

# Promising Source of Phytochemicals, Antioxidants, and Antibacterial Actions of Pomegranate (*Punica granatum* L.) Juice Extracts Using Different Solvents

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## ABSTRACT

**Background:** The highly nutritious pomegranate (*Punica granatum* L.) is valued for its numerous bioactive substances, such as phenols, flavonoids, and tannins, which provide to its numerous health-enhancing properties. **Objectives:** The nutritional composition, antioxidant properties, and antimicrobial properties of pomegranate fruit juice preparations were examined in this work by using solvents with different polarity (aqueous ethanol, ethyl acetate and hexane). **Materials and Methods:** Qualitative and quantitative analyses confirmed that aqueous ethanol extracts consist the highest levels of total phenolics and flavonoids, which strongly correlated with antioxidant activity. **Results:** Antioxidant assays revealed that aqueous ethanol extracts demonstrated the strongest radical scavenging capacity ( $IC_{50} = 15.20 \mu\text{g/mL}$ ) and highest ferric reducing power ( $13.1 \pm 0.6 \text{ mg AAE/g DW}$ ) compared with other extracts. In antibacterial screening, aqueous ethanol extracts displayed broad-spectrum inhibitory effects, particularly against *Staphylococcus aureus* and *Klebsiella pneumoniae*. **Conclusion:** These findings highlight that solvent choice plays a critical role in extracting phytochemicals from pomegranate juice and that aqueous ethanol yields extracts with potent antioxidant and antimicrobial properties. This study exposes the possible outcomes of pomegranate juice as a natural basis of functional biologically active substances for therapeutic and nutraceutical applications.

**Keywords:** Antibacterial, Antioxidant, Flavonoids, Pomegranate, Polyphenols.

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## INTRODUCTION

Pomegranate (*Punica granatum* L.) has long been recognized as a rich source of biological active phytochemicals, particularly polyphenols such as punicalagins, punicalins, ellagic acid, anthocyanins, ellagitannins, and tannins, that are primarily present in the peel, husk, and juice (Amyrgialaki *et al.*, 2014; De Souza *et al.*, 2020; Brighenti *et al.*, 2017). The methods of extraction and solvent utilised have a significant effect on the composition and output of these chemical substances. Conventional methods such as Soxhlet extraction with methanol or ethanol yield high levels of polyphenols, with methanol often proving slightly more effective (Ahmetovic *et al.*, 2025). In recent years, advanced techniques which include microwave-assisted,

ultrasound-assisted, enzyme-assisted, and supercritical fluid extraction have demonstrated improved extraction efficiency, shorter processing times, and higher-quality extracts, though they require greater optimization and investment for large-scale applications (Pm *et al.*, 2024; Jauhar *et al.*, 2018). Solvent polarity plays a decisive role: ethanol-water mixtures are particularly effective for hydrophilic compounds, while the inclusion of acid (e.g., citric or hydrochloric acid) further enhances the extraction of punicalins and ellagic acid (Amyrgialaki *et al.*, 2014; Brighenti *et al.*, 2017). Despite their simplicity, methods such as maceration are less efficient, underscoring the importance of optimized extraction conditions including temperature, solvent-to-solid ratios, and particle size (Li 011, De Souza *et al.*, 2020). The development of eco-friendly extraction strategies combining green solvents and novel technologies holds promise for maximizing polyphenolic yields for dietary supplements and natural health products (Pm *et al.*, 2024; Jauhar *et al.*, 2018).

Pomegranate phytochemicals contain powerful antioxidant properties as they include a large quantity of compounds such as flavonoids, tannins, gallic acid and other components that



