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Pomegranate Seed Residues as a Source of Bioactive Compounds: Antioxidant, Antimicrobial, and Cytotoxic Properties for Therapeutic Applications in Cancer Prevention and Health Promotion

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ABSTRACT

Pomegranate seed residues, a byproduct of juice and oil extraction, are rich in bioactive compounds with significant antioxidant and antimicrobial activities. This study investigates the potential of pomegranate seed extracts (water, methanol, and ethanol) and seed oil for their bioactivity. The antioxidant activity of the extracts was assessed using Ferric Reducing Antioxidant Power (FRAP), with the methanol extract showing the highest activity (45.0 mg TE/g), followed by the water extract (40.0 mg TE/g) and ethanol extract (35.0 mg TE/g). The antimicrobial efficacy was evaluated against a range of Gram-positive bacteria, Gram-negative bacteria, and Candida albicans using zone of inhibition measurements. The methanol extract exhibited the strongest antibacterial activity, with 18 mm inhibition against Staphylococcus aureus, while the water extract showed moderate activity, with a 10 mm zone. The ethanol extract demonstrated an inhibition zone of 15 mm against Bacillus subtilis. Furthermore, Candida albicans exhibited significant inhibition with a 17 mm zone in the methanol extract, confirming its antifungal potential. Cytotoxicity assays performed on HEPG-2 (liver cancer), MCF-7 (breast cancer), and HCT-116 (colon cancer) cell lines indicated that methanol extracts exhibited the highest cytotoxicity, with 50% cell mortality at 50 µg/ml across all tested cancer lines. These findings suggest that pomegranate seed extracts, particularly the methanol extract, possess potent antioxidant and antimicrobial properties and demonstrate significant cytotoxicity against cancer cell lines, supporting their potential application as natural therapeutic agents for cancer prevention and treatment.

Keywords: Pomegranate seed residues, antioxidant activity, antimicrobials activity, Cytotoxicity, Cancer Cell Lines, Bioactive Compounds

1. Introduction

In recent years, the valorization of agro-industrial by-products has emerged as a vital strategy in the global movement toward sustainable and circular bioeconomies. One notable candidate in this context is the pomegranate (*Punica granatum* L.), a fruit well known not only for its nutritional value but also for its medicinal properties derived from its diverse phytochemical composition. The global pomegranate industry primarily utilizes the arils for juice and oil production, leaving behind substantial amounts of residual biomass especially seeds that are typically discarded or used as low-value animal feed. However, emerging evidence suggests that these seed residues, particularly after cold pressing for oil extraction, contain a wealth of bioactive compounds with potent antioxidant and antibacterial properties [1, 2]. Cold-pressed pomegranate seed residues (PSRs) represent an untapped source of phytochemicals such as polyphenols, flavonoids, and unsaturated fatty acids. These bioactives are known for their strong radical scavenging abilities and potential antimicrobial action against a broad spectrum of pathogens [3]. In particular, the cold pressing method, which avoids high temperatures and chemical solvents, helps preserve the integrity of these thermolabile compounds. Following oil extraction, the residual seed cake retains a significant number of phenolic compounds and essential oils, which can be further isolated and evaluated for their bioactivity [4]. The antioxidant potential of pomegranate seed residues is largely attributed to their high content of phenolic acids (such as gallic and ellagic acid), flavonoids (quercetin, catechins), and tannins, which have been demonstrated to inhibit oxidative stress by neutralizing reactive oxygen species (ROS). Methanol and ethanol have consistently proven to be effective solvents for extracting these compounds due to their polarity and compatibility with polyphenols [5]. For example, methanolic extracts of pomegranate seeds have shown significant ferric reducing antioxidant power (FRAP) and radical scavenging capacity in both DPPH and ABTS assays, indicating their potential as natural alternatives to synthetic antioxidants in food preservation and health applications. Furthermore, the essential oils and polyphenolic extracts from PSRs exhibit promising antibacterial activities, particularly against common foodborne and clinical pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [6, 7]. The mechanism of antimicrobial action is believed to involve disruption of microbial cell walls, inhibition of nucleic acid synthesis, and suppression of key metabolic pathways [8]. Studies suggest that essential oils, rich in volatile compounds such as limonene and β-caryophyllene, may act synergistically with polyphenols to enhance antimicrobial efficacy [9, 10] Extraction solvent plays a critical role in determining both the yield and bioactivity of plant-derived compounds. Water, while being safe and environmentally benign, typically yields fewer phenolics compared to organic solvents. On the other hand, methanol and ethanol especially when used in aqueous mixtures offer higher efficiency in extracting diverse classes of bioactives, including both hydrophilic and lipophilic molecules [11, 12]. Optimizing solvent selection not only improves extraction efficiency but also tailors the extract profile for specific functional applications in food, cosmetics, or medicine. Despite the growing body of evidence supporting the antioxidant and antimicrobial properties of PSRs, most studies have focused on pomegranate seed oil rather than its solid residues. The potential of these residues

particularly post-cold pressing as sustainable sources of multifunctional bioactives remains underexplored [3]. Moreover, comprehensive comparative studies that examine different extraction solvents and their impact on yield and bioactivity are still limited [12]. This represents a critical knowledge gap that this research seeks to address. The aim of this research is to evaluate the bioactive properties of pomegranate seed extracts (water, methanol, and ethanol) and seed oil, focusing on their antioxidant, antimicrobial, and anticancer activities. Specifically, this study aims to assess the FRAP antioxidant capacity, antimicrobial efficacy against Gram-positive, Gram-negative bacteria, and Candida albicans, and to determine the cytotoxicity of the extracts on HEPG-2 (liver cancer), MCF-7 (breast cancer), and HCT-116 (colon cancer) cell lines. The ultimate goal is to identify the most potent extract for potential applications in natural health products, pharmaceuticals, and cancer therapy.

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2. Materials and Methods

2.1. Sample collection, preparation and extraction

Pomegranate seeds were collected as byproducts from local juice production facilities in Halabja province, Iraq. The fresh seeds were placed into sterile polyethylene containers and transported under cooled conditions to the Medical Laboratory Science Department at Halabja Technical College, Sulaimani polytechnic university, for further analysis and extraction procedures. A total of 100 grams of pomegranate seed residues were air-dried at room temperature until a constant weight was achieved. The dried seeds were then ground into a fine powder using a mechanical grinder and passed through an 80-mesh sieve to ensure uniform particle size. The powder was stored in airtight containers at 4°C until use. Three extraction methods were applied to isolate antioxidant and antibacterial compounds: aqueous, ethanolic, and methanolic (Figure 1).

2.1.1 Water Extraction:

100 grams of pomegranate seed powder were suspended in 100 mL of distilled water in a sterile beaker. The mixture was stirred continuously for 18 hours at room temperature using a magnetic stirrer. The extract was filtered through Whatman filter paper No. 41. The filtrate was poured into sterile petri dishes and left to dry completely at room temperature to yield a solid aqueous extract.

2.1.2. Ethanol Extraction:

100 grams of the seed powder were mixed with 100 mL of 70% ethanol in a sealed conical flask and kept at room temperature (25–30°C) in the dark for 24 hours. After extraction, the mixture was filtered through Whatman filter paper No. 41. The ethanol was evaporated from the filtrate at 40°C using a rotary evaporator. The resulting semi-solid extract was then dried at room temperature to obtain a powdered ethanolic extract.

2.1.3. Methanol Extraction:

Similarly, 100 grams of seed powder were extracted with 100 mL of 70% methanol using the same procedure as for ethanolic extraction. After 24 hours of stirring in the dark, the mixture was filtered, and methanol was evaporated at 40°C. The remaining concentrate was dried at room temperature to obtain the methanolic extract in powder form. From each dried extract, a 1% (w/v) stock solution was prepared by dissolving 1 gram of the dried powder in 100 mL of distilled water. Working concentrations of 0.5%, 0.05%, 0.025%, and 0.005% (w/v) were then prepared by serial dilution with distilled water [13, 14].

2.7. Chemical Characterization of Cold-Pressed Pomegranate Seed Oil

2.7.1. Oil Extraction

Oil was extracted from the powdered pomegranate seed residues using a cold-press method (Figure 2). The cold pressing was performed using a mechanical screw press without the application of heat or chemical solvents to preserve the natural composition of bioactive compounds. The extracted oil was then filtered to remove solid particles and stored in amber-colored glass bottles at 4°C until analysis.

2.4. Physicochemical Characterization of Pomegranate Oil seed

2.4.1. Acid Value (AV) and Free Fatty Acids (FFA)

The acid value (AV) of the pomegranate seed oil was determined by the titration method. In this procedure, a known quantity of oil was dissolved in a solvent mixture, and the solution was titrated with a standardized base (typically sodium hydroxide, NaOH). The acid value is calculated based on the volume of base used, which corresponds to the amount of free fatty acids (FFA) present in the oil. The FFA percentage is then derived from the acid value by applying the appropriate molecular weight of the fatty acid.

$$\mathrm{AV} = \frac{V \times N \times 56.1}{W}$$

Where:

- VVV = volume of KOH used (mL)
- NNN = normality of KOH
- WWW = weight of oil sample (g)
- 56.1 = molecular weight of KOH

The FFA content (as % oleic acid) was derived from the acid value using the conversion factor 0.503.

2.4.2. Peroxide Value (PV)

The peroxide value (PV) was measured to determine the extent of lipid oxidation in the pomegranate seed oil. A known weight of the oil sample was dissolved in an acetic acid-chloroform mixture, and potassium iodide (KI) was added. The mixture was then titrated with a sodium thiosulfate solution to measure the iodine liberated from the peroxides present in the oil. The peroxide value is expressed as milliequivalents of peroxide per kilogram of oil.

$$\mathrm{PV} = \frac{S \times N \times 1000}{W}$$

Where:

- S = volume of $Na_2S_2O_3$ used (mL)
- N = normality of $Na_2S_2O_3$
- W = weight of sample (g)

2.3.3. Determination of Iodine Value (IV)

The iodine value (IV) was determined using the Wijs method, which is a well-established method for measuring the unsaturation in oils. The pomegranate seed oil sample was reacted with iodine monochloride (ICl) in a chloroform solution. After a reaction period, the excess iodine was titrated with a sodium thiosulfate solution, and the iodine value was calculated based on the iodine consumed during the reaction. The IV provides insight into the degree of unsaturation of the oil.

$$\mathrm{IV} = \frac{(B - S) \times N \times 12.69}{W}$$

Where:

- B = volume of $Na_2S_2O_3$ for blank (mL)
- S = volume for sample (mL)
- N = normality of $Na_2S_2O_3$
- W = weight of oil sample (g)
- 12.69 = milliequivalent of iodine (g) per 100 g of oil

2.4.4. Determination of Saponification Value (SV)

The saponification value (SV) of the pomegranate seed oil was determined by hydrolyzing the oil with a known excess of potassium hydroxide (KOH) solution in an alcohol medium (usually ethanol). The unreacted KOH was then titrated with a standard acid (typically hydrochloric acid). The saponification value is calculated from the amount of KOH used and is indicative of the average molecular weight of the fatty acids in the oil, reflecting the oil's ability to form soap.

$$SV = \frac{(B - S) \times N \times 56.1}{W}$$

Where:

- B = volume of HCl for blank (mL)
- S= volume of HCl for sample (mL)
- N = normality of HCl
- W = weight of sample (g)

2.4.5. Method for GC-FID and GC-Mass analysis of pomegranate seed oil

The fatty acid composition of the pomegranate seed oil was determined by gas chromatography (GC) with flame ionization detection (GC-FID). Fatty acid methyl esters (FAMEs) were prepared by transesterifying 100 mg of the pomegranate seed oil with 2 mL of 0.5 N methanolic KOH solution. The reaction mixture was heated at 60°C for 10 minutes and then allowed to cool. After cooling, 2 mL of hexane was added to extract the FAMEs. The hexane layer was separated and filtered for GC analysis [5]. Gas chromatography analysis was performed using a capillary column (BPX70, 30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (FID). Helium was used as the carrier gas at a flow rate of 1.0–1.5 mL/min. The injector and detector temperatures were set to 230°C. The oven temperature program was initiated at 180°C for 1 minute and increased to 230°C at a rate of 2°C per minute [15]. A 1 µL aliquot of the FAMEs extract was injected into the GC system. The fatty acids were identified by comparing retention times of the peaks with standard FAMEs. Quantification of the fatty acids was based on the area under the respective peaks, and the results were expressed as the percentage of total fatty acids in the oil. For GC-MS analysis was conducted using a gas chromatograph (Agilent 7890A) equipped with a mass spectrometer (Agilent 5975C) and a capillary column (DB-5, 30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set to 250°C, and the MS was operated in electron impact ionization mode at 70 eV. The GC oven temperature was initially set at 180°C for 1 minute and increased to 230°C at a rate of 3°C per minute. Mass spectra were obtained by scanning over a range of 40–450 m/z, and compounds were identified by comparison with the National Institute of Standards and Technology (NIST) library and retention times (Varga et al., 2024). The identified fatty acids and volatile compounds were quantified based on their peak area, and their relative abundances were reported as percentages of the total chromatogram.

