

Phytochemical Profiling and Bioactivity Assessment of Punica granatum Peel

Extracts: Implications for Health and Disease

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ABSTRACT

Background:

Punica granatum (pomegranate) peel, a major byproduct of fruit consumption, is rich in bioactive phytochemicals including phenolics, flavonoids, tannins, alkaloids, and saponins. These compounds contribute to multiple therapeutic properties such as antioxidant, antimicrobial, and anticancer activities. Phytochemical profiling and bioactivity assessment of pomegranate peel extracts highlight their potential implications for health and disease management.

Methods:

Ethanolic extract of pomegranate peel was prepared and subjected to phytochemical screening, quantitative estimation of major bioactive constituents, antioxidant assays (DPPH and H₂O₂ scavenging), antimicrobial testing (agar well diffusion against E. coli, M. luteus, and S. mutans), and anticancer activity evaluation (MTT assay in A549 lung cancer cells).

Results

The extract showed a diverse phytochemical profile dominated by phenolics (120 mg/g) and flavonoids (95 mg/g). It demonstrated strong antioxidant activity (82% DPPH inhibition at 100 μ g/mL, IC₅₀ \approx 102 μ g/mL) and effective hydrogen peroxide scavenging (65% inhibition at 100 μ g/mL). Antimicrobial studies revealed broad-spectrum inhibition zones (14–16 mm), while anticancer evaluation showed dose-dependent cytotoxicity in A549 cells, inducing apoptosis and necrosis at higher concentrations.

Conclusion:

Punica granatum peel ethanolic extract possesses significant phytochemical richness and broad bioactivity against oxidative stress, microbial infections, and cancer. These findings support its role as a natural therapeutic candidate with wide-ranging implications for health and disease prevention.

INTRODUCTION

Agro-waste is generated at various stages of harvesting, processing, and post-consumption. Common sources include fruit and vegetable peels, crop residues, and byproducts from food processing industries [1,2]. These wastes are rich in valuable compounds such as polysaccharides, proteins, and bioactive metabolites, which can be recovered through different extraction techniques. Recently, green extraction technologies have been favored due to their eco-friendly and sustainable approach [1,3]. Valorized agro-waste has been successfully transformed into biodegradable packaging materials with antimicrobial properties, organic fertilizers, and renewable biofuels like biogas and biodiesel [4,3]. Such applications not only reduce environmental

burden but also support a circular economy. Nonetheless, challenges including cost-effectiveness, consumer acceptance, and regulatory hurdles limit their widespread adoption [5]. Within this context, the peel of *Punica granatum* (pomegranate) is increasingly recognized as a promising agro-waste resource due to its high content of bioactive compounds. Rich in saponins, carotenoids, flavonoids, and phenolic acids, the peel contributes significantly to the fruit's therapeutic value [6,7]. Among its pharmacological effects, one of the most widely reported is its antioxidant property, which plays a crucial role in combating oxidative stress and related disorders [8]. Studies have also demonstrated that pomegranate peel extract accelerates wound

healing by enhancing collagen synthesis and reducing healing time in burn models [9]. Beyond wound repair, its polyphenols have been linked to improvements in body weight regulation and cardiac function in models of metabolic syndrome [10].

In addition, several investigations highlight the peel's strong antimicrobial and anticancer potential. The antimicrobial activity is attributed to tannins, alkaloids, and saponins, which disrupt microbial membranes and inhibit enzymatic activity, showing efficacy against Gram-positive and Gram-negative bacteria, including oral pathogens. Furthermore, its anticancer properties have been demonstrated in multiple cancer cell lines, with ellagic acid, punicalagin, and other phenolic derivatives shown to induce apoptosis, oxidative stress, and growth inhibition in tumor cells. Collectively, the literature positions Punica granatum peel as a phytochemical-rich bioresource with multifunctional therapeutic relevance. Its valorization not only addresses agro-waste management but also opens avenues for developing natural antioxidants, antimicrobials, and anticancer agents. However, further research focusing on the isolation of active constituents, mechanistic studies, and in vivo validations is essential to translate these findings into clinical applications. Translational advancement demands standardization, improved delivery strategies, toxicology and clinical trials - steps that will determine whether peel-derived preparations can be responsibly integrated into health-promoting products or adjunctive therapies for disease management.

