

Phytochemical Screening and Biological Activity of Pomegranate Extracts

B. Premkumar^{1,*}, K. Vinothkumar², V. Mugilan², P. Nandhini², P. Venkatesan²

Abstract

Pomegranates are renowned for their abundance of bioactive compounds that exhibit potent antioxidant, anti-inflammatory, and antimicrobial properties, making them a subject of growing interest in natural medicine. This study aimed to compare the biological activities of ethanolic extracts derived from different parts of the pomegranate plant – specifically, the bark, peel, and leaves. A comprehensive investigation was conducted to evaluate their phytochemical composition and assess their antimicrobial, antioxidant, and anticancer properties. The antimicrobial activity of each extract was tested against Staphylococcus aureus, Klebsiella pneumoniae, and Aspergillus niger using the Kirby–Bauer well diffusion method, with zones of inhibition measured to determine efficacy. Antioxidant capacity was evaluated through total antioxidant activity, hydrogen peroxide (H₂O₂) scavenging assays, and Ferric Reducing Antioxidant Power (FRAP) assays. Antitumor study was assessed using the MTT assay. Among the three extracts, the bark extract demonstrated the most significant biological activity and was subsequently chosen for advanced anticancer studies on liver cell lines, where it showed marked cytotoxic effects. These findings suggest that pomegranate bark holds considerable promise as a source of therapeutic agents, particularly in the context of cancer treatment. Continued research may pave the way for the development of novel health-promoting formulations derived from pomegranate.

Keywords: Pomegranate, phytochemical, Kirby–Bauer well diffusion, antioxidant, antimicrobial, MTT assay

INTRODUCTION

Phytotherapy, the use of plant-derived products for the prevention and treatment of diseases, has been an integral part of healthcare since the earliest human civilizations [1]. Medicinal plants continue to play a vital role in modern pharmaceutical development, contributing bioactive compounds that serve as the basis for numerous therapeutic agents [2]. Using medicines made from plants has been linked to longer life and less need for man-made antibiotics [3, 4]. In recent years, growing concerns over the

high costs and side effects of allopathic medicine, along with the emergence of drug-resistant microbial strains, have intensified scientific interest in plant-based therapeutics [5]. The pomegranate (*Punica granatum L.*), a deciduous tree belonging to the *Lythraceae* family, is one such plant that has garnered considerable attention. Thought to have originated in Iran, it has been cultivated since antiquity in Northern India and throughout the Mediterranean region [6]. The pomegranate fruit is differentiated by its dense red rind, crown-like calyx, and arils containing seeds covered by a white, spongy pericarp. It is full of natural compounds that are known to fight bacteria, reduce inflammation, act as antioxidants, and help prevent cancer [7, 8].

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Received Date: June 12, 2025

Accepted Date: June 25, 2025

Published Date: July 03, 2025

Citation: B. Premkumar, K. Vinothkumar, V. Mugilan, P. Nandhini, P. Venkatesan. Phytochemical Screening and Biological Activity of Pomegranate Extracts. Research and Reviews: Journal of Pharmacognosy. 2025; 12(2): 64–79p.

Scientific interest in pomegranate as a medicinal resource has grown significantly, with numerous studies highlighting its therapeutic potential. In traditional Indian medicine, pomegranate has been used as an antiparasitic agent and a remedy for gastrointestinal ailments, such as ulcers and diarrhea [9, 10]. The Unani system of medicine has recognized its value in managing diabetes [11]. Modern research supports these traditional uses, revealing that pomegranate constituents can modulate key signalling pathways involved in inflammation, cell proliferation, angiogenesis, and the initiation and progression of cancer [12, 13]. Particularly, pomegranate peel shows a strong ability to stop the growth of different harmful microbes, such as bacteria, fungi, Molds, and viruses [14]. It also shows promise in treating chronic inflammatory conditions, such as ulcerative colitis, particularly by alleviating inflammation in the gastrointestinal tract [15]. The entire pomegranate fruit – including its peel, seeds, and other by-products – can be utilized to produce value-added compounds with applications in medicine, industry, and cosmetics [16]. Among the many global health challenges, chronic respiratory diseases remain the leading causes of mortality, often accompanied by long-term complications, adverse drug reactions, and psychosocial burdens [17]. Risk factors, such as tobacco use, infections, radiation, environmental pollutants, obesity, and alcohol consumption, contribute to the development of these diseases, which are often characterized by uncontrolled cell proliferation and complex etiologies involving genetic and environmental components [18, 19]. Despite medical advances, respiratory disorders continue to claim millions of lives annually, with incidence and mortality rates on the rise [20, 21]. Likewise, digestive system diseases are very common and cause a lot of illness and high healthcare expenses around the world [22]. These conditions are triggered by various internal and external factors, including harmful dietary components, pathogens, and lifestyle habits, such as physical inactivity and poor diet [23].

ORIGIN AND GEOGRAPHICAL DISTRIBUTION

Pomegranate (*Punica granatum*), believed to have originated in the regions of present-day Afghanistan and Iran, holds deep symbolic and mythological significance across various ancient civilizations [24, 25]. From its place of origin, it was gradually introduced and cultivated throughout Asia, Africa, and Europe. The fruit was first introduced to Spanish America by Spanish adventurers in the late 1500s and eventually brought to California in 1769. At present, pomegranate is extensively grown in the Mediterranean region, dry areas of Southeast Asia, North and Tropical Africa, South and Central Asia, West Asia, and the Caucasus.

