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Phytochemical Profiling And Biological Evaluation Of Parrotia Persica

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ABSTRACT

Parrotia persica (Persian ironwood) is an underexplored medicinal plant with significant potential as a source of bioactive compounds. The present study aimed to evaluate its phytochemical composition and biological activities using various in vitro assays. Plant materials (leaves and bark) were extracted using solvents of different polarities, including n-hexane, ethyl acetate, methanol, and water. Qualitative phytochemical screening revealed the presence of flavonoids, phenols, tannins, saponins, and terpenoids, with methanolic and aqueous extracts showing a richer profile. Quantitative analysis demonstrated that the methanolic extract possessed the highest total phenolic content (85.2 mg GAE/g) and total flavonoid content (72.8 mg QE/g). The antioxidant activity assessed through the DPPH assay indicated that the methanolic extract exhibited the strongest radical scavenging activity with the lowest IC_{50} value (42.7 µg/mL), comparable to the standard ascorbic acid. Enzyme inhibitory studies showed significant prolyl endopeptidase (PEP) inhibition, suggesting potential neuroprotective applications. Additionally, antimicrobial activity evaluated using the

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agar well diffusion method revealed that methanolic extract produced the highest zones of inhibition against both Gram-positive and Gram-negative bacterial strains. Overall, the findings demonstrate a strong correlation between phytochemical content and biological activity, with polar extracts exhibiting superior efficacy. This study highlights *Parrotia persica* as a promising natural source of antioxidant, antimicrobial, and enzyme inhibitory agents, supporting its potential use in pharmaceutical and therapeutic applications. Further in vivo and clinical studies are recommended to confirm its safety and efficacy.

Keywords: *Parrotia Persica*, Phytochemical Profiling, Antioxidant Activity, Flavonoids, Enzyme Inhibition

INTRODUCTION

Medicinal plants have long served as a cornerstone in the discovery and development of therapeutic agents, contributing significantly to modern pharmacology and drug design. A substantial proportion of currently available drugs are derived either directly or indirectly from natural sources, particularly plants, which synthesize a wide array of structurally diverse secondary metabolites. These phytochemicals including alkaloids, flavonoids, terpenoids, phenolic acids, and glycosides exhibit a broad spectrum of biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective effects (Farhadpour et al., 2023). In recent years, there has been a renewed global interest in plant-based medicines, driven by the increasing prevalence of chronic diseases, drug resistance, and the demand for safer and more sustainable therapeutic alternatives (Rezadoost et al., 2023).

Among the lesser-explored medicinal plants, *Parrotia persica* (Persian ironwood), belonging to the family Hamamelidaceae, has emerged as a species of considerable scientific interest. Native to the Hyrcanian forests of Iran and parts of the Caucasus region, *P. persica* is primarily recognized for its ornamental value due to its vibrant seasonal foliage and strong, dense wood (Djapic, 2022). Despite its ecological and horticultural importance, the plant has received limited attention in terms of its phytochemical composition and pharmacological potential. This underexplored status presents an opportunity to investigate and uncover novel bioactive compounds that may contribute to future drug discovery (Ali et al., 2020).

The genus *Parrotia* is relatively small, yet it holds significant promise due to its capacity to produce diverse secondary metabolites. Preliminary phytochemical investigations have revealed that *P. persica* is particularly rich in flavonoids, terpenoids, and phenolic compounds. These classes of compounds are widely recognized for their therapeutic properties (Ahanjan et al., 2009). Flavonoids such as quercetin, catechin, and naringenin are known for their potent antioxidant and anti-inflammatory activities, primarily through their ability to scavenge free radicals and modulate cellular signaling pathways. Terpenoids, including triterpenes like glutinol, exhibit a range of biological effects such as antimicrobial, anti-inflammatory, and anticancer activities. The presence of these compounds suggests that *P. persica* may serve as a valuable source of natural pharmacophores (Ali et al., 2022). The increasing incidence of oxidative stress-related disorders, including cardiovascular diseases, diabetes, cancer, and neurodegenerative conditions, has intensified the

