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Original Article

HPLC-Based Phytochemical Profiling and Untargeted Proteomics with Cytotoxicity Assessment of Ethanolic Extracts of *Punica granatum* on MCF-7 and C6 Cancer Cell Lines

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ABSTRACT

Background and Aim: To perform HPLC-based phytochemical profiling and untargeted proteomic analysis of ethanolic extracts from *Punica granatum*, and evaluate their cytotoxic activity against MCF-7 and C6 cancer cell lines. The study aims to identify key bioactive compounds in the extract, analyze their molecular mechanisms through proteomic changes, and assess their potential anticancer properties. **Materials and Methods:** Pomegranate peels extracts were prepared using ethanol analyzed for phytochemical content, including alkaloids, flavonoids, and saponins. Cell viability was evaluated through the MTT assay. Advanced techniques like HPLC and LC-HRMS were used to analyze chemical compositions, while proteomics assays identified and quantified proteins through processes such as chromatography, and mass spectrometry. **Results:** RP-HPLC analysis revealed a greater diversity of compounds in inner *Punica granatum* peels across different wavelengths compared to outer peels. Comparative analysis of retention times and phytocomponents demonstrated both unique and shared compounds between the two peel types. LC-HRMS analysis of the outer peel identified 36 secondary metabolites, including important compounds like gallic acid, citric acid, and syringic acid. Finally, proteomic data highlighted significant changes in protein expression profiles in both MCF-7 (cancer) and HEK (normal) cells, suggesting potential biological effects of the pomegranate peel extracts on cellular processes. **Conclusion:** Anticancer activity was confirmed on MCF-7 (breast cancer) and C6 (glioblastoma) cell lines, with no significant toxicity on HEK 293 (non-cancerous) cells. These findings suggest potential for further cancer therapy development, with additional proteomic analysis planned.

Keywords: Breast Cancer, *Punica granatum*, Glioblastoma, HEK Cell lines, Metabolomics, LC-HRMS, RP-HPLC.

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INTRODUCTION

A recent WHO report indicates that cancer was responsible for nearly 10 million deaths globally in 2020, making it the leading cause of approximately one in six deaths. The most prevalent cancers include breast cancer (2.26 million cases), lung cancer (2.21 million cases), colorectal cancer (1.93 million cases), and prostate cancer (1.41 million cases).[1] Since 2022, the cancer burden has grown significantly, with an estimated 20 million new cases and 9.7 million deaths due to cancer globally. This is a significant increase from past years, highlighting the increasing burden of cancer on the health of the world.[2] In India too, the cancer scenario is changing dramatically. The National Cancer Registry Programme (NCRP) estimates the age-adjusted incidence rate for all cancers put together to be around 130 per 100,000 population. Breast, cervical, and oral cancers are most common in women, and lung, colorectal, and stomach cancers are most common in men.[3] Current studies have also emphasized the anticancer activity of *Punica granatum* (pomegranate). Pomegranate extracts have been shown to have anti-proliferative activities against numerous cancer cell lines, such as breast and prostate cancers, by mainly inducing apoptosis and arresting cell cycle.[4] Nevertheless, these observations are preliminary, and additional clinical trials are needed to confirm the efficacy and safety of pomegranate-based treatments in the management of cancer.[5] Approximately 30% of cancer cases in low- and

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lower-middle-income countries are linked to cancer-causing infections, such as Human Papillomavirus (HPV) and hepatitis.[6] Many tumors are curable if detected early and treated effectively. Cancer patients today have significantly more treatment options compared to a decade ago.[7] In certain cases, these treatments can completely eliminate tumors even when cancer has spread throughout the body. However, nearly all existing treatments share a major drawback: they eventually become ineffective for many patients. This issue, known as treatment resistance, remains one of the most challenging obstacles for both cancer researchers and patients.[8] When cancer cells develop resistance to treatment drugs, they can continue to grow and form new tumors, a process referred to as recurrence or relapse.[9] Sometimes resistance develops quickly, within a matter of weeks of starting treatment.[10] In other cases, it develops months, or even years, later. There is a chance that your cancer won't respond to the medications you're taking, despite the fact that treatment is effective in reducing or stabilising tumor growth. Cancer drug resistance, or chemotherapy resistance, occurs when cancers that previously responded to a drug or treatment begin to spread or grow again.[11] Polyphenols and flavonoids in fruits and vegetables are linked to suppressing genes, proteins, and signaling pathways involved in tumor growth,[12,13] making them promising therapeutic agents for cancer. Among these, pomegranate (*Punica granatum*), rich in bioactive phytochemicals, has gained significant attention for its anti-cancer properties.[14] Its seeds are particularly notable, containing polyphenolic compounds like ellagitannins, anthocyanins, and flavonoids with antioxidant, anti-inflammatory, and anti-carcinogenic effects.[15] Breast cancer is a diverse and complex disease, comprising various subtypes with unique molecular profiles and clinical behaviors.[16] Some examples of commonly studied cell lines include MCF-7, derived from human breast adenocarcinoma cells, because its molecular details are well described and therefore sensitive to hormone manipulation-a representative model of hormone responsive human breast cancer and an exceptionally valuable resource to explore therapy options.[17] Despite significant advancements in understanding breast cancer biology and the development of targeted therapies, the search for effective and reliable treatments remains a critical challenge.[18] Natural compounds, such as pomegranate fruits peel extract, have emerged as promising candidates in breast cancer treatment.[19] Pomegranate-derived compounds' multi-targeted actions, such as inhibiting angiogenesis, inducing apoptosis, and inhibiting cell proliferation, highlight their promise as therapeutic adjuvants or as sole therapeutic drugs for treating breast cancer.[20,21] The MCF-7 cell line, a common breast cancer cell line, was used in the current study to test the effects of *Punica granatum* peel extract. To learn more about its potential therapeutic use in the treatment of breast cancer, a number of in vitro tests were conducted to assess its impact on cell viability, proliferation, and cytotoxicity mechanisms.[22] However, despite the numerous studies on the effects of pomegranate fruit and its polyphenolic derivatives on various cancers, no comprehensive study has been conducted specifically examining the effects of these individual compounds across different cancer types.[23] However, while various chemotherapeutic agents and treatment modalities may initially prove effective, the development of drug resistance remains a major obstacle in cancer therapy. Chemoresistance either intrinsic or acquired can lead to treatment failure, disease recurrence, and ultimately, increased mortality.

MATERIALS AND METHODS

Plant material identification and collection

The peels of *Punica granatum* were collected from Hajipur, district Vaishali, Bihar. These were cut into small pieces and shade-dried at room temperature for 15 days and then ground into a fine powder. 300 g of the powdered material was used for extraction with 70% ethanol for 24 hr. After complete elimination of alcohol under reduced pressure, a semi-solid extract was obtained, yielding 16%. The extract containing polar and nonpolar phytocomponents was preserved in the refrigerator for further use as illustrated Figure 1.

Cytotoxity assay

The MTT assay involves seeding cells in a 96-well plate (5,000-10,000 cells per well), incubating overnight at 37°C in 5% CO_2 for adhesion, and treating with plant extracts dissolved in DMSO at various concentrations (≤0.1-0.5% v/v). After 24-48 hr, MTT solution is added, incubated to form formazan crystals, and dissolved in DMSO for absorbance measurement at 570 nm. 23 Cell viability (%) is calculated as the ratio of treated to control absorbance, with data analyzed to determine cytotoxicity or protective effects across different extract concentrations.[24] Cytotoxicity activity of all extract was tested against cancerous cell line like MCF-7 (breast cancer), C6 (Glioblastoma) and non-cancerous cell line i.e., HEK (Human Embryonic Kidney) cells. Firstly, 100000 cells/well of cells were sown in 96-well plates, and after cell incubation for 24 hr.[43] Cells were exposed to extracts of S. americanum and *Punica granatum* in varying doses, and they were again incubated for the following 24 hr Result in Figures 2-6. Then 0.5 mg/mL of MTT dye was added to each well and further incubated for 4 hr and further followed by the addition of DMSO and the absorbance was recorded at 590 nm wavelength with the help of a Multimode reader synergy biotek and Medium alone is used as control.[44]

Extraction procedure

250 g of the powdered leaf sample of *Punica granatum* was extracted using 70% aqueous methanol by reflux technique for four 4 hr as illustrated. The extract was filtered using Whatman No.1.25 The filtrate was transferred into an evaporating dish,

allowed to dry under reduced pressure and temperature, and then stored in a desiccator.[26]

Phytochemical screening

Phytochemical alkaloids, anthraquinones, cardenolides, cardiac glycosides, flavonoids, and saponins, among others, were screened for presence or absence using the conventional methods outlined by numerous experts as shown in Table 11.[27]

Sample preparation of *Punica granatum* for analytical development:

10 mg/mL concentration of each of the extract of *Punica granatum* were prepared in methanol and vortex for 30 min then filtered through syringe filters (Nylon 0.45 um). The filtered solution was used for the analysis.[28]

RP-HPLC profiling of the extracts of *P. granatum*

HPLC profiling was done with the help of Agilent 1260 Infinity II HPLC system comprising of 1260 MCT column; 1260 Vial sampler; 1260 Quat Pump VL; three Detectors: 1260 FLD spectra, 1260 DAD WR, 1260 RID. Software Open lab chem station, and column, C18 column 5 um and dimensions (250*4.6 mm).[28] Solvent A of the mobile phase was HPLC grade water with 0.2% acetic acid, and solvent B was HPLC grade acetonitrile. The gradient mode and the flow of 1 mL/min was applied for both solvents and the injection volume was set at 20uL of 10 mg/mL solution of extract for both the inner and outer peel and detection was made at the wavelength of 210, 254, 290, 230, 260, 360 and 320 nm (DAD). The column oven was kept at 25°C. It lasted for a total of 80 min.[29] (Results illustrated in Figures 7-14).