Taxonomical Classification

- Kingdom: *Plantae*.
- Division: *Magnoliophyte*.
- Class: *Magnoliopsida*.
- Subclass: *Rosidae*.
- Order: *Myrtales*.
- Family: *Punicaceae*.
- Genus: *Punica*.
- Species: *Granatum*.

MATERIALS AND METHODS

Collection and Processing of Sample

The medicinal plant *Punica granatum* was collected in and around Coimbatore. The plant was authenticated by Dr. S. S. Hameed, Scientist “F” & Head of Office, Southern Regional Centre, T. N. A. U. Campus, Coimbatore. The Pomegranate fruit was collected from the local market in the Coimbatore area. From collected fruits, the peel was removed, and from the plant, the bark and leaves were used as samples. The plant parts were washed several times with running water. The plant parts were shade dried at room temperature for about 10–15 days. After drying, the leaves, bark, and peel were ground into fine powder using the grinding machine.

Preparation of Plant Extract

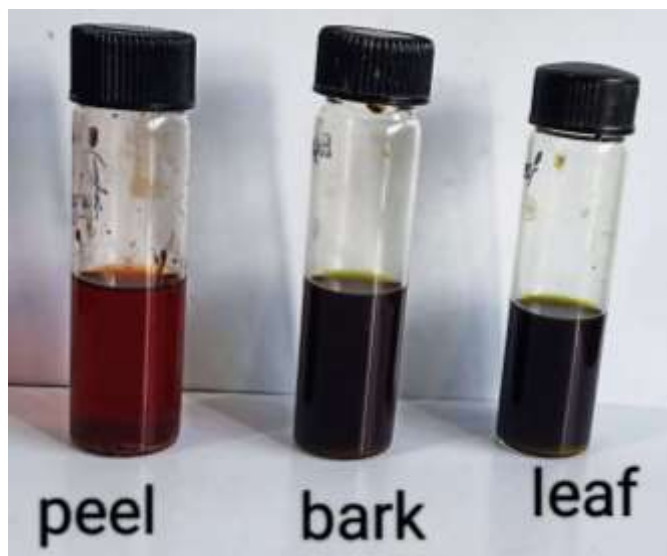


Figure 1. Ethanol extract of peel, bark & leaf.

A total of 30 grams of the powdered plant material was subjected to hot continuous extraction using ethanol as the solvent in a Soxhlet apparatus. Extraction was continued until the liquid in the thimble turned clear, showing that all the useful compounds had been fully removed. The resulting extract was then concentrated, and a few drops were collected for preliminary observation. The final filtrate was stored under refrigeration for subsequent analyses (Figure 1).

Preliminary Phytochemical Screening

Ethanol extracts of pomegranate peel, bark, and leaves were subjected to qualitative phytochemical screening to identify the presence of various bioactive compounds, such as alkaloids, flavonoids, steroids, tannins, glycosides, phenols, and others. The tests and their respective procedures are detailed below

- *Test for Alkaloids (Mayer's Test):* Mayer's reagent was prepared by dissolving 0.113 g of mercuric chloride in 5 mL of distilled water. 1 mL of the extract was added with a few drops of Mayer's reagent showed that alkaloids were present.
- *Test for Terpenoids:* 1 mL of concentrated sulfuric acid was added to 1 mL of the extract, and the mixture was heated for about 2 minutes. The presence of terpenoids was observed by a greyish color.
- *Test for Phenols:* Ferric chloride solution (2%) was prepared by dissolving 0.1 g of ferric chloride in 5 mL of distilled water. 1 mL of the extract was mixed with 1 mL of ferric chloride solution in equal proportions. The appearance of a blue-green or black color confirmed the presence of phenolic compounds.
- *Test for Reducing Sugars (Fehling's Test):* Fehling's A: To dissolve 0.35 g of copper sulfate, 5 mL of distilled water was used. Distilled water was used for dissolving Fehling's B: 1.75 g of potassium sodium tartrate and 0.5 g of sodium hydroxide. The mixture was heated for 2 minutes after the addition of 1 mL of extract with Fehling's A and B solutions. A red precipitate indicated reducing sugars.
- *Test for Saponins:* After adding 1–2 mL of water to 1 mL of the extract, the mixture was shaken well. The formation of a stable foam layer approximately 1 cm thick indicated the presence of saponins.
- *Test for Flavonoids:* Small pieces of magnesium ribbon were added to 1 mL of the extract, followed by a few drops of concentrated hydrochloric acid. After a few minutes, a pink or scarlet color confirmed the presence of flavonoids.

- **Test for Quinones:** A 2% sodium hydroxide solution was made by dissolving 0.1 g of sodium hydroxide in 5 mL of distilled water. 1 ml each of extract and sodium hydroxide solution was mixed. The quinones were confirmed by blue–green or red coloration.
- **Test for Proteins:** A Few drops of mercuric chloride or nitric acid were added to 1 ml of the extract. The yellow colour confirmed the presence of proteins.
- **Test for Steroids:** 1 mL of chloroform was added to 1 mL of the extract, followed by gently layering 1 mL of concentrated sulfuric acid along the side of the test tube. The presence of steroids indicated a red colour formation at the interface of the chloroform layer [26].