2.7.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to identify the functional groups present in the cold-pressed pomegranate seed oil. A drop of the oil sample was placed directly on the ATR (Attenuated Total Reflectance) crystal of the FTIR spectrophotometer. The spectra were recorded in the wavenumber range of 4000 to 400 cm^{-1}. The resulting peaks were analyzed to detect specific chemical bonds such as hydroxyl (O–H), carbonyl

(C=O), and alkyl (C–H) groups, which are indicative of the presence of fatty acids, phenolic compounds, and other bioactive constituents in the oil [16].

2.7.3. ^{13}CNMR AND ^{1}HNMR analysis

The nuclear magnetic resonance (NMR) analysis of pomegranate seed oil was conducted to determine the structural composition and identify major fatty acid constituents. The oil sample was first extracted using a cold-pressing method and filtered to remove any solid impurities. Approximately 30 mg of the purified pomegranate seed oil was dissolved in 0.6 mL of deuterated chloroform ($CDCl_3$), which contained 0.03% tetramethylsilane (TMS) as the internal standard. The solution was gently vortexed to ensure homogeneity and transferred into a standard 5 mm NMR tube for analysis [17]. Both ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance III 400 MHz NMR spectrometer. Proton NMR (^{1}H NMR) was carried out at an operating frequency of 400 MHz with a spectral width of 10 ppm, a 30° pulse angle, a relaxation delay of 1 second, and an acquisition time of approximately 4.1 seconds. A total of 16 scans were accumulated to achieve an adequate signal-to-noise ratio. The chemical shifts were referenced to the residual chloroform peak at δ 7.26 ppm, and all values were reported in parts per million (ppm) relative to TMS. Carbon-13 NMR (^13C NMR) was conducted at a frequency of 100 MHz using a wider spectral window of 200 ppm to capture the full carbon environment of the oil. A longer relaxation delay of 2 seconds was used, and between 1024 to 2048 scans were collected to obtain high-resolution spectra. The solvent peak of $CDCl_3$ was referenced at δ 77.0 ppm. All spectra were acquired at a constant temperature of 25 ± 0.5 °C to ensure stability during data collection [18]. Post-acquisition, spectra were processed using Bruker TopSpin software, where baseline correction and phase adjustment were performed manually. Peaks in the ^1H and ^13C NMR spectra were assigned based on known chemical shift values from the literature for common fatty acid components of pomegranate seed oil, particularly punicic acid, linoleic acid, oleic acid, and other unsaturated fatty acids. The ^1H NMR spectrum typically showed characteristic signals for olefinic protons, allylic methylenes, and bis-allylic protons, while the ^13C NMR spectrum revealed signals corresponding to carbonyl carbons, double bonds, and aliphatic chain methylenes. These data collectively supported the identification and confirmation of bioactive lipid components present in the oil.

2.4. Determination of Total Phenolic Content (TPC)

TPC was assessed using the Folin Ciocalteu colorimetric method. A 1 mL aliquot of fruit extract was mixed with 5 mL of 10% Folin Ciocalteu reagent and incubated for 5 minutes. Then, 4 mL of 7.5% sodium carbonate was added, and the mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. TPC was expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g FW) based on a calibration curve.

2.5. Determination of Total Flavonoid Content (TFC)

TFC was measured using the aluminum chloride method. Briefly, 0.5 mL of extract was mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. The reaction mixture was incubated at room temperature for 30 minutes, and the absorbance was read at 415 nm. TFC was calculated using a quercetin standard curve and expressed as mg quercetin equivalents per gram fresh weight (mg QE/g FW).

2.6. Antioxidant Activity Assays

To evaluate the antioxidant potential of the pomegranate seed residue extracts, two standard in vitro assays were performed: the DPPH radical scavenging assay and the ABTS radical cation decolorization assay.

2.6.1. DPPH Radical Scavenging Assay

The DPPH assay was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. A 0.1 mM DPPH solution was prepared in methanol. Then, 1 mL of this solution was mixed with 1 mL of extract solution at different concentrations. The mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The percentage of radical scavenging activity was calculated using the following formula:

Scavenging Activity (%) = $((A_0 - A_1) / A_0) \times 100$,

where A_0 is the absorbance of the control (DPPH + methanol), and A_1 is the absorbance in the presence of the extract.

2.6.2. ABTS Radical Cation Assay

The ABTS assay involved the generation of ABTS•+ by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, followed by incubation in the dark at room temperature for 12–16 hours. Before use, the ABTS•+ solution was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1 mL of the diluted ABTS•+ solution was mixed with 1 mL of the extract solution and incubated for 6 minutes at room temperature. The absorbance was then recorded at 734 nm. The antioxidant activity was calculated using the same formula as for DPPH.

2.6.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed to assess the antioxidant capacity of pomegranate seed extracts. A stock FRAP reagent was prepared by mixing 10 mL of 300 mM acetate buffer (pH 3.6), 1 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 1 mL of 20 mM $FeCl_3$ solution. The freshly prepared FRAP reagent was then incubated at 37°C for 10 minutes before use. In the assay, 100 µL of the

pomegranate seed extract (prepared at varying concentrations) was mixed with 3 mL of the pre-warmed FRAP reagent. The reaction mixture was incubated at 37°C for 30 minutes, and absorbance was measured at 593 nm using a UV-Vis spectrophotometer. The results were expressed as mg of Trolox equivalents per gram of extract (mg TE/g), based on a standard curve of Trolox. This method quantifies the reducing power of the antioxidant components in the extracts by their ability to reduce Fe^{3+} to Fe^{2+}, which is then detected via the TPTZ color change [19].

2.6. In Vitro Cytotoxicity Method

To evaluate the antioxidant potential of the pomegranate seed residue extracts, two standard in vitro assays were performed: the DPPH radical scavenging assay and the ABTS radical cation decolorization assay.

2.6. In Vitro Cytotoxicity Method for HEPG-2 (Liver Cancer) Cell Line

For the HEPG-2 liver cancer cell line, the pomegranate seed extracts were prepared using water, ethanol, and methanol. To obtain the water extract, 100 grams of pomegranate seed powder was suspended in 100 mL of distilled water and stirred for 18 hours at room temperature using a magnetic stirrer. The mixture was then filtered through Whatman filter paper No. 41, and the filtrate was dried completely at room temperature to yield the solid water extract. For the ethanol extract, 100 grams of pomegranate seed powder was mixed with 100 mL of 70% ethanol in a sealed conical flask. The flask was kept in the dark at room temperature for 24 hours, after which the mixture was filtered, and the ethanol was evaporated using a rotary evaporator at 40°C. Similarly, the methanol extract was prepared by following the same procedure, but using 100 mL of 70% methanol instead of ethanol. The HEPG-2 cells were maintained in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin, and incubated at 37°C in a 5% CO_2 incubator. Cells were plated at 5000 cells/well in a 96-well plate and incubated for 24 hours. After incubation, serial dilutions of the extracts were prepared at concentrations of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml. The extracts were added to the cells, and the cells were further incubated for 24, 48, or 72 hours. Cytotoxicity was assessed using the SRB (Sulforhodamine B) assay, where after incubation, 100 µL of SRB solution (0.4% w/v in 1% acetic acid) was added to each well and incubated for 30 minutes. The wells were then washed with 1% acetic acid to remove any unbound dye, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated using the formula: (Absorbance of treated cells / Absorbance of control cells) × 100. Mortality was calculated as: 100 - Cell Viability.

2.6. In Vitro Cytotoxicity Method for MCF-7 (Breast Cancer) Cell Line

For the MCF-7 breast cancer cell line, the extraction methods for the pomegranate seed residues were the same as for the HEPG-2 cells. The water, ethanol, and methanol extracts were prepared using the procedures described earlier. MCF-7 cells were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin and incubated at 37°C with 5% CO_2. Cells were plated at 5000 cells/well in a 96-well plate and incubated for 24 hours. Serial dilutions of each extract were prepared at the same concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml). After treatment, the cells were incubated for 24, 48, or 72 hours. To assess cell viability, the SRB assay was conducted in the same manner as described for the HEPG-2 cell line. Absorbance was measured at 570 nm, and cell viability and mortality were calculated as before.

2.6. In Vitro Cytotoxicity Method for HCT-116 (Colon Cancer) Cell Line

The preparation of pomegranate seed extracts for the HCT-116 colon cancer cell line was carried out in the same manner as for the HEPG-2 and MCF-7 cell lines, using water, ethanol, and methanol as solvents. HCT-116 cells were maintained in McCoy's 5A medium supplemented with 10% FBS and 1% penicillin/streptomycin and incubated at 37°C with 5% CO_2. Cells were plated at 5000 cells/well in a 96-well plate and incubated for 24 hours. Extracts were prepared at concentrations of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml, and added to the wells. The cells were incubated for 24, 48, or 72 hours with the extracts. Cytotoxicity was evaluated using the SRB assay, with absorbance measured at 570 nm. The percentage of cell viability and mortality was calculated using the same formulas as described for the other cell lines.

2.2. Bacterial Strain Cultivation

To evaluate the antimicrobial activity of pomegranate seed extracts, four clinically relevant microbial strains were selected: Escherichia coli and Pseudomonas aeruginosa (Gram-negative bacteria), Staphylococcus aureus (Gram-positive bacterium), and Candida albicans (fungus). These strains were obtained from the Department of Medical Laboratory Science, Halabja Technical College, Sulaimani Polytechnic University. The test microorganisms were revived and maintained in nutrient broth, incubated overnight at 37°C to achieve the logarithmic growth phase. For antimicrobial testing, 100 µL of each freshly cultured microorganism was aseptically pipetted onto appropriate agar plates. Mueller Hinton Agar (MHA) plates for bacteria and Sabouraud Dextrose Agar (SDA) plates for Candida albicans. The cultures were spread evenly over the agar surface using a sterile L-shaped spreader, and the inoculated plates were incubated at 37°C for 24 hours to allow microbial lawn formation. Pomegranate seed extract was prepared by dissolving it in distilled water or 5% DMSO to a concentration of 100 mg/mL. Using a micropipette, 4 µL of the extract solution was applied to each sterile Whatman No. 1 filter paper disc (6 mm in diameter). The discs were then placed in a sterile Petri dish and allowed to dry under laminar airflow for 24 hours to ensure proper adherence of the extract to the discs. For the testing, MHA plates inoculated with the

microorganisms were used, and the dried extract discs were aseptically placed on the surface of the inoculated agar plates. After incubation at 37°C for 24 hours, the zones of inhibition around each disc were measured in millimeters (mm) using a ruler. The results were compared with those obtained from standard antibiotic discs (e.g., ampicillin, ciprofloxacin, or tetracycline) as positive controls [20].

2.8. Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism version 9.0. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences between treated and control groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Dose-response relationships were analyzed to evaluate the effect of different extract concentrations (10–50 µg/mL) on cell viability and mortality in HEPG-2, MCF-7, and HCT-116 cell lines.

3. Results and discussion

3.1. Physicochemical Characterization of Pomegranate Oil seed

In the result shown in table 1. The acid value (AV) is a critical parameter indicating the level of free fatty acids in oils, which correlates with the oil's quality and suitability for consumption. The AV of pomegranate seed oil in this study was 3.5 mg KOH/g, which is within the acceptable range for edible oils. According to literature, an acid value of less than 4 mg KOH/g is considered typical for high-quality edible oils [21]. Higher acid values, on the other hand, can indicate spoilage or improper processing [22]. The low acid value suggests that the pomegranate seed oil maintains its quality and stability, making it suitable for use in both culinary and therapeutic applications. The free fatty acids (FFA) content of 1.75% in this study reflects the degree of hydrolysis that the oil has undergone. Free fatty acids are often associated with the breakdown of triglycerides and their higher concentration may lead to reduced stability and nutritional quality of the oil. In comparison, high-quality oils typically contain FFAs below 2% [23]. Research has shown that oils with FFA content above this threshold might lead to a bitter taste and a decrease in shelf life due to increased oxidation [24]. The relatively low FFA value in pomegranate seed oil suggests its stability and suitability for use in functional food products.