Gradient mode condition for Pomegranate (*Punica granatum*)

The gradient conditions for the HPLC analysis were as follows: at 0 min, A% (Water) was 95 and B% (Acetonitrile) was 5; at 10 min, A% remained at 95 and B% at 5; at 30 min, A% was 70 and B% was 30; at 45 min, A% was 20 and B% was 80; at 60 min, A% was 5 and B% was 95; and from 60 to 80 min, A% stayed at 5 and B% remained at 95, ensuring a continuous gradient flow throughout the analysis as per HPLC procedures as illustrated Tables 1-7.[30]

Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS)

Sample preparation of Punica granatum

1 mg/mL concentration of each of the extract of *Punica granatum* were prepared in methanol and vortex for 30 min then filtered through syringe filters (Nylon 0.45 um). The filtered solution was used for the analysis.[31]

LC-HRMS profiling of the extracts

Analysis was done with the help of UHPLC- Thermo scientific ultimate Dionex 300; MS-Thermo scientific Orbitrap Exploris 240; Softwares- UHPLC- Thermo scientific Xcalibur and chromeleon;

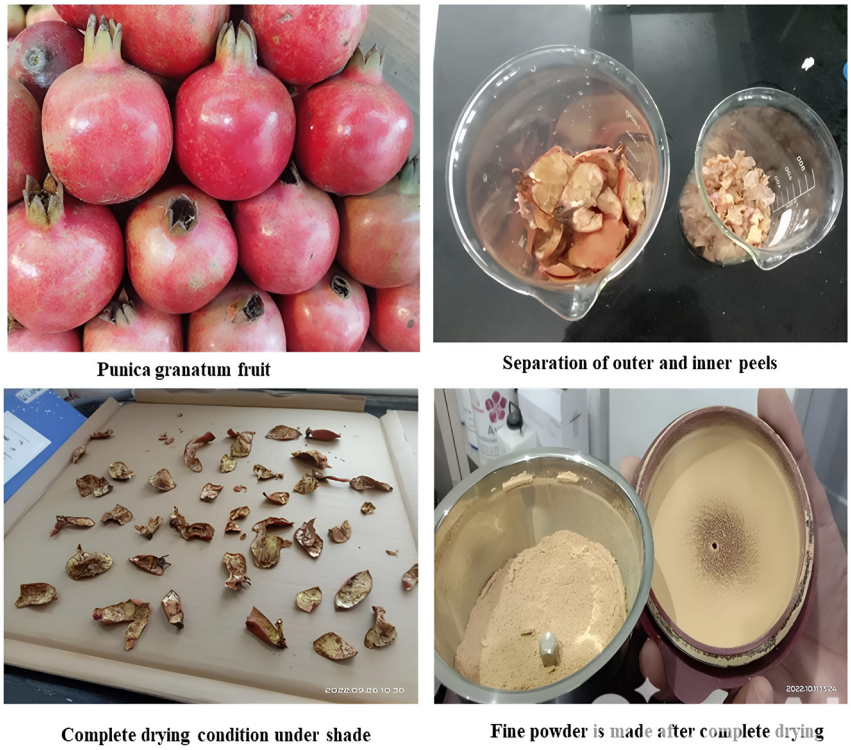

Figure 1: Depict images of *Punica granatum* peels collection process.

MS- Orbitrap Exploris Tune; Data interpretation-Compound Discover.[32]

The metabolomics analysis was performed using LC-HRMS under optimized MS conditions Model-thermos scientific Orbitrap Exploris 240

The ion source that was applied was H-ESI with a spray voltage positive at 3500 V and negative at 2500 V. The sheath gas was set at 45 arbitrary units, the auxiliary gas at 10 arbitrary units, and sweep gas at 1 arbitrary unit. The temperature at the ion transfer tube was at 300°C, and the vaporizer at 320°C. The scan type was full scan and ddMS2, with a scan range of 40-1500 m/z and resolution at 60,000 for full scan and 30,000 for ddMS2. Microscans were set to 1, RF lens to 70, and polarity set to full scan (both positive and negative modes) and ddMS2 (positive and negative modes). Fragmentation was done at HCD collision energies of 30, 50, and 150 Ev.[33]

LC conditions

The Thermo Scientific Ultimate Dionex 3000 system, with a Hypersil GOLD column (100×2.1 mm, 1.9 µm), 40 min runtime, and 10 L injection volume, was used. Mobile phases A, B, C, and D were 100% MS grade water, 0.1% formic acid; 100% MS grade acetonitrile, 0.1% formic acid; 100% MS grade methanol, 0.1% formic acid; and 80% MS grade methanol, respectively. The UV absorbance was set at 210 nm, 254 nm, 280 nm, and 320 nm with column temperature set at 40°C and autosampler at 5°C. The flow rate was maintained at 0.3 mL/min, and gradient was set as follows: for the period of 1 to 5 min, 5% B; from 6 to 15 min, 30% B; from 16 to 35 min, 95% B; and from 31 to 40 min, 5%. The results were (Table 8).[33]

The proteomics assay commenced with sample preparation, with a primary focus on efficient protein extraction

Following protein extraction, protein concentration was determined using the Bradford assay/BCA, and then acetone was added in a 1:4 ratio for protein precipitation.[34,35]

In-solution digestion

A total of 100-300 µg of protein is extracted from denaturing buffer and digested using in- solution digestion for further conversion of protein to peptide. Membrane enriched and soluble protein pellets were reconstituted in 100 uL of 7M Urea, 50 mM ammonium bicarbonate solution (pH 7.5 to 8.0) for denaturation for 10 min.[36] Reducing the presence of 5 uL 200 mM DTT in 50 M Ammonium bicarbonate for 1 hr followed by alkylation in darkness with the presence of 20 uL 200 mM iodoacetamide in 50

RESULTS

Table 1: The retention times and number of compounds detected in the inner and outer peel extracts of *Punica granatum* were analyzed using RP-HPLC at detection wavelengths of 210, 230, 254, 290, 320, and 360 nm.

Extracts	210 nm	230 nm	254 nm	260 nm	290 nm	320 nm	360 nm
Outer *Punica granatum*	49	42	30	32	29	17	12
Inner *Punica granatum*	78	68	63	62	47	16	13

Table 2: The retention times (tR) and UV spectra of phytocomponents from the inner and outer peel extracts of *Punica granatum* were detected at 210 nm.

λ_{max}	*Punica granatum* inner part peel (Aril) (t_R)	*Punica granatum* Outer part (t_R)
210 nm	5.589	Absent
	17.246	Absent
	Absent	7.694
	Absent	11.543

Table 3: The retention times (tR) and UV spectra of phytocomponents from the inner and outer peel extracts of pomegranate (*Punica granatum*) were detected at 254 nm.

λ_{max}	*Punica granatum* inner part peel (Aril) (t_R)	*Punica granatum* Outer part (t_R)
254 nm	5.589	Absent
	17.246	Absent
	22.705	Absent
	44.147	Absent
	48.510	Absent
	Absent	7.708
	Absent	11.541
	Absent	46.207
	Absent	46.778
	Absent	47.683

Table 4: The retention times (tR) and UV spectra of phytocomponents from the inner and outer peel extracts of *Punica granatum* were detected at 290 nm.

λ_{max}	*Punica granatum* inner part peel (Aril) (t_R)	*Punica granatum* Outer part (t_R)
290 nm	5.577	Absent
	17.247	Absent
	22.705	Absent
	Absent	7.710
	Absent	11.540

Table 5: Retention times (tR) and UV spectra of phytocomponents from the inner and outer peel extracts of *Punica granatum* detected at 230 nm.

λ_{max}	*Punica granatum* inner part peel (Aril) (t_R)	*Punica granatum* Outer part (t_R)
230 nm	17.247	Absent
	22.705	Absent
	Absent	7.705
	Absent	11.538
	Absent	47.685

Table 6: Retention times (tR) and UV spectra of phytocomponents from the inner and outer peel extracts of *Punica granatum* detected at 260 nm.

λ_{max}	*Punica granatum* inner part peel (Aril) (t_R)	*Punica granatum* Outer part (t_R)
260 nm	5.589	Absent
	17.246	Absent
	22.705	Absent
	44.147	Absent
	48.510	Absent
	Absent	7.708
	Absent	11.542
	Absent	46.207
	Absent	47.684

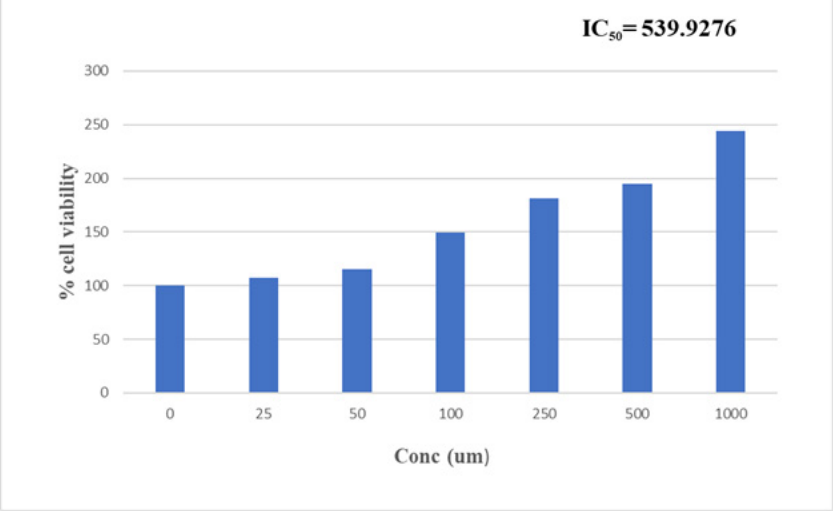

Figure 2: Biological activity of *Punica granatum* outer peel extract in human embryonic kidney cell lines, demonstrating its effects on cell viability and response under experimental conditions.

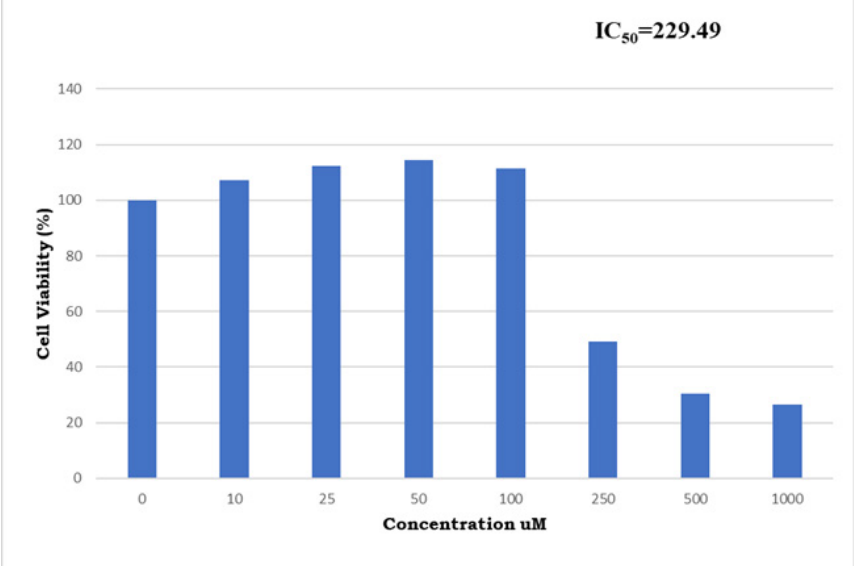

Figure 3: Biological activity of *Punica granatum* outer peel extract in MCF-7 breast cancer cell lines, illustrating its effects on cell viability and proliferation under experimental conditions.

Table 7: Retention times (tR) and UV spectra of phytocomponents from the inner and outer peel extracts of *Punica granatum* detected at 254 nm.