Antioxidant Study of *Punica granatum*

FRAP: Ferric Reducing/Antioxidant Power

The antioxidant power of *Punica granatum* (pomegranate) extracts was tested using the FRAP method. In this test, 0.5 mL of the extract was mixed with 1 mL of phosphate buffer and 1 mL of a 0.1% potassium ferricyanide solution. The mixture was heated in a water bath at 50°C for 20 minutes. After it cooled down to room temperature, 1 mL of 10% trichloroacetic acid was added, followed by 1 mL of distilled water and 0.5 mL of freshly made 0.1% ferric chloride solution. The mixture's color change was measured using a spectrophotometer at 700 nm. A control sample without the extract was also prepared for comparison. The antioxidant activity was determined by how well the extract could turn iron ions (Fe^{3+}) into a more stable form (Fe^{2+}) [27].

Reducing Power (%) =

$$\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Absorbance of Control

Hydrogen Peroxide Scavenging Assay

The ability of *Punica granatum* (pomegranate) extract to remove hydrogen peroxide was tested using a standard method. The total volume was made by adding 0.5 ml of extract and 50mM of phosphate buffer. Then, 2 mL of a 2 mM hydrogen peroxide solution was added. The mixture was mixed well and left to sit at room temperature for 10 minutes. After that, the absorbance (how much light the solution absorbs) was measured at 230 nm using a UV-Visible spectrophotometer. By using a standard formula, the H_2O_2 removed from the extract was known [28].

The percentage of hydrogen peroxide (H_2O_2) scavenging activity is calculated by subtracting the absorbance of the sample from that of the control, dividing the result by the control's absorbance, and then multiplying by 100.

Total Antioxidant Activity

The overall antioxidant capacity of *Punica granatum* (pomegranate) extracts was tested using the phosphomolybdenum method, with some small changes to earlier procedures. A reagent solution was prepared by combining 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After that, 0.5 mL of the extract was added to 0.5 mL of this prepared solution. The mixture was heated at 50°C for 90 minutes along with a blank sample (without extract). After heating, the samples were cooled to room temperature, and the absorbance (light absorbed) was measured at 695 nm using a spectrophotometer. Ascorbic acid (vitamin C) was used as a reference to calculate the total antioxidant activity, shown in milligrams per gram (mg/g) [29, 30].

Antimicrobial Properties of Pomegranate Extracts

Antibacterial Assay

The antibacterial activity was carried out by the Kirby–Bauer test. Nutrient broth cultures of *Staphylococcus aureus* and *Klebsiella pneumoniae* were incubated at 37°C for 24 hours. After incubation, 70 μL of each bacterial culture was spread onto sterile Mueller–Hinton agar plates (prepared

by dissolving 39 g of Mueller–Hinton agar in 1000 mL of distilled water and sterilizing by autoclaving at 121°C for 15 minutes).

Wells were created using a cork borer, and the extracts were added to the wells along with appropriate controls: dimethyl sulfoxide (DMSO) as the negative control and antibiotic disc as the positive control. The incubation time for the plates was 37°C for 24 hours. By measuring the diameter of the inhibition zones in millimeters, the antibacterial activity was determined.

Antifungal Activity

Malt extract agar was prepared by dissolving 45 g of malt extract in 1000 mL of distilled water and sterilized via autoclaving. After pouring into petri dishes and solidification, an antibiotic was added to prevent bacterial contamination. An 80 µL fungal suspension of *Aspergillus niger* was spread on the plates using a sterile cotton swab. Wells were made using a cork borer, and the extracts were added. At 30°C the plates were held for 3–5 days. Fluconazole (5 µL at a concentration of 10 mg/mL) was used as the reference antifungal agent. Antimycotic activity was assessed by measuring the diameter of the inhibition zones in millimeters [31].

Anticancer Activity (MTT Assay)

3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

Liver cancer cell lines (HepG2) were used to evaluate the anticancer activity. These were procured from the National Centre for Cell Science (NCCS), Pune, India, and maintained at the Centre for Bioscience and Nanoscience Research Laboratory, Coimbatore, Tamil Nadu, India. RPMI medium was used for cell culture. Cultures were incubated in a CO₂ incubator at 37°C with 70–80% humidity and pH 7–7.5 for 24–72 hours. An inverted microscope was used to observe and confirm cell growth. The MTT assay was executed by placing the cells in 96-well plates and maintaining them for 24 hours at 37°C with 5% CO₂ incubator. Cells were then treated with various concentrations of the extracts, along with DMSO (blank), untreated cells (control), and Doxorubicin (12.5 microgram per milliliter) as the standard drug. After 24 hours of treatment, DMSO was used for washing the cells, and then treated with trypsin. Next, 20 µL of MTT dye was added to each well, gently mixed, and the plates were incubated again for another 24 hours at 37°C. Following incubation, the media were removed, and formazan crystals formed by viable cells were solubilized with 100 µL of DMSO per well. The absorbance of the developed purple colour was recorded at 570 nm using a 96-well ELISA plate reader (Robotnik, India).

RESULTS AND DISCUSSION

The current study examined the phytochemical content, antioxidant capacity, antimicrobial properties, and anticancer effects of ethanol extracts derived from the peel, bark, and leaves of *Punica granatum*.