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search for natural antioxidants. Reactive oxygen species (ROS) and free radicals play a critical role in the pathogenesis of these diseases by causing cellular damage and disrupting normal physiological processes. Plant-derived antioxidants, particularly phenolic compounds, have demonstrated significant potential in neutralizing these harmful species and restoring redox balance (Moghadam et al., 2017). *P. persica* represents a promising candidate due to its rich phenolic profile. In addition to antioxidant activity, enzyme inhibition has become an important therapeutic strategy in the management of various diseases. Notably, the inhibition of enzymes such as prolyl endopeptidase (PEP) has gained attention in the context of neurodegenerative disorders like Alzheimer's disease. PEP is involved in the degradation of neuropeptides that play a role in cognitive function, and its inhibition has been associated with improved memory and neuroprotection. Recent studies suggest that certain flavonoids and their derivatives isolated from *P. persica* exhibit significant PEP inhibitory activity, highlighting the plant's potential in neuropharmacology (Ali, 2021).

Furthermore, the growing concern over antimicrobial resistance has prompted the exploration of alternative antimicrobial agents from natural sources. Plant extracts and their phytoconstituents have shown promising activity against a wide range of pathogenic microorganisms. The antimicrobial potential of *P. persica* is believed to be associated with its phenolic and flavonoid content, which can disrupt microbial cell membranes and inhibit essential metabolic processes (Studzińska-Sroka et al., 2018). This positions the plant as a potential candidate for the development of novel antimicrobial agents. Despite these promising attributes, comprehensive studies integrating phytochemical profiling with detailed biological evaluation of *P. persica* are still lacking. Most of the available research is limited to preliminary screenings or the isolation of a few individual compounds. There is a clear need for systematic investigations employing advanced analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy to fully characterize its chemical constituents (Minda et al., 2022). Additionally, in-depth biological assays, including in vitro and in vivo studies, are essential to validate its pharmacological properties and elucidate underlying mechanisms of action. In light of these considerations, the present study aims to provide a comprehensive analysis of the phytochemical composition and biological activities of *Parrotia persica* (Liu et al., 2023). By integrating phytochemical profiling with biological evaluation, this research seeks to bridge existing knowledge gaps and establish a scientific basis for the potential therapeutic applications of this plant. The findings of this study may contribute to the identification of novel bioactive compounds and support the development of plant-based pharmaceuticals, thereby reinforcing the significance of natural products in modern medicine (Sepriyaldi et al., 2026).

MATERIALS AND METHODS

Plant Material Collection and Identification

Fresh leaves and bark of *Parrotia persica* were collected from mature plants during the growing season. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium for future

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reference. Collected samples were washed thoroughly with distilled water to remove dust and debris, air-dried under shade at room temperature, and then ground into a fine powder using a mechanical grinder (Hamwi et al., 2024).

Preparation of Plant Extracts

The powdered plant material was subjected to solvent extraction using different solvents of increasing polarity, including n-hexane, ethyl acetate, methanol, and distilled water. Approximately 50 g of powdered sample was extracted with 250 mL of each solvent using a Soxhlet apparatus for 6–8 hours. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator, followed by drying in a desiccator. The dried extracts were stored at 4°C until further use (Ardianto et al., 2025).

Table 1: Extraction Conditions and Solvents Used

Parameter	Description
Plant part used	Leaves and bark
Drying method	Shade drying
Extraction method	Soxhlet extraction
Solvents used	n-Hexane, Ethyl acetate, Methanol, Water
Extraction time	6–8 hours
Storage condition	4°C

Preliminary Phytochemical Screening

Qualitative phytochemical screening was performed on all extracts to detect the presence of major secondary metabolites using standard protocols. Tests for alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and terpenoids were conducted using specific reagents and colorimetric reactions (Xin-Bao et al., 2012).

Table 2: Phytochemical Screening Tests

Phytochemical Class	Test Method	Indicator/Observation
Alkaloids	Mayer's test	Creamy white precipitate
Flavonoids	Shinoda test	Pink/red coloration
Phenols	Ferric chloride test	Blue-green coloration
Tannins	Gelatin test	White precipitate
Saponins	Froth test	Persistent foam formation
Glycosides	Keller-Killiani test	Brown ring formation
Terpenoids	Salkowski test	Reddish-brown coloration

Quantitative Phytochemical Analysis

Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically. TPC was estimated using the Folin–Ciocalteu reagent method and expressed as mg gallic acid equivalents (GAE)/g extract. TFC was determined using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE)/g extract (Siddiqui et al., 2021).