λ_{max}	*Punica granatum* inner part peel (Aril) (t_R)	*Punica granatum* Outer part (t_R)
320 nm	5.579	Absent
	Absent	3.374
	Absent	3.607
	Absent	66.662

mM Ammonium bicarbonate for 1 hr. Add 20 ul of 200 mM DTT in 50mM Ammonium bicarbonate (pH- 8.0) and incubate the mixture for 1 hr at room temperature in the dark to consume any unreacted iodoacetamide. Add 50mM ammonium bicarbonate and 1mM calcium chloride (pH 7.6) to reduce the concentration of urea to 0.5M. Enzymatic digestion was initiated by addition of Trypsin at a final ratio of 1:50 w/w trypsin: protein or 1:25, vortexed and incubated for 16 hr at 37°C. The peptide sample was then acidified with formic acid to a final concentration of 0.1% to deactivate the trypsin, excess salts were removed using C18 spin columns, and material was dried in a speed-vac before presentation for MS analysis.[37]

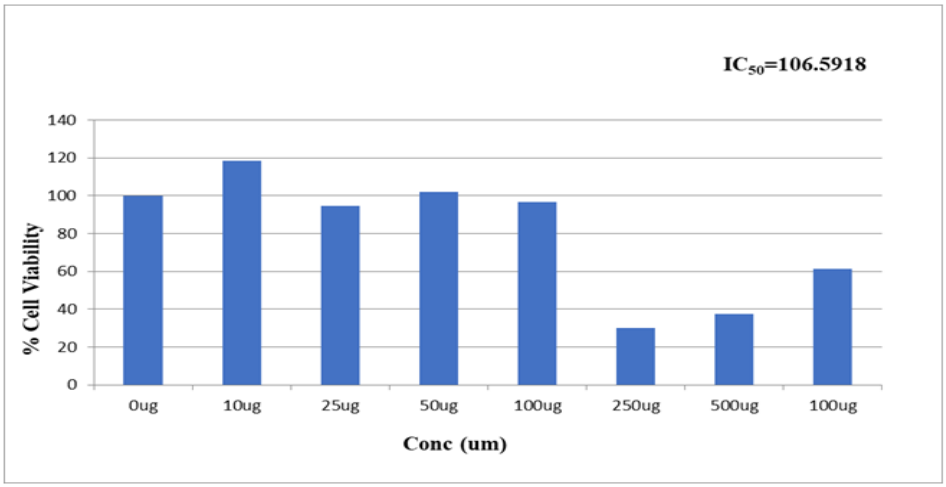

Figure 4: Biological activity of *Punica granatum* outer peel extract in C6 glioma cell lines, demonstrating its impact on cell viability and growth under experimental conditions.

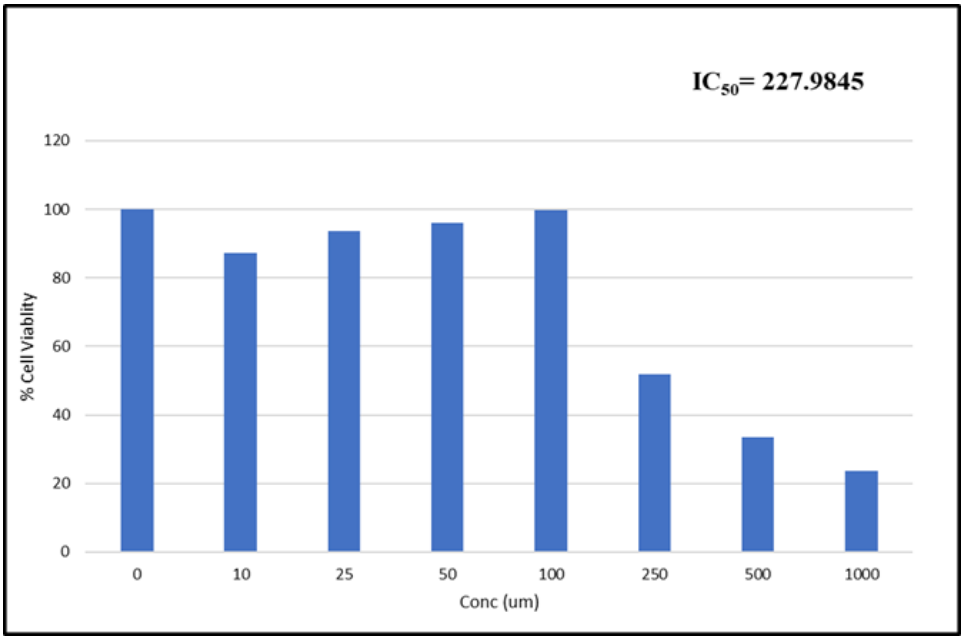

Figure 5: Biological activity of *Punica granatum* inner peel extract in MCF-7 breast cancer cell lines, showing its effects on cell viability and proliferation under experimental conditions.

Table 8: Secondary metabolites identified from the outer peel of *Punica granatum* (pomegranate) using LC-HRMS analysis under positive ion mode ESI conditions.

Sl. No.	Metabolites	M.F	m/z	RT	Area	Calc.MW
1.	Gallic acid	$C_7 H_6 O_5$	169.0143	1.384	53226504.2	170.0216
2.	€-p-coumaric acid	$C_9 H_8 O_3$	165.054	1.693	8062622.12	164.04749
3.	D-(-)-Quinic acid	$C_7 H_{12} O_6$	269.088	0.938	11108790.1	210.07401
4.	Protocatechuic acid	$C_7 H_6 O_4$	153.0193	35.398	81127.4707	154.02661
5.	Cinnamic acid	$C_9 H_8 O_2$	149.0598	34.958	9953667.05	148.05254
6.	Benzoic acid	$C_7 H_6 O_2$	105.0335	0.765	46655434.1	122.03678
7.	Syringic acid	$C_9 H_{10} O_5$	197.0457	11.052	11700149.2	198.05295
8.	Glucose	$C_{13} H_{16} O_{10}$	331.0673	1.212	116963642	332.07459
9.	Ascorbic acid	$C_6 H_6 O_6$	173.0092	2.882	850077.523	174.01645
10.	D-(+)-Malic acid	$C_4 H_6 O_5$	133.0143	1.191	91584608.1	134.02157
11.	Citric acid	$C_6 H_8 O_7$	191.0198	1.212	75874826.9	192.02707
12.	ACETYL PROLINE	$C_7 H_{11} N O_3$	158.0812	4.23	81215580.3	157.07392
13.	N-Acetylvaline	$C^7 H_{13} N O_3$	160.0968	0.996	3112133.43	159.08954
14.	2-Aminopalmitic acid	$C_{16} H_{33} N O_2$	272.2585	19.331	11503463.5	271.25124
15.	Succinic acid	$C_4 H_6 O_4$	117.0194	1.236	18760769.9	118.02664
16.	L-(-)-Serine	$C_3 H_7 N O_3$	106.05	0.828	34414994	105.0427
17.	DL-Glutamine	$C_5 H_{10} N_2 O_3$	147.0765	0.97	1.18E+08	146.0692
18.	4-Azidophenylalanine	$C_9 H_{10} N_4 O_2$	207.0876	23.894	878048.9	206.0803
19.	Allysine	$C_6 H_{11} N O_3$	178.1074	1.001	7955538	145.0739
20.	DL-Histidine	$C_6 H_9 N_3 O_2$	156.0768	0.923	4732031	155.0695
21.	DL-tyrosine	$C_9 H_{11} N O_3$	182.0813	1.217	8157769	181.074
22.	(+)-7-Iso-Jasmonyl-L-isoleucine	$C_{18} H_{29} N O_4$	324.2171	0.069	4109851	323.2098
23.	vinylglycine	$C_4 H_7 N O_2$	102.055	0.626	55340377	101.0477
24.	Valproic acid	$C_8 H_{16} O_2$	145.1225	2.299	1001386.407	144.1153
25.	Umbelliferone	$C_9 H_6 O_3$	163.039	14.782	2072960.918	162.0317
26.	Triethylamine	$C_6 H_{15} N$	102.1279	2.107	1367775	101.1206
27.	tranexamic acid	$C_8 H_{15} N O_2$	158.1176	1.236	16435581	157.1103
28.	Suberic acid	$C_8 H_{14} O_4$	173.082	11.715	674197.5	174.0893
29.	Solasodine	$C_{27} H_{43} N O_2$	414.3375	13.941	2767670	413.3302
30.	rolipram	$C_{16} H_{21} N O_3$	276.1595	18.382	1164147	275.1522
31.	Quinuclidinol	$C_7 H_{13} N O$	128.107	20.511	514887.4	127.0997
32.	Pyridoxal	$C_8 H_9 N O_3$	168.0656	14.973	4836649	167.0583
33.	putrescine	$C_4 H_{12} N_2$	89.10733	34.281	8850521	88.10005
34.	Protocatechuic acid	$C_7 H_6 O_4$	153.0193	35.398	81127.47	154.0266
35.	Propionic acid	$C_3 H_6 O_2$	75.04409	1.212	4.14E+08	74.03681
36.	Procarbazine	$C_{12} H_{19} N_3 O$	220.1465	16.895	2691662	221.1538

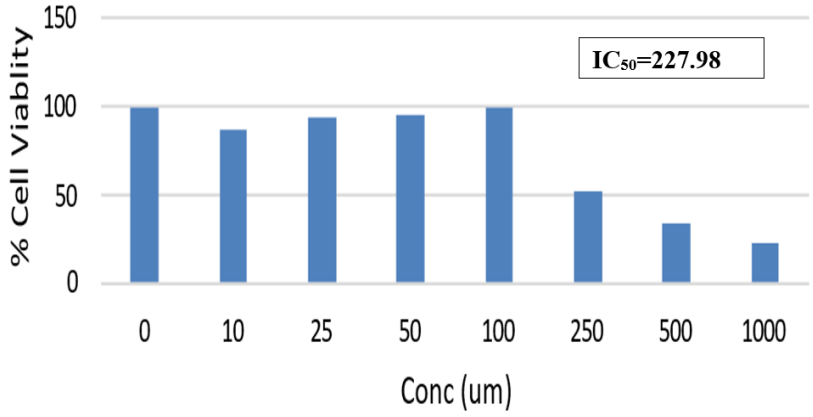

Figure 6: Biological activity of *Punica granatum* inner peel extract in C6 glioma cell lines, demonstrating its effects on cell viability and proliferation under experimental conditions.

