RESEARCH PAPER

Screening of *Punica granatum* Peel Extracts for Antioxidant and Anticancer Activity Against Skin Cancer

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ABSTRACT

Punica granatum is a rich source of bioactive compounds exhibiting diverse pharmacological properties. In the present study, crude extracts obtained from pomegranate peel (PPE) were phytochemically characterized and evaluated for their antioxidant and anticancer potential against skin cancer. The phytochemical composition of the extracts was analyzed using Thin Layer Chromatography (TLC), High-Performance Thin Layer Chromatography (HPTLC), and High-Performance Liquid Chromatography (HPLC) techniques.

The antioxidant potential of the extracts was assessed using standard in vitro assays, demonstrating significant free radical scavenging activity, particularly in the ethanolic peel extract. The in vitro cytotoxic activity was further evaluated against the A431 human skin cancer cell line. Among the tested extracts, the ethanolic extract exhibited pronounced cytotoxic effects, with a lower IC_{50} value, indicating higher efficacy.

HPLC analysis confirmed the presence of key bioactive compounds, including ellagic acid, which is well-known for its antioxidant and anticancer properties. Overall, the findings suggest that the ethanolic extract of *Punica granatum* peel possesses potent antioxidant and anticancer activities, highlighting its potential for therapeutic and industrial applications.

Keywords: *Punica granatum*, phytocompounds, antioxidant activity, anticancer activity, skin cancer, A431 cell line, molecular docking

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1. Introduction

Skin cancer is one of the most common global public health problems, with increased mortality rates and morbidity and treatment costs annually [1]. Despite the abundance of data on the presentation of skin cancer in white individuals, there is a paucity of data regarding disease morphology and risk factors in darker-skinned individuals because the incidence of skin cancer is relatively higher in white individuals [2]. Recently, there have been many traditional treatments for cancer control, including chemotherapy, surgery, radiotherapy, and immunotherapy. Still, unfortunately, there are some important limitations to their use due to lack of safety, efficiency, undesired side effects, high cost, and availability [3].

There has been a growing interest in the use of complementary and alternative medicines, due to the disadvantages associated with conventional cancer chemotherapies and the supposed advantages of more natural treatment option. Phytochemical compounds from extracts of plant roots, bulbs, barks, leaves, stems and others have shown promising potential as anti-cancer drugs, or for serving as lead compounds in the synthesis of new drugs [4]. Phytochemicals including curcumin, sulforaphane, resveratrol, quercetin, and epigallocatechin gallate (EGCG) have shown their potential against the development of melanoma [5]. *Punica granatum* L. commonly referred to as pomegranate, is a small shrub tree that is native to the Mediterranean regions [6]. The fruit of pomegranate can be divided into three parts, which are peels, juice, and seeds. Usually, pomegranates are consumed fresh or processed into juice. When

processed into pomegranate juice, a large amount of waste is generated, in which peels take up about 26–30% of the total weight. It is worth noting that pomegranate peels contain many bioactive compounds such as polyphenols, dietary fiber, vitamins, minerals, etc. [7] *P. granatum* specifically its peel, is a potent source of bioactive compounds—including ellagitannins, punicalagin, and ellagic acid—that exhibit significant anti-inflammatory, antioxidant, and anti-cancer properties suitable for skin cancer prevention and treatment [8].

Ellagic acid, a major polyphenol from *P. granatum* peel, has shown significant potential in skin cancer treatment [9]. The potential of ellagic acid as inhibitors for UV-mediated skin carcinogenesis is confirmed through this silico study involving seven protein kinases [10]. In metastatic melanoma cell lines (1205Lu, WM852c, A375), ellagic acid reduced cell viability in a dose-dependent manner (25–100 μM), induced G1 cell cycle arrest, promoted apoptosis, and suppressed inflammatory mediators such as IL-1β and IL-8 [11]. It also inhibited NF-κB signaling, suggesting a key mechanism underlying its anticancer effects. These findings highlight its potential as a therapeutic and chemopreventive agent in melanoma.

Therefore, the present study aims to phytochemically characterize *P. granatum* peel extracts and evaluate their antioxidant and anticancer potential against skin cancer.