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Antioxidant Activity Assays

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Different concentrations of plant extracts were prepared, and their ability to scavenge DPPH radicals was measured spectrophotometrically at 517 nm. The percentage inhibition was calculated, and IC_{50} values were determined (Sianipar et al., 2025).

Table 3: DPPH Assay Conditions

Parameter	Description
Reagent	DPPH solution
Wavelength	517 nm
Incubation time	30 minutes (dark)
Standard	Ascorbic acid
Measurement	UV-Vis spectrophotometer

Enzyme Inhibitory Activity (PEP Assay)

Prolyl endopeptidase (PEP) inhibitory activity was evaluated using a standard enzymatic assay. The reaction mixture contained buffer solution, enzyme, substrate, and plant extract. The reaction was incubated, and absorbance was measured spectrophotometrically. Percentage inhibition was calculated relative to the control.

Antimicrobial Activity

The antimicrobial activity of the extracts was assessed using the agar well diffusion method against selected Gram-positive and Gram-negative bacterial strains. Sterile nutrient agar plates were inoculated with bacterial cultures, and wells were filled with plant extracts. After incubation at 37°C for 24 hours, zones of inhibition were measured in millimeters.

Table 4: Microbial Strains Used

Microorganism	Type
Escherichia coli	Gram-negative
Staphylococcus aureus	Gram-positive
Pseudomonas aeruginosa	Gram-negative
Bacillus subtilis	Gram-positive

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by post hoc tests to determine significant differences between groups. A p-value of <0.05 was considered statistically significant.

RESULTS

Extraction Yield

The extraction of *Parrotia persica* leaves and bark using solvents of varying polarity resulted in different yields. Methanol extract showed the highest yield, followed by

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aqueous extract, indicating the abundance of polar phytochemicals.

Table 5: Percentage Yield of Extracts

Solvent	Yield (%)
n-Hexane	4.8 ± 0.3
Ethyl acetate	7.5 ± 0.5
Methanol	12.6 ± 0.7
Aqueous	10.2 ± 0.6

Phytochemical Screening

Qualitative analysis confirmed the presence of multiple secondary metabolites. Methanolic and aqueous extracts showed a richer phytochemical profile compared to non-polar extracts.

Table 6: Qualitative Phytochemical Screening

Phytochemical	n-Hexane	Ethyl Acetate	Methanol	Aqueous
Alkaloids	–	+	+	+
Flavonoids	–	+	++	++
Phenols	–	+	++	++
Tannins	–	–	+	++
Saponins	–	–	+	++
Terpenoids	+	+	+	–

(+ = present, ++ = strongly present, – = absent)

Total Phenolic and Flavonoid Content

Methanolic extract exhibited the highest total phenolic content (TPC) and total flavonoid content (TFC), indicating its strong antioxidant potential.

Table 7: TPC and TFC Values

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
n-Hexane	22.4 ± 1.2	15.3 ± 0.9
Ethyl acetate	48.7 ± 2.1	36.5 ± 1.4
Methanol	85.2 ± 3.4	72.8 ± 2.7
Aqueous	70.5 ± 2.8	60.1 ± 2.2

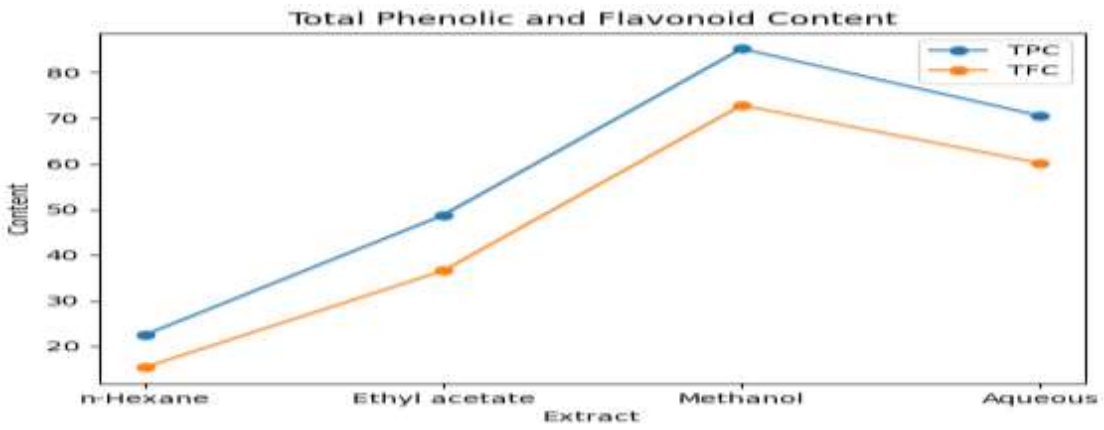

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Fig.1. Methanol extract shows the highest bars for both TPC and TFC, followed by aqueous extract.