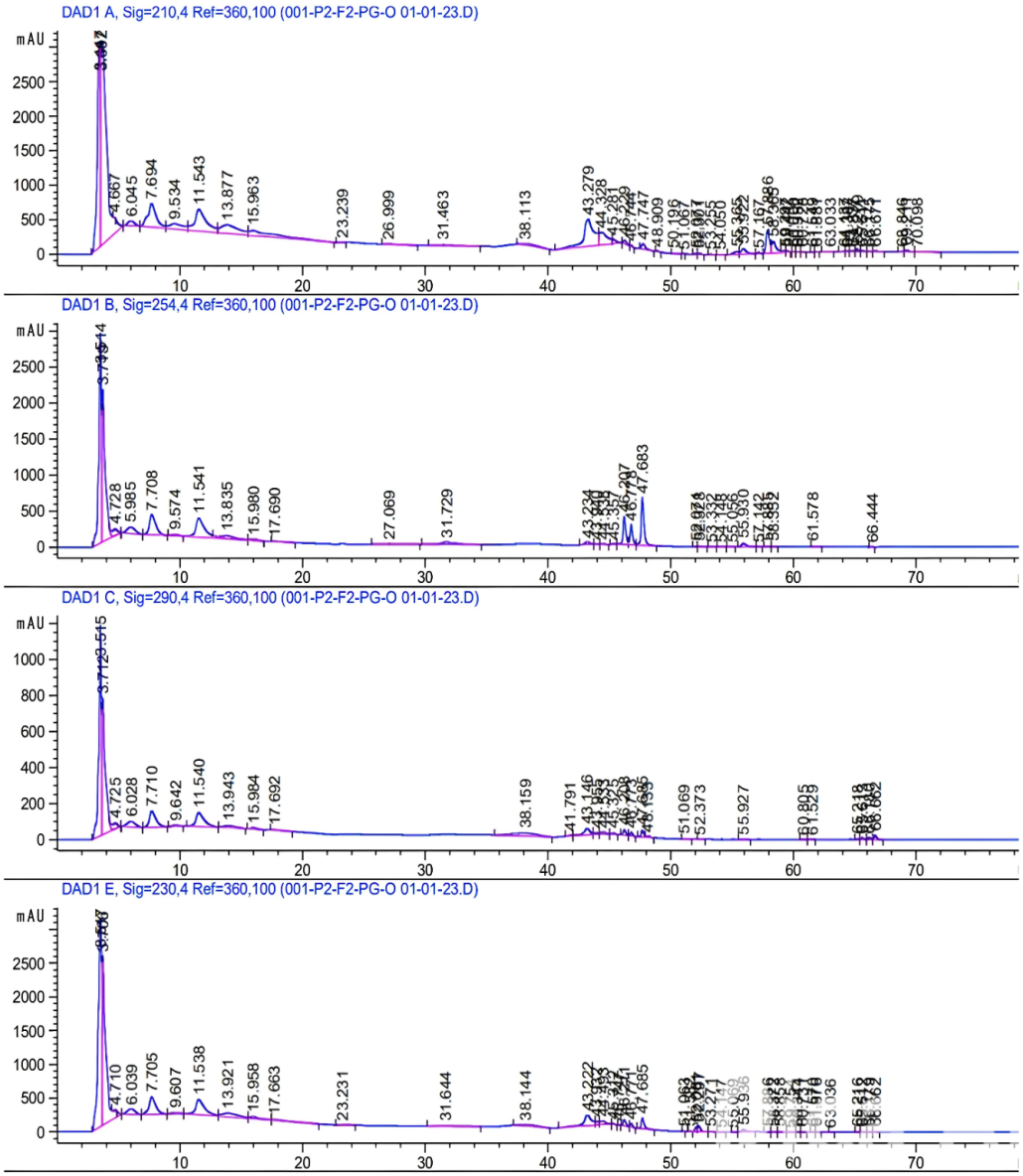

Figure 7: High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD) chromatogram of the ethanolic extract from the outer peel of *Punica granatum*. The peaks represent various phytochemical constituents detected under optimized conditions.

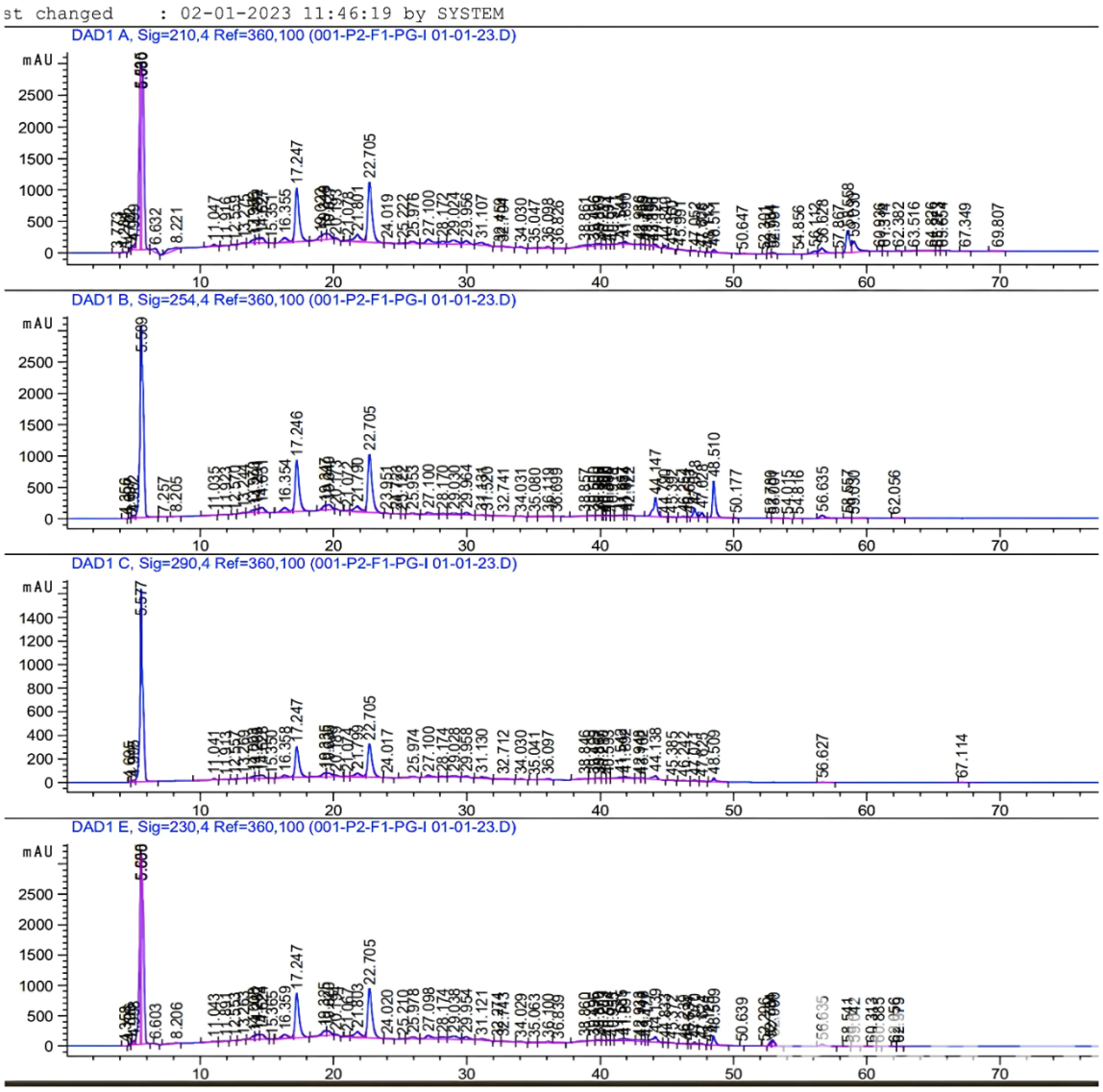

Figure 8: High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD) chromatogram of the ethanolic extract from the inner peel of *Punica granatum*. The detected peaks indicate the presence of fluorescent phytochemical constituents under the applied analytical conditions.

Table 9: MCF-7 Cell Protein Regulation by Ethanolic Extract of *Punica granatum*.

Concentration	MCF-7 Upregulation	MCF-7 Downregulation
50 µg/mL	Fibulin-1	Mediator of RNA polymerase II transcription subunit 23
	Envelope glycoprotein (Fragment)	Protein SCAF11
100 µg/mL	Fibulin-1	Mediator of RNA polymerase II transcription subunit 23
	Envelope glycoprotein (Fragment)	Patatin-like phospholipase domain containing 8
200 µg/mL	Fibulin-1	SAMD4B protein (Fragment)
	Zinc finger protein-like 1 (Fragment)	Dolichyl-phosphate-mannose--protein mannosyltransferase

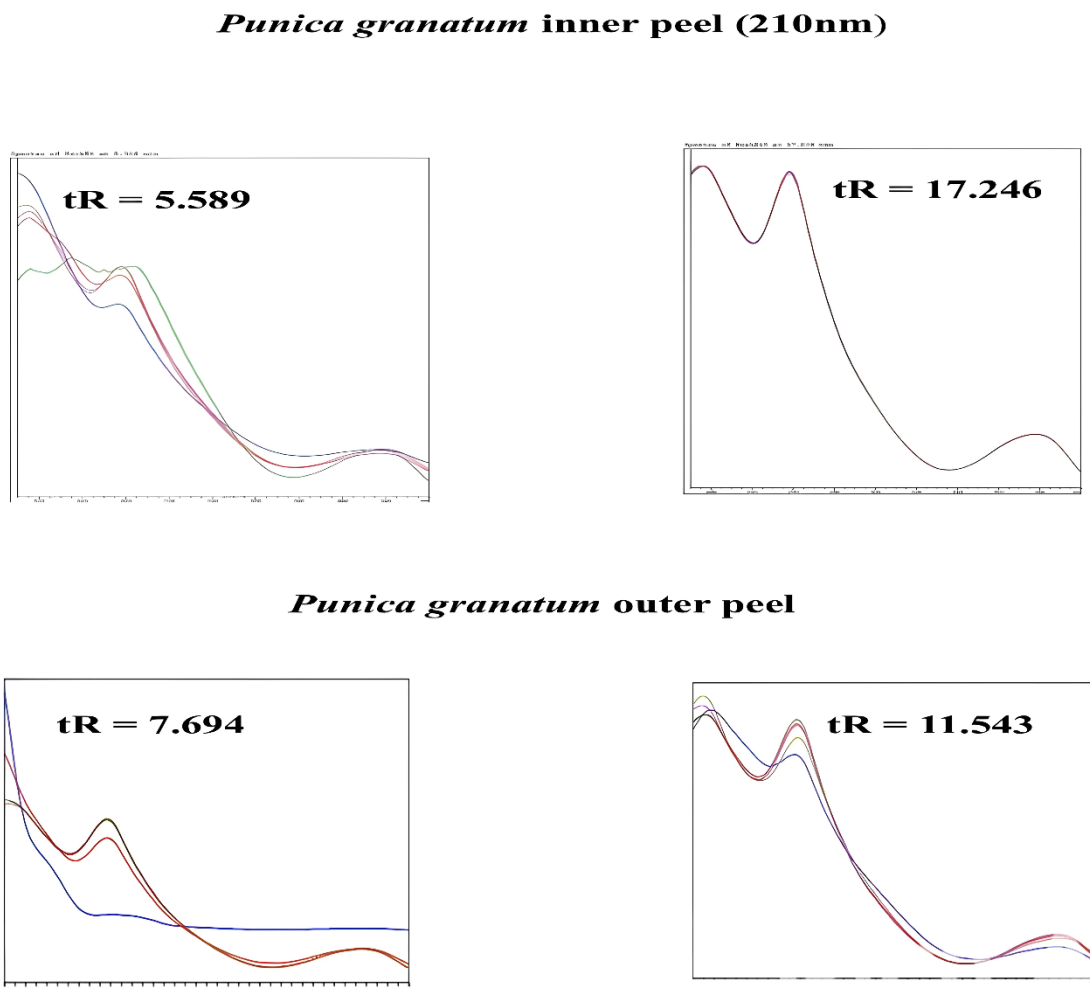

Figure 9: High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) profile of the total extract from *Punica granatum* (combined outer and inner peels), showing distinct peaks with varying retention times at 210 nm, indicative of diverse phytochemical constituents.

Table 10: HEK Cell Protein Regulation by Ethanolic Extract of *Punica granatum*.

