish brown
12	Powdered drug + Liquor Ammonia [NH_3]	Golden brown	Black	Black
13	Powdered drug + Sulphuric Acid [H_2SO_4]	Black	Black	Black
14	Powdered drug + Hydrochloric Acid [HCL]	Greenish	Black	Black
15	Powdered drug + Nitric Acid [HNO_3]	Dark orange	Violet	Black
16	Powdered drug + Sodium Hypochlorite [NaClO]	Golden yellow	Dark olive green	Black
17	Powdered drug + Gram Iodine [I_3K]	Brown	Black	Black
18	Powdered drug + Distilled Water [H_2O]	Brownish	Dark olive green	Black

light. The results of the fluorescence of *Punica granatum* L. stem bark are presented in Table 2.

Elemental analysis

Elemental analysis was used to ascertain the relative amounts of elements by subjecting the stem bark of *Punica granatum* L. to the analysis in terms of percentages of weight (Figure 4). The presence of the component element in curld on the bark of *Punica granatum* L. stems implies that it possesses nutritional and medicinal value. The presence of high concentrations of the following components, carbon, oxygen, and hydrogen, was evidenced by high peaks. The initial peak, whose retention time is 0.783, has no real component and a percentage of 0.000% as its element. The second peak that has a retention time of 1.200 is carbon, where the element percentage is 40.850%. The third peak, with a 1.575 retention time, is the oxygen element, which has a percentage of 32.151%. The fourth peak, whose retention time is 4.058, is that of the hydrogen whose element proportion is 5.186 (Figure 4).

This whole component has various metabolic functions. The measure of contaminations C, H, N, O, and S helps to determine the purity of a crude medication. It also provides information on the basic composition of the drug, and this is vital in understanding the composition and properties of the drug. As an illustration, oxygen plays a vital role in herbal remedies, as it works as a potential degradation agent and, at the same time, is a required component in most therapeutic applications. It also serves as a primary medication remedy towards the treatment of oxygen deficiency and the maintenance of many physiological functions. Tissue healing and inflammation are affected by oxygen. High-dose Oxygen Therapy (HBO) has been used in the treatment of diverse diseases over a period of over 40 years. Carbon is a key ingredient in their organic compounds, a route of administration and purification, and it is a basic building block of organic molecules, which are the main ingredient of herbal medicine. Carbon dots can be used to alter herbal medicines and increase their therapeutic efficacy, and help overcome obstacles such as the blood-brain barrier, since often herbal medicines are complex mixtures of compounds. Hydrogen is essential to the life of plants as it is one of the key building blocks, a source of energy, and the controller of many physiological mechanisms. Other vitamins, like the (+)-biotin, are also produced through hydrogenation.

DISCUSSION

Punica granatum L. is very popular in Ayurveda and Unani traditions of holistic health and herbal medicine. To fulfil the requirements of safety, efficacy, and quality of herbal medicine