Phytochemical Screening

The preliminary phytochemical screening revealed the presence of bioactive constituents, such as alkaloids, flavonoids, tannins, phenols, steroids, saponins, and terpenoids in all three parts tested. Among them, the bark extract showed the highest concentration of secondary metabolites, particularly phenolic compounds, flavonoids, and alkaloids, making it the most potent extract in terms of biological activity (Table 1 and Figures 2–4).

- *Tube 1*: Mayer's test.
- *Tube 2*: Salkowski's test.
- *Tube 3*: Ferric chloride test.
- *Tube 4*: Sugar test.
- *Tube 5*: Foam test.
- *Tube 6*: Flavonoid test.
- *Tube 7*: Quinine test.
- *Tube 8*: Folin–Lowry's test.

- *Tube 9*: Sulphuric acid test.

Table 1. Photochemical analysis of pomegranate ethanolic extracts: peel, bark, and leaf.

Phytochemical Test	Peel	Bark	Leaves
Mayers Test	+	++	+++
Salkowski's Test	+++	+	–
Ferric Chloride Test	+++	+++	++
Sugar Test	+++	++	++
Foam Test	–	–	–
Flavonoid's Test	+++	+++	+++
Quinines Test	+++	++	++
Folin–Lowry's Test	–	+	–
Sulphuric Acid Test	–	–	–

**Figure 2.** Phytochemical test for peel.

Previous studies have demonstrated that the peel of *Punica granatum* is particularly rich in polyphenolic compounds, such as ellagic acid and punicalagins, which are largely responsible for its notable antioxidant and antimicrobial activities. The bark, especially when subjected to ethanolic extraction, yields high concentrations of phenolic compounds and flavonoids, enhancing its therapeutic potential, particularly in antimicrobial and anti-inflammatory applications. The leaves contain alkaloids and flavonoids, which contribute to their therapeutic effects. Overall, the phytochemical profiling of different parts of the pomegranate plant confirms the presence of various bioactive constituents that support its traditional and contemporary medicinal uses.

**Figure 3.** Phytochemical test for leaf:

- *Tube 1*: Mayer's test.
- *Tube 2*: Salkowski's test.
- *Tube 3*: Ferric chloride test.
- *Tube 4*: Sugar test.
- *Tube 5*: Foam test.
- *Tube 6*: Flavonoid test.
- *Tube 7*: Quinine test.
- *Tube 8*: Folin–Lowry's test.
- *Tube 9*: Sulphuric acid test.



Figure 4. Phytochemical test for bark.

- *Tube 1*: Mayer's test.
- *Tube 2*: Salkowski's test.
- *Tube 3*: Ferric chloride test.
- *Tube 4*: Sugar test.
- *Tube 5*: Foam test.
- *Tube 6*: Flavonoid test.
- *Tube 7*: Quinine test.
- *Tube 8*: Folin–Lowry's test.
- *Tube 9*: Sulphuric acid test.

Antioxidant Activities

Total Antioxidant Capacity

The overall antioxidant capacity of the pomegranate extracts was tested using the phosphomolybdenum method, and the absorbance was measured at 695 nm. The results revealed that the bark extract exhibited the highest antioxidant activity, recording a value of 420 micrograms per gram, followed by the peel with 346 micrograms per gram, and the leaf extract with 278 micrograms per gram. These findings emphasize the potent free radical-scavenging ability of pomegranate extracts, particularly the bark, and support their potential use as natural antioxidants in health-promoting formulations (Table 2).

Table 2. Total antioxidant activity of pomegranate ethanolic extracts: bark, peel, and leaves.

Total Antioxidant Activity	Total Antioxidant [mg/ml]
Bark	420
Peel	346
Leaf	278

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide (H_2O_2) scavenging activity of *Punica granatum* extracts – derived from the peel, bark, and leaves – serves as a crucial indicator of their antioxidant potential and ability to mitigate oxidative stress. Although H_2O_2 is relatively stable, it can generate highly reactive hydroxyl radicals through Fenton-type reactions, contributing to cellular damage and various degenerative diseases. The antioxidant efficacy of pomegranate extracts is largely attributed to their high content of bioactive compounds, such as polyphenols, flavonoids, tannins, and alkaloids.

Quantitative analysis revealed that the bark extract demonstrated the highest H_2O_2 scavenging activity at 34.84%, followed by the leaf extract at 24.27%, and the peel extract at 23.4%. These results indicate that the bark possesses superior antioxidant capabilities in neutralizing hydrogen peroxide, further supporting its potential application in oxidative stress-related therapeutic interventions (Table 3 and Figure 5).

Table 3. H_2O_2 activity of pomegranate ethanolic extracts: bark, leaf, and peel.