Concentration	HEK Upregulation	HEK Downregulation
50 µg/mL	NADH-ubiquinone oxidoreductase chain 3	RBR-type E3 ubiquitin transferase (Fragment)
	cDNA FLJ14648 fis, clone NT2RP2002046, highly similar to Protein FAM48A	Crn-related protein kim1
100 µg/mL	NADH-ubiquinone oxidoreductase chain 3	Acetyl-coenzyme A transporter 1
	cDNA FLJ14648 fis, clone NT2RP2002046, highly similar to Protein FAM48A	Crn-related protein kim1
200 µg/mL	NADH-ubiquinone oxidoreductase chain 3	Mitochondrial ribosomal protein L27
	cDNA FLJ14648 fis, clone NT2RP2002046, highly similar to Protein FAM48A	cDNA FLJ60209, highly similar to Transcriptional repressor p66 alpha

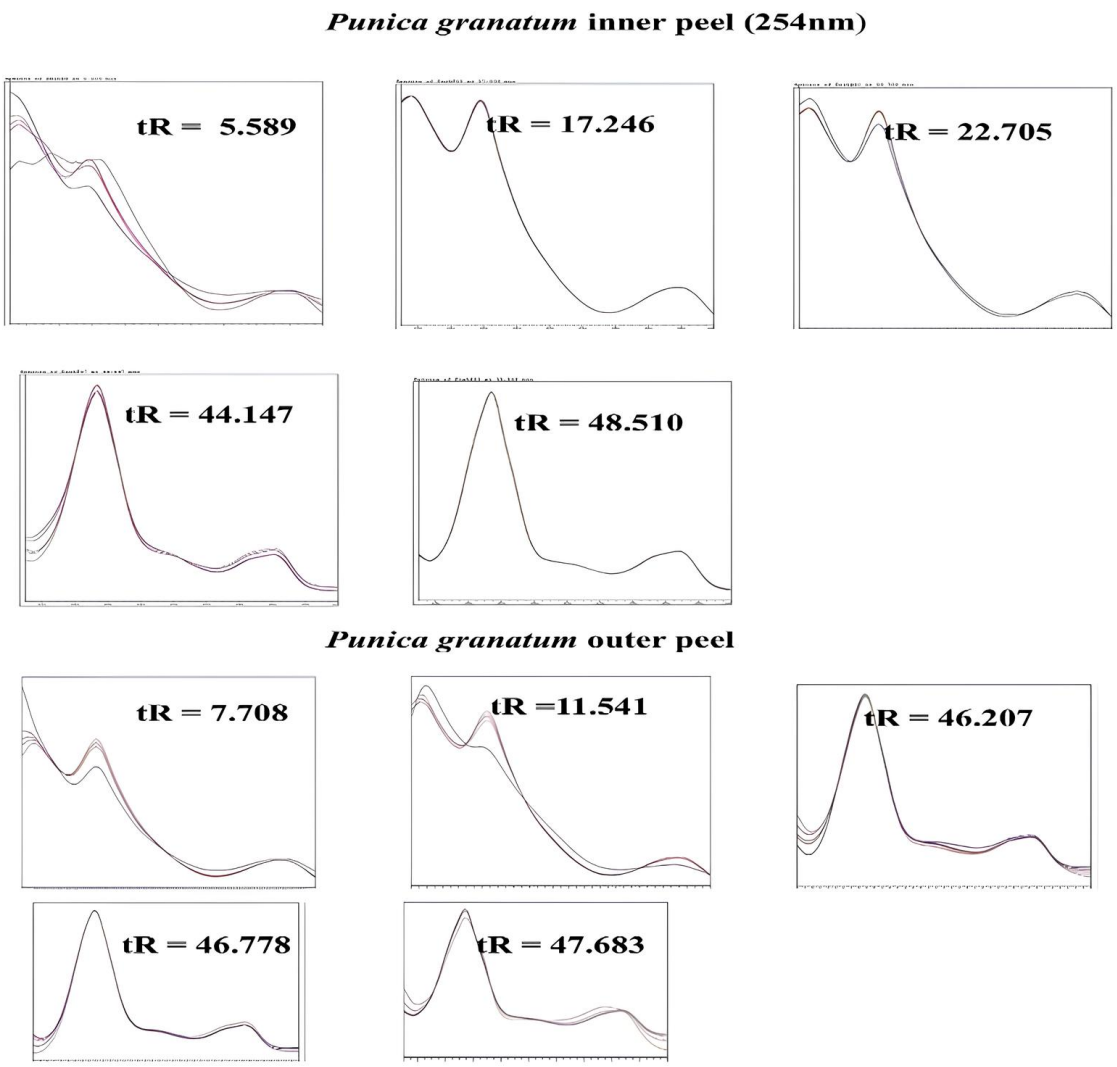

Figure 10: High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) profile of *Punica granatum* peel extract (combined outer and inner peels), displaying multiple peaks with varying retention times at 254 nm, indicating the presence of diverse phytochemical compounds.

Table 11: Phytochemical screening results of *Punica granatum* peel extracts.

Sl. No.	Test	Compound detects	Observation
1	Molish test	Carbohydrate	+
2	Killer Killani	Cardiac glycoside	+
3	Ninhydrin	Protein	+
4	Xanthoproteic test	Protein	+
5	Ferric chloride test	Tannin	+
6	Wagner's test	Alkaloid	+
7	Froth test	Saponin	-
8	Salkowski's test	Phytosterol	+

Sample cleanup and data analysis

The sample solution from trypsin digestion contains peptides and contaminants like salts, which can harm MS equipment and reduce sensitivity.[38] To avoid this, samples are cleaned using desalting tips before LC-MS/MS injection, preventing clogging and maintaining MS performance. Protein samples are desalted with a C18 spin column, dried, and either stored or reconstituted for later use. Raw data is processed with Proteome discoverer and analyzed using the Rattus norvegicus uniprot database through Label Free Quantification (LFQ).[39]

LC-MS configure ration

Tryptic peptides were separated by nanoscale LC using a RSLC Nano system (Thermoscientific Ultimate Dionex 3000) that is equipped with RSLC pump (Figures 14,15) (Loading and Nano pumps), RS autosampler, Acclaim PepMap 100, 75 umx2 cm, c18, 3 um, 100A trap/loading column and an analytical RP column

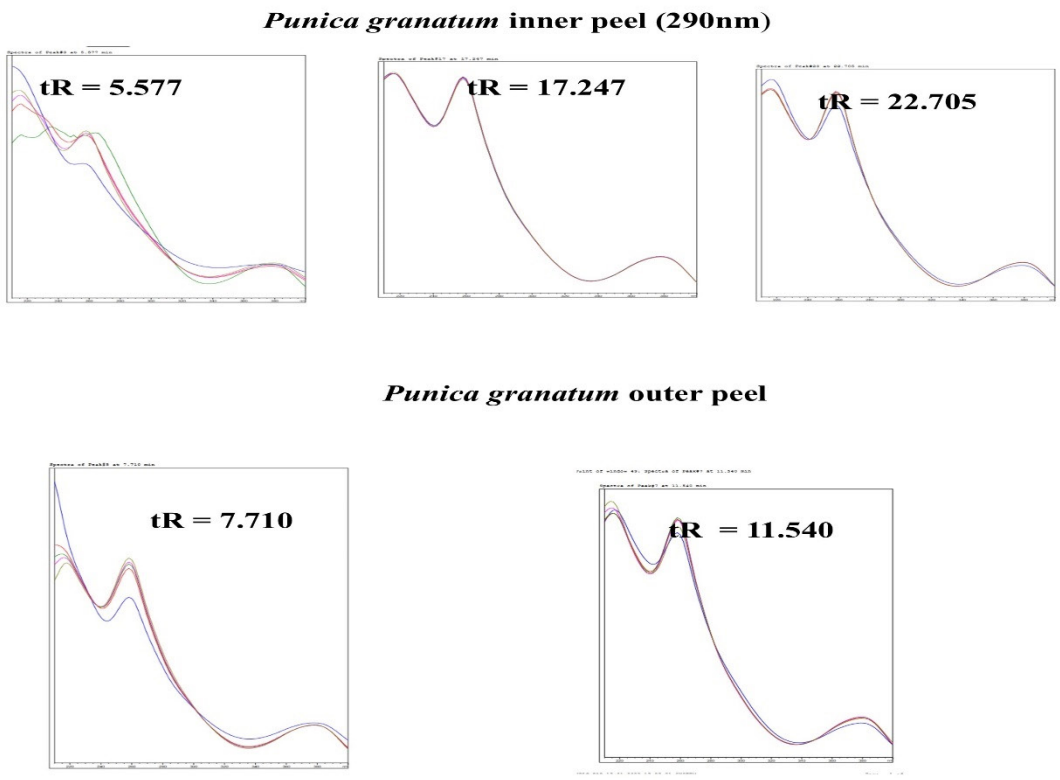

Figure 11: High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) chromatogram of *Punica granatum* peel extract (combined outer and inner peels), recorded at 290 nm. The chromatogram exhibits multiple peaks with varying retention times, reflecting the presence of diverse UV-absorbing phytochemical constituents.

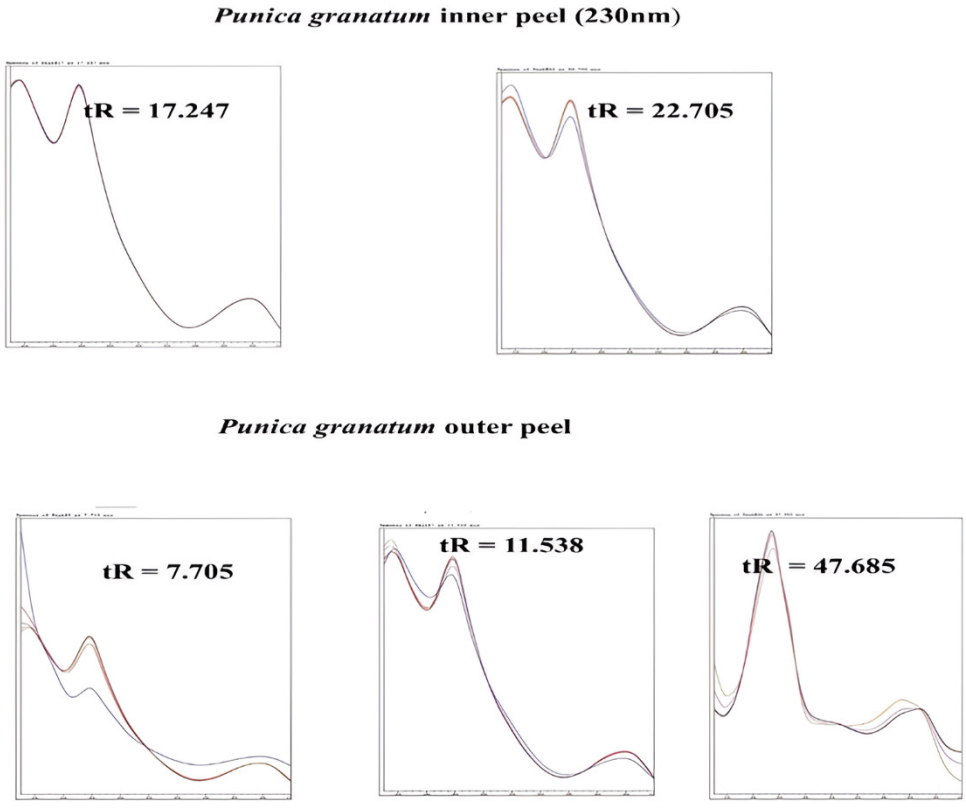

Figure 12: High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) chromatogram of *Punica granatum* peel extract (combined outer and inner peels), recorded at 230 nm. The chromatogram displays multiple peaks with varying retention times, indicating the presence of a diverse range of UV-absorbing phytochemicals.