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scavenge free radicals and avoid lipid peroxidation (Singh *et al.*, 2002; Yasoubi *et al.*, 2007; Turkmen *et al.*, 2021; Wijanti *et al.*, 2023). DPPH radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and lipid oxidation inhibition assays are frequently used to assess their antioxidant activity; greater efficacy is demonstrated by reduced IC<sub>50</sub> levels (Chasanah, 2021; Turkmen *et al.*, 2021). Among different parts of the fruit, the peel consistently demonstrates the maximum total phenolic content and antioxidant potential, often more active than the seeds and juice (Singh *et al.*, 2002; Yasoubi *et al.*, 2007; Turkmen *et al.*, 2021). Methanol and acetone, particularly when combined with ultrasound-assisted methods, are especially effective for extracting phenolics from the peel, producing extracts with superior antioxidant activity compared to water or ethanol alone (Turkmen *et al.*, 2021; Chasanah, 2021). Emerging methods such as hydrodynamic cavitation and decoction have further enhanced antioxidant yields, with hydrodynamic cavitation offering high activity under mild conditions (Kaneria *et al.*, 2012; Minutolo *et al.*, 2023). Although juice and aril extract also display considerable phenolic content, their antioxidant activities are generally weaker than peel extracts, reflecting compositional differences (Lantzouraki *et al.*, 2016; Büdienė *et al.*, 2021).

In addition to their antioxidant activities, pomegranate polyphenols exhibit significant antibacterial action against bacteria of all kinds, including multidrug-resistant strains (Banu, 2019; Mendes *et al.*, 2023; Nuamsetti *et al.*, 2012; Kumar *et al.*, 2023; Bouneb *et al.*, 2021). Peel extracts have shown antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* when prepared by solvents like ethanol, methanol water, or acetone (Nuamsetti *et al.*, 2012; Hazzani *et al.*, 2013; Gosset-Erard *et al.*, 2021). Gallic acid, Punicalagin, ellagic acid, and different water-soluble tannins are the bioactive compounds primarily responsible for these outcomes. These compounds exhibit antibacterial effects by a different mechanism, such as breakdown of bacteria's membranes, hindering proteins that are linked to bacterial resistance, restriction of efflux pumps, and also structural breakdown of bacterial cell wall (Mendes *et al.*, 2023; Dey *et al.*, 2012). Importantly, some extracts enhance the activity of antibiotics like ciprofloxacin against resistant Gram-negative bacteria, suggesting synergistic applications (Dey *et al.*, 2012).

Pomegranate polyphenols were associated with valuable cardioprotective qualities in contrast to their antimicrobial properties. They improve endothelial function, decrease the synthesis of macrophage foam cells, restrict the oxidative damage of Low-Density Lipoprotein (LDL), and retard the development of plaque formation (Aviram *et al.*, 2002; Delgado *et al.*, 2020; Aviram and Rosenblat, 2013; Benchagra *et al.*, 2021). Clinical and preclinical studies further indicate that pomegranate polyphenols lower blood pressure, enhance Paraoxonase 1 (PON1) activity,

and reduce oxidative stress, thereby reducing cardiovascular risk (Viuda-Martos *et al.*, 2010; Saporbekova *et al.*, 2022; Alami *et al.*, 2023; Siddiqui *et al.*, 2024). Their combined antioxidant and antimicrobial activities also support potential applications as natural food preservatives and therapeutic agents (Kumar *et al.*, 2018; Viuda-Martos *et al.*, 2010). Nevertheless, while *in vitro* and animal studies strongly support these effects, evidence from clinical trials remains limited and occasionally inconsistent, underscoring the need for further translational research (Ferrara *et al.*, 2022; Alami *et al.*, 2023).

Considering the above context, the focus of this study was to evaluate both the qualitative and quantitative phytochemical profiling of pomegranate fruit juice preparations prepared with three different solvents used. In addition to their antimicrobial capacity against both Gram-positive as well as Gram-negative bacteria was studied and their ability to scavenge free radicals was checked using 2,2-diphenyl-1-picrylhydrazyl assay and Ferric Reducing Antioxidant Power assays. By linking solvent-dependent extraction efficiency with biological activities, this study goals to contribute to the optimization of pomegranate polyphenol utilization for functional food and therapeutic applications.

## MATERIALS AND METHODS

### Sample- Plant Material

In April 2025, freshly picked pomegranate fruits were collected from pomegranate bushes. Healthy fruits free of biological contamination or notable defects in appearance were accepted. Using double-distilled water (ddH<sub>2</sub>O) fruits were washed twice and disinfected with 150 µL/L sodium hypochlorite solution for 1 min. Arils were manually detached and processed for juice extraction (Cite: standard disinfection and preparation protocols).