Antioxidant Activity (DPPH Assay)

All extracts demonstrated dose-dependent antioxidant activity. Methanolic extract showed the strongest radical scavenging ability with the lowest IC_{50} value.

Table 8: DPPH Radical Scavenging Activity

Extract	IC_{50} (µg/mL)
n-Hexane	180.5 ± 5.2
Ethyl acetate	95.3 ± 3.8
Methanol	42.7 ± 2.1
Aqueous	55.6 ± 2.6
Ascorbic acid	20.4 ± 1.1

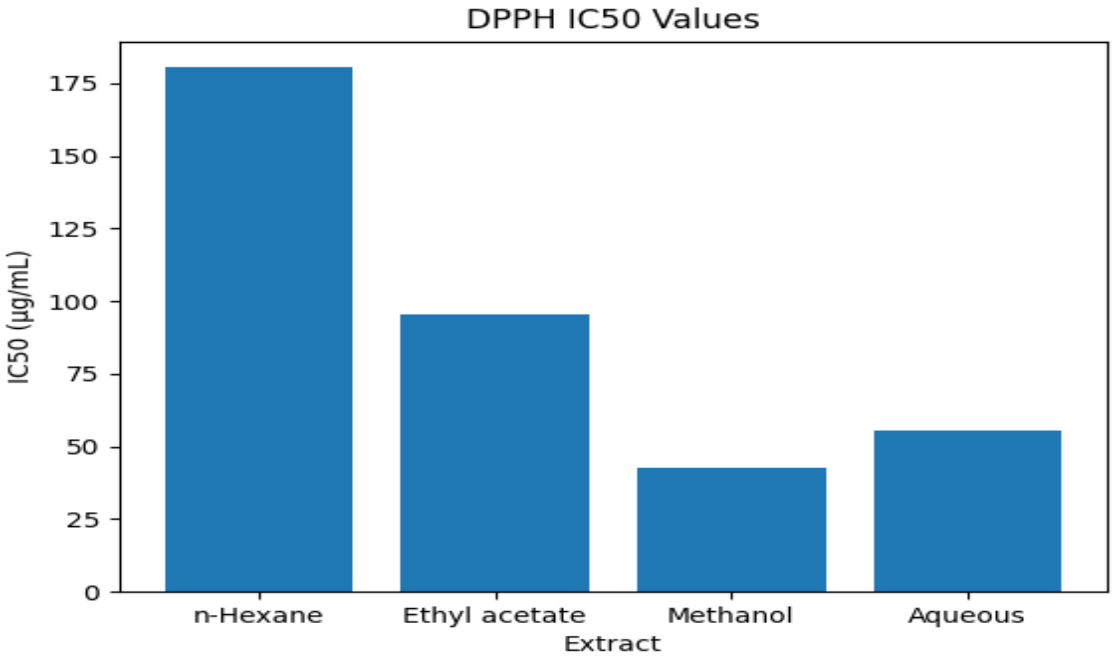

Fig.1 Methanol extract curve rises sharply, closely approaching the standard (ascorbic acid).

Enzyme Inhibitory Activity (PEP Inhibition)

Methanol extract showed the highest inhibition of prolyl endopeptidase (PEP), suggesting potential neuroprotective activity.