2. Materials and Methods

2.1 Collection, Preparation, and Extraction of Fruit Peels

Initially, *P. granatum* peels were procured from local vendors in Noida, Uttar Pradesh. The peels underwent preliminary processing, which included washing, air-drying at room temperature, rinsing with distilled water, and then shade-drying for a week. Subsequently, the dried peels were ground into a fine powder and kept in an airtight container for future experiments [12].

Ethanolic extraction, Methanolic extraction, Chloroform extraction, Petroleum ether extraction, Ethyl acetate extraction: The peel powder (10g) of *P. granatum* was weighed and soaked in 100 ml of different solvents for 72 hrs at 25˚ C separately. The extracts were filtered and concentrated and were stored for further research [13].

Aqueous extraction: 10 gm of powdered peels were dissolved in 250 ml of water and boiled on water bath for 6 hrs. Then, the mixture was filtered with blotting paper and then with Whattman no. 2 filters paper. After that, the filtrate was poured in glass petriplates and allowed to dry at ambient temp. of 50°C. The dried extracts were scraped down and stored in eppendorfs at 4°C until further use [14].

2.2 Qualitative Phytochemical Analysis

Phytochemical screening of extracts of *P. granatum* peel samples were performed for the qualitative detection of different phytochemicals such as, carbohydrates, proteins, alkaloids, phenols, anthocyanins, coumarins, sterols, terpenoids, tannins, and flavonoids. All phytochemical tests were performed according to the standard methods [15] [16] [17] [18] [19] [20].

2.3 Quantitative Phytochemical Analysis

Total phenolic content: The total phenolic content was estimated according to the method of Ayele et al., 2022 [21], and the results were expressed as gallic acid equivalent in μg/mg of extract.

Total flavonoid content: A total flavonoid content of all the extracts was determined by the method of Sulastri et al., 2018 [22], and the values were expressed as quercetin equivalent in μg/mg of extract.

Total tannin content: The total phenolic content was estimated according to the method of Ayele et al., 2022 [21], and the results were expressed as gallic acid equivalent in μg/mg of extract.

2.4 Thin Layer Chromatography for Ellagic Acid Identification [23]

In accordance with previous literature reports indicating ellagic acid as a major bioactive compound of *P. granatum* peel, TLC was performed to confirm its presence in the extracts. TLC was employed for the identification of ellagic acid in methanolic, ethanolic, and aqueous extract. Samples and standard ellagic acid were applied on RP silica gel 60 TLC plates as bands and developed using a mobile phase of toluene: ethyl acetate: formic acid (5:5:1). The chromatogram was developed in a pre-saturated chamber and visualized under UV light at 265 nm. Ellagic acid was identified by comparing the Rf value and spot characteristics of the sample with the standard, confirming its presence in the extract.

2.5 High Performance Thin Layer Chromatography for Ellagic Acid Identification [24]

HPTLC analysis was performed using a CAMAG ATS4 automatic TLC sampler equipped with a 100 μL Hamilton syringe. Samples and standard solutions were applied as 8 mm bands on precoated silica gel 60 F254 aluminium TLC plates (20 × 10 cm, 250 μm thickness), with RP plates used for ellagic acid analysis. Plate development was carried out in a CAMAG glass twin-trough chamber saturated with the mobile phase for 20 minutes. The mobile phases employed were methanol: water: formic acid (3.8:4.7:0.8) for ellagic acid scanning was performed at 254 nm using CAMAG vision CATS software, with a scanning speed of 20 mm/s and slit dimensions of 6 mm × 0.45 mm. Standard stock solutions (1 mg/mL) were prepared in methanol, while sample extracts (50 mg) were

dissolved in methanol, centrifuged, and diluted to volume prior to analysis.

2.6 High-Performance Liquid Chromatography [25]

Ellagic acid was analyzed using HPLC with a Shimadzu system equipped with a UV detector. The standard solution was prepared in HPLC-grade water and diluted to a final concentration of 3 µg/5 ml. The sample (0.125 g) was extracted using HPLC-grade water, sonicated for 30 minutes, filtered, and passed through a 0.45 µm membrane filter. Chromatographic separation was carried out on a C18 column (250 × 4.6 mm, 5 µm) using acetonitrile:5 mM KH_2PO_4 buffer (95:5, v/v; pH 2.5) as the mobile phase. The analysis was performed at a flow rate of 1.5 mL/min with a run time of 18 minutes, and detection was carried out at 254 nm.