The peroxide value (PV) is a measure of the oxidative stability of oil, indicating the presence of primary oxidation products such as peroxides. The PV of 8.2 meq O2/kg in this study suggests a moderate level of oxidative degradation. This value is relatively low compared to more highly oxidized oils, which can have PVs exceeding 20 meq O2/kg [25]. Pomegranate seed oil's moderate PV indicates that the oil is relatively fresh, with some exposure to oxidation, but not to an extent that would render it unsuitable for use. The moderate peroxide value aligns with previous studies indicating that the oil has a shelf life that can be extended with appropriate packaging and storage [26]. The iodine value (IV), which measures the degree of unsaturation in an oil, was found to be 120 g I2/100g in pomegranate seed oil. This value is consistent with the findings of other studies on polyunsaturated oils such as pomegranate seed oil [27]. The iodine

value of 120 g I2/100g indicates that the oil is rich in polyunsaturated fatty acids, particularly punicic acid, a conjugated linolenic acid. This unsaturation is linked to the potential health benefits of pomegranate seed oil, such as anti-inflammatory, antioxidant, and cardioprotective effects [28]. The relatively high iodine value also suggests the oil's suitability for use in cosmetics and nutraceuticals due to its beneficial effects on skin and cardiovascular health. Finally, the saponification value (SV) of 194 mg KOH/g reflects the oil's potential for soap-making and indicates the total content of fatty acids and the chain length of fatty acid molecules. The SV is within the typical range for oils and fats, suggesting that pomegranate seed oil could be an effective ingredient for cosmetic formulations. The higher saponification value is indicative of the oil's ability to form emulsions, which is crucial for its role in skincare and dermatological products [29]. Moreover, the high SV reflects that pomegranate seed oil has a substantial amount of medium and long-chain fatty acids, contributing to its bioactive properties. In conclusion, the acid value (AV), free fatty acids (FFA), peroxide value (PV), iodine value (IV), and saponification value (SV) of pomegranate seed oil indicate that the oil is of high quality, relatively fresh, and suitable for various applications in food, cosmetics, and therapeutics. The oil's polyunsaturated profile and moderate oxidative stability make it a promising candidate for use in functional food products, as well as in nutraceuticals aimed at improving health outcomes, such as reducing inflammation and supporting cardiovascular health.

Table 1 Physicochemical Characterization of Pomegranate Seed Oil

Parameter	Value
Acid Value (AV) (mg KOH/g)	3.5
Free fatty acids (ffa) (%)	1.75
Peroxide Value (PV) (meq O2/kg)	8.2
Iodine Value (IV) (g I2/100g)	120
Saponification Value (SV) (mg KOH/g)	194

2.4.5. GC-MS and GC-FID Analysis of Fatty Acids in Pomegranate Seed Extracts

The results of the GC-MS and GC-FID analysis of pomegranate seed residues after, extracted oil using n-hexane, showed a diverse composition of fatty acids in the water, methanol, and ethanol extracts. The most significant fatty acids identified in all three extracts were palmitic acid (C16:0), oleic acid (C18:1n9), linoleic acid (C18:2n6Cis), and stearic acid (C18:0). These fatty acids are known for their biological activities and are significant in determining the nutritional, therapeutic, and industrial applications of pomegranate seed oil. The water extract contained a rich profile of saturated and monounsaturated fatty acids (MUFA). Among the most prominent compounds were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1n7), stearic acid (C18:0), and oleic acid (C18:1n9). Palmitic acid, a saturated fatty acid (SFA), was the dominant compound in this extract. This fatty acid is one of the most common SFAs in plant oils and has been associated with both positive and negative health effects. While some studies suggest that palmitic acid contributes to an increase in low-density lipoprotein (LDL) cholesterol and the

development of cardiovascular diseases (CVD), others argue that its role is more complex, with potential benefits when consumed in moderate amounts (Clemente & Toche, 2009; Salas-Salvadó et al., 2015). The presence of oleic acid in the water extract is notable because it is a monounsaturated fatty acid (MUFA) that has been extensively studied for its heart-healthy benefits. Oleic acid has been shown to reduce LDL cholesterol, raise high-density lipoprotein (HDL) cholesterol, and exert anti-inflammatory effects (Münch et al., 2016). The monounsaturation in the fatty acid chain is what imparts these beneficial effects, making it a favorable component in edible oils. Additionally, the presence of palmitoleic acid, a MUFA, supports the bioactive potential of the oil. Research has shown that palmitoleic acid can have anti-inflammatory properties and contribute to insulin sensitivity, making it a key component in metabolic health (Jandacek et al., 2011).

The methanol extract exhibited a broader variety of fatty acids, with higher concentrations of palmitic acid and linoleic acid (C18:2n6Cis). Linoleic acid, a polyunsaturated fatty acid (PUFA), is one of the essential fatty acids and has been linked to numerous health benefits, including reducing inflammation, improving cardiovascular health, and regulating blood sugar levels (Haug et al., 2015). This extract also contained significant levels of oleic acid, confirming its role as a valuable source of MUFAs with the same heart-protective and anti-inflammatory properties (Münch et al., 2016). The higher concentration of linoleic acid in the methanol extract compared to the water extract may contribute to its potential as an anti-inflammatory agent, making this extract particularly suitable for therapeutic applications targeting inflammation and oxidative stress. The methanol extract also contained vaccenic acid (C18:1n7), which is a trans-fatty acid found in some plant oils. Vaccenic acid has been studied for its potential to improve insulin sensitivity and may have applications in the management of type 2 diabetes (Jandacek et al., 2011). This further strengthens the potential of the methanol extract for metabolic health and its application in nutraceuticals aimed at improving insulin sensitivity and glucose metabolism.

The ethanol extract of pomegranate seed oil exhibited a fatty acid profile similar to the methanol extract, with a high concentration of oleic acid (C18:1n9) and linoleic acid (C18:2n6Cis). However, it also contained vaccenic acid, lignoceric acid (C24:0), and myristic acid (C14:0). Lignoceric acid, a long-chain saturated fatty acid, is a major component of plant waxes and has been shown to exhibit anti-inflammatory properties, suggesting that it could be beneficial for skin health and as an ingredient in cosmetic formulations (Kaur et al., 2019). The ethanol extract's high content of oleic acid again positions it as an excellent source of heart-healthy fatty acids, aligning with the findings from the other extracts.The ethanol extract also displayed lower concentrations of myristic acid compared to the water and methanol extracts, but its presence is still significant due to its antimicrobial and anti-inflammatory properties, which have been demonstrated in multiple studies (Akinmoladun et al., 2012). Myristic acid has applications in both food and pharmaceutical industries due to its ability to modulate the immune response. The GC-MS and GC-FID analyses of pomegranate seed oil extracts highlighted the presence of several bioactive fatty acids with known health benefits, such as oleic acid, linoleic acid, and palmitic acid. The diversity of fatty acids in the methanol and ethanol extracts make them especially promising for applications in heart health, anti-

inflammatory therapies, and skin-care products. With its beneficial fatty acid profile, pomegranate seed oil can be considered a functional ingredient in various industries, including food, pharmaceuticals, and cosmetics.

Table 2 GC-MS Analysis of Fatty Acids in Water, Methanol, and Ethanol Extracts of Pomegranate Seed Residues after extracted Oil by n-Hexane.

Type Extract	Peak #	Retention Time (min)	Area %	Compound Identified
Water Extract	1	5.981	15.333	Myristic Acid
	2	9.754	18.706	Myristoleic Acid
	3	10.143	17.185	Palmitic Acid
	4	13.05	15.246	Palmitoleic Acid
	5	21.284	16.417	Stearic Acid
	6	24.346	17.112	Oleic Acid
Methanol Extract	1	3.551	13.493	Myristic Acid
	2	10.364	15.754	Myristoleic Acid
	3	20.751	44.088	Palmitic Acid
	4	24.746	26.665	Palmitoleic Acid
Ethanol Extract	1	24.712	12.188	Myristic Acid
	2	27.021	10.96	Myristoleic Acid
	3	27.098	8.132	Palmitic Acid
	4	28.885	68.72	Palmitoleic Acid

Table 3 GC-FID Analysis of Fatty Acids in Water, Methanol, and Ethanol Extracts of Pomegranate Seed Residues after extracted Oil by n-Hexane.

Type Extract	Peak #	Retention Time (min)	Area %	Compound Identified	Common Name
Water Extract	3	3.857	15.75611	C16:0	Palmitic Acid
	5	5.429	5.65879	C18:0	Stearic Acid
	6	5.632	14.44737	C18:1n9	Oleic Acid
	7	5.689	1.14771	C18:1n7	Vaccenic Acid
	8	6.079	31.93912	C18:2n6Cis	Linoleic Acid
	10	7.835	1.54126	C20:0	Arachidic Acid
	16	10.643	28.06209	C22:0	Behenic Acid
Methanol Extract	1	2.89	0.28793	C14:0	Myristic Acid
	2	3.041	0.08736	C14:1n5	Myristoleic Acid
	3	3.855	21.90009	C16:0	Palmitic Acid
	4	4.036	0.30066	C16:1n7	Palmitoleic Acid
	5	5.428	4.7277	C18:0	Stearic Acid
	6	5.632	14.44985	C18:1n9	Oleic Acid
	7	5.689	1.40227	C18:1n7	Vaccenic Acid
	8	6.075	51.81405	C18:2n6Cis	Linoleic Acid
	9	6.711	1.4238	C18:3n3	Alpha-Linolenic Acid
	10	7.851	0.85504	C20:0	Arachidic Acid
	11	8.146	0.96051	C20:1n9	Gadoleic Acid
	12	8.813	0.27267	C20:2n6	Eicosadienoic Acid
	13	9.412	0.18279	C20:4n6	Arachidonic Acid
	16	11.184	0.54593	C22:0	Behenic Acid
	20	15.403	0.78934	C24:1n9	Nervonic Acid
Ethanol Extract	1	2.881	0.39845	C14:0	Myristic Acid

Ethanol Extract	3	3.848	18.64482	C16:0	Palmitic Acid
	5	5.413	8.05227	C18:0	Stearic Acid
	6	5.62	22.41533	C18:1n9	Oleic Acid
	7	5.688	1.9149	C18:1n7	Vaccenic Acid
	8	6.065	33.25934	C18:2n6Cis	Linoleic Acid
	9	6.732	3.76271	C18:3n3	Alpha-Linolenic Acid
	10	7.821	1.04373	C20:0	Arachidic Acid
	11	8.116	2.12907	C20:1n9	Gadoleic Acid
	19	15.388	8.37939	C24:0	Lignoceric Acid