### Extraction of Juice and Phytochemicals

Pomegranate juice was prepared manually, and extracts were obtained using three solvents of varying polarity: aqueous ethanol (70% ethanol:30% water), hexane and ethyl acetate. The solvent and juice combinations were mixed in an incubator (Stuart TM, SBS40, China) at 100 revolutions per minute over 4 hr at 35°C. Upon filtering the extracted substances, solvents are allowed to dry at the usual temperature. For further examination, the dried extracts are kept in air-tight containers at 4°C (Turkyilmaz *et al.*, 2013; Idris & Atif, 2017).

## Phytochemical Analysis

### Qualitative Screening

Qualitative phytochemical analysis was accomplished to detect major phytochemical groups (alkaloids, flavonoids, tannins, saponins, etc.) using standard procedures described by Evans WC (2009), Yang *et al.*, (2009).

## Total Phenolic Content (TPC)

By using Folin-Ciocalteu test is employed to identify total phenolic count Y Li *et al.*, (2006); Li, (2011). 0.3 mL of extracts and 1.7 mL 10% of the Folin-Ciocalteu reagent were added together and the resulting mixture then underwent incubation for 10 min at room temperature A UV-vis spectrophotometer was deployed to identify absorbance at 760 nm. Results are presented as milligram Gallic Acid Equivalents per gram of Dry Weight (mg GAE/g DW) based on calibration curve a gallic acid. All outcomes were made in triplicate.

## Total Flavonoid Content (TFC)

Total flavonoid count was evaluated by using Li, (2011) and Yang *et al.*, (2009). extract solution. following 40 min of room temperature incubation, absorbance was taken at the wavelength of 415 nm.

The calibration graph was developed by quercetin, then the outcomes were presented as milligrams of quercetin equivalents per gramme of dry weight (mg QuE/g DW).

## Antioxidant Activity

### DPPH Radical Scavenging Assay

The DPPH method serves to calculate the extracts' free radical ability. Sweidan *et al.*, (2023). 3 mL of a 0.1 millimolar solution of DPPH mixture in methanol were added to extracts (50-250 µg/mL). Absorbance was detected at the wavelength of 517 nm after a 30 min dark incubation time at room temperature. The following equation were utilised to calculate the radical scavenging action:

$$\text{Per cent inhibition} = \frac{\text{Blank} - \text{Sample}}{\text{Blank}} \times 100$$

## Ferric Reducing Antioxidant Power (FRAP)

An FRAP method was carried out according to Benzie and Strain (1996). Ardekani *et al.*, 2011 or equivalent standard. In brief, 500 µL of plant extract was dissolved in 1 mL of 1% K<sub>3</sub>Fe (CN) with 1 mL of a buffered phosphate (0.2 M, pH= 6.6). The reaction fluid absorbance was detected at 700 nm upon a 15-min incubation time at 37°C. Outcomes were expressed as mg ascorbic acid equivalents per gramme of dry mass (mg AAE/g DW), using ascorbic acid acting being the standard for measurement.

## Antibacterial activity

### Microbial Strains and Culture Conditions

Gram-positive organisms (*Staphylococcus aureus* ATCC 25923) and gram-negative organisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 35657) were tested to examine the extracts' antibacterial properties. prior to their use, isolates were grown in nutrient broth at 37°C and stored in glycerol stocks at -80°C. The drug used as the positive reference is fluoroquinolone like ciprofloxacin (Cite: CLSI guidelines for antimicrobial assays).

## Agar Diffusion Assay

The agar-well diffusion technique was adopted to determine the antibacterial effect. In short, Mueller-Hinton agar plates were coated with overnight culture strains of bacteria that were adjusted by about  $4 \times 10^2$  CFU/mL. Aseptic wells about a width of 1.5 mL were stocked with 100 µL of extract mixtures (Turkyilmaz *et al.*, 2013; Betanzos-Cabrera *et al.*, 2015). Plates were incubated over between 16 and 18 hr at 37°C. Bacterial growth inhibition is estimated in millimetres. with included both negative (solvent) and positive vehicle controls.