H_2O_2 Activity	% of Scavenging Activity
Bark	34.84%
Leaf	24.27%
Peel	23.4%

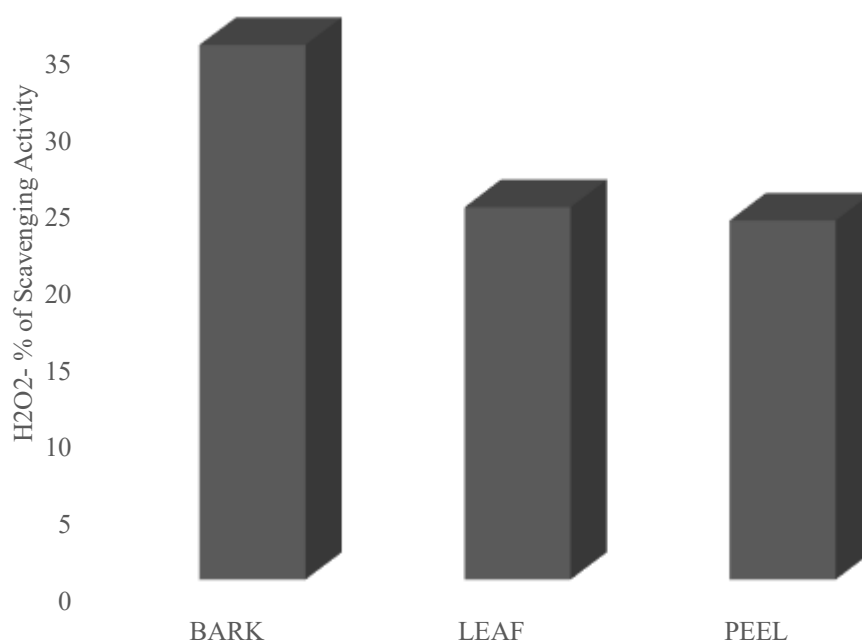


Figure 5. Graphical representation of H_2O_2 .

FRAP: Ferric Reducing/Antioxidant Power

The FRAP assay is widely used to determine the antioxidant capacity of plant extracts by measuring their ability to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. This reduction occurs in the presence of the FRAP reagent, which consists of 2,4,6-tris(1-pyridyl)-5-triazine (TPTZ) dissolved in sodium acetate buffer at pH 3.6. The development of blue ferrous-TPTZ complex is measured using a spectrophotometer at 593 nm.

In this study, the antioxidant capacities of *Punica granatum* extracts were quantified as follows: peel extract exhibited 98 micrograms per gram, leaf extract showed 125 micrograms per gram, and bark extract demonstrated 104 micrograms per gram. These values highlight the potent ferric-reducing ability across different pomegranate parts, with the leaf extract showing the highest activity (Table 4 and Figure 6).

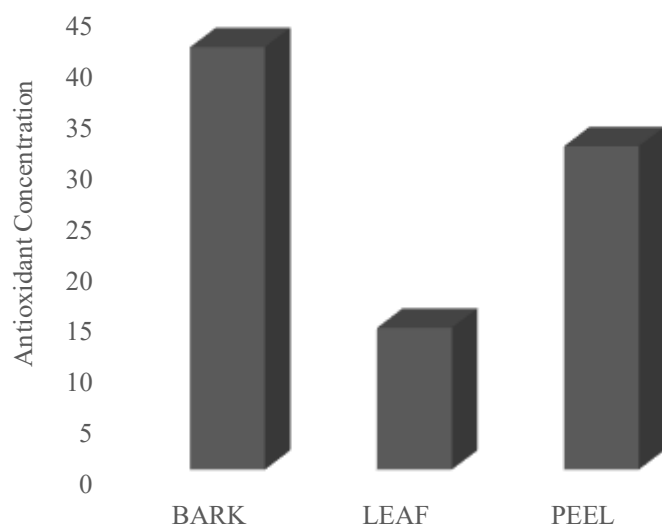


Figure 6. Graphical representation of FRAP.

Table 4. FRAP activity of pomegranate ethanolic extracts: bark, leaf and peel.

FRAP Activity	Reducing Power Assay %
Bark	41.4%
Leaf	13.9%
Peel	31.69%

Anti-Microbial Studies

Antibacterial and antifungal evaluations are essential for assessing the ability of natural or synthetic compounds to inhibit the growth of harmful microorganisms. These studies are typically performed in vitro, using methods, such as a petri dish or broth cultures. Antibacterial testing often targets pathogens, like *Staphylococcus aureus* and *Klebsiella pneumoniae*, examining how compounds interfere with bacterial cell walls, enzymatic functions, or biofilm formation.

Likewise, antifungal studies target pathogens, like *Aspergillus* species, examining mechanisms like disruption of fungal cell membranes, inhibition of spore germination, and disruption of essential biological functions. Natural extracts from *Punica granatum* (pomegranate) have demonstrated notable antibacterial and antifungal effects, attributed to their rich phytochemical profile – including polyphenols, tannins, flavonoids – and antioxidant activities measured by assays like H₂O₂ scavenging, FRAP, and total antioxidant capacity.

These studies are critical for discovering alternative therapies against antibiotic- and antifungal-resistant strains, advancing the development of novel pharmaceutical and nutraceutical agents.

Zone of Inhibition

The antibacterial and antifungal activities of shade-dried *Punica granatum* bark, peel, and leaf extracts were evaluated against human pathogenic microorganisms. The pomegranate extracts were

assessed for their antimicrobial activity against the fungal species *Aspergillus niger* and the bacterial organisms, such as *Staphylococcus aureus* and *Klebsiella pneumoniae* using the Kirby–Bauer method.

The efficacy of each extract was determined by measuring the diameter of the zones of inhibition around the wells, indicating their antimicrobial potency (Table 5 and Figures 7–10).

Table 5. Antibacterial activity of pomegranate ethanolic extracts: bark, leaf, and peel.