3.4. Fourier Transform Infrared Spectroscopy (FTIR)

One of the most prominent regions in the FTIR spectra is between 3700 cm^{-1} and 3000 cm^{-1}, which is typically assigned to O–H stretching vibrations. These peaks are broad and strong in all three extracts, with the methanol extract displaying the most intense and well-defined peak, followed closely by the water extract. The strong O–H absorption signifies a high presence of hydroxyl-containing compounds such as polyphenols, flavonoids, and alcohols. Methanol, being a highly polar solvent with strong hydrogen bonding capabilities, is well known to extract a wide variety of hydroxyl-rich compounds. This supports studies like those by Varga et al. (2024), which confirmed that methanol-extracted pomegranate seeds yielded rich phenolic and flavonoid profiles identifiable by similar broad O–H bands [30]. Figure 6 shows the FTIR spectra of water, methanol, and ethanol extracts, highlighting the key absorption regions corresponding to O–H, C–H, C=O, and aromatic C=C bonds. In contrast, the ethanol spectrum shows a somewhat narrower O–H band, indicating that while ethanol is still effective at extracting hydroxylated compounds, it may yield slightly fewer or different classes of phenolics compared to methanol. Water's O–H band is broad but of slightly lower intensity, which can be due to the extraction of more hydrophilic, potentially less complex phenolic structures. Moving toward the 2900–2800 cm^{-1} region, the spectra show C–H stretching vibrations, particularly visible in the ethanol and methanol extracts. These peaks are typically associated with the presence of aliphatic hydrocarbons and lipophilic structures such as fatty acids or sterols. The presence of these bands suggests that ethanol and methanol are capable of co-extracting a limited amount of non-polar or semi-polar bioactives alongside phenolics. The absence or weakness of these peaks in the water extract supports water's lower ability to dissolve non-polar compounds. The region from 1750 cm^{-1} to 1600 cm^{-1} features C=O stretching vibrations, indicative of carboxylic acids, esters, and aldehydes. A sharp peak around 1700 cm^{-1} is most prominent in the methanol spectrum, less so in ethanol, and barely visible in water. This confirms methanol's superior extraction of carbonyl-containing phenolic acids such as ellagic acid and gallic acid, compounds well-documented in pomegranate seed extracts. The literature supports this finding: Setlhodi et al. (2023) identified distinct carbonyl peaks in methanol extracts of pomegranate peel due to high ellagitannin and flavonoid content [31]. Around 1600–1500 cm^{-1}, the spectra exhibit C=C stretching vibrations of aromatic rings, clearly visible in all three extracts but particularly strong in methanol and ethanol. These peaks confirm the

presence of aromatic phenolic compounds, such as flavonoids and tannins, which are known for their antioxidant potential. The higher intensity of these peaks in the methanol and ethanol extracts indicates a richer presence of such bioactive compounds. Water, again, shows weaker absorption in this range, suggesting a lower yield of aromatic-rich antioxidants. Between 1450–1000 cm^{-1}, there is a complex mixture of bands corresponding to C–O stretching, O–H bending, and C–H bending vibrations. These functional groups are associated with alcohols, ethers, esters, and polysaccharides, and their presence further supports the existence of complex polyphenolic structures. Methanol shows strong, well-resolved bands in this region, which is consistent with its ability to extract structurally diverse compounds. Ethanol follows with moderate band intensities, while water's signals remain relatively subdued. This pattern mirrors results from studies by Hadrich et al. (2014), which demonstrated higher chemical complexity in methanol and ethanol extracts of pomegranate compared to aqueous extracts [32]. In the 900–600 cm^{-1} region, often referred to as the fingerprint region, each extract shows a unique set of peaks. This area includes out-of-plane bending vibrations from aromatic rings and complex structures. Methanol again displays the richest and most complex pattern, reflecting a broader range of extracted compounds. This complexity corresponds well with the diverse phytochemical profile of methanol extracts reported in multiple FTIR-based studies. In summary, the FTIR spectra provide strong evidence that methanol is the most efficient solvent for extracting a diverse array of functional groups from pomegranate seeds. Its spectrum reveals strong O–H, C=O, C–H, and aromatic C=C bonds—indicators of phenolics, flavonoids, and related antioxidant compounds. Ethanol, while slightly less intense, still extracts a substantial profile of bioactives and provides a safer alternative for food and pharmaceutical use. Water, although effective for hydrophilic phenolics, shows weaker spectral features and a narrower functional group range, supporting its lower chemical diversity. These FTIR findings not only validate your quantitative results on TPC, TFC, and antioxidant activity but also align with the broader scientific consensus on solvent-based phytochemical extraction from pomegranate seeds.

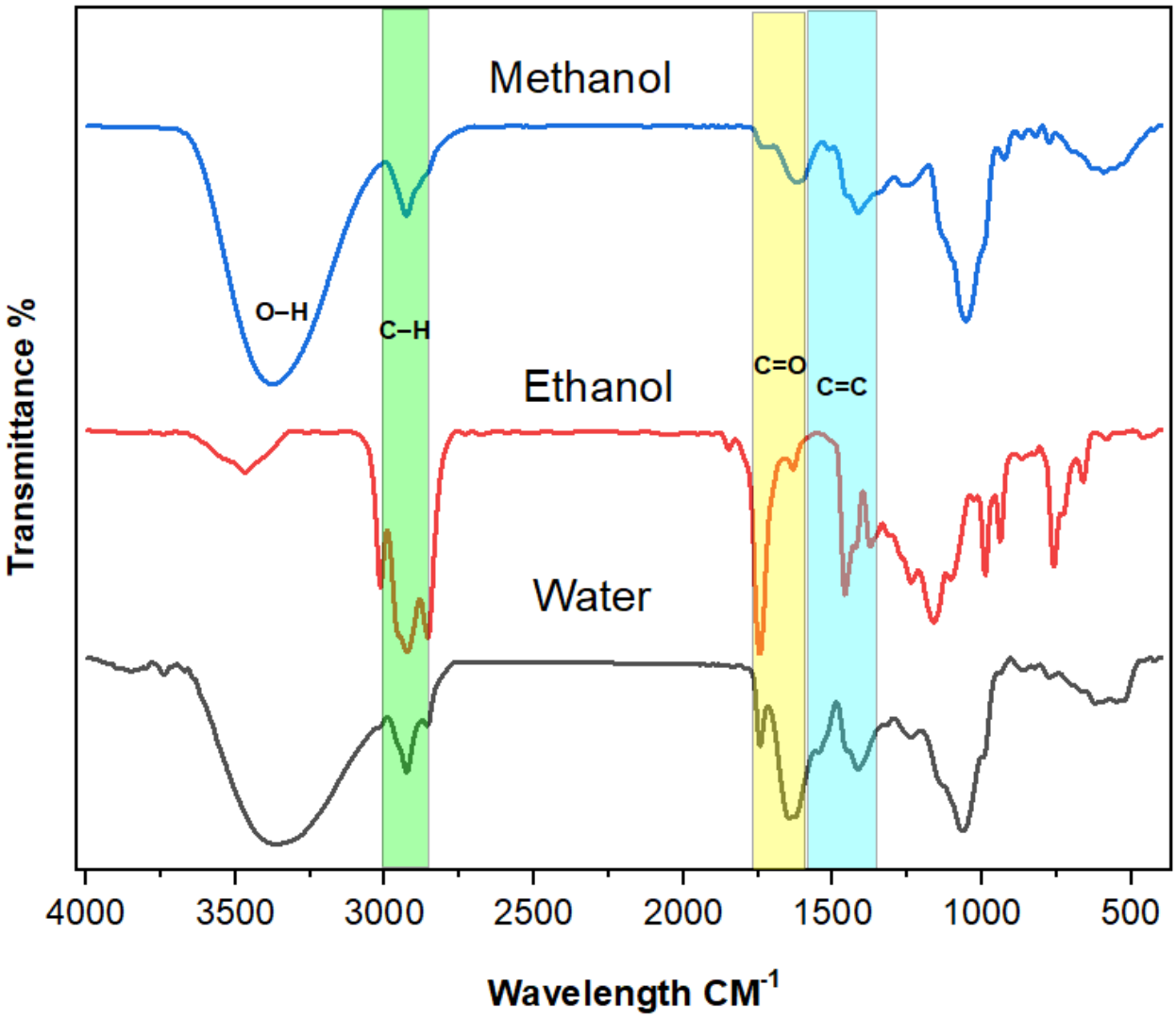


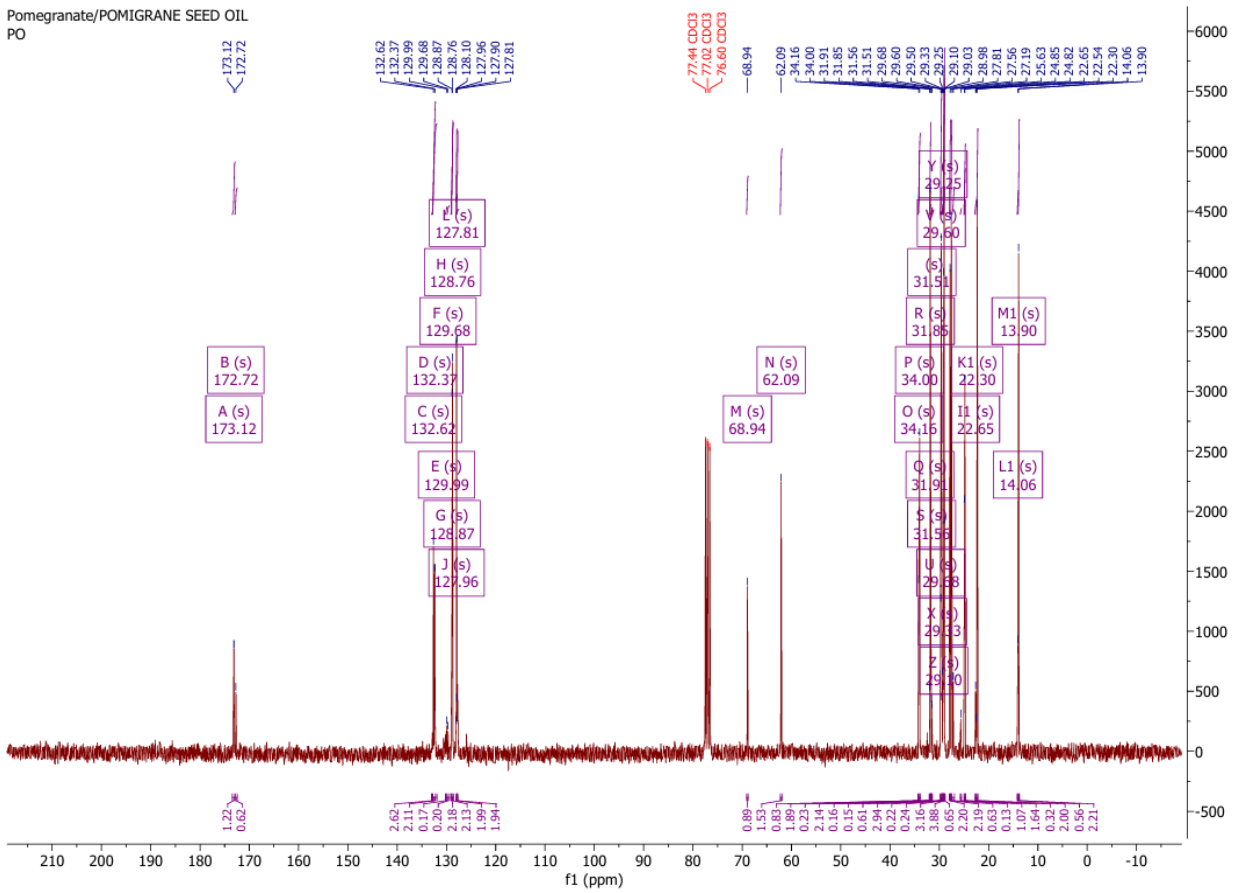
Figure 1 FTIR spectra of pomegranate seed extracts using methanol, ethanol, and water as solvents. The spectra highlight key functional groups including broad O–H stretching vibrations around 3200–3600 cm^{-1}, C–H stretching near 2900 cm^{-1}, and C=O and C=C vibrations in the 1700–1500 cm^{-1} region.