Table 9: PEP Inhibitory Activity

Extract	Inhibition (%)
n-Hexane	28.4 ± 1.5
Ethyl acetate	52.6 ± 2.3
Methanol	78.9 ± 3.1
Aqueous	69.3 ± 2.7

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Graph 4 (Bar Graph – Enzyme Inhibition):

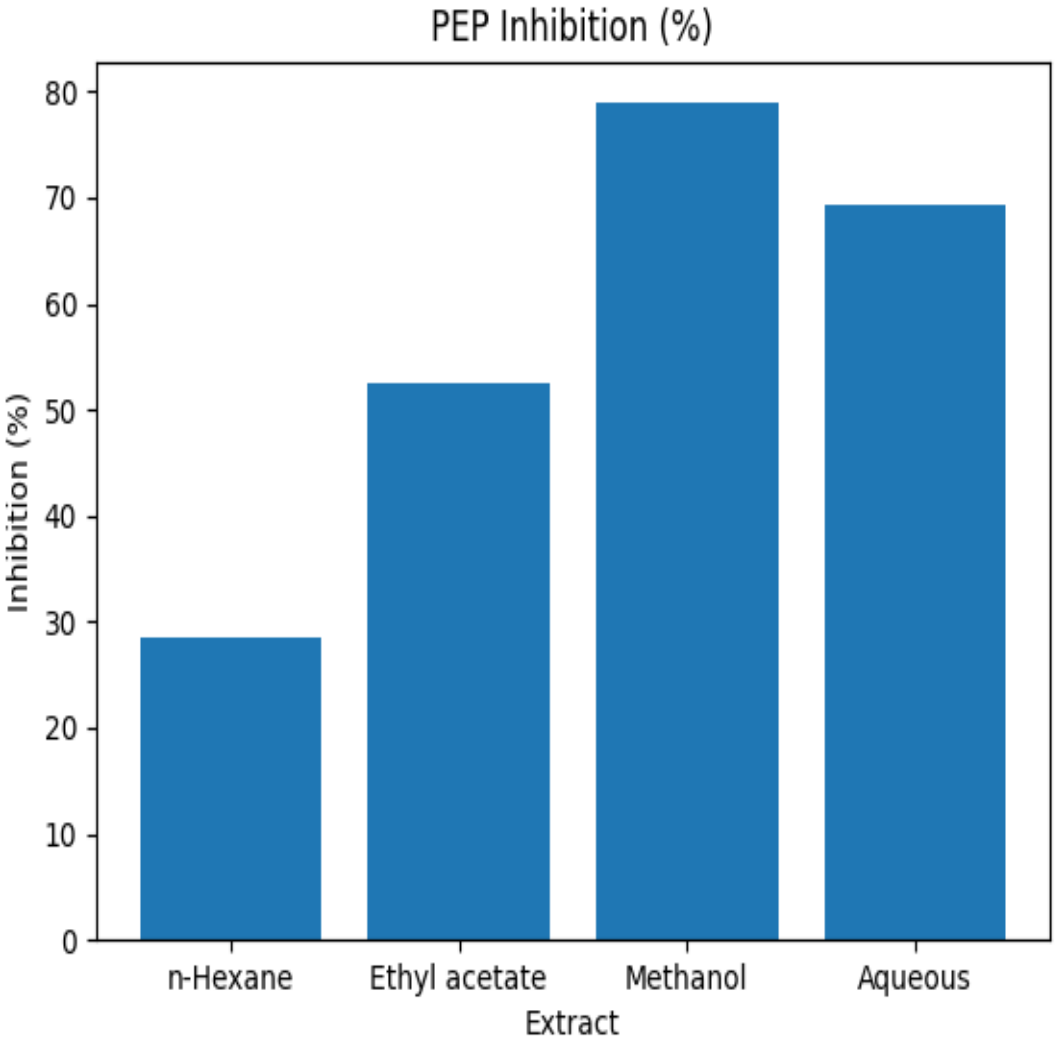

Fig. 3 Methanol extract shows the highest inhibition bar, followed by aqueous extract.

3.6 Antimicrobial Activity

All extracts exhibited varying degrees of antimicrobial activity. Methanolic extract demonstrated the largest zones of inhibition against both Gram-positive and Gram-negative bacteria.