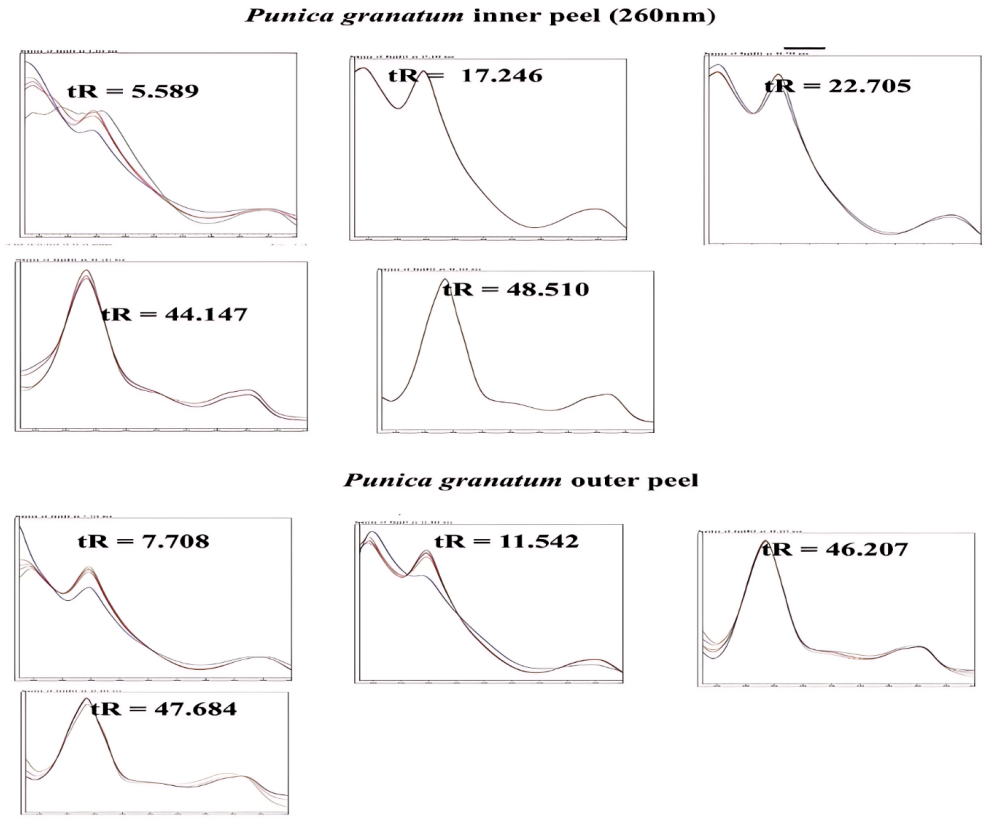

Figure 13: High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) chromatogram of *Punica granatum* peel extract (combined outer and inner peels), recorded at 260 nm. The chromatogram reveals multiple peaks with distinct retention times, suggesting the presence of various UV-absorbing phytochemical constituents.

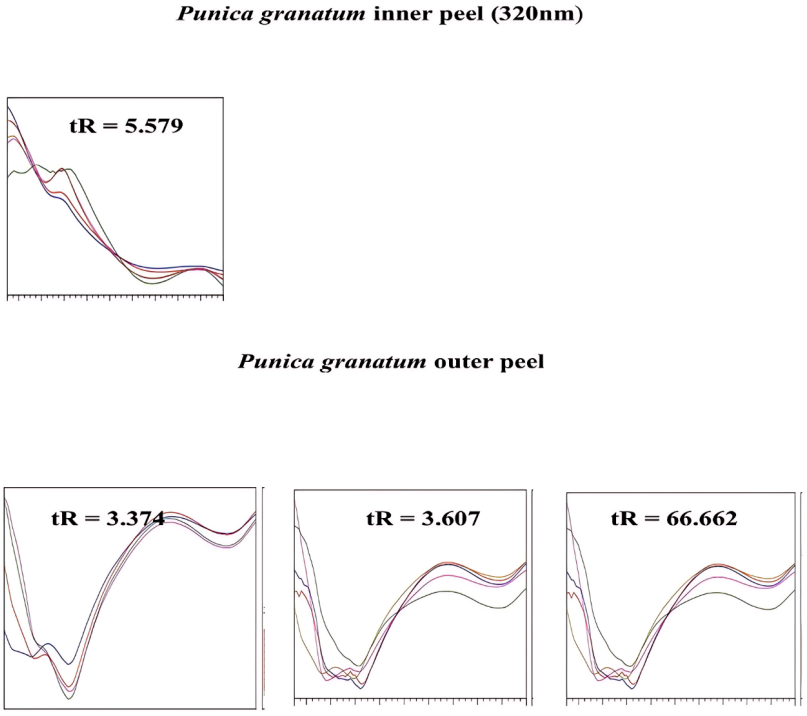

Figure 14: High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) chromatogram of *Punica granatum* peel extract (combined outer and inner peels), recorded at 320 nm. The chromatogram shows multiple peaks with varying retention times, indicating the presence of diverse phytochemicals absorbing at this wavelength.

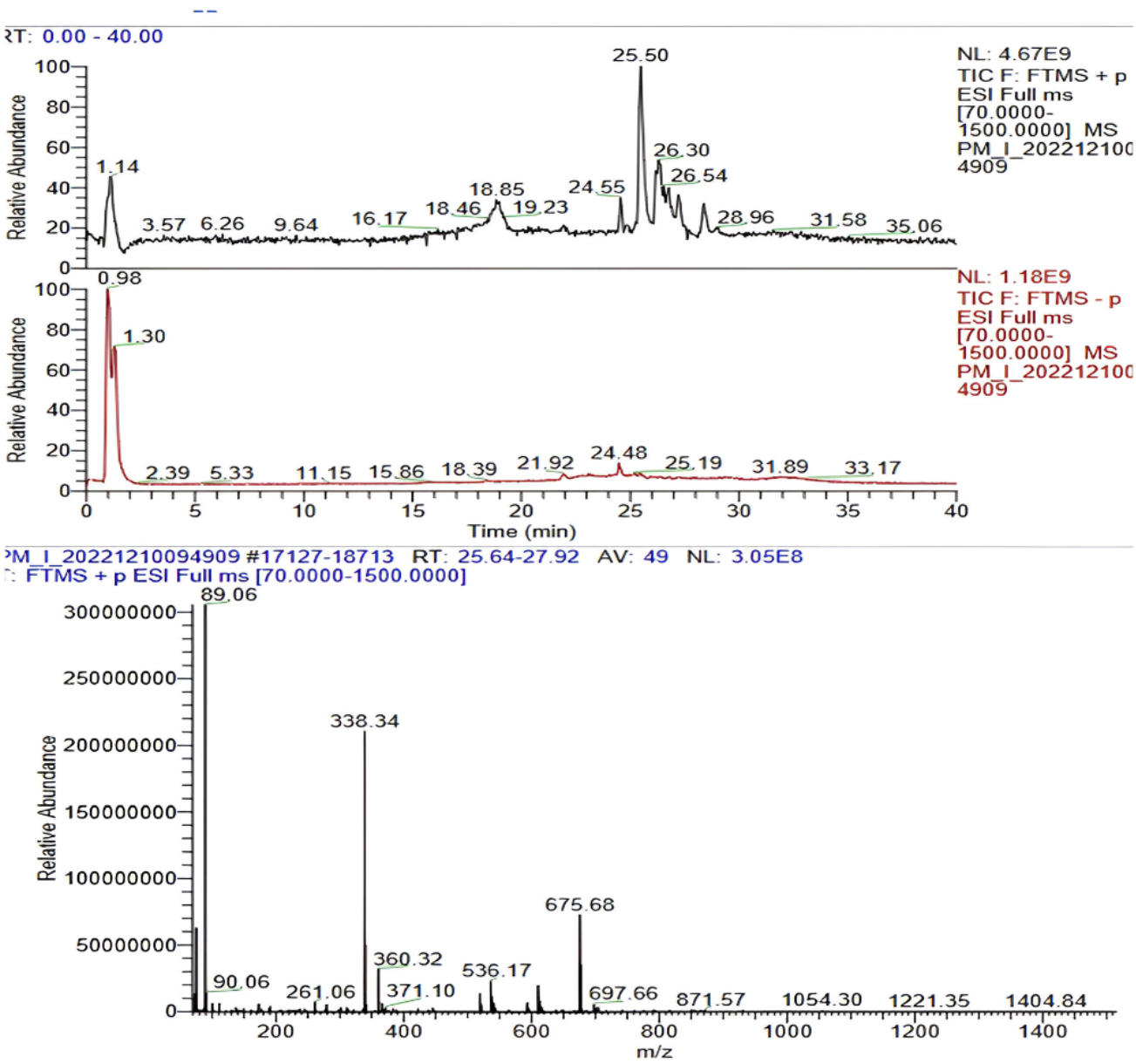

Figure 15: Liquid Chromatography-High-Resolution Tandem Mass Spectrometry (LC-HRMS/MS) chromatograms of *Punica granatum* extract obtained in both negative and positive ionization modes, illustrating the comprehensive metabolomic profile and diversity of detected compounds.

PepMap RSLC C18, 75 umx25 cm, 2 um, 100A. Mobile phase A consisted of 0.1% formic acid in Water and mobile phase B consisted of 0.1% formic acid in ACN for nano pump. 0.1% formic acid in water used as loading pump peptide was separated using a gradient of 0 to 20 min, 5%B; 21 to 60 min, 50%B; 61 to 130 min, 95%B; 121 to 130 min-5%B at 0.3 ul/min flow.[40] The column and MS were equilibrated for 20 min, with the column at 40°C and the autosampler at 5°C. Samples were transferred with a 100% water, 0.1% formic acid solution at 5 µL/min for 4 min, with a 2 µL injection. MS settings included a positive spray voltage of 1900 V, negative voltage of 600 V, and ion transfer tube temperature at 290°C. Full MS scans were conducted from 350 to 2000 m/z at a 60,000 resolution, followed by MS2 scans at 15,000 resolutions in DDA mode (Tables 9 and 10). The 20 most intense peaks were fragmented using HCD with a collision energy of 30% Figure 15.[41] Dithiothreitol (DTT) and Iodoacetamide (IAA) were purchased from G Biosciences. Water and Acetonitrile (ACN), both of MS grade with purity >99.9%, were obtained from JT Baker. Urea was obtained from SRL, ammonium bicarbonate from Sigma-Aldrich, and trypsin and formic acid from Fisher Chemical (Figures 16 and 17).[42-44]

DISCUSSION

According to the World Health Organization (WHO), nearly 80% of the global population depends on herbal medicine as a primary source of healthcare. For centuries, medicinal plants have played a vital role in the treatment of various human diseases.[45] Cancer continues to be a major global health challenge,