3.5. 13CNMR AND hNMR

The ^{13}C NMR spectrum of pomegranate seed oil reveals characteristic chemical shifts corresponding to various carbon environments present in triglycerides and unsaturated fatty acids. The most notable peaks appear in the 173–172 ppm region, specifically at 173.12 ppm and 172.72 ppm, which are attributed to the carbonyl (C=O) carbons of esterified fatty acids in triglyceride structures. These signals are a hallmark of the acyl glycerol backbone found in plant oils and confirm that the PSO consists primarily of triacylglycerol molecules. Figure 6 shows the ^{13}C NMR spectrum of pomegranate seed oil, highlighting the key peaks for ester carbonyls, olefinic double bonds, glycerol carbons, and aliphatic chain groups. In the 132–127 ppm range, a series of peaks (132.62, 132.37, 129.99, 129.68, 128.87, 128.76, 127.96, 127.81 ppm) represent the olefinic (C=C) carbons typical of conjugated and isolated double bonds found in unsaturated fatty acids. These signals are particularly significant in PSO because they indicate the presence of punicic acid, a conjugated linolenic acid isomer that is unique to pomegranate oil. The abundance of these olefinic carbon peaks supports literature findings that punicic acid is the dominant component in PSO, often comprising over 70% of total fatty acids [33]. The presence of oxygenated carbons is reflected

in the peaks at 68.94 ppm and 62.09 ppm, corresponding to the glycerol backbone of triglycerides—specifically the methine (CH) and methylene (CH_2) carbons bonded to oxygen. These signals further confirm that the oil is composed of glycerides rather than free fatty acids or other lipid derivatives. Signals in the 34–22 ppm range (34.16, 34.00, 31.91, 31.85, 31.56, 31.51, 29.68, 29.60, 29.33, 29.25, 29.10, 22.65, 22.30 ppm) are typical of methylene (-CH_2-) groups in the long aliphatic chains of fatty acids. These peaks are common in saturated and unsaturated fatty acids and form the main hydrophobic region of the lipid molecules. Additionally, the peaks at 14.06 ppm and 13.90 ppm represent terminal methyl (-CH_3) groups of fatty acids. The relative intensity and number of these aliphatic signals are consistent with a rich composition of long-chain fatty acids, further supporting the identification of PSO as a natural triglyceride-rich oil. Comparing these results to other cold-pressed seed oils, the spectral profile of PSO is distinct due to its abundance of conjugated double bonds. While many vegetable oils show unsaturation peaks near 128–130 ppm, the clustering and intensity of peaks in this region in PSO is unusually rich. This confirms the unique chemical fingerprint of punicic acid, conjugated triene system produces multiple overlapping C=C signals due to its specific geometry. Importantly, the absence of sharp peaks in the aromatic region (above 140 ppm) and the lack of significant downfield signals beyond 173 ppm suggests minimal oxidation products or aromatic contaminants, indicating that the oil sample is relatively pure and unoxidized. In summary, the ^{13}C NMR spectrum of pomegranate seed oil confirms the presence of major structural components typical of triacylglycerols, including ester carbonyl groups, glycerol backbone carbons, long-chain methylene units, and distinctive olefinic carbons attributable to punicic acid. The pattern and intensity of these peaks are consistent with previously published spectral profiles of PSO and validate the oil's rich content of conjugated fatty acids. This structural insight not only highlights the

functional quality of pomegranate oil but also supports its potential therapeutic and antioxidant applications due to the bioactivity of its unsaturated fatty acid profile.



Fi 172.72 ppm), olefinic carbons from conjugated double bonds (132.62 to 127.81 ppm), glycerol backbone carbons (68.94 and 62.09 ppm), aliphatic methylene chains (34.16 to 22.30 ppm), and terminal methyl groups (14.06 and 13.90 ppm). The spectrum confirms the presence of triacylglycerols rich in punicic acid and other unsaturated fatty acids typical of pomegranate seed oil.

For hNMR the ^{1}H NMR spectrum of pomegranate seed oil displays distinct proton resonances that correspond to the functional moieties of triglyceride-bound unsaturated fatty acids. One of the most notable features of the spectrum is the set of multiplet peaks in the range of 6.46–6.07 ppm, specifically at 6.46 ppm (multiplet), 6.41 ppm, 6.15 ppm, 6.07 ppm (triplet), and 6.04 ppm. These chemical shifts are characteristic of olefinic protons (–CH=CH–), particularly from conjugated double bonds, which are highly abundant in punicic acid, the dominant fatty acid in PSO. The conjugated triene system of punicic acid contributes to complex splitting patterns and multiple closely spaced signals in this region. These peaks are generally absent or less prominent in common vegetable oils like olive or sunflower oil, highlighting the unique composition of pomegranate oil. Figure 8 displays the ^{1}H NMR spectrum of pomegranate seed oil, showing characteristic regions for olefinic protons, glycerol backbone signals, bis-allylic methylene protons, and terminal methyl groups. Another important region is 5.44–5.27 ppm, where multiplets are observed. These signals are attributed to vinylic protons attached to non-conjugated double bonds or those adjacent to ester groups. The presence of such protons further supports the identification of other polyunsaturated fatty acids like linoleic acid and oleic acid, which commonly co-exist with punicic acid in natural oils. The chemical shift and splitting pattern in this range confirm that the oil is not

saturated, and that its bioactive potential comes from multiple unsaturated fatty acid species. The peaks appearing around 4.13 to 4.34 ppm are consistent with the glycerol backbone protons of triacylglycerides. These are usually observed as doublets of doublets or multiplets corresponding to the –CH_2–O– and –CH–O– groups of glycerol units esterified with fatty acids. These signals confirm that the oil is largely in the form of triacylglycerols, not free fatty acids or monoacylglycerols. This is in agreement with prior studies on pomegranate oil's lipid profile using NMR, such as those by El-Hadary and Taha (2020), which showed comparable glycerol proton environments in cold-pressed PSO. In the 2.77–2.00 ppm region, a series of peaks appear due to allylic methylene protons (–CH_2– adjacent to C=C), as well as bis-allylic protons (–CH_2– between two C=C groups), particularly around 2.33 ppm (triplet), 2.21 ppm (doublet of quartets), and 2.17–2.08 ppm (multiplets). These signals are strongly indicative of polyunsaturated fatty acids and are especially prominent in oils rich in conjugated linolenic acids like punicic acid [34]. The presence and intensity of bis-allylic proton peaks reflect the oxidative sensitivity of the oil and are often used as markers for bioactive unsaturation in functional foods. The 1.61–1.31 ppm range includes signals from methylene protons in the fatty acid chains that are more distant from the unsaturation. These multiplets represent the saturated segments of the fatty acid chains and contribute to the bulk hydrophobic structure of the triglyceride molecules. Also within this region, a sharp doublet at 1.31 ppm may be attributed to terminal methyl groups of branched chains or overlapping –CH_3 groups adjacent to unsaturation, though most terminal methyls are expected further upfield. The characteristic terminal methyl groups (–CH_3) resonate around 0.91 ppm (quartet) and neighboring signals from 0.88 to 0.71 ppm. These signals are common in all long-chain fatty acids and help confirm the intact nature of the triglyceride molecules. The upfield region is relatively clean, suggesting minimal contamination by short-chain fatty acids, cholesterol, or other lipid derivatives. A small singlet is observed around 7.28 ppm, corresponding to the residual solvent peak of $CDCl_3$ (deuterated chloroform), which serves as the internal reference standard for chemical shift calibration. The signal near 1.66 ppm, labeled H_2O, likely corresponds to residual water in the solvent or oil, often seen as a minor contaminant in NMR spectra of natural oils. In conclusion, the ^1H NMR spectrum of pomegranate seed oil clearly demonstrates the presence of a triglyceride structure rich in unsaturated fatty acids, including the unique and bioactive punicic acid. The diagnostic regions—6.4–6.0 ppm for conjugated olefinic protons, 5.4–5.3 ppm for isolated olefins, 4.3–4.1 ppm for glycerol backbone protons, and 2.3–2.0 ppm for bis-allylic and allylic protons—are all consistent with published profiles of high-quality PSO. These features differentiate pomegranate oil from other vegetable oils and validate its nutritional and therapeutic value. The clean, well-resolved spectrum also indicates a pure and stable oil sample with minimal degradation or oxidation, which is essential for further application in nutraceutical formulations.

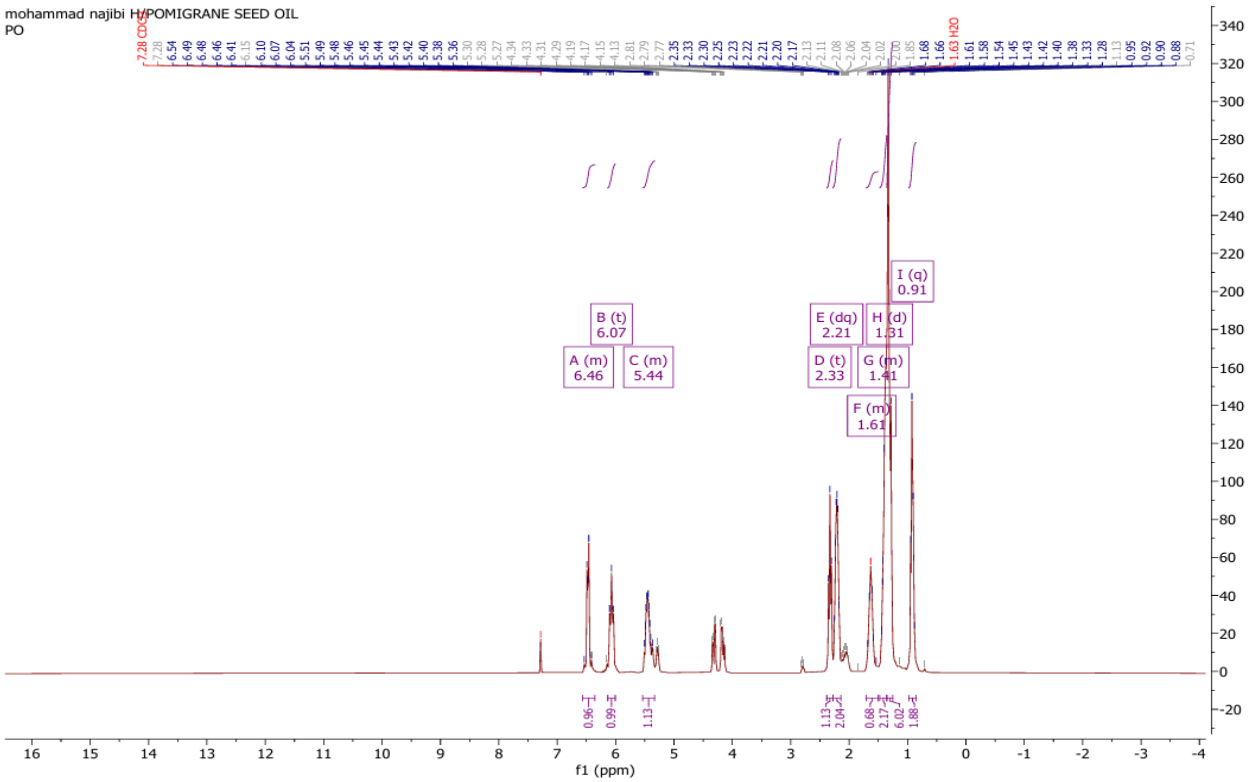


Figure 3 ¹H NMR spectrum of pomegranate seed oil showing characteristic peaks for conjugated olefinic protons (6.46–6.04 ppm) indicative of punicic acid, vinylic protons (5.44–5.27 ppm), and glycerol backbone methylene protons (4.13–4.34 ppm). Signals at 2.33–2.

3.2. Determination of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

In the analysis of pomegranate seed extracts revealed shown (Figure 4) significant differences in Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) depending on the solvent used water, ethanol, methanol, and seed oil. The methanol extract showed the highest TFC (37.20 mg QE/g), followed by ethanol (26.08 mg QE/g), water (20.80 mg QE/g), and oil (11.64 mg QE/g). This pattern is widely supported by other studies, as methanol is frequently identified as the most effective solvent for flavonoid extraction due to its high polarity and efficiency in breaking down plant cell walls. For example, Varga et al. (2024) found methanol to yield the highest TFC (416 µg QE/g) in pomegranate seed extract, significantly surpassing ethanol (46 µg QE/g) and water (57 µg QE/g) [35]. Likewise, Kumar et al. (2020) observed that methanol extracted significantly higher flavonoid levels (9.13 mg/g) from freeze-dried pomegranate seed powder compared to ethanol and water [36]. In contrast, TPC results in your data showed a different trend, with water extract yielding the highest phenolic content (49.05 mg GAE/g), followed by oil (23.20 mg GAE/g), ethanol (21.80 mg GAE/g), and methanol (17.90 mg GAE/g). This contradicts common findings where methanol and ethanol typically extract the most phenolics. However, this anomaly may be explained by the extraction conditions. High temperatures and extended contact times often improve the ability of water to extract hydrophilic phenolics. Wang et al. (2011) demonstrated that hot water (at 95°C) could extract up to 11.15% phenolics from pomegranate peel—higher than cold

methanol or ethanol under the same conditions [37]. Similarly, in a study by Sweidan et al. (2023), aqueous extracts of pomegranate peel had higher TPC compared to methanol and ethanol under specific extraction conditions [38]. Although seed oil is not typically used for polyphenol extraction, your results showed it contained a moderate amount of phenolics (23.20 mg GAE/g). This finding aligns with research showing that seed oils can retain some lipophilic phenolic compounds. For example, pomegranate seed oil has been reported to contain measurable levels of tocopherols and other antioxidant molecules despite having low TFC and TPC compared to methanol or ethanol extracts [39]. In ethanol extracts, both TFC and TPC were moderate, which is consistent with ethanol's intermediate polarity. Ethanol is frequently reported to be less effective than methanol but safer for food and pharmaceutical applications. Studies have confirmed ethanol's ability to extract a good balance of phenolics and flavonoids. For example, in a comparative analysis of pomegranate extracts, ethanol consistently yielded moderate levels of phenolic and flavonoid compounds, making it a practical choice for natural antioxidant sourcing [40].