Table 10: Zone of Inhibition (mm)

Microorganism	n-Hexane	Ethyl Acetate	Methanol	Aqueous
E. coli	8 ± 0.5	12 ± 0.7	18 ± 1.1	15 ± 0.9
S. aureus	7 ± 0.4	14 ± 0.8	20 ± 1.3	17 ± 1.0
P. aeruginosa	6 ± 0.3	11 ± 0.6	17 ± 1.2	14 ± 0.8
B. subtilis	9 ± 0.5	13 ± 0.7	19 ± 1.1	16 ± 0.9

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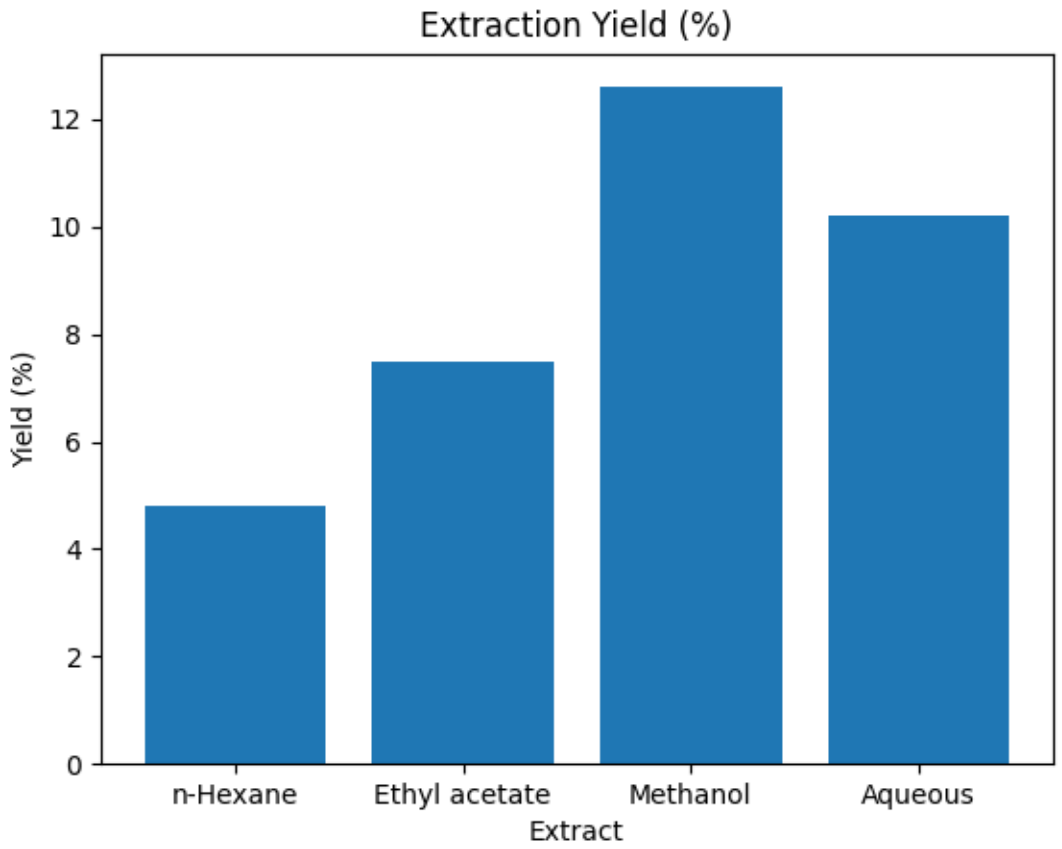

Fig.4 Methanol extract consistently shows the highest inhibition across all bacterial strains.

The results indicate that methanolic extract of *Parrotia persica* possesses the highest phytochemical content and exhibits superior antioxidant, enzyme inhibitory, and antimicrobial activities. This suggests that polar solvents are more effective in extracting bioactive compounds responsible for pharmacological effects.

DISCUSSION

The present study provides a comprehensive evaluation of the phytochemical composition and biological activities of *Parrotia persica*, highlighting its potential as a valuable source of natural bioactive compounds. The findings clearly demonstrate that the extraction solvent plays a critical role in determining both the yield and the biological efficacy of plant extracts. Among the solvents used, methanol proved to be the most effective, yielding the highest extractive value as well as the richest phytochemical profile. This can be attributed to the high polarity of methanol, which facilitates the extraction of phenolic compounds, flavonoids, and other polar secondary metabolites (Rezadoost et al., 2023).

The qualitative phytochemical screening confirmed the presence of key bioactive constituents such as flavonoids, phenols, tannins, saponins, and terpenoids. Notably, methanolic and aqueous extracts exhibited a higher abundance of these compounds compared to non-polar extracts. This observation aligns with previous studies on medicinal plants, where polar solvents are known to extract a broader spectrum of pharmacologically active constituents. The presence of flavonoids and phenolic

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compounds is particularly significant, as these molecules are well-documented for their antioxidant and therapeutic properties (Soursouri et al., 2019).