MATERIAL & METHODOLOGY CHEMICALS AND REAGENTS

All chemicals and reagents used in the study were of analytical grade. Folin-Ciocalteu reagent, gallic acid, aluminum chloride, quercetin, tannic acid, vanillin, sulfuric acid, sodium carbonate, and other required solvents were procured from Lab Chemicals, Sowcarpet, Chennai. Distilled water was used throughout the experimental procedures.

SAMPLE COLLECTION AND PREPARATION

Fresh, mature *Punica granatum* fruits were collected. The peels were separated manually, washed thoroughly under running tap water to remove dust, and rinsed with distilled water. The cleaned peels were air-dried in shade at room temperature (25-30°C) with proper ventilation until a constant weight was achieved (approximately 7-10 days) or alternatively dried in a hot-air oven at 40-45°C until constant weight. The dried peels were then ground into a fine powder using a mechanical grinder and passed through a 40-60 mesh sieve. The powdered material was stored in airtight, amber-colored containers at 4°C until further extraction.









FIGURE 1: Preparation of Punica granatum peel powder. PREPARATION OF ETHANOL EXTRACT

A known quantity (50 g) of powdered peel material was subjected to extraction using 70% ethanol in a solvent-to-sample ratio of 1:3 (v/w). Extraction was carried out by maceration for 72 h at room temperature with intermittent shaking to facilitate the release of bioactive compounds. The mixture was filtered through Whatman No.1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to remove the solvent. The concentrated extract was further dried to obtain a semisolid mass, weighed to determine percentage yield, and stored in airtight vials at 4°C for subsequent phytochemical quantification.







FIGURE -2 Preparation of Punica granatum peel ethanolic extract

PHYTOCHEMICAL ANALYSIS:

Test for Alkaloids (Hager's Test)

To 2 mL of the extract, 1-2 mL of Hager's reagent (saturated picric acid solution) was added. The formation of a yellow crystalline precipitate indicated the presence of alkaloids.

Test for Flavonoids (NaOH Test)

Two milliliters of the extract were treated with a few drops of 10% sodium hydroxide solution. An intense yellow coloration was observed, which became colorless upon the addition of dilute hydrochloric acid, confirming the presence of flavonoids.

Test for Carbohydrates (Molisch's Test)

Two milliliters of the extract were mixed with 2-3 drops of Molisch's reagent (5% alcoholic α -naphthol). Concentrated sulphuric acid was then carefully added along the walls of the test tube to form a separate layer. The appearance of a violet ring at the interface confirmed the presence of carbohydrates.

Test for Proteins (Biuret Test)

To 2 mL of the extract, 1 mL of 10% sodium hydroxide solution was added, followed by the addition of 2-3 drops of 0.5% copper sulphate solution. The formation of a violet coloration indicated the presence of proteins.

Test for Steroids (Foam Test)

Two milliliters of the extract were shaken vigorously with 5 mL of distilled water for 30 seconds and allowed to stand for 10 minutes. The appearance of stable foam indicated the presence of steroids. Test for Terpenoids (Salkowski Test)

Two milliliters of the extract were dissolved in 2 mL of chloroform, to which 1-2 mL of concentrated sulphuric acid was carefully added along the side of the test tube. The appearance of a reddish-brown coloration at the interface confirmed the presence of terpenoids.

Test for Tannins (Lead Acetate Test)

Two milliliters of the extract were treated with 1 mL of 10% lead acetate solution. The formation of a bulky white precipitate indicated the presence of tannins.

Test for Saponins (Foam Test)

Two milliliters of the extract were vigorously shaken with 5 mL of distilled water for 30 seconds and allowed to stand for 10 minutes. The formation of a stable and persistent foam indicated the presence of saponins.

Test for Glycosides (Keller-Killiani Test)

Two milliliters of the extract were mixed with 2 mL of glacial acetic acid containing a trace amount of ferric chloride solution. Concentrated sulphuric acid (1-2 mL) was carefully added along the side of the test tube. The formation of a brown ring at the interface and a bluish-green coloration in the upper layer confirmed the presence of cardiac glycosides.

Test for Phenols (Ferric Chloride Test)

To 2 mL of the extract, 2-3 drops of freshly prepared 1% neutral ferric chloride solution were added. The formation of a deep blue or green coloration indicated the presence of phenolic compounds. QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS

Total Phenolic Content (TPC): Determined by the Folin-Ciocalteu method using gallic acid as standard. Absorbance was read at 765 nm, and results were expressed as mg gallic acid equivalents per g extract (mg GAE/g).