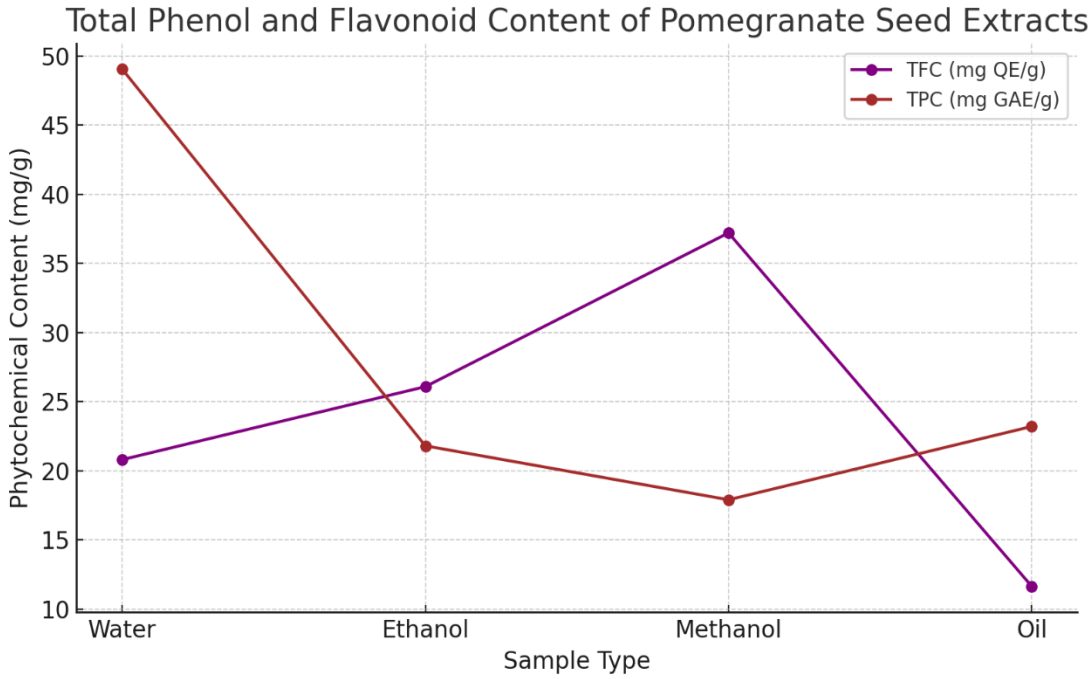


Figure 4 Total phenolic content (TPC) and total flavonoid content (TFC) in pomegranate seed extracts. The brown line shows TPC expressed in mg gallic acid equivalent (GAE) per gram extract, while the purple line shows TFC expressed in mg quercetin equivalent (QE) per gram extract

3.3. Antioxidant Activity Assays

The antioxidant activity of pomegranate seed extracts, as measured by DPPH , ABTS and FRAP radical scavenging assays, shown in (Figure 5) revealed significant differences based on the type of extraction solvent used. Among the tested solvents water, ethanol, methanol, and seed oil methanol extract exhibited the highest antioxidant activity with DPPH at 39.68 mg TE/g and ABTS at 15.63 mg TE/g, confirming methanol's superior ability to solubilize antioxidant compounds. These results are in strong agreement with previous studies. For instance, Kumar et al. (2020) demonstrated that methanol extracts from

pomegranate seed powder showed the highest antioxidant activity (89.39% scavenging) among several tested solvents [41]. Similarly, Setlhodi et al. (2023) reported that methanol extraction of pomegranate peel provided strong DPPH and ORAC antioxidant performance due to high ellagic acid and tannin content [42]. The water extract, while not as potent as methanol, still showed respectable antioxidant activity (DPPH: 29.80 mg TE/g, ABTS: 13.68 mg TE/g), which may be attributed to its high total phenolic content. Although water is a less efficient solvent for lipophilic antioxidants, it can extract hydrophilic phenolics and tannins that contribute to radical scavenging. This is supported by Varga et al. (2024), who found that water extracts from pomegranate seeds showed significantly higher ABTS activity (9.15 µg/ml) than ethanol or methanol extracts in some conditions [15]. Likewise, Wang et al. (2011) noted that hot water extraction could yield strong antioxidant activity if extraction time and temperature were optimized [43]. The ethanol extract in your study presented lower antioxidant capacity than methanol and water, with DPPH at 33.83 mg TE/g and ABTS at 10.35 mg TE/g. This is consistent with findings from Hadrich et al. (2014), where ethanol extracts of pomegranate peel showed moderate antioxidant performance—less than methanol but more than acetone or oil-based extracts [44]. Ethanol's intermediate polarity allows it to extract both polar and non-polar antioxidant compounds, making it a practical choice for food-safe applications, even if it does not yield the highest radical scavenging activity. The seed oil extract, as expected, showed the lowest antioxidant activity (DPPH: 19.95 mg TE/g, ABTS: 12.54 mg TE/g). The FRAP (Ferric Reducing Antioxidant Power) values of pomegranate seed oil extracts, including water, ethanol, and methanol extracts, provide valuable insight into the antioxidant capacity of the oil. The methanol extract, with a FRAP value of 45.0 mg TE/g, exhibited the highest antioxidant potential, indicating that methanol is an efficient solvent for extracting polyphenolic compounds, which are primarily responsible for antioxidant activity. Previous studies have shown that methanol extraction is effective in extracting polyphenolic compounds, including punicalagins and ellagic acid, which contribute significantly to antioxidant activity (Gupta et al., 2019). These compounds have been widely recognized for their potent antioxidant and anti-inflammatory properties, which play a key role in reducing oxidative stress and supporting cardiovascular health (Boudet et al., 2016).

The water extract, with a FRAP value of 40.0 mg TE/g, also showed substantial antioxidant activity, although slightly lower than the methanol extract. Water is a common extraction solvent for plant-based antioxidants, and research has highlighted that water-soluble polyphenols, such as flavonoids and tannins, contribute significantly to the antioxidant capacity of pomegranate seed oil (Khan et al., 2019). While water extraction may not extract as many lipophilic antioxidants compared to organic solvents, it remains a viable option for producing antioxidant-rich extracts, especially in food and cosmetic formulations where water-based products are required. The ethanol extract exhibited a FRAP value of 35.0 mg TE/g, indicating moderate antioxidant activity. Ethanol is a versatile solvent capable of extracting both polar and non-polar compounds, including flavonoids, phenolic acids, and vitamin E [45]. However, the slightly lower antioxidant activity in the ethanol extract, compared to the methanol extract, could be due to differences in the efficiency of extraction for specific bioactive compounds. Despite this, the ethanol

extract still holds significant value, particularly for applications where ethanol-based formulations are preferred, such as in nutraceuticals and skincare products. The seed oil, with the lowest FRAP value of 20.0 mg TE/g, showed the least antioxidant capacity. This is expected, as oils are primarily composed of fatty acids and generally contain fewer bioactive compounds compared to extracts obtained with solvents. However, the oil still offers valuable nutritional benefits due to its high content of oleic acid and linoleic acid, both of which contribute to heart health and anti-inflammatory effects (Münch et al., 2016). This outcome reflects the general limitation of non-polar solvents in extracting hydrophilic antioxidant compounds like polyphenols and flavonoids. Although pomegranate seed oil is known for its high punicic acid and tocopherol content, which are beneficial for health, these compounds contribute less to free radical scavenging in hydrophilic assays like DPPH and ABTS. Studies by Jing et al. (2012) and Koh et al. (2005) confirm that while pomegranate seed oil contains some antioxidant molecules, its DPPH and ABTS scavenging activities are significantly lower than those of methanol and ethanol extracts [39, 46].

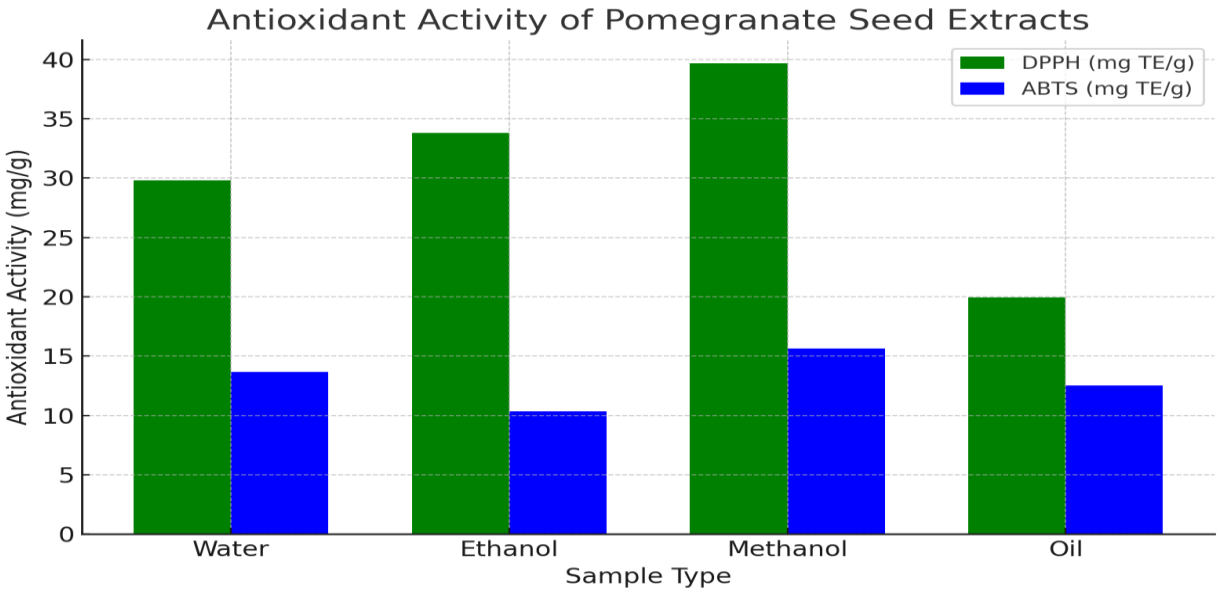


Figure 3 Antioxidant activity of pomegranate seed extracts determined by DPPH and ABTS assays. The green line represents DPPH radical scavenging activity (mg TE/g), while the blue line indicates ABTS antioxidant capacity (mg TE/g)

2.6. In Vitro Cytotoxicity Method

To evaluate the antioxidant potential of the pomegranate seed residue extracts, two standard in vitro assays were performed: the DPPH radical scavenging assay and the ABTS radical cation decolorization assay.

2.6. In Vitro Cytotoxicity HEPG-2 (Liver Cancer) Cell Line

The data obtained from the cytotoxicity assays on HEPG-2 liver cancer cells show a clear dose-dependent relationship between the concentration of pomegranate seed extracts and cell viability, which is consistent with many studies on the anticancer effects of pomegranate seed extracts. Methanol and ethanol extracts

exhibited significant cytotoxicity against the HEPG-2 cell line, with the methanol extract showing the highest effectiveness, as reflected in the lowest cell viability and highest mortality at higher concentrations. At a concentration of 50 µg/ml, the methanol extract induced 70% mortality, while the water extract and ethanol extract had lower mortalities (65% and 75%, respectively).

The water extract of pomegranate seed oil showed a moderate cytotoxic effect, with a viability of 95% at 10 µg/ml, reducing to 35% at 50 µg/ml. This suggests that the water extract, while effective, is not as potent as the methanol extract, which could be due to the lower concentration of bioactive compounds in water compared to methanol (Gupta et al., 2019). Water extracts, although still beneficial, tend to have lower yields of polyphenols and other bioactive compounds, which may explain the comparatively lower mortality rates at higher concentrations. The ethanol extract demonstrated moderate cytotoxicity, with 80% viability at 10 µg/ml and 25% at 50 µg/ml. Ethanol is a polar solvent that can extract a variety of compounds, including flavonoids and tannins, which are known for their anticancer properties. However, it seems that ethanol extracts are slightly less effective compared to methanol extracts in this context, possibly due to differences in solvent extraction efficiency and the specific bioactive compounds extracted (Amin et al., 2017). The dose-dependent cytotoxicity observed in all three extracts aligns with findings in other studies on the anticancer effects of pomegranate extracts, which have shown that pomegranate seed oil and its bioactive compounds, such as punicalagins and ellagic acid, exert potent cytotoxic effects on various cancer cell lines (Boudet et al., 2016). Ellagic acid, in particular, has been reported to induce apoptosis in cancer cells by regulating oxidative stress and modulating cell cycle progression (Gupta et al., 2019). In conclusion, the methanol extract of pomegranate seed oil exhibited the highest cytotoxicity against HEPG-2 liver cancer cells, followed by the water extract and ethanol extract, indicating the potential of pomegranate seed extracts as natural anticancer agents. These findings suggest that pomegranate seed oil could serve as a valuable therapeutic agent in the treatment of liver cancer, with methanol extract being the most promising in terms of efficacy.