2.7 Antioxidant Assay [26]

2.7.1 DPPH Radical Scavenging Assay

The antioxidant activity was determined using the DPPH assay following Zhang et al. with minor modifications. A 0.06 mM DPPH solution in methanol (2 mL) was mixed with 50 µL of extract and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm.

$$\% \text{ Inhibition} = \frac{(A_0 - A_S)}{A_0} \times 100$$

where A_0 is the control absorbance and A_S is the sample absorbance. Ascorbic acid served as the positive control. IC_{50} values were calculated from the inhibition curve. Experiments were performed in triplicate.

2.7.2 Hydrogen Peroxide (H_2O_2) Scavenging Assay

H_2O_2 scavenging activity was assessed using the method of Ruch et al. A 40 mM H_2O_2 solution in 50 mM phosphate buffer (pH 7.4) was prepared. The reaction mixture containing 500 µL of extract or ascorbic acid and 1 mL H_2O_2 was incubated for 30 min, and absorbance was measured at 230 nm against a blank.

$$\% \text{ Scavenging} = \frac{(OD_{control} - OD_{test})}{OD_{control}} \times 100$$

All experiments were conducted in triplicate.

2.8 Cytotoxic Effect of *P. granatum* Peel Extract on A431 Cell Lines [27]

The cytotoxic activity of *P. granatum* peel extract against A431 cells was assessed using the MTT assay. Cells were seeded in a 96-well plate at a density of 3×10^4 cells/well and allowed to adhere overnight. The cells were then treated with varying concentrations of the extract (0.2–5 µg/mL) for 16 h.

Following treatment, 10 µl of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3 h in a CO_2 incubator. The resulting formazan crystals were dissolved by adding 110 µl of DMSO, followed by incubation in the dark at room temperature for 10 min. Absorbance was measured at 570 nm using an ELISA plate reader

3. Results and Discussion

3.1 Phytochemical Screening

Phytochemical screening is used to evaluate the constituents of the plant extracts and their predomination, along with the search for bioactive constituents that may be helpful in the production of therapeutic drugs. In the current study, the qualitative phytochemical analysis of the plant extracts revealed the presence of various bioactive compounds across different solvents (see Table 1). The study demonstrated that the methanolic, ethanolic, and aqueous extracts exhibited a higher diversity of phytoconstituents compared to the chloroform, ethyl acetate, and petroleum ether extracts.

Similarly, Sweidan et al. reported comparable findings, demonstrating through qualitative analysis that the ethanolic peel extract of *P. granatum* contained the highest diversity of phytochemicals [12]. *P. granatum* exhibits significant anticancer activity due to its polyphenol-rich extracts, which exert antiproliferative and anti-inflammatory effects, highlighting its potential as a dietary agent for cancer prevention [28].

Consequently, the methanolic, ethanolic, and aqueous extracts were selected for further quantitative phytochemical analysis due to their richer bioactive compound profiles. This variation in phytochemical composition across solvents highlights the differential solubility and extractability of these bioactive compounds, which may influence their potential pharmacological properties.

Table 1: Qualitative analysis of *P. granatum* extracts

3.2 Quantitative Analysis

Among the six extracts, the ethanolic, methanolic, and aqueous extracts demonstrated a higher diversity of phytoconstituents; thus, these were selected for quantitative analysis. The total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) of these extracts are detailed in Table 2. The ethanolic extract displayed the highest concentrations of phenolic (30.43±0.18 mg GAE/g), flavonoid compounds (24.87±0.10 mg QE/g), and tannin levels (19.34±0.26mg GAE/g) was higher in the methanolic extract. In contrast, the