## Ethical Statement

This study is performed *in vitro* lab and there is no participation of animals and humans in any manner.

## Statistical Analysis

The tests were carried out in triplicate, in addition the data is presented as average and Standard Deviation (SD). SPSS v.20 (IBM Corp., Armonk, NY, USA) was adopted for data analysis. 2 factor ANOVA were employed to analyse variance between groups, and  $p < 0.05$  was chosen for the statistical significance range.

## RESULTS

### Qualitative Phytochemical analysis

Primary phytochemical screening of pomegranate juice extracts revealed the existence of some important macromolecules (Table 1). Extracts obtained with aqueous ethanol, ethyl acetate, and hexane exhibited distinct phytochemical profiles, reflecting the impact of polar nature of solvent on the extraction process.

Overall, phenols, flavonoids, tannins, terpenoids, and resins were consistently detected across most extracts, whereas alkaloids, steroids, saponins, coumarins, carboxylic acids, quinones, and fats were absent. Carbohydrates were present in both aqueous ethanol and ethyl acetate extracts, while amino acids were detected only in the aqueous ethanol extract. Notably, tannins were present in aqueous ethanol and hexane extracts but absent in the ethyl acetate fraction. Among the identified groups, phenolic compounds and flavonoids were emphasized due to their well-documented antioxidant, antimicrobial, and cardioprotective properties. These findings provide preliminary evidence of the broad phytochemical diversity in pomegranate juice and justify further quantitative and functional assays to determine their biological relevance.

### Total Phenolic and Flavonoid Content

Depending on the used solvent, pomegranate juice extracts' Total Flavonoid Content (TFC) total phenolic count varied markedly (Table 2). Aqueous ethanol extracts exhibited the maximum levels of flavonoid and phenolic constituents out of all studied solvents, with average levels of 20.12±12.20 mg QuE/g DW and

**Table 1: Preliminary qualitative analysis of phytochemicals.**

Test	Inference		
	Aqueous ethanol	Ethyl acetate	Hexane
Steroid	-	-	-
Alkaloid	-	-	-
Carbohydrate	+	+	-
Tannin	+	-	+
Terpenoid	+	+	+
Saponins	-	-	-
Flavonoids	+	+	+
Coumarins	-	-	-
Resin	+	+	+
Carboxylic acid	-	-	-
Amino acids	+	-	-
Phenols	+	+	+
Quinone	-	-	-
Fats	-	-	-

(+) = present; (-) = negative.

27.55±3.12 mg GAE/g DW, respectively. In contrast, ethyl acetate and hexane extracts yielded comparatively lower values.

Ethyl acetate extracts recorded 14.46±16.86 mg GAE/g DW for phenolics and 13.89±16.12 mg QuE/g DW for flavonoids, while hexane extracts contained 13.72±17.34 mg GAE/g DW and 5.97±3.52 mg QuE/g DW. The observed differences highlight the role of solvent polarity in determining extractability, with aqueous ethanol proving most effective for both phenolics and flavonoids.

The calibration curve for TPC was established using gallic acid ( $y = 0.0077x - 0.0635$ ,  $R^2 = 0.939$ ), while TFC was quantified based on a quercetin calibration curve ( $y = 0.0094x - 0.069$ ,  $R^2 = 0.9245$ ). The reliability of both standard curves confirms the robustness of the quantitative measurements.

### Antioxidant activities

Pomegranate juice extracts' antioxidant potential was determined using DPPH radical scavenging and FRAP tests, with gallic acid acting as the standard reference (Table 3).

The aqueous ethanol extract exhibited net worthy free radical scavenging activity in a concentration-dependent manner, with an  $IC_{50}$  value of 15.20 µg/mL. Although gallic acid exhibited stronger activity ( $IC_{50} = 13.09$  µg/mL), the extract retained considerable potency, corresponding to approximately 1.16-fold lower activity than the standard.