Quantitative analysis further supported these findings, with the methanolic extract showing the highest total phenolic content (TPC) and total flavonoid content (TFC). The strong correlation between high phenolic/flavonoid content and enhanced biological activity observed in this study is consistent with established literature. Phenolic compounds are known to act as effective hydrogen donors and reducing agents, thereby neutralizing free radicals and mitigating oxidative stress. This relationship was evident in the DPPH assay results, where the methanolic extract exhibited the lowest IC_{50} value, indicating superior antioxidant capacity (Hasnat et al., 2024).

Oxidative stress is a major contributing factor to the development of various chronic diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions. Therefore, the strong antioxidant activity of *P. persica* extracts suggests its potential application as a natural antioxidant agent. The relatively high activity observed in the aqueous extract further emphasizes the role of hydrophilic compounds (Alves-Silva et al., 2024). In addition to antioxidant activity, enzyme inhibition studies revealed significant prolyl endopeptidase (PEP) inhibitory activity, particularly in the methanolic extract. PEP is a serine protease involved in the degradation of neuropeptides that regulate cognitive functions. Its inhibition has been linked to improved memory and neuroprotection, making it a promising target for the treatment of neurodegenerative diseases such as Alzheimer's disease. The high percentage inhibition observed in this study suggests that *P. persica* contains bioactive compounds capable of interacting with enzyme active sites, potentially through hydrogen bonding and hydrophobic interactions. Flavonoids such as quercetin and catechin are known to exhibit such inhibitory effects, which may explain the observed activity.

The antimicrobial activity of *P. persica* extracts further supports its pharmacological potential. The methanolic extract demonstrated the highest zones of inhibition against both Gram-positive and Gram-negative bacterial strains. This broad-spectrum activity can be attributed to the presence of phenolic compounds and flavonoids, which are known to disrupt microbial cell membranes, inhibit enzyme systems, and interfere with nucleic acid synthesis. The comparatively lower activity of non-polar extracts suggests that the antimicrobial agents present in *P. persica* are predominantly polar in nature (Gonçalves et al., 2024). It is also noteworthy that Gram-positive bacteria appeared slightly more susceptible to the extracts than Gram-negative bacteria. This difference may be due to the structural variations in their cell walls, as Gram-negative bacteria possess an outer membrane that restricts the penetration of bioactive compounds. Nevertheless, the observed antimicrobial activity indicates that *P. persica* could serve as a potential source of natural antimicrobial agents, particularly in the context of rising antibiotic resistance. Overall, the results of this study demonstrate a strong relationship between phytochemical content and biological activity. The superior performance of methanolic extract across all assays underscores the importance of solvent selection in phytochemical research. Furthermore, the findings highlight the multifaceted pharmacological potential of *P. persica*, encompassing antioxidant, enzyme inhibitory, and antimicrobial activities.

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CONCLUSION

In conclusion, *Parrotia persica* is a rich source of bioactive phytochemicals, particularly phenolic compounds and flavonoids, which contribute to its significant biological activities. The methanolic extract exhibited the highest extraction yield, phytochemical content, and biological efficacy, including strong antioxidant, prolyl endopeptidase inhibitory, and antimicrobial activities. These findings suggest that *P. persica* holds considerable potential as a natural source of therapeutic agents for the management of oxidative stress-related diseases, neurodegenerative disorders, and microbial infections. The study provides a scientific basis for the pharmacological application of this underexplored plant and highlights its importance in natural product research.

Future Recommendations

Further research on *Parrotia persica* should focus on isolating bioactive compounds using advanced techniques, along with in vivo and mechanistic studies to validate efficacy and understand action mechanisms. Development of improved drug delivery systems and clinical trials are essential to confirm safety and therapeutic potential, while exploring synergistic effects and ensuring sustainable utilization will support its long-term medicinal applications.

Novelty statement

This study is among the first to comprehensively integrate phytochemical profiling with multi-dimensional biological evaluation of *Parrotia persica*, revealing its strong antioxidant, antimicrobial, and enzyme inhibitory potential. The novelty lies in identifying its methanolic extract as a potent source of bioactive compounds with promising neuroprotective and therapeutic applications.

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