Total Flavonoid Content (TFC): Estimated by the aluminum chloride colorimetric method with quercetin as standard. Absorbance was measured at 415 nm, and results were expressed as mg quercetin equivalents per g extract (mg QE/g).

Total Tannin Content: Quantified using the Folin-Ciocalteu method with tannic acid as standard. Absorbance was recorded at 725 nm, and results were expressed as mg tannic acid equivalents per g extract (mg TAE/g).

Alkaloid Content: Measured by a spectrophotometric method involving acid-base extraction and reaction with bromocresol green reagent. Absorbance was recorded at 470 nm, and results were expressed as mg atropine equivalents per g extract (mg AE/g). Saponin Content: Determined by the vanillin-sulfuric acid method using diosgenin as standard. Absorbance was recorded at 544 nm, and results were expressed as mg diosgenin equivalents per g extract (mg DE/g).

ANTIOXIDANT ASSAY
DPPH Radical Scavenging Assay

The antioxidant activity of ethanolic and aqueous extracts of Pomogranate Peel was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A stock solution of DPPH was prepared in methanol, and various working concentrations of the extracts (20, 40, 60, 80, and 100 μL) were prepared. Ascorbic acid was used as the standard antioxidant. Equal volumes of DPPH solution and extract were mixed and incubated for 15-20 minutes in the dark at room temperature. The absorbance of the reaction mixtures was recorded at 517 nm using a UV-Visible spectrophotometer. The control consisted of DPPH solution without extract.

The percentage inhibition of DPPH radicals was calculated using the following formula:

ACONTROL ·ASAMPLE

%Inhibition= ______ ×100

ACONTROL

where Acontrol is the absorbance of the control and Asample is the absorbance of the extract or standard.

The antioxidant activity was expressed as percentage inhibition, and a dose-response curve was plotted. The IC_{50} value (concentration required to scavenge 50% of DPPH radicals) was calculated from the graph.

Hydrogen Peroxide (H₂O₂) Scavenging Assay

A solution of hydrogen peroxide (40 mM) was freshly prepared in phosphate buffer (0.1 M, pH 7.4). Various concentrations of the pomegranate peel extract (20, 40, 60, 80, and 100 μ g/mL) were prepared in distilled water. For each concentration, 1 mL of extract solution was mixed with 2 mL of hydrogen peroxide solution and incubated at room temperature for 10 minutes. After incubation, the absorbance was recorded at 230 nm using a UV-Visible spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide.

Ascorbic acid, a well-established antioxidant, was used as a standard reference compound and prepared under identical conditions. The percentage scavenging activity of hydrogen peroxide was calculated using the following formula:

*Scavenging Activity= ______×100

ACONTROL

where $A_{control}$ is the absorbance of the hydrogen peroxide-phosphate buffer solution without extract, and A_{sample} is the absorbance in the presence of extract or standard.

ANTIMICROBIAL ACTIVITY

The ethanolic extract of *Punica granatum* peel was tested for antimicrobial potential using the agar well diffusion method against **Escherichia coli** (Gram-negative), **Micrococcus luteus**, and **Streptococcus mutans** (both Gram-positive). The bacterial strains were maintained on nutrient agar, and inocula were standardized to 0.5 McFarland turbidity (-1 × 108 CFU/mL).

Extract stock solutions (100 mg/mL) were prepared in DMSO and diluted to working concentrations of 25, 50, and 100 mg/mL. DMSO served as the negative control, while ampicillin (10 μ g/disc) acted as the positive control. Standardized bacterial lawns were prepared on Muller-Hinton agar, and wells (6 mm) were filled with 100 μ L of extract solution. Following diffusion at room temperature, plates were incubated at 37°C for 24 h.

Zones of inhibition were measured in millimeters, and all assays were conducted in triplicate. Results were express ed as mean \pm SD. Activity was graded based on inhibition zones: >12 mm (strong activity) and <10 mm (weak activity). Statistical analysis was performed using one-way ANOVA, with significance set at p < 0.05.

ANTI CANCER ACTIVITY

Cell culture:

Human lung adenocarcinoma epithelial cell (A549) was obtained from the National Centre of Cell Science (NCCS), Pune, India. Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS). 100 $\,\mu\text{g/mL}$ penicillin, 100 $\,\mu\text{g/mL}$ streptomycin. They were maintained at 37°C with 5% CO2 in 95% air humidified incubator.