Similarly, Ferric Reducing Antioxidant Power (FRAP) analysis confirmed the antioxidant potential of the aqueous ethanol extract, which achieved 13.1±0.6 mg AAE/g DW, surpassing the activity of gallic acid (11.4±1.0 mg AAE/g DW). These outcomes

**Table 2: Total phenolic content and flavonoid content of extracts.**

Plant Extract	Polyphenols (mg GAE/g dw)	Flavonoids (mg QuE/g dw)
Aqueous ethanol	27.55±3.12	20.12±12.2
Ethyl acetate	14.46±16.86	13.89±16.12
Hexane	13.72±17.34	5.97±3.52

Values are Mean±SD ( $n=3$ ), expressed as gallic acid equivalents (GAE) and quercetin equivalents (QuE), respectively.

indicate that phenolic-rich extracts of pomegranate juice possess strong antioxidant activity, and suggest that phenolic content is a major determinant of variation in antioxidant capacity among extracts.

### Antibacterial Activity

The agar well-diffusion technique was applied for evaluating the antibacterial effectiveness of pomegranate juice extracts against both Gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli*) and Gram-positive (*S. aureus*) bacteria. The results (Table 4 and Figure 1) revealed broad-spectrum antibacterial activity, although the degree of inhibition varied across extracts and test organisms.

The aqueous ethanol extract demonstrated the most effective activity, producing inhibition zones ranging from 10.3±0.3 mm against *E. coli* to 13.2±0.9 mm against *Klebsiella pneumoniae*. Interestingly, it exhibited greater inhibition against *S. aureus* (12.45±0.5 mm) compared with the positive control (11.3±0.3 mm). In contrast, *n-butyl alcohol* and hexane extracts showed minimal or negligible inhibitory effects, with inhibition zones generally below 1 mm. Positive control (chloramphenicol)

produced consistent inhibition across all strains, while negative (nutrient broth) and vehicle (DMSO) controls were inactive, confirming the activity was specific to the bioactive constituents of pomegranate extracts. These findings suggest that phenolic-rich aqueous ethanol extract contributes substantially to the antibacterial properties of pomegranate juice.

## DISCUSSION

Pomegranate (*Punica granatum* L.) is widely consumed as a nutritious fruit and has long been admired in conventional medicine for its therapeutic properties. Its fruits are known to be rich in bioactive phytochemicals, particularly phenolic compounds, which contribute to their diverse biological effects, including antioxidant and antimicrobial activities. However, the composition and concentration of these compounds are strongly influenced by agricultural practices, cultivar type, geographic origin, maturation stage, and extraction methodology (Mphahlele *et al.*, 2014).

In the current investigation, qualitative screening discovered the occurrence of major phytochemical groups such as phenols, flavonoids, tannins, terpenoids, amino acids, and resins in pomegranate aril extracts. Among the solvents tested, aqueous ethanol extract demonstrated the most comprehensive phytochemical profile, confirming its efficiency in extracting polar bioactive compounds. These findings align with earlier reports that phenolics, flavonoids, and tannins are the predominant phytochemical classes in pomegranate pulp and juice (Beaulieu

*et al.*, 2015). The variation in solubility and extraction efficiency highlights the importance of solvent polarity, as different phytoconstituents partition differently depending on solvent properties.

The antioxidant potential of pomegranate juice extracts, assessed by DPPH and FRAP assays, further established the strong free radical scavenging and reducing power of the aqueous ethanol extract (Dey *et al.*, 2014). The IC<sub>50</sub> value obtained in the DPPH assay indicated substantial radical scavenging activity, comparable to gallic acid, a standard phenolic antioxidant. This is consistent with the known role of phenolic compounds as hydrogen donors and electron donors, neutralizing free radicals and breaking radical chain reactions.

Our FRAP results also highlighted the superior reducing capacity of the aqueous ethanol extract, reflecting its richness in electron-donating phenolic compounds. These findings are in line with previous studies showing that ethanol-water extracts of pomegranate peels and arils exhibit the highest phenolic content and antioxidant activity (Kumar *et al.*, 2023). Moreover, differences between solvents emphasize that antioxidant efficacy depends not only on total phenolic concentration but also on structural variations and synergistic interactions among individual compounds.