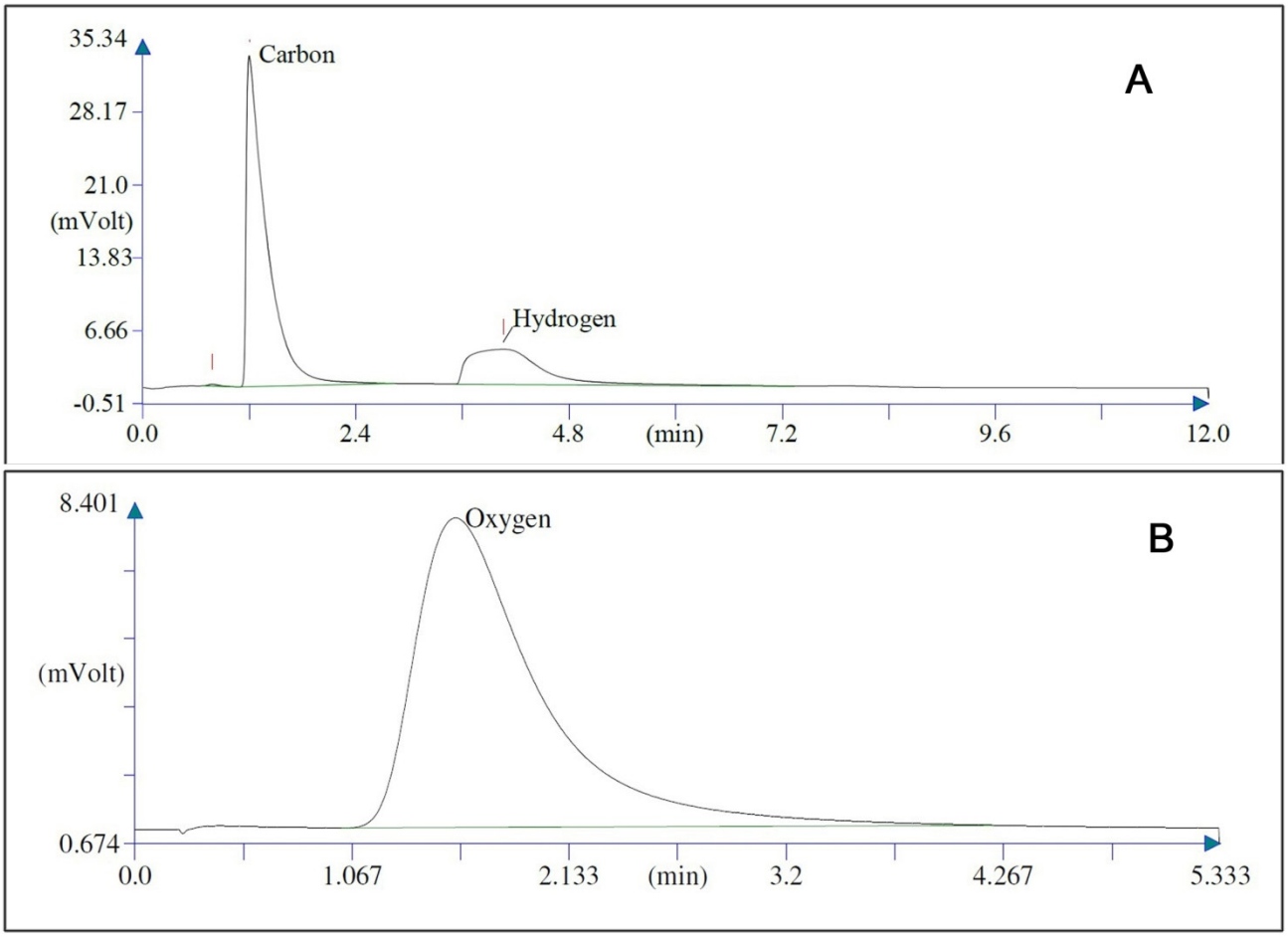

Figure 4: Composition of elements % of hydrogen, carbon and oxygen in the powdered stem bark of *Punica granatum* L.

preparations, it is important to collect, identify, authenticate, and, most importantly, ensure that the purity of the crude drug is upheld. In this work, the pharmacognostical and physicochemical characteristics of *Punica granatum* L. stem bark were researched in detail. The standardisation was done by use of macroscopic, microscopic, and powder microscopic analysis- basic parameters used to make assessments of the plant-based drugs. To eliminate any potential adulterants or substitutes, quantitative microscopy and physicochemical analysis were employed to ensure that quality is attained. Several chemical reagents were applied to the powdered medication, and it was examined under the daylight and ultraviolet background (254 nm and 366 nm). The resulting colour changes that are represented in Table 2 are unique and could aid in the recognition of the stem bark. C, H, and O are the elements that are found in *Punica granatum* L. stem bark and facilitate various physiological processes. The findings are important in providing the essential information to ensure the effective identification and verification of *Punica granatum* L. so that the chances of adulteration of herbal products can be reduced at a minimal threshold. However, more studies are being conducted to isolate, purify and characterise the therapeutically active compounds in its aqueous extract, which will undergo pharmacological research in order to establish the mechanism of action which they perform. The area of future research ought to involve the investigation of the bioactive compounds in *Punica granatum* L. and their medicinal effects to confirm the conventional uses of this plant even further.

CONCLUSION

The qualitative and quantitative data provided by the stem barks of *Punica granatum* L. are assessed pharmacognostically, and the physicochemical parameters are used to provide data necessary to identify, purify, and provide an account of the medicinal value of the *Punica granatum* L. stem barks. The characteristic features of the species in terms of anatomy were proven by macroscopic and microscopic features, which ensured the reliable authenticity of the crude drug. Some of the physicochemical characteristics within acceptable limits were moisture content, total ash, acid-insoluble ash, water-soluble ash and extractive values, indicating a low degree of adulteration and quality.

Such findings demonstrate the abundance of the phytoconstituents in the stem bark that can lead to its traditional medicinal properties and establish important demands on it. Altogether,

the paper proves the correct basis of safe and effective application of *Punica granatum* stem bark as a herbal preparation and further phytochemical or pharmacological studies through the statement of the pharmacognostic identity of this substance and physicochemical integrity.

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ABBREVIATIONS

C_7H_8: Toluene; **$C_4H_8O_2$:** Ethyl Acetate; **$Pb(C_2H_3O_2)_2$:** Lead Acetate; **C_6H_{14}:** Petroleum Ether; **$CHCl_3$:** Chloroform; **CH_3COCH_3:** Acetone; **C_2H_6O:** Ethanol; **CH_3OH:** Methanol; **NH_3:** Liquor Ammonia; **HCl:** Hydrochloric Acid; **$FeCl_3$:** Ferric chloride; **H_2SO_4:** Sulphuric Acid; **CH_2O_2:** Formic Acid; **CH_3COOH:** Acetic Acid; **HNO_3:** Nitric Acid; **I_2:** Iodine Solution; **UV:** Ultra Violet; **mL:** Milliliter; **H_2O:** Distilled Water; **NaClO:** Sodium Hypochloride; **WHO:** World Health Organization; **CHNSO:** Carbon, Hydrogen, Nitrogen, Sulfur, Oxygen; **LOD:** Loss on Drying; **UV:** Ultraviolet; **nm:** Nanometers; **HBO:** High-dose oxygen therapy.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest related to this manuscript.

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AUTHOR CONTRIBUTIONS

Amit Rishi: Writing – original draft, Formal Analysis, Conceptualization. Harpreet Singh: Writing-review and Editing, Supervision, Conceptualization, Visualization. Both the author have reviewed and approved the final version of the manuscript.

SUMMARY

The current research combines traditional knowledge with current quality control procedures in order to establish a reliable reference profile for the stem bark of *Punica granatum* L. By considering the results of macroscopic, microscopic, and physicochemical characterization, the current research establishes a useful framework for the discrimination of authentic material versus possible adulterants in traditional herbal medicines. The identified structural characteristics and quality attributes combine in order to highlight that traditional pharmacognostic approaches could be harmonized with current quality requirements. Apart from verifying the traditional use of the stem bark, the current research provides a current data point for future comparative analysis in traditional medicines. The current research verifies the general aim of enhancing the safety, credibility, and scientific status of traditional plant medicines.

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