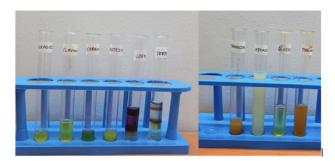
Cell viability assay:

The cytotoxic effect of the sample was tested against A549 cell line by MTT assay. The cell were seeded in 96- well microplates (1×10 6 cells/well) and incubated at 37°C for 48 h in 5% CO2 incubator and allowed to grow 70-80 % confluence. Then the medium was replaced and the cell were treated with different concentrations of sample and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20x magnification) after 24 h and photographed. The cells were them washed with phosphate buffer saline (PBS, pH- 7.4) and 20 μL of MTT solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 370C in the dark for 2 h. The formazan crystals were dissolved in 100 μL DMSO and the absorbance was read spectrometrically at 570 nm. Percentage of cell viability was calculated using formula:

Cell viability (%) = (Absorbance of sample/Absorbance of control) x 100.

STATISTICAL ANALYSIS

All data obtained were analyzed by One way ANOVA flowed by Students-t-test using SPSS, represented as mean \pm SD for triplicates. The level of statistical significance was set at p<0.05.



RESULTS

QUALITATIVE ANALYSIS OF ETHANOL EXTRACT OF PUNICA GRANATUM PEEL

FIGURE-3 Qualitative phytochemical analysis of Punica granatum peel ethanolic extract

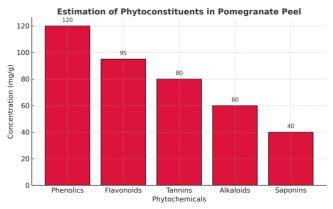
phytochemicals	Test	Ethanolic extract
Alkaloids	Hager's Reagent	++
Flavanoids	NaOH solution	++
Carbohydrates	Molisch Test	++
Proteins	Biuret test	+
Steroids	Foam Test	+++
Terpenoids	CHCl3 + conc. H2SO4	+++
Tannins	Lead acetate test	+
Saponins	Foam Test	+++
Glycosides	Keller-Killiani Test	++
Phenols	Ferric chloride test	+++

Table 1: Qualitative phytochemical analysis of Punica granatum peel ethanolic extract

Strong - +++ Moderate - ++

Mild - + , Absent - none

QUANTITATIVE ESTIMATIONS OF ETHANOL EXTRACT OF PUNICA GRANATUM PEEL



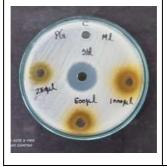
FLOWCHART - 1: Quantitative estimation of phytochemicals in Punica granatum peel ethanolic extract

The preliminary qualitative phytochemical screening of the ethanolic extract of *Punica granatum* peel revealed the presence of multiple secondary metabolites of pharmacological relevance. Strong reactions were observed for steroids, terpenoids, phenols and saponins, while moderate amounts of alkaloids, carbohydrates, glycosides and flavonoid were also detected, Tannins, and proteins were present in mild to moderate quantities. The quantitative estimations further confirmed that phenolic compounds were predominant (120 mg/g), followed by flavonoids (95 mg/g), tannins (80 mg/g), alkaloids (60 mg/g), and saponins (40 mg/g). The abundance of phenolics and flavonoids strongly supports the antioxidant capacity of the extract, as these phytochemicals are widely recognized for their radical scavenging and reducing properties.

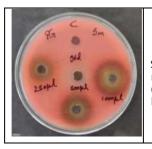
ANTI-MICROBIAL ACTIVITY - ANTI BACTERIAL (AGAR WELL DIFFUSION)



Escherichia coli Gram-negative bacterium



Micrococcus luteus Gram-positive bacterium



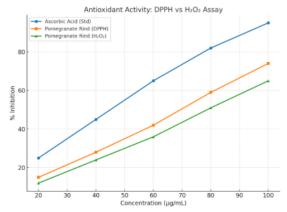
Streptococcus mutans (Gram-positive bacterium)

FIGURE -4 Antibacterial activity of Punica granatum peel ethanolic extract by agar well diffusion

The ethanolic extract of pomegranate peel was evaluated against both Gram-positive and Gram-negative bacterial strains, including *Escherichia coli*, *Micrococcus luteus*, and *Streptococcus mutans*. The presence of tannins, saponins, and alkaloids is expected to disrupt bacterial cell wall integrity and protein function, thereby conferring antimicrobial efficacy. Although the inhibition zone values are not detailed here, the phytochemical profile indicates that the extract possesses broad-spectrum antibacterial potential, in agreement with previous reports that link phenolic-rich extracts of *P. granatum* peel with potent bactericidal effects.