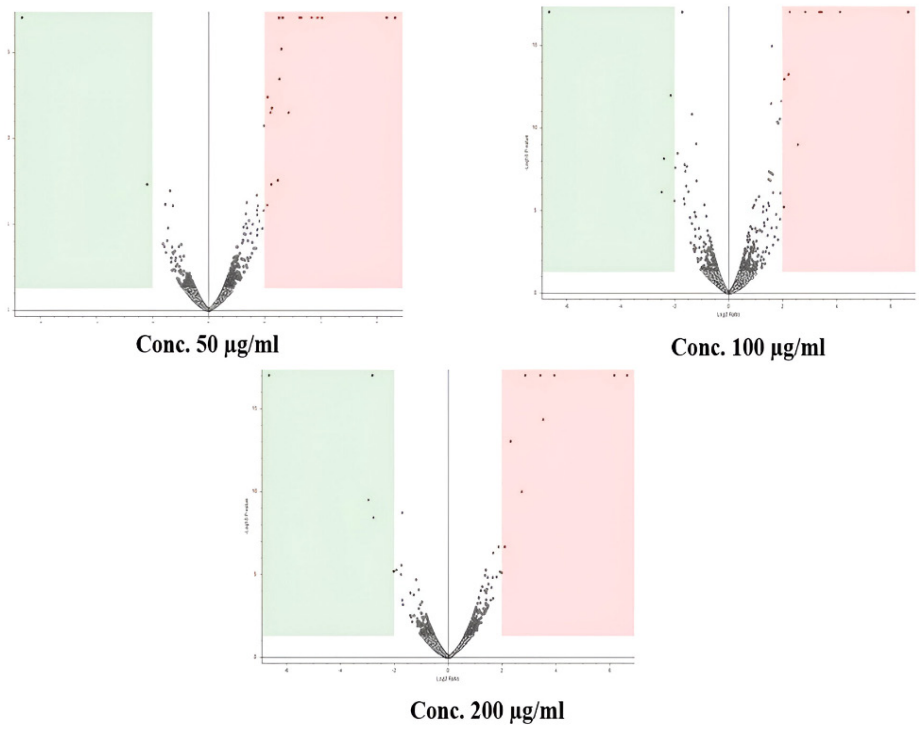

Figure 16: Differential protein expression in MCF-7 cell lines showing upregulation and downregulation patterns, as determined through proteomic analysis. The results reflect the cellular response to treatment conditions.

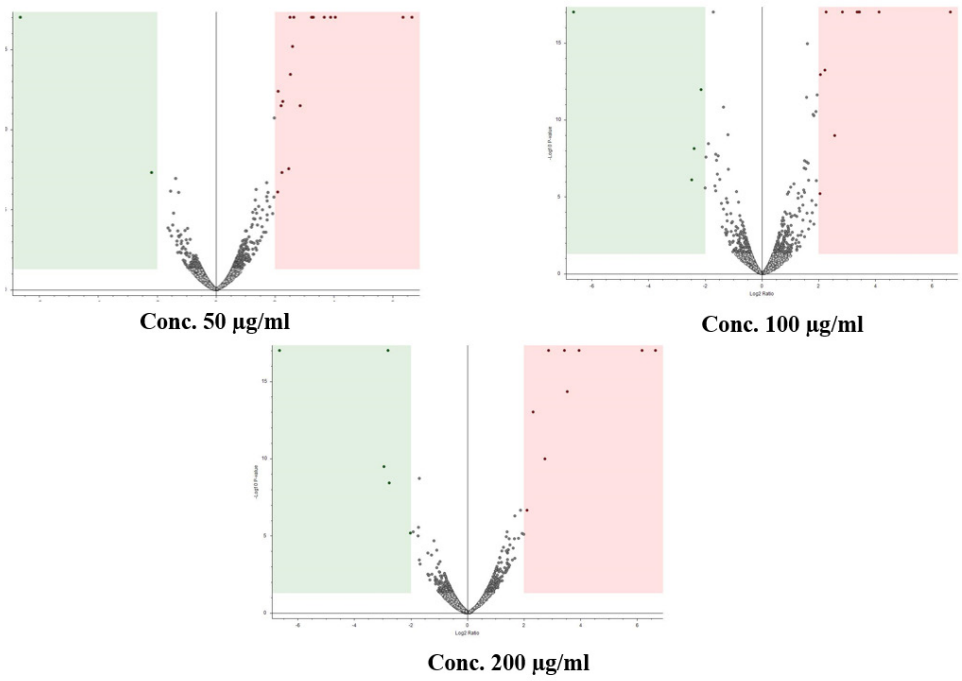

Figure 17: Protein expression analysis in human embryonic kidney cell lines depicting patterns of upregulation and downregulation, indicating cellular responses under specified experimental conditions.

with current chemotherapy treatments offering limited efficacy and causing severe side effects. This underscores the growing interest in alternative therapies, particularly those derived from natural sources. In this study, we evaluated the cytotoxic effects of ethanolic extracts from *Punica granatum* (pomegranate) peels on MCF-7 (breast cancer) and C6 (glioblastoma) cell lines. The results demonstrated significant anticancer activity, with minimal toxicity observed in normal HEK 293 cells.[46] Phytochemical analysis identified key bioactive compounds, including gallic acid, citric acid, and syringic acid, known for their antitumor properties. Additionally, proteomic analysis revealed substantial alterations in proteins related to apoptosis and cell proliferation. These findings suggest that *Punica granatum* peel extract holds promise as a potential anticancer agent, warranting further investigation into its mechanisms and clinical applicability.[47]

The main objective of this study was to evaluate the effect of ethanolic extracts from *Punica granatum* (outer and inner peels) on proliferation and apoptosis induction in MCF-7 breast cancer, C6 glioblastoma, and normal HEK 293 cell lines.[48,49] The MTT assay results indicated that the extracts of the outer and inner peels of *Punica granatum* demonstrated a high degree of activity against the cell lines MCF-7 and C6.49 The results of this study suggest that medicinal plants, such as *Punica granatum*, could serve as a valuable source of promising anticancer agents, potentially leading to the development of novel treatments for various types of cancer. These findings provide a strong basis for further investigations into the mechanisms through which plant-derived compounds induce cytotoxicity in cancer cells. Notably, our LC-HRMS analysis of ethanolic extracts revealed over 3,000 metabolites, highlighting the complexity and diversity of bioactive compounds present in *Punica granatum*. This extensive metabolite profile opens avenues for future research to identify and characterize specific compounds responsible for the observed anticancer effects.[50] By comparing with standards and MS data from the literature, approximately more than 30 secondary metabolites were identified and described.[51] According to above Result shown Table 11, these include steroid alkaloids, steroid saponins, flavonols, amino acids, caffeoylquinic acids, and other substances presence.[52] According to UHPLC-Q Orbitrap MS characterization, the most prevalent peaks in the ion chromatograms of extracts from the outer peel of *Punica granatum* were located in the positive and negative modes.[53]

CONCLUSION

This study highlights the strong anticancer potential of ethanolic extracts from *Punica granatum* (pomegranate) peels. Phytochemical profiling using RP-HPLC and LC-HRMS revealed a rich composition of bioactive secondary metabolites particularly in the inner peel including gallic acid, syringic acid, and citric acid, all of which are known for their therapeutic properties. The extract exhibited marked cytotoxicity against MCF-7 (breast cancer) and C6 (glioblastoma) cell lines, while showing minimal toxicity toward normal HEK 293 cells, indicating selective anticancer activity. Proteomic analysis further demonstrated significant alterations in the expression of proteins related to key cellular processes such as apoptosis, proliferation, and stress response, suggesting that the extract actively modulates molecular pathways associated with cancer progression.

These findings position *Punica granatum* peel extract as a promising candidate for further preclinical evaluation, either as a complementary agent or a standalone therapeutic in cancer treatment. Future investigations including targeted proteomic studies and in vivo experiments are essential to fully elucidate the mechanisms of action and assess the clinical relevance of these bioactive constituents.

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ABBREVIATIONS

HPLC: High-performance liquid chromatography; **MTT:** [3-(4,5-dimethylthiazol-2-yl); **LC-HRMS:** Liquid Chromatography High resolution Mass Spectrometry; **LC-MS:** Liquid Chromatography- Mass Spectrometry; **LC-HRMS/MS:** Liquid chromatography- High Resolution Mass Spectrometry/ Mass Spectrometry; **RP-HPLC:** Reverse-phase high-performance liquid chromatography; **HEK:** Human embryonic kidney; **WHO:** World Health Organization; **DMSO:** Dimethyl sulfoxide; **DAD:** Diode Array Detector; **FLD:** Fluorescence Detector; **UHPLC:** Ultra-performance liquid chromatography; **ESI:** Electrospray ionization; **HCD:** Higher-energy collisional dissociation; **LC:** Liquid chromatography; **UV:** Ultraviolet radiation; **BCA:** Bicinchoninic acid assay; **LFQ:** Label Free Quantification; **ACN:** Acetonitrile; **DTT:** Dithiothreitol; **IAA:** Iodoacetamide; **WHO:** World Health Organisation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Mr. Pawan Kumar Goswami was primarily responsible for the conceptualization and investigation of the study, as well as for writing the original draft. Mr. Sandip Chatterjee contributed to data curation, formal analysis, and project administration. Both Mr. Pawan Kumar Goswami and Mr. Sandip Chatterjee collaboratively developed the methodology and worked on the software and visualization aspects of the research. Supervision of the overall project was carried out by Mr. Sandip Chatterjee. Validation was jointly performed by both authors. The review and editing of the manuscript were also conducted collaboratively by Mr. Sandip Chatterjee and Mr. Pawan Kumar Goswami.

ETHICAL APPROVAL

We hereby affirm that the content of this manuscript is original. Furthermore, it has not been published elsewhere fully or partially in any language. It has not been submitted for publication (fully or partially) elsewhere simultaneously. We also affirm that all the authors have seen and agreed to the submission of paper and their inclusion of name(s) as co-author(s). We am looking forward to hear from you.

SUMMARY

The study examines *Punica granatum* (pomegranate) ethanolic extracts for their anti-cancer potential. Using HPLC, it identifies bioactive compounds, while untargeted proteomics analyzes their effects on protein expression. Cytotoxicity tests reveal the extracts' ability to inhibit growth or induce cell death in MCF-7 (human breast cancer) and C6 cell lines, highlighting their therapeutic potential.