Name of Organism	Zone of Inhibition (in mm)			
	Bark	Leaf	Peel	Disc
<i>S. aureus</i>	13	10	11	23
<i>K. pneumoniae</i>	12	8	10	22

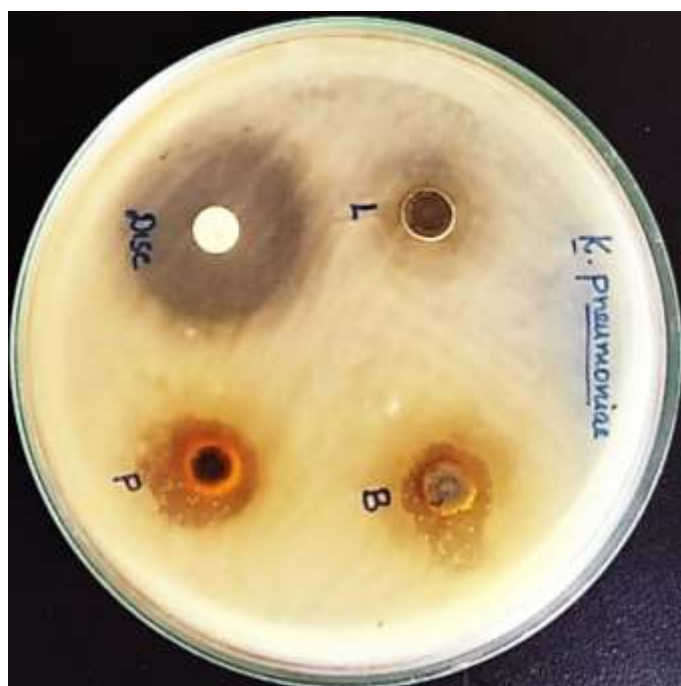


Figure 7. Anti-bacterial activity against *S. Aureus*.

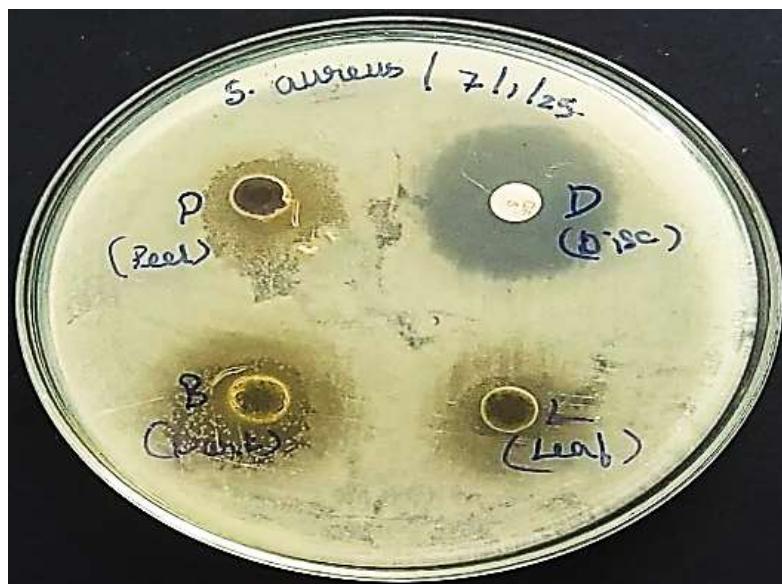


Figure 8. Anti-bacterial activity against *K.*

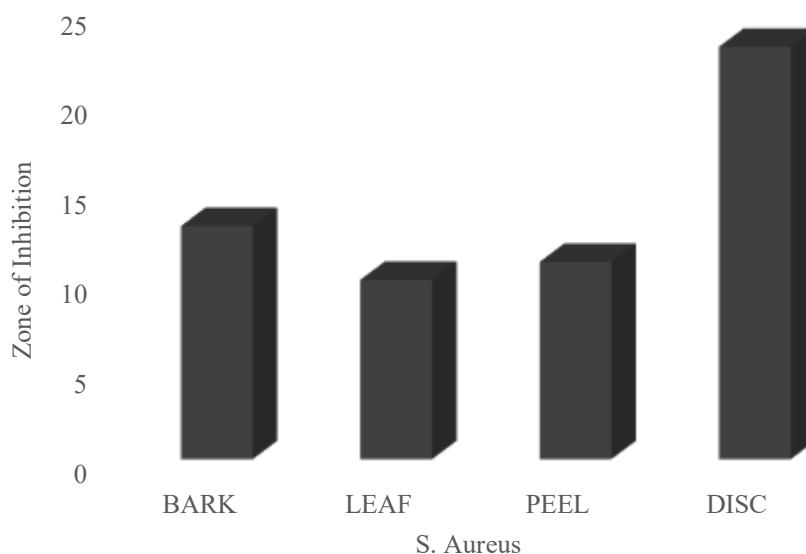


Figure 9. Graphical representation of anti-bacterial activity against *S. Aureus*.