Phytochemicals		Petroleum ether extract	Ethyl acetate extract	Chloroform extract	Methanolic extract	Ethanolic extract	Aqueous extract
1. Carbohydrates	Molisch test	+	-	+	-	-	-
	Benedict test	-	-	-	+	+	+
	Fehling test	-	-	-	+	+	+
2. Proteins	Millon's test	-	-	-	-	-	-
3. Alkaloids	Wagner's test	-	-	-	+	+	+
4. Phenolic compounds	Zinc-hydrochloride reduction test	-	-	-	-	-	-
	Ferric chloride test	-	-	-	+	+	+
5. Anthocyanins		-	-	-	-	-	-
6. Coumarins		-	+	-	++	++	+
7. Sterols		-	-	+	++	++	+
8. Triterpenoids		+	-	-	-	-	+
9. Tannins	Ferric chloride test	-	+	-	++	++	+
	Alkaline reagent test	-	-	-	+	++	-
10. Flavonoids	Zinc-hydrochloride reduction test	-	+	-	+	+	-
	Alkaline reagent test	+	+	+	+	+	-
11. Quinones		-	-	+	-	+	+

aqueous extract demonstrated the lowest concentrations of phenolic, and tannin compounds. Kumaran et al., 2025 also reported that the ethanolic extract contained a higher phenolic content, with comparatively lower levels of flavonoids and tannins, following a pattern similar to that observed in our study [29]. In a study by Sarojini et al.,2020 the ethanolic extract was reported to contain a high phenolic content (115.21 ± 1.32 mg GAE/g) and a comparatively lower flavonoid content (18.71 ± 1.74 mg quercetin/g), which is consistent with the values observed in our study [30].

Table 2. Total phenol, flavonoid and tannin content

Extracts	TP (mg GAE/g extract)	TF (mg QE/g extract)	TTC (mg GAE/g extract)
***P. granatum* methanolic extract**	22.53±0.19	11.63±0.90	23.84±0.43
***P. granatum* ethanolic extract**	30.43±0.18	24.87±0.10	13.38±0.21
***P. granatum* aqueous extract**	16.48±0.30	17.10±0.35	19.34±0.26

3.3 Thin Layer Chromatography for Ellagic Acid Identification

The present study aimed to separate phytoconstituents and identify ellagic acid (standard biomarker) in methanolic, ethanolic, and aqueous extracts of *P. granatum* peel. TLC profiling was performed using a common solvent system for both the standard and extracts. After multiple trials with different mobile phases, the optimized system produced sharp and well-resolved bands, particularly in the ethanolic extract. The Rf value of standard ellagic acid was observed at 0.45. The ethanolic extract exhibited two peaks, of which the second peak (Rf = 0.43) closely matched the standard, confirming the presence of ellagic acid. In contrast, no corresponding peaks were detected in the methanolic and aqueous extracts (Figure 2). A

comparable study by Bagade et al. (2014) on *P. granatum* roots using HPTLC reported an Rf value of 0.45 for ellagic acid, which is consistent with the present findings and further supports the presence of phenolic compounds in the ethanolic extract [23].

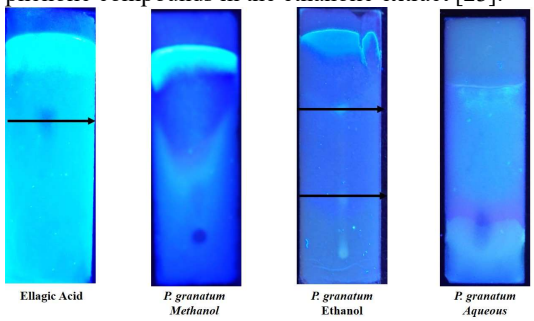

Figure 1: TLC of standard (Ellagic acid) and extracts at UV 265 nm in the visible range

3.4 High Performance Thin Layer Chromatography for Ellagic Acid Identification

Based on the TLC findings, the ethanolic extract exhibited an Rf value comparable to that of ellagic acid and was therefore selected for further HPTLC analysis. The chromatographic profile of the test sample showed peaks closely aligning with the standard ellagic acid having Rf value of 0.17 with a peak area of 12068.6 (Figure 2). In the ethanolic extract, three distinct peaks were observed, among which the third peak (Rf = 0.17; peak area: 313) corresponded closely to the standard, indicating the presence of ellagic acid (Figure 3). Visualization of the HPTLC plate at 254 nm revealed multiple bands in the extract, with one band matching the Rf of the ellagic acid standard, thereby confirming its presence (Figure 4).