Alongside antioxidant effect, aqueous ethanol extracts confirmed superior antibacterial activity against both Gram-negative bacteria (*E. coli* and *Klebsiella pneumoniae*) and Gram-positive (*S. aureus*), with inhibition zones comparable or superior to those of the positive control. In contrast, *n-butyl alcohol* and hexane extracts showed negligible inhibitory effects. These findings suggest that phenolic-rich extracts are primarily responsible for antibacterial activity. Previous studies similarly reported moderate to strong antibacterial and antifungal activity of ethanol extracts of pomegranate peel and juice against pathogenic microorganisms (Dahham *et al.*, 2010; Rosas-Burgos *et al.*, 2017).

The antibacterial effect of pomegranate extracts has been credited to polyphenols, particularly ellagitannins and flavonoids,

**Table 3: Antioxidant activity compared with gallic acid standard.**

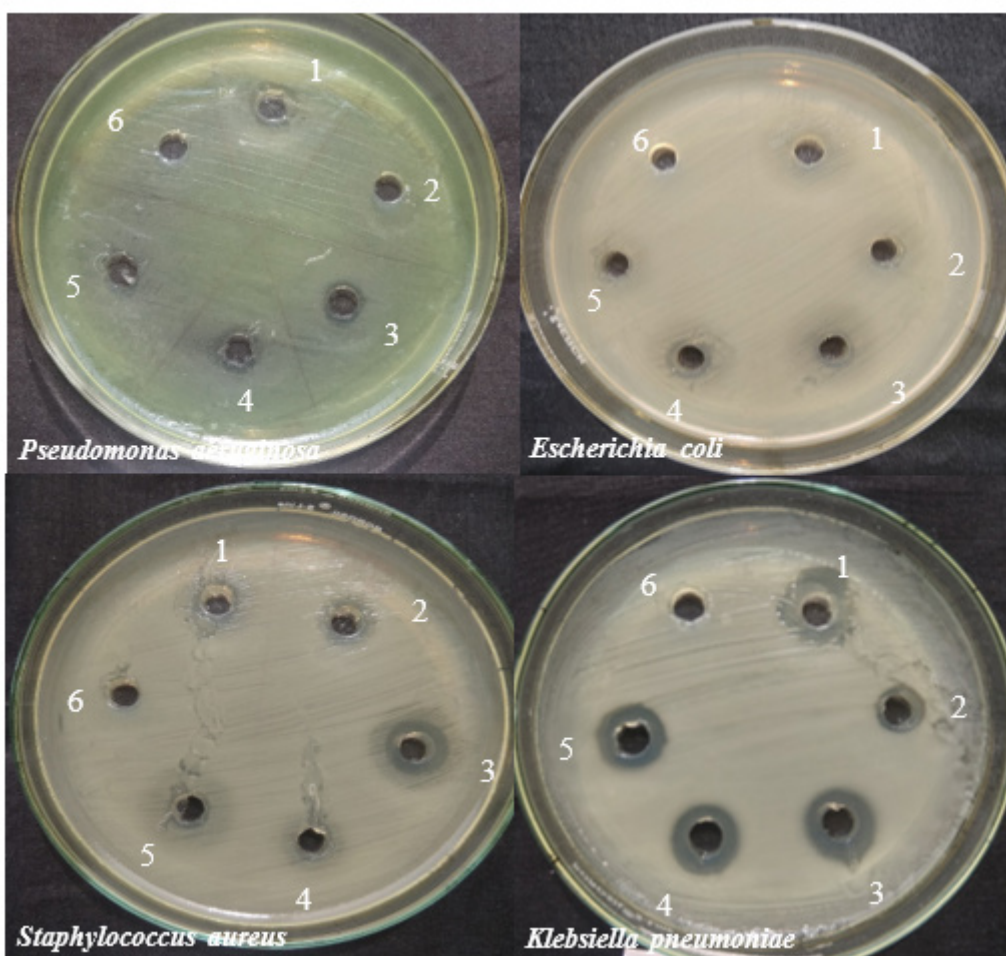
	Antioxidant activities	
	DPPH (IC <sub>50</sub> values, µg/mL)	FRAP (mg AAE/g dw)
Aqueous ethanol	15.20	13.1±0.6
Standard gallic acid	13.09	11.4±1.0

Values are mean±SD (n=3).