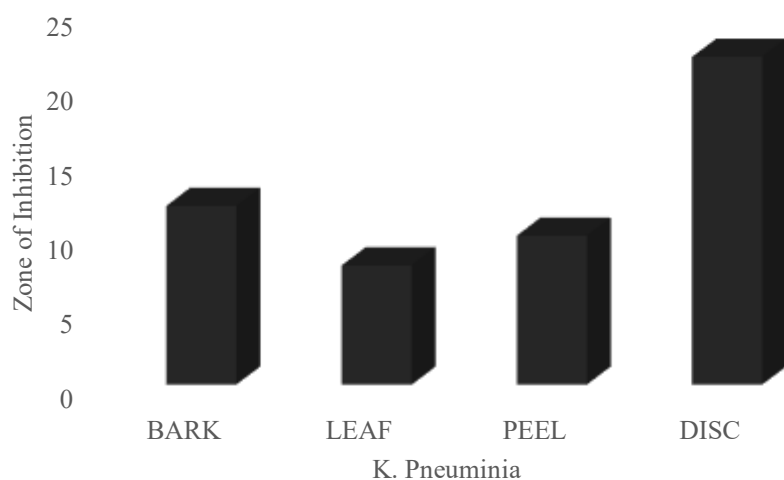


Figure 10. Graphical representation of anti-bacterial activity against *K. pneumoniae*.

Pomegranate extracts demonstrate notable antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, primarily due to their high levels of bioactive compounds, such as polyphenols, flavonoids, and tannins. Research indicates that the extract effectively inhibits *S. aureus*, a common cause of skin infections and foodborne illnesses, by damaging bacterial cell walls and disrupting key enzymatic functions. Against *K. pneumoniae*, a pathogen responsible for respiratory and bloodstream infections, pomegranate extract impedes bacterial adhesion and biofilm formation, thereby limiting infection.

Anti-Fungal Studies

The antifungal efficacy of pomegranate extracts is attributed to specific bioactive constituents, including alkaloids, phenols, flavonoids, terpenes, ketones, amines, and sulfides, which work synergistically to inhibit fungal growth and pathogenicity (Table 6 and Figures 11 and 12).



Figure 11. Antifungal activity against *A. Niger*.

Table 6. Antifungal activity of pomegranate ethanolic extracts: bark, leaf, and peel.

Anti-Fungal Activity	Zone of Inhibition
Fluconazole	3mm
Bark	3mm
Leaf	1mm
Peel	2mm

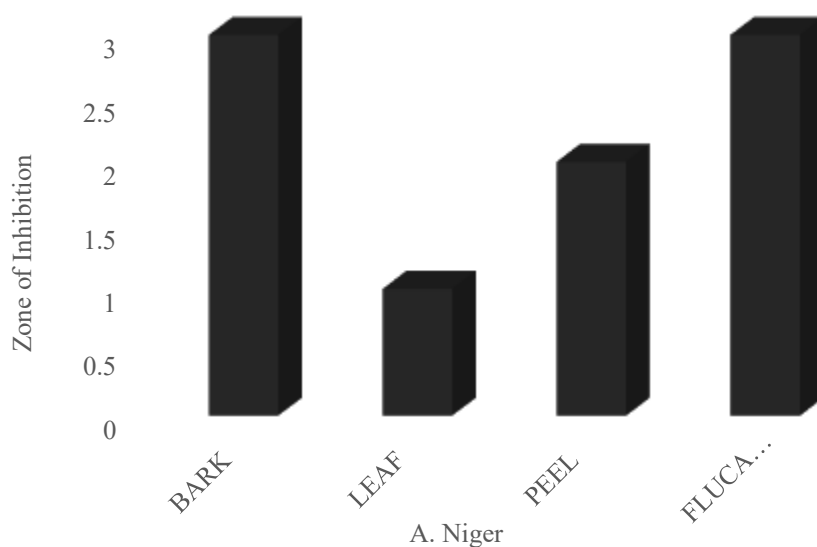


Figure 12. Graphical representation of antifungal activity against *A. Niger*.

Anti-Cancer Activity

Cancer remains one of the leading causes of death in the twenty-first century. Given its profound impact on human health and the significant burden on healthcare systems, there is growing interest in developing novel therapies aimed at preventing, slowing, or inhibiting cancer progression (Table 7 and Figures 13–15).

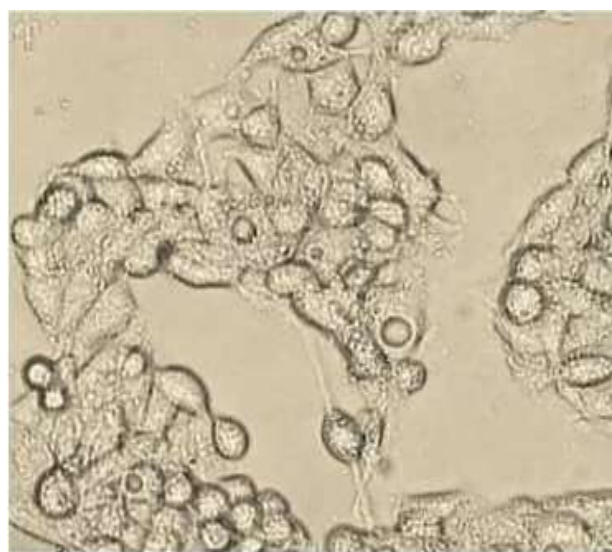


Figure 13. MTT assay.