REFERENCES

1. Cao W, Chen H-D, Yu Y-W, Li N, Chen W-Q. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chinese medical journal. 2021;134(07):783-91.
2. Bray F, Laversanne M, Ferlay J, *et al*. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(6):229-263.
3. Knaul FM, Arreola-Ornelas H, Kwete XJ, Bhadelia A, Rosa WE, Touchton M, *et al*. The evolution of serious health-related suffering from 1990 to 2021: an update to The Lancet Commission on global access to palliative care and pain relief. The Lancet Global Health. 2025;13(3):e422-36.
4. Asthana S, Khenchi R, Labani S. Incidence of colorectal cancers in India: A review from population-based cancer registries. Current Medicine Research and Practice. 2021;11(2):91-6.
5. Esmaeli M, Dehabadi MD, Ghanbari A. Anticancer effects of pomegranate extract on breast cancer cell lines: a systematic review of the literature. Discover Medicine. 2025 ;2(1):1-0.
6. Gelband H, Sankaranarayanan R, Gauvreau CL, Horton S, Anderson BO, Bray F, *et al*. Costs, affordability, and feasibility of an essential package of cancer control interventions in low-income and middle-income countries: key messages from Disease Control Priorities. The Lancet. 2016;387(10033):2133-44.
7. Dickman PW, Adami HO. Interpreting trends in cancer patient survival. Journal of internal medicine. 2006;260(2):103-17.
8. Efferth T, Saeed ME, Mirghani E, Alim A, Yassin Z, Saeed E, *et al*. Integration of phytochemicals and phytotherapy into cancer precision medicine. Oncotarget. 2017;8(30):50284.
9. Leary M, Heerboth S, Lapinska K, Sarkar S. Sensitization of drug resistant cancer cells: a matter of combination therapy. Cancers. 2018;10(12):483.
10. Goldie JH, Coldman AJ. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. Cancer research. 1984;44(9):3643-53.
11. Kuczynski EA, Sargent DJ, Grothey A, Kerbel RS. Drug rechallenge and treatment beyond progression-implications for drug resistance. Nature reviews Clinical oncology. 2013;10(10):571-87.
12. Martin RC, Joshi J, Robbins K, Tomalty D, Bosnjakovik P, Derner M, *et al*. Hepatic intra-arterial injection of drug-eluting bead, irinotecan (DEBIRI) in unresectable colorectal liver metastases refractory to systemic chemotherapy: results of multi-institutional study. Annals of Surgical Oncology. 2011;18:192-8.
13. Alamgir ANM. Medicinal, non-medicinal, biopesticides, Color- and Dye-Yielding Plants; Secondary Metabolites and drug Principles; Significance of medicinal plants; Use of medicinal plants in the systems of Traditional and Complementary and Alternative Medicines (CAMs). In: Progress in drug research [Internet]. 201; 1(1): 61-104.
14. Ranjha MM, Shafique B, Wang L, Irfan S, Safdar MN, Murtaza MA, Nadeem M, Mahmood S, *et al*. A comprehensive review on phytochemistry, bioactivity and medicinal value of bioactive compounds of pomegranate (*Punica granatum*). Advances in Traditional Medicine. 2021; 23(3):1-21.
15. Ismail T, Calcabrini C, Diaz AR, Fimognari C, Turrini E, Catanzaro E, *et al*. Ellagitannins in cancer chemoprevention and therapy. Toxins. 2016;8(5):151.
16. Testa U, Castelli G, Pelosi E. Breast cancer: a molecularly heterogenous disease needing subtype-specific treatments. Medical Sciences. 2020;8(1):18.
17. Andreou M, Jąkalski M, Duzowska K, Filipowicz N, Kostecka A, Davies H, Horbacz M, *et al*. Prelude to malignancy: A gene expression signature in normal mammary gland from breast cancer patients suggests pre-tumorous alterations and is associated with adverse outcomes. International Journal of Cancer. 2024;155(9):1616-28.
18. Masoud V, Pagès G. Targeted therapies in breast cancer: New challenges to Figureht against resistance. World journal of clinical oncology. 2017;8(2):120.
19. Hussein L, Gouda M, Buttar HS. Pomegranate, its components, and modern deliverable formulations as potential botanicals in the prevention and treatment of various cancers. Current Drug Delivery. 2021;18(10):1391-405.
20. Abuelmakarem HS, Aly Saad Aly M, Aly SH, Ayman S, Al Mamun MS, *et al*. Investigating the Application of Pomegranate-Loaded Chitosan Nanoparticles as Contrast Agents for Enhancing Breast Cancer Detection via Diffuse Reflectance Spectroscopy. Chemosensors. 2025;13(2):73
21. Mgbonyebi OP, Russo J, Russo IH. Roscovitine induces cell death and morphological changes indicative of apoptosis in MDA-MB-231 breast cancer cells. Cancer research. 1999;59(8):1903-10.
22. Turrini E, Ferruzzi L, Fimognari C. Potential effects of pomegranate polyphenols in cancer prevention and therapy. Oxidative medicine and cellular longevity. 2015;2015(1):938475.
23. Rajput S, Malviya R, Srivastava S, Ahmad I, Rab SO, Uniyal P. *In vivo* Evaluation of Apoptosis-Inducing Herbs for the Treatment of Breast Cancer: Recent Developments and Mechanism of Action. Current Nutrition and Food Science. 2025;21(3):282-94.
24. Boncler M, Różalski M, Krajewska U, Podsędek A, Watala C. Comparison of PrestoBlue and MTT assays of cellular viability in the assessment of anti-proliferative effects of plant extracts on human endothelial cells. Journal of pharmacological and toxicological methods. 2014;69(1):9-16.
25. Bouziane T, Daouia H, Soumia A. In vitro antifungal activity of the extracts of *Punica granatum* L obtained by reflux method against Fusarium oxysoprum albedenis in south west of Algeria. Advances in Biology and Earth Sciences. 2022;7(3).
26. Rojo-Gutiérrez E, Carrasco-Molinar O, Tirado-Gallegos JM, Levario-Gómez A, Chávez-González ML, Baeza-Jiménez R, *et al*. Evaluation of green extraction processes, lipid composition and antioxidant activity of pomegranate seed oil. Journal of Food Measurement and Characterization. 2021;15:2098-107.
27. Kaur G, Jabbar Z, Athar M, Alam MS. *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food and chemical toxicology. 2006;44(7):984-93.
28. Fischer UA, Carle R, Kammerer DR. Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MSn. Food chemistry. 2011;127(2):807-21.
29. Middha SK, Usha T, Pande V. HPLC evaluation of phenolic profile, nutritive content, and antioxidant capacity of extracts obtained from *Punica granatum* fruit peel. Advances in Pharmacological and Pharmaceutical Sciences. 2013;2013(1):296236.
30. Yisimayili Z, Chao Z. A review on phytochemicals, metabolic profiles and pharmacokinetics studies of the different parts (juice, seeds, peel, flowers, leaves and bark) of pomegranate (*Punica granatum* L.). Food Chemistry. 2022;395:133600.
31. Karagecili H, İzol E, Kirecci E, Gulcin İ. Determination of antioxidant, anti-alzheimer, antidiabetic, antiglaucoma and antimicrobial effects of zivzik pomegranate (*Punica granatum*)-a chemical profiling by LC-MS/MS. Life. 2023;13(3):735.
32. Jain R, Aishwarya D, Wankhade S, Anupriya, Kumarasamy M, Peraman R. Identification and in vitro genotoxicity assessment of forced degradation products of glimepiride and glyburide using HEK cell-based COMET assay. Biomedical Chromatography. 2024;38(12):e6025.
33. Panara A, Gikas E, Tzavellas I, Thomaidis NS. Comprehensive HRMS Chemical Characterization of Pomegranate-Based Antioxidant Drinks via a Newly Developed Suspect and Target Screening Workflow. Molecules. 2023;28(13):4986.
34. Kecojević I, Đekić S, Lazović M, Mrkajić D, Baošić R, *et al*. Evaluation of LC-MS/MS methodology for determination of 179 multi-class pesticides in cabbage and rice by modified QuEChERS extraction. Food Control. 2021;123:107693.
35. Thomas SL, Thacker JB, Schug KA, Maráková K. Sample preparation and fractionation techniques for intact proteins for mass spectrometric analysis. Journal of Separation Science. 2021;44(1):211-46.
36. Thomas SL, Thacker JB, Schug KA, Maráková K. Sample preparation and fractionation techniques for intact proteins for mass spectrometric analysis. Journal of Separation Science. 2021;44(1):211-46.
37. Kim SC, Chen Y, Mirza S, Xu Y, Lee J, Liu P, *et al*. A clean, more efficient method for in-solution digestion of protein mixtures without detergent or urea. Journal of proteome research. 2006;5(12):3446-52.
38. Zhou JY, Dann GP, Shi T, Wang L, Gao X, Su D, *et al*. Simple sodium dodecyl sulfate-assisted sample preparation method for LC-MS-based proteomics applications. Analytical chemistry. 2012;84(6):2862-7.
39. Chiariello AM, Abraham A, Bianco S, Esposito A, Fontana A, Vercellone F, *et al*. Multiscale modelling of chromatin 4D organization in SARS-CoV-2 infected cells. Nature Communications. 2024;15(1):4014.
40. Bian Y, Zheng R, Bayer FP, Wong C, Chang YC, Meng C, *et al*. Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC-MS/MS. Nature communications. 2020;11(1):157.

41. Smith, M.P.W, Wood S.L, Zougman A, Ho J.T., Peng, J, Jackson D, *et al*. A systematic analysis of the effects of increasing degrees of serum immunodepletion in terms of depth of coverage and other key aspects in top-down and bottom-up proteomic analyses. Proteomics, 11(11), pp.2222-2235.
42. Marcus K, Schäfer H, Klaus S, Bunse C, Swart R, Meyer HE. A new fast method for nanoLC– MALDI-TOF/TOF– MS analysis using monolithic columns for peptide preconcentration and separation in proteomic studies. Journal of Proteome Research. 2007 ;6(2):636-43.
43. Fakudze N, Sarbadhikary P, Abrahamse H, George BP. Phytochemical analysis and anticancer activity of fruit extracts of Southern African pomegranate (*Punica granatum*)'Wonderful'cultivar. South African Journal of Botany. 2024;172:747-67.
44. Alqahtani RA, Almutairi B, Al-Zharani M, Alkahtane AA, AL-Johani NS, Alkeraishan N *et al*. The Potential Therapeutic Efficacy of Pomegranate (*Punica granatum*) Seeds on MCF-7 Breast Cancer Cell Line. Natural Product Communications. 2024;19(12):1934578X241302555.
45. Soriano S. Wholeness and Interconnection as the Foundation for a New Epistemology of Health. Global Advances in Integrative Medicine and Health. 2025;14:27536130251328636.
46. Pellegrino C, Stone EF, Valentini CG, Teofili L. Fetal Red Blood Cells: A Comprehensive Review of Biological Properties and Implications for Neonatal Transfusion. Cells. 2024;13(22):1843.
47. Noreen S, Hashmi B, Aja PM, Atoki AV. Phytochemicals and pharmacology of pomegranate (*Punica granatum* L.): nutraceutical benefits and industrial applications: a review. Frontiers in Nutrition. 2025;12:1528897.
48. Fakudze NT, Sarbadhikary P, George BP, Abrahamse H. Ethnomedicinal Uses, Phytochemistry, and Anticancer Potentials of African Medicinal Fruits: A Comprehensive Review. Pharmaceuticals. 2023;16(8):1117.
49. Panth N, Manandhar B, Paudel KR. Anticancer activity of *Punica granatum* (pomegranate): a review. Phytotherapy research. 2017;31(4):568-78.
50. Beteinakis S, Papachristodoulou A, Stathopoulos P, Mikros E, Halabalaki M. A multilevel LC-HRMS and NMR correlation workflow towards foodomics advancement: Application in table olives. Talanta. 2024;280:126641.
51. Kildgaard S, Mansson M, Dosen I, Klitgaard A, Frisvad JC, *et al*. Accurate dereplication of bioactive secondary metabolites from marine-derived fungi by UHPLC-DAD-QTOFMS and a MS/HRMS library. Marine Drugs. 2014 ;12(6):3681-705.
52. Yang M, Sun J, Lu Z, Chen G, Guan S, Liu X, *et al*. Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry. Journal of Chromatography A. 2009 ;1216(11):2045-62.
53. Kiran S, Aslam A, Parveen A, Dilshad M, Hussain S. Phytochemistry of *Punica granatum* Fruit: Its Nutritional and Biological Potential. Bioactivities. 2024 ;2(1):57-73.

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