In a validated HPTLC study, ellagic acid from pomegranate peel extract showed a characteristic Rf value (~0.19) that closely matched the standard, confirming its suitability as a marker compound for phytochemical standardization [24]. Additionally, previous phytochemical investigations have established that pomegranate peel is a rich source of polyphenolic compounds, particularly ellagitannins that hydrolyze to release ellagic acid [31].

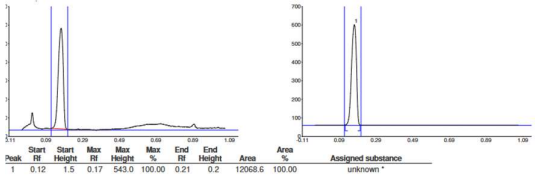

Figure 2: HPTLC chromatogram of ellagic acid obtained using optimized chromatographic condition at 254 nm

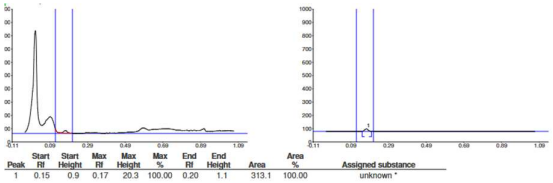

Figure 3: HPTLC chromatogram of *P. granatum* ethanolic extract obtained using optimized chromatographic condition at 254 nm

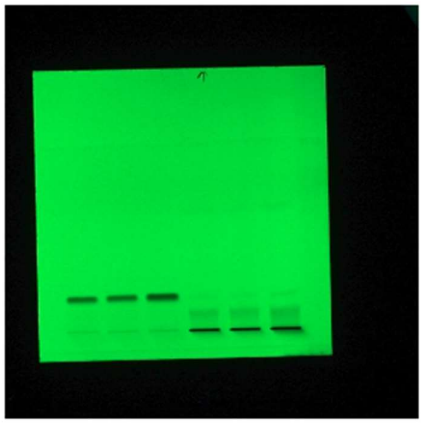

Figure 4: HPTLC fingerprints of *P. granatum* ethanolic extract

3.5 High-Performance Liquid Chromatography for ellagic acid

The HPLC chromatogram confirmed the presence of ellagic acid in the ethanolic extract of *P. granatum*, with a retention time (16.60 min) closely matching the standard (16.64 min). The ellagic acid content (0.15%) corresponds to approximately 1.5 mg/g of extract, indicating its presence in a low but quantifiable concentration (Figure 5). These results correlate well with the HPTLC findings, where phenolic compounds were identified based on matching Rf values with standards. Together, both techniques validate the presence of bioactive phenolics in the ethanolic extract.

Kumar et al., 2022 also reported the presence of ellagic acid in *P. granatum* peel using HPLC analysis, where it was detected at a retention time of approximately 4 minutes [32]. This finding is consistent with the present study, as the identification of ellagic acid was similarly confirmed through chromatographic comparison with the standard. The close agreement in retention time across studies further supports the reliability of HPLC methods for the detection and characterization of phenolic compounds in pomegranate peel extracts.

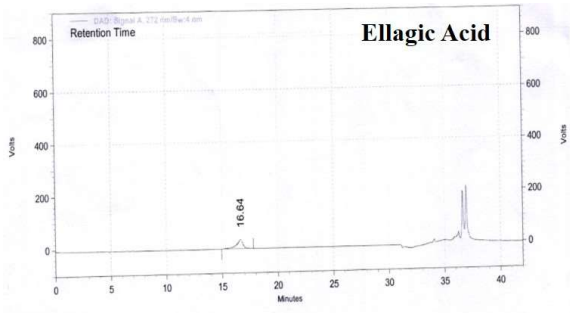

Figure 5: HPLC chromatogram of *Punica granatum* ethanolic extract recorded at 254 nm

3.6 Antioxidant Activity

The antioxidant activity of *P. granatum* peel extracts was assessed using DPPH and H_2O_2 scavenging assays, expressed as IC_{50} values. Ascorbic acid showed the strongest activity (26.84 ± 0.29 µg/mL for DPPH; 54.55 µg/mL for H_2O_2). Among the extracts, the ethanolic extract exhibited better DPPH scavenging (80.27 ± 0.67 µg/mL) and H_2O_2 scavenging (91.19 µg/mL) than the aqueous extract Overall, both extracts demonstrated notable antioxidant potential, with high linearity (R^2 = 0.98–0.99), indicating reliable results (Table 3).