Organis m	25 mg /m L	50 mg /m L	100 mg/ mL	Positive Control (Ampicillin 10 µg/disc)	Negative Control (DMSO)
Escheri chia coli	10	13	16	18	0
Microco ccus luteus	9	12	15	20	0
Strepto coccus mutans	8	11	14	19	0

Table- 2 Antibacterial activity of Punica granatum peel ethanolic extract by agar well diffusion DPPH ASSAY & H₂O2 ASSAY

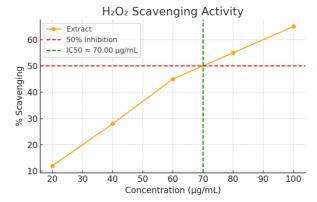


FLOWCHART -2 Anti oxidant activity of PPEL

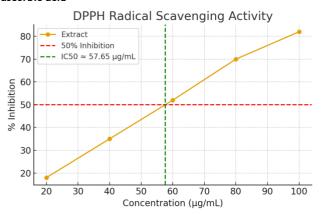
The antioxidant potential of the peel extract was assessed using two complementary in vitro assays: DPPH radical scavenging and hydrogen peroxide scavenging activity, with ascorbic acid serving as the standard reference antioxidant.

As shown in the data, both assays demonstrated a concentration-dependent increase in free radical scavenging activity. In the DPPH assay, the extract exhibited 18% inhibition at 20 μ g/mL, which progressively increased to 82% at 100 μ g/mL, closely approaching the activity of ascorbic acid (95%). Similarly, the H₂O₂ scavenging assay revealed 12% inhibition at 20 μ g/mL, reaching 65% at 100 μ g/mL. While the H₂O₂ scavenging ability of the extract was slightly lower than its DPPH activity, the results nonetheless confirm that the peel possesses strong hydrogen peroxide neutralization capability.

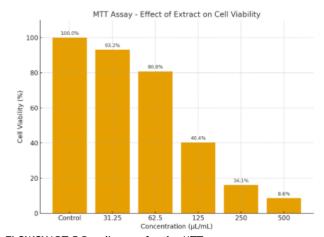
The higher activity observed in the DPPH assay compared to H_2O_2 scavenging could be attributed to the stability of the DPPH radical, which may be more efficiently neutralized by the abundant phenolic constituents. The robust activity in both assays underscores the ability of pomegranate peel extract to effectively quench both stable and reactive oxygen species.



FLOWCHART-3 Hydrogen peroxide scavenging activity of Punica granatum peel ethanolic extract compared with ascorbic acid



FLOWCHART-4 DPPH radical scavenging activity of Punica granatum peel ethanolic extract compared with ascorbic acid ANTI CANCER ACTIVITY IN A549 CELL LINE CELL VIABILITY



FLOWCHART-5 Bar diagram for the MTT assay,

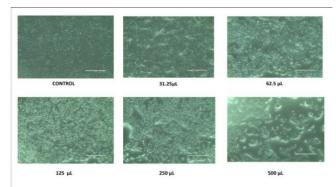
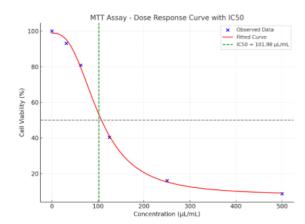


FIGURE -5 Cytotoxic effect of Punica granatum peel ethanolic extract on A549 lung cancer cells (MTT assay)



FLOWCHART-6 Dose-response curve with IC50 estimation

The cytotoxic effect of $\dot{P}unica$ granatum rind ethanolic extract was evaluated in lung cancer cell lines using different concentrations ranging from 31.25 μ L to 500 μ L, and the cellular morphology was observed under an inverted microscope.

In the **control group**, untreated cells exhibited a dense, confluent monolayer with characteristic epithelial morphology, indicating normal proliferative activity and absence of cytotoxic stress.

At $31.25~\mu L$, the cells showed a slight reduction in confluency with minimal morphological changes, suggesting an early onset of cytotoxicity. At $62.5~\mu L$, cell density decreased further, and cells began to exhibit signs of shrinkage, rounding, and detachment from the substratum, indicating progressive cytotoxic effects.