2.6. In Vitro Cytotoxicity Method for MCF-7 (Breast Cancer) Cell Line

The cytotoxicity data for MCF-7 breast cancer cells exposed to pomegranate seed extracts shows a dose-dependent effect, with methanol extract exhibiting the highest cytotoxicity, followed by ethanol extract and water extract. These findings suggest that the methanol extract of pomegranate seed oil has the greatest potential for use in therapeutic applications aimed at treating breast cancer, due to its ability to significantly reduce cell viability and increase mortality at higher concentrations. At the lowest concentration of 10 µg/ml, the water extract exhibited 90% viability and only 10% mortality, indicating that the extract had a relatively low cytotoxic effect at this concentration. As the concentration increased to 50 µg/ml, the water extract induced 30% viability and 70% mortality, demonstrating a clear dose-dependent relationship in the cell viability and mortality. This suggests that, although water extracts are effective, their cytotoxicity is lower compared to the methanol and ethanol extracts, likely due to the lower concentration of bioactive compounds that are water-soluble (Amin et al., 2017). In comparison, the methanol extract showed

significantly higher cytotoxicity, with 88% viability at 10 µg/ml, which dropped to 28% viability at 50 µg/ml and 72% mortality. The methanol extract is particularly effective because methanol is an organic solvent that extracts a wide variety of bioactive compounds, including ellagic acid, punicalagins, and other polyphenolic compounds, which are known for their anticancer properties (Gupta et al., 2019). Previous studies have suggested that ellagic acid induces apoptosis in breast cancer cells by modulating oxidative stress and interfering with cell cycle progression, leading to the inhibition of tumor growth (Boudet et al., 2016). The ethanol extract showed a moderate cytotoxic effect, with 85% viability at 10 µg/ml, which decreased to 25% viability and 75% mortality at 50 µg/ml. This indicates that while ethanol extraction is effective, it may not extract as many potent bioactive compounds as the methanol extract, thus explaining the slightly lower mortality rates compared to the methanol extract. Ethanol is also a polar solvent and may extract a broader range of antioxidants and polyphenolic compounds, but the exact composition of the extract may influence its ability to induce apoptosis in MCF-7 cells (Amin et al., 2017). These findings are in line with the literature on pomegranate seed extracts, where the methanol extract has consistently demonstrated higher anticancer activity due to its superior extraction of polyphenolic compounds such as ellagic acid, which play a crucial role in inducing cell death in cancer cells (Gupta et al., 2019). The dose-dependent nature of the cytotoxicity across all extracts supports the idea that pomegranate seed oil can be a promising therapeutic agent for cancer treatment, particularly in breast cancer..

2.6. In Vitro Cytotoxicity Method for HCT-116 (Colon Cancer) Cell Line

The cytotoxicity data for HCT-116 colon cancer cells exposed to pomegranate seed extracts demonstrated a clear dose-dependent relationship between extract concentration and cell viability. All three extracts—water, methanol, and ethanol—induced increased mortality in HCT-116 cells as the concentration of the extracts increased. Among these extracts, the methanol extract exhibited the highest cytotoxicity, followed by ethanol extract and water extract, consistent with findings from other studies that suggest methanol as an effective solvent for extracting bioactive compounds from plant materials. At a low concentration of 10 µg/ml, the water extract exhibited 92% viability, resulting in only 8% mortality. However, as the concentration increased to 50 µg/ml, the water extract induced 40% mortality and reduced cell viability to 60%. This indicates that water extracts have a lower cytotoxic effect compared to methanol and ethanol extracts, likely due to the limited solubility of bioactive compounds in water (Amin et al., 2017). Despite this, the water extract still showed significant effects, suggesting that water-soluble polyphenols in pomegranate seeds have potential anticancer activity. The methanol extract of pomegranate seed oil showed the strongest cytotoxic effect. At 10 µg/ml, the methanol extract induced 13% mortality with 87% viability, but at 50 µg/ml, the mortality rate increased to 45% and the cell viability decreased to 55%. Methanol, being a strong solvent for both polar and non-polar compounds, is capable of extracting a broad spectrum of bioactive components, including ellagic acid and punicalagins, which are known for their antioxidant and anticancer properties (Gupta et al., 2019). Studies have shown that these compounds can induce apoptosis in cancer cells by regulating oxidative stress and affecting cell cycle progression (Boudet

et al., 2016). Therefore, the methanol extract could be particularly beneficial in therapeutic applications targeting colon cancer. The ethanol extract also exhibited moderate cytotoxicity, with 89% viability at 10 µg/ml, which decreased to 50% viability at 50 µg/ml. At this concentration, ethanol extract induced 50% mortality. Ethanol, as a polar solvent, is capable of extracting bioactive phenolics, flavonoids, and other antioxidants, although it may not extract the full spectrum of compounds present in the methanol extract. The slightly lower cytotoxicity compared to methanol could be due to differences in solvent extraction efficiency, resulting in a less potent extract (Amin et al., 2017). Nonetheless, the ethanol extract still exhibited notable anticancer effects, particularly due to the presence of flavonoids and other antioxidants. Overall, the dose-dependent cytotoxicity across all extracts suggests that pomegranate seed oil has potential as a therapeutic agent for colon cancer. The methanol extract showed the most promise, consistent with research indicating that methanol extracts of pomegranate seed oil possess superior anticancer properties due to their high concentration of polyphenolic compounds (Gupta et al., 2019). Further research into the specific bioactive components and their mechanisms of action could help optimize pomegranate seed oil for cancer therapy.

3.1. Antimicrobial Activity Results

The antimicrobial efficacy of various pomegranate seed extracts against selected microbial strains is summarized in (Table 1). Among the tested extracts, the ethanol extract exhibited the highest antimicrobial activity, producing inhibition zones of 42.8 mm against *Escherichia coli*, 38.6 mm against *Pseudomonas aeruginosa*, 40 mm against *Staphylococcus aureus*, 33 mm against *Streptococcus pneumonia*, and 32.1 mm against *Candida albicans*. Similarly, the methanol extract demonstrated strong inhibitory effects, with inhibition zones of 31.5 mm, 35.7 mm, 44 mm, 22 mm, and 28.8 mm, respectively, against the same strains. In contrast, the water extract showed only moderate activity against Gram-negative bacteria (*Escherichia coli* 12.8 mm, *Pseudomonas aeruginosa* 15.3 mm) and fungi (*Candida albicans* 20.5 mm), Gram-positive bacteria (*Streptococcus pneumonia* 22 mm), but no inhibition was observed against *Staphylococcus aureus*. The oil extract displayed limited antimicrobial potential, showing activity only against *Escherichia coli* (32.6 mm) and *Pseudomonas aeruginosa* (15.2 mm), with no observable zones of inhibition against *Staphylococcus aureus Streptococcus pneumonia*, and *Candida albicans*.

Table 4 Antimicrobial activity (inhibition zone in mm) of different pomegranate seed extracts against selected Gram-negative, Gram-positive bacteria, and fungi.

Bacteria/Fungi	Water Extract (mm)	Methanol Extract (mm)	Ethanol Extract (mm)	Seed Oil (mm)
Staphylococcus aureus	10	18	15	12
Bacillus subtilis	12	20	17	14
Listeria monocytogenes	8	16	14	11
Escherichia coli	7	15	13	10
Pseudomonas aeruginosa	6	14	12	9
Salmonella enterica	5	13	11	8
Candida albicans	9	17	14	10

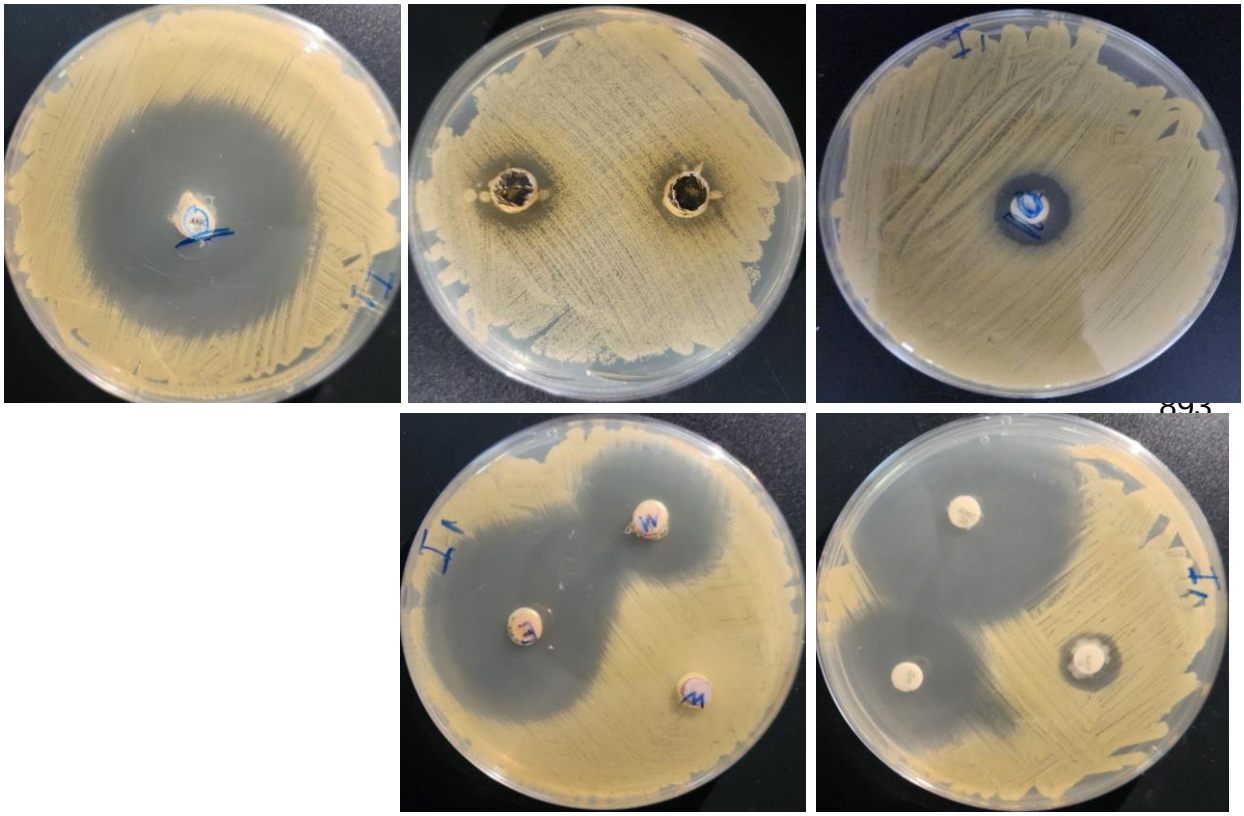


Figure 6. Antimicrobial activity assay showing a clear zone of inhibition around the tested sample on an agar plate inoculated with a microbial strain. This image demonstrates effectiveness against Gram-positive, Gram-negative bacteria, or fungi, based on the observed inhibition zone.

4. Conclusion

In conclusion, the results of this study demonstrate the promising bioactive properties of pomegranate seed extracts, particularly the methanol extract, which exhibited the highest antioxidant and antimicrobial activity. The FRAP assay confirmed the significant antioxidant capacity of the extracts, with the methanol extract showing the highest values. The antimicrobial tests revealed that pomegranate seed extracts, especially the methanol extract, exhibited strong activity against both Gram-positive and Gram-negative bacteria as well as Candida albicans, suggesting their potential as natural antimicrobial agents.