Further supporting the present findings, previous studies have consistently reported that organic solvent extracts of *Punica granatum* peel exhibit superior antioxidant activity compared to aqueous extracts. Methanolic extracts, for instance, showed higher DPPH scavenging activity than aqueous extracts, with a strong correlation to total phenolic content. Similarly, hydroalcoholic extracts demonstrated enhanced antioxidant potential over water extracts, emphasizing their suitability for nutraceutical applications [33].

Table 3: Antioxidant activity (IC_{50}) of methanolic, ethanolic and aqueous extract of *P. granatum* peel along with the standard ascorbic acid

Samples Name	DPPH (IC_{50} µg/ml)	H_2O_2 (IC_{50} µg/ml)	R_2
Ascorbic Acid (AA)	26.84 ±0.29	54.55±0.24	0.98
P. granatum Ethanol	80.27 ±0.67	91.19 ±0.37	0.99
P. granatum *A*queous	93.31 ±0.56	124.37±0.17	0.99

3.7 Cytotoxic Effect of *P. granatum* Peel Extract on A431 Cell Lines

The graph demonstrates a dose-dependent decrease in cell viability with increasing concentration of *P. granatum* peel extract. At lower concentrations (0.5–1 µg/mL), cell viability remained close to 100%, indicating minimal cytotoxicity. However, a gradual decline was observed with increasing concentration, with viability reducing to approximately 60–80% at 2–3 µg/mL, and further decreasing to ~55% and 42% at 5 and 10 µg/mL, respectively. This indicates significant inhibition of cell proliferation at higher concentrations. These findings confirm that the extract exhibits concentration-dependent anticancer activity against A431 skin cancer cells (Figure 6).

In agreement with the present study, previous research has reported that *P. granatum* peel extract obtained using hybrid ultrasound–microwave-assisted extraction exhibits significant anticancer activity against HeLa and HepG2 cell lines. The enhanced extraction method improves polyphenol yield, leading to inhibition of cell proliferation and induction of apoptosis, primarily attributed to bioactive compounds such as ellagic acid and punicalagin [34].

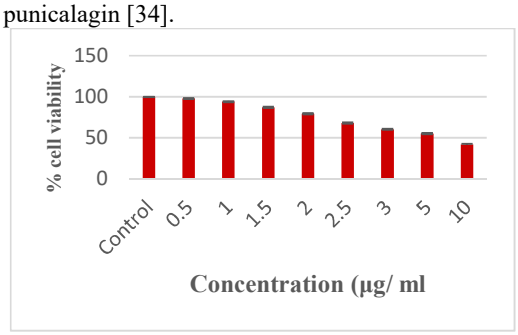

Figure 6: Cytotoxicity effect of *P. granatum* ethanolic extracts (0.5-10 µg/mL, 16 h) on A431 cell lines

3.8 Conclusion

In conclusion, the present study demonstrates that *Punica granatum* peel is a rich source of bioactive phytoconstituents with significant antioxidant and anticancer potential. Phytochemical screening and chromatographic analyses (TLC, HPTLC, and HPLC) confirmed the presence of key polyphenolic compounds, particularly ellagic acid, in the ethanolic extract. Quantitative analysis further revealed that the ethanolic extract possesses higher phenolic and flavonoid content, which correlates with its enhanced biological activity.

The antioxidant assays (DPPH and H_2O_2) indicated notable free radical scavenging activity, with the ethanolic extract showing superior performance compared to the aqueous extract. Additionally, the extract exhibited dose-dependent cytotoxic effects against A431 skin cancer cells, confirming its anticancer efficacy.

Overall, the findings highlight that the ethanolic extract of *P. granatum* peel holds promising potential as a natural source of antioxidant and anticancer agents. This study supports its prospective application in pharmaceutical and nutraceutical formulations, although further in vivo studies and mechanistic investigations are required to validate its therapeutic efficacy.

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