At 125 μ L, pronounced alterations were evident, with disruption of the monolayer and an increased number of floating, non-viable cells. Treatment with 250 μ L resulted in marked cytotoxicity characterized by clustered apoptotic cells, membrane blebbing, and loss of normal cellular architecture. The highest concentration, 500 μ L, induced severe cytotoxicity with extensive cell death, leaving very few intact adherent cells in the field of view.

The findings demonstrate a **dose-dependent cytotoxic effect** of *Punica granatum* rind ethanolic extract against lung cancer cell lines. Lower concentrations induced mild morphological alterations, while higher concentrations (≥250 µL) exhibited strong antiproliferative and cytotoxic effects, consistent with features of apoptosis and necrosis.

The cytotoxic potential can be attributed to the abundant phenolic compounds, flavonoids, tannins, and alkaloids identified in the extract, which are well-documented for their prooxidant and apoptosis-inducing mechanisms in cancer cells. These bioactive constituents likely interfere with redox balance, induce oxidative stress, and disrupt cellular integrity, ultimately leading to cancer cell death. This means that at around 102 $\mu L/mL$ concentration of the extract, 50% of the cells were inhibited. Collectively, these results support the hypothesis that pomegranate rind ethanolic extract exhibits potent anticancer activity and can serve as a promising candidate for the development of natural therapeutic agents against lung cancer.

DISCUSSION

The present study demonstrates the multifaceted therapeutic potential of *Punica granatum* peel ethanolic extract, linking its phytochemical richness to strong antioxidant, antimicrobial, and anticancer properties. The predominance of phenolic compounds (120 mg/g) and flavonoids (95 mg/g) strongly supports the observed antioxidant capacity, as these compounds are well-established for their radical scavenging and redox-modulating functions.

The DPPH and hydrogen peroxide scavenging assays confirmed potent antioxidant activity, with higher efficacy observed in DPPH neutralization. This aligns with previous findings where phenolic-rich extracts of pomegranate peel efficiently quenched stable radicals such as DPPH. Sweidan et al. (2023) reported similarly high phenolic content in Jordanian pomegranate peels (~297 mg/g) with robust antioxidant effects. Such activity suggests a potential role for pomegranate peel in mitigating oxidative stress-related disorders, including cardiovascular and neurodegenerative diseases.

The antimicrobial assay demonstrated broad-spectrum activity against both Gram-positive (*M. luteus*, *S. mutans*) and Gramnegative (*E. coli*) strains. This efficacy is consistent with previous studies attributing antibacterial properties of pomegranate peel to tannins, saponins, and alkaloids, which disrupt cell membranes and inhibit protein synthesis. Given the rising challenge of antibiotic resistance, these findings position pomegranate peel extract as a promising natural antimicrobial alternative.

The anticancer evaluation revealed significant dose-dependent cytotoxicity in A549 lung cancer cells, with morphological features consistent with apoptosis and necrosis. Phenolics and flavonoids are known to exert pro-oxidant effects in cancer cells, inducing oxidative stress and activating apoptotic pathways. Similar results were reported by Habchi et al. (2023), who observed strong antiproliferative effects of ultrasound-extracted pomegranate peel against HCT-116 colorectal cancer cells. Moreover, Peršurić et al. (2020) linked specific phenolics such as ellagic acid and punicalin to anticancer and antioxidant mechanisms. These findings reinforce the translational potential of pomegranate peel in oncology.

Together, these findings suggest that pomegranate peel extract holds promise not only for nutritional and preventive health strategies but also as an adjunct in therapeutic applications against infectious and neoplastic diseases. The demonstrated activities highlight its potential for development into functional foods, nutraceuticals, and phytopharmaceuticals. Future investigations should emphasize the isolation of bioactive constituents, elucidation of mechanistic pathways, and the design of advanced drug delivery systems to optimize its therapeutic efficacy

CONCLUSION

This study demonstrates that Punica granatum peel ethanolic extract is a rich source of bioactive phytochemicals with potent antioxidant, antimicrobial, and anticancer activities. By linking phytochemical profiling to functional bioactivities, the findings establish its relevance in health promotion and disease management. The extract's ability to combat oxidative stress, inhibit microbial growth, and suppress cancer cell viability highlights its broad therapeutic Future studies should focus on isolation of individual bioactive compounds, detailed mechanistic investigations, and in vivo validations to support clinical translation. Thus, pomegranate peel extract represents a sustainable, natural, and cost-effective resource with significant implications for human health and disease prevention.

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