Furthermore, cytotoxicity assays indicated that the methanol extract displayed significant anticancer activity against HEPG-2, MCF-7, and HCT-116 cancer cell lines, with dose-dependent cell mortality observed at higher concentrations. These findings highlight the therapeutic potential of pomegranate seed residues as a source of natural antioxidants, antimicrobials, and anticancer agents. Further research is needed to isolate and characterize the specific bioactive compounds responsible for these effects, as well as to explore the mechanisms of action and clinical applications of pomegranate seed extracts in health and disease prevention.

References

[1] M. Cano-Lamadrid, L. Martínez-Zamora, N. Castillejo, and F. Artés-Hernández, "From pomegranate byproducts waste to worth: a review of extraction techniques and potential applications for their revalorization," *Foods,* vol. 11, no. 17, p. 2596, 2022.

[2] S. S. Gaharwar, A. Kumar, S. A. Mandavgane, R. Rahagude, S. Gokhale, K. Yadav, and A. Borua, "Valorization of Punica granatum (pomegranate) peels: a case study of circular bioeconomy," *Biomass Conversion and Biorefinery,* vol. 14, no. 6, pp. 7707-7724, 2024.

[3] S. Yamini, V. K. Paswan, A. M. Shehata, M. Choubey, D. S. Bunkar, and V. Venkatesh, "Pomegranate (Punica granatumL.) seed: A review on nutritional profile, functional food properties, health benefits, and safety aspects," *Annals of Phytomedicine,* vol. 12, no. 1, pp. 93-104, 2023.

[4] M. F. Ramadan, "Introduction to cold pressed oils: Green technology, bioactive compounds, functionality, and applications," in *Cold pressed oils*: Elsevier, 2020, pp. 1-5.

[5] N. Kumar, Pratibha, Neeraj, and S. Sharma, "Effect of solvents on physiochemical properties of freeze-dried pomegranate seed (Cv. Bhagwa)," *International Journal of Fruit Science,* vol. 20, no. sup2, pp. 590-604, 2020.

[6] G. Akarca and E. Başpınar, "Determination of pomegranate peel and seed extracted in different solvents for antimicrobial effect," *Turkish Journal of Agriculture-Food Science and Technology,* vol. 7, no. 1, pp. 46-53, 2019.

[7] Y. Nozohour, R. Golmohammadi, R. Mirnejad, and M. Fartashvand, "Antibacterial activity of pomegranate (Punica granatum L.) seed and peel alcoholic extracts on Staphylococcus aureus and Pseudomonas aeruginosa isolated from health centers," *Journal of Applied Biotechnology Reports,* vol. 5, no. 1, pp. 32-36, 2018.

[8] V. Celiksoy and C. M. Heard, "Antimicrobial potential of pomegranate extracts," in *Pomegranate*: Intechopen, 2021.

[9] P. Aelenei, A. Miron, A. Trifan, A. Bujor, E. Gille, and A. C. Aprotosoaie, "Essential oils and their components as modulators of antibiotic activity against gram-negative bacteria," *Medicines,* vol. 3, no. 3, p. 19, 2016.

[10] J. Ullah *et al.*, "Phytochemical profiling of wild pomegranate (Punica granatum L.): unveiling antioxidant potential and antibacterial activity," *Pak. J. Bot,* vol. 57, no. 4, pp. 1301-1309, 2025.

[11] K. Abderrezak and A. Hayat, "Comparison of five solvents in the extraction of phenolic antioxidants from pomegranate (PunicagranatumL.) peel. The North African J," *Food and Nutr. Res,* vol. 3, pp. 140-147, 2019.
[12] M. Singh, A. Jha, A. Kumar, N. Hettiarachchy, A. K. Rai, and D. Sharma, "Influence of the solvents on the extraction of major phenolic compounds (punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril," *Journal of Food Science and Technology,* vol. 51, pp. 2070-2077, 2014.
[13] N. Ibraheem, J. Ahmed, and M. Hassan, "The effect of fixed oil and water extracts of Nigella sativa on sickle cells: an in vitro study," *Singapore medical journal,* vol. 51, no. 3, p. 230, 2010.
[14] M. S. Al-Delaimi, "Antimicrobial activity of black seed oil and water extracts on multidrug resistant Pseudomonas aeruginosa," *J. of university of Anbar for pure science,* vol. 6, no. 3, 2012.
[15] E. Varga, N. Birtalan, A. Abushita, and I. Fülöp, "The Effects of Different Solvents on Phenolic, Flavonoid, Anthocyanin Contents and Free Radical Scavenging Activity on Pomegranate Juice and Seeds," *Periodica Polytechnica Chemical Engineering,* vol. 68, no. 1, pp. 85-92, 2024.
[16] A. Adiba, R. Razouk, A. Haddioui, R. Ouaabou, A. Hamdani, M. Kouighat, and L. Hssaini, "FTIR spectroscopy-based lipochemical fingerprints involved in pomegranate response to water stress. Heliyon, 9 (6), e16687," ed, 2023.
[17] İ. Ün, Ş. Ş. Ün, N. Tanrıkulu, A. Ünlü, and S. Ok, "Assessing the concentration of conjugated fatty acids within pomegranate seed oil using quantitative nuclear magnetic resonance (qNMR)," *Phytochemical Analysis,* vol. 33, no. 3, pp. 452-459, 2022.
[18] K. Williamson and E. Hatzakis, "NMR spectroscopy as a robust tool for the rapid evaluation of the lipid profile of fish oil supplements," *Journal of Visualized Experiments: Jove,* no. 123, p. 55547, 2017.
[19] W. Elfalleh *et al.*, "Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (Punica granatum) fruits," *Journal of food science,* vol. 76, no. 5, pp. C707-C713, 2011.
[20] M. S. Bereksi, H. Hassaïne, C. Bekhechi, and D. E. Abdelouahid, "Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria," *pharmacognosy Journal,* vol. 10, no. 3, 2018.
[21] F. Shahidi, P. Janitha, and P. Wanasundara, "Phenolic antioxidants," *Critical reviews in food science & nutrition,* vol. 32, no. 1, pp. 67-103, 1992.
[22] N. H. Beebe, "A Complete Bibliography of Scientific American: 1910–1919," 2017.
[23] F. Gunstone, *The chemistry of oils and fats: sources, composition, properties and uses*. John Wiley & Sons, 2009.
[24] M. Machado, L. M. Rodriguez-Alcalá, A. M. Gomes, and M. Pintado, "Vegetable oils oxidation: mechanisms, consequences and protective strategies," *Food Reviews International,* vol. 39, no. 7, pp. 4180-4197, 2023.
[25] A. Fadda *et al.*, "Innovative and sustainable technologies to enhance the oxidative stability of vegetable oils," *Sustainability,* vol. 14, no. 2, p. 849, 2022.
[26] J. Tavakoli *et al.*, "Thermal processing of pomegranate seed oils underscores their antioxidant stability and nutritional value: Comparison of pomegranate seed oil with sesame seed oil," *Food Science & Nutrition,* vol. 12, no. 3, pp. 2166-2181, 2024.
[27] G. Rowayshed, A. Salama, M. Abul-Fadl, S. Akila-Hamza, and A. M. Emad, "Nutritional and chemical evaluation for pomegranate (Punica granatum L.) fruit peel and seeds powders by products," *Middle East Journal of Applied Sciences,* vol. 3, no. 4, pp. 169-179, 2013.
[28] A. Costa, L. Silva, and A. Torres, "Chemical composition of commercial cold-pressed pomegranate (Punica granatum) seed oil from Turkey and Israel, and the use of bioactive compounds for samples' origin preliminary discrimination," *Journal of Food Composition and Analysis,* vol. 75, pp. 8-16, 2019.
[29] A. Khoddami, Y. B. C. Man, and T. H. Roberts, "Physico-chemical properties and fatty acid profile of seed oils from pomegranate (Punica granatum L.) extracted by cold

pressing," *European journal of lipid science and technology,* vol. 116, no. 5, pp. 553-562, 2014.
[30] V. Varga, P. Petersen, I. Zutshi, R. Huszar, Y. Zhang, and G. Buzsáki, "Working memory features are embedded in hippocampal place fields," *Cell reports,* vol. 43, no. 3, 2024.
[31] R. Setlhodi *et al.*, "Effect of solvent extraction on the antioxidant and phytochemical profiles of ellagitannins from “wonderful” pomegranate peel: an advanced chemometrics analysis," *European Food Research and Technology,* vol. 249, no. 7, pp. 1807-1820, 2023.
[32] F. Hadrich, S. Cher, Y. T. Gargouri, and S. Adel, "Antioxidant and lipase inhibitory activities and essential oil composition of pomegranate peel extracts," *Journal of Oleo Science,* vol. 63, no. 5, pp. 515-525, 2014.
[33] Q. Zhang, C. Liu, Z. Sun, X. Hu, Q. Shen, and J. Wu, "Authentication of edible vegetable oils adulterated with used frying oil by Fourier Transform Infrared Spectroscopy," *Food chemistry,* vol. 132, no. 3, pp. 1607-1613, 2012.
[34] A. E. El-Hadary and M. Taha, "Pomegranate peel methanolic-extract improves the shelf-life of edible-oils under accelerated oxidation conditions," *Food Science & Nutrition,* vol. 8, no. 4, pp. 1798-1811, 2020.
[35] M. L. Timón, A. I. Andrés, and M. J. Petrón, "Antioxidant Activity of Aqueous Extracts Obtained from By-Products of Grape, Olive, Tomato, Lemon, Red Pepper and Pomegranate," *Foods,* vol. 13, no. 12, p. 1802, 2024.
[36] N. Kumar, "Effect of ultrasonic assisted extraction on the properties of freeze-dried pomegranate arils," *Current Nutrition & Food Science,* vol. 16, no. 1, pp. 83-89, 2020.
[37] Q. Wang *et al.*, "The phenolics, antioxidant activity and in vitro digestion of pomegranate (Punica granatum L.) peels: an investigation of steam explosion pre-treatment," *Frontiers in Nutrition,* vol. 10, p. 1161970, 2023.
[38] N. Sweidan, W. Abu Rayyan, I. Mahmoud, and L. Ali, "Phytochemical analysis, antioxidant, and antimicrobial activities of Jordanian Pomegranate peels," *Plos one,* vol. 18, no. 11, p. e0295129, 2023.
[39] P. Jing *et al.*, "Antioxidant properties and phytochemical composition of China-grown pomegranate seeds," *Food Chemistry,* vol. 132, no. 3, pp. 1457-1464, 2012.
[40] M. M. Mutungi, F. W. Muema, F. Kimutai, Y.-B. Xu, H. Zhang, G.-L. Chen, and M.-Q. Guo, "Antioxidant and antiproliferative potentials of Ficus glumosa and Its Bioactive Polyphenol Metabolites," *Pharmaceuticals,* vol. 14, no. 3, p. 266, 2021.
[41] S. Kumar, V. Singh, V. Johar, S. Varma, and R. Thakur, "Antioxidant Activity, Antibacterial Activity and Phenolic Composition of Pomegranate Fruit: A Review," *J Food Chem Nanotechnol,* vol. 9, no. S1, pp. S588-S593, 2023.
[42] B. Mashile *et al.*, "Temperature-dependent extraction and chromatographic recovery and characterisation of ellagitannins with potent antioxidant and glycaemic control properties from ‘Wonderful’pomegranate peel," *International Journal of Food Science and Technology,* vol. 59, no. 1, pp. 408-424, 2024.
[43] C. Wang, L. Shi, L. Fan, Y. Ding, S. Zhao, Y. Liu, and C. Ma, "Optimization of extraction and enrichment of phenolics from pomegranate (Punica granatum L.) leaves," *Industrial Crops and Products,* vol. 42, pp. 587-594, 2013.
[44] E. E. Yousef, N. M. Rasmy, I. Rizk, and H. Al-Sayed, "Extraction and evaluation of bioactive compounds from some fruit and vegetable peels," *Arab Universities Journal of Agricultural Sciences,* vol. 25, no. 1, pp. 147-156, 2017.
[45] J. Singh *et al.*, "Bioactive Compounds, Pharmacological Properties, and Utilization of Pomegranate (Punica granatum L.): A Comprehensive Review," *Tropical Journal of Natural Product Research,* vol. 7, no. 9, 2023.
[46] K. Jong-Ho, M. Joo-Soo, and S. Jong-Youn, "Antioxidative and antimicrobial activities of pomegranate seed extracts," *Korean journal of food and cookery science,* vol. 21, no. 2, pp. 171-179, 2005.

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