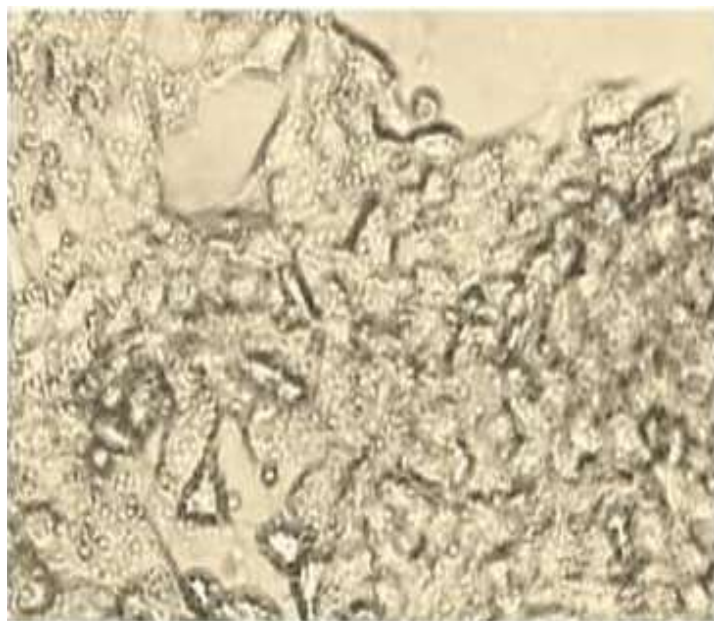
The study focused on the most promising extract, the bark, as identified through phytochemical analysis, antioxidant assays, and antibacterial and antifungal tests. The anticancer potential of the pomegranate bark extract was evaluated against a liver cancer cell line, with results illustrated through corresponding figures and graphs. Using concentrations ranging from 5 to 25 μg , the in vitro assay demonstrated a dose-dependent increase in cell death, ranging from 6.9% to 39.7%, confirming the significant anticancer activity of the pomegranate bark extract.



Control – untreated cells



Low concentration – 5 μg



High concentration – 25 µg

Figure 14. Cell death of HepG2 (liver cancer).

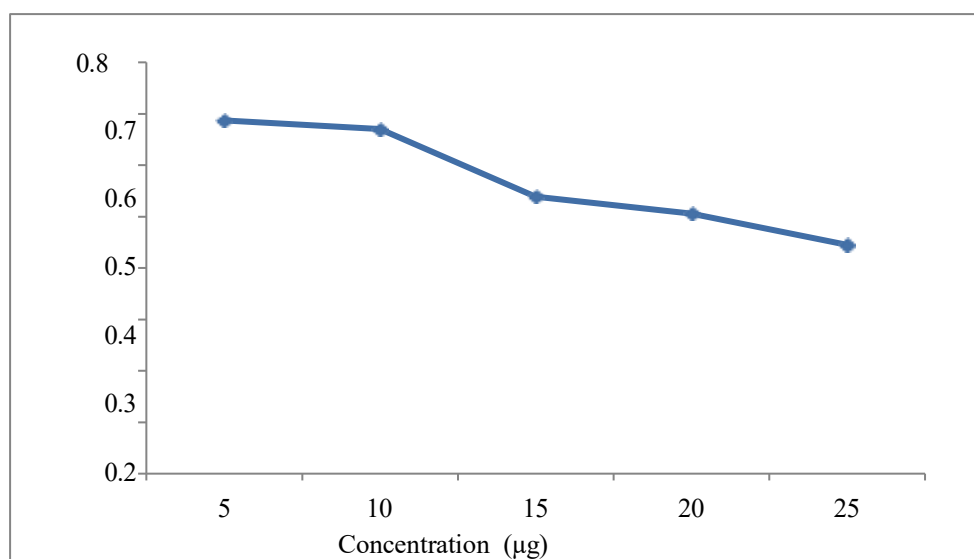


Figure 15. Graphical representation of anticancer activity.

Table 7. Anticancer activity of pomegranate ethanolic extracts: bark, leaf, and peel.

Concentration (µg/ml)	% of Cell Death
5	0.687
10	0.670
15	0.539
20	0.506
25	0.445

CONCLUSIONS

Pomegranate bark, peel, and leaf extracts demonstrate significant potential as rich sources of nutraceuticals, owing to their abundant phytochemical content and nutritional value. The bioactive compounds present – such as polyphenols, flavonoids, and tannins – especially in the peel, contribute

to a wide array of health benefits, including antioxidant, antibacterial, antifungal, and anticancer activities. Numerous studies have underscored the therapeutic promise of pomegranate peel extract, particularly its antimicrobial and anticancer effects. The extraction of these compounds from pomegranate peel, juice, and seeds has attracted growing interest for their applications in nutraceutical development and cancer research. Due to its high phytochemical concentration, pomegranate extract holds great potential for enhancing dietary supplements, functional foods, beverages, and cancer treatment formulations.

The study explored the phytochemical composition and biological activities of extracts derived from various parts of the pomegranate plant, including the bark, peel, and leaves. Researchers aimed to identify and quantify key bioactive compounds, such as polyphenols, flavonoids, and ellagitannins in each extract. Among the samples tested, the bark extract exhibited the most potent biological activity, showing notable antimicrobial, antioxidant, and anticancer effects. Due to these promising results, the bark extract was selected for further in vitro studies targeting liver cancer. These studies revealed a significant inhibitory effect of the extract on liver cancer cells. Building on these findings, the research team envisions future development of innovative health products that harness the bark extract's therapeutic potential. Such products could offer natural support for disease prevention and overall health, capitalizing on its strong bioactive properties.

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