**Table 4: Antibacterial activity (Zone of inhibition).**

Samples	Test Organisms			
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
	Diameter of zoi (mm)			
Positive	12.4±0.2	11.3±0.3	10.4±0.1	13.2±0.2
Negative	0.1	0	0.1	0.2
Vehicle	0.2	0.1	0.1	0.1
Aqueous ethanol	10.3±0.3	12.45±0.5	13.2±0.9	0.3±0.3
n- butyl alcohol	0.2	0.5±0.3	0.4±0.2	0.4±0.1
Hexane	0.4±0.2	0.3±0.2	0.2±0.4	0.2

Values are mean±SD (n=3). CP: Chloramphenicol (positive control); NB: Nutrient broth (negative control); DMSO: Vehicle control.



**Figure 1:** Antibacterial action of various extracts on different bacterial strains by agar-well diffusion assay with numbered labels for controls and solvents. (1-positive control, 2- Negative control, 3- Vehicle control, 4- Aqueous ethanol, 5- Ethyl acetate, 6- Hexane).

which may disrupt bacterial cell walls, interfere with enzyme activity, or inhibit nucleic acid synthesis (Dey *et al.*, 2012). The broad-spectrum activity observed in this study supports their potential application as natural alternatives to synthetic antimicrobial agents.

## IMPLICATIONS AND FUTURE DIRECTIONS

Overall, our findings confirm that aqueous ethanol is the most suitable solvent for extracting bioactive compounds from pomegranate arils, yielding extracts with strong radical scavenging and antimicrobial activities. This reinforces the potential of pomegranate juice as a functional food ingredient with therapeutic benefits. However, future work should involve:

- Structural characterization of active compounds using advanced techniques (HPLC, LC-MS, NMR).
- Mechanistic studies to clarify modes of action of individual phytochemicals.

- *In vivo* studies to validate bio-efficacy and safety for clinical applications.

## CONCLUSION

This study demonstrated that pomegranate juice extracts are a rich source of phytochemicals, particularly flavonoids, phenols, and tannins, which help significantly to their antioxidant and antimicrobial activities. Among the tested solvents, aqueous ethanol verified to be the most effective for extracting these bioactive compounds, yielding the highest levels of total phenolic and flavonoid content as well as superior antioxidant and antibacterial activities. The strong association between phytochemical content and biological activity confirms the role of phenolic compounds as major contributors to the health-promoting properties of pomegranate. Nevertheless, the findings also suggest that other bioactive components may be involved, warranting further investigation. Overall, these results reinforce the potential of pomegranate juice as a natural

functional food and provide a basis for future research into its therapeutic applications and compound-specific mechanisms.

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## ABBREVIATIONS

**TPC:** Total Phenol Count; **TFC:** Total Flavonoid Count; **ddH<sub>2</sub>O:** Double Distilled Water; **FRAP:** Ferric Reducing Antioxidant Power; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **PONI:** Paraoxonase 1; **CP:** Chloramphenicol (Positive Control); **NB:** Nutrient Broth (Negative Control); **DMSO:** Dimethyl Sulfoxide; **ZOI:** Zone of Inhibition; **IC<sub>50</sub>:** Half Maximal Inhibitory Concentration; **AAE:** Ascorbic Acid Equivalents; **DW:** Dry Weight; **GAE:** Gallic Acid Equivalents; **QuE:** Quercetin Equivalents; **CFU:** Colony-Forming Unit; **SD:** Standard Deviation; **ANOVA:** Analysis of Variance; **ATCC:** American Type Culture Collection.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## AUTHOR CONTRIBUTIONS

**RPC:** Study designing, conceptualization, manuscript drafting, data collection, and resources; **SRD:** Study design and Conceptualization; **MBM:** Manuscript drafting and data analysis; **SS:** Data analysis, manuscript drafting and Data collection; **TJ:** Supervision and approval of final draft of manuscript.

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