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Phytochemical profile, antioxidant and antibacterial activities of methanolic extract of *Peperomia pellucida* leaves revealed by GC-MS and molecular docking

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Abstract. *Salnus S, Wahab AW, Muharram, Arfah RA, Kasim S, Rajab A, Arif AR, Armah Z, Irfandi R, Alam MN, Yasser M. 2026. Phytochemical profile, antioxidant and antibacterial activities of methanolic extract of* Peperomia pellucida *leaves revealed by GC-MS and molecular docking. Biodiversitas 27 (2): d270219. https://doi.org/10.13057/biodiv/d270219*. A comprehensive analysis of the methanol extract of *Peperomia pellucida* leaves was conducted using various analytical techniques, revealing a complex profile of bioactive compounds with potential therapeutic applications. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 43 bioactive compounds within the extract, with sesquiterpenoids emerging as the predominant class of compounds. The major components identified were dillapiole (41.77%), N,N'-diacridin-9-yl-hydrazine (7.44%), and caryophyllene (6.94%), highlighting the diverse chemical composition of the extract. This extract demonstrated significant antibacterial activity against several bacterial strains, indicating its potential as a natural antimicrobial agent. Notably, it exhibited moderate effects against *Klebsiella pneumoniae.* The largest inhibition zone was observed against *Staphylococcus aureus* (14 mm), followed by *Salmonella typhi* (12 mm), *Shigella* sp. (12 mm), and *Escherichia coli* (11 mm). The smallest inhibition zone was recorded for *K. pneumoniae* (10 mm), indicating a moderate susceptibility, suggesting its possible application in treating infections caused by this pathogen. Furthermore, the extract showed remarkable antioxidant activity with an IC_{50} value of 4.30 µg/mL, underscoring its potential as a natural source of antioxidants. Molecular docking analysis provided insights into the mechanism of action of the components of the extract. Dillapiole, the most abundant compound (41.77%), displayed a strong binding affinity (-70.53 kJ/mol) for *S. aureus* proteins, suggesting its potential as a lead compound for developing novel antibacterial agents, particularly against *S. aureus* infections. Fourier-Transform Infrared (FT-IR) spectroscopy confirmed the presence of polyphenolic functional groups in the extract, further supporting its antioxidant properties. Overall, these results demonstrate that *P. pellucida* possesses potent antimicrobial and antioxidant activities linked to its diverse phytochemical composition. These findings highlight *P. pellucida* as a promising source of natural antioxidants and antimicrobials that support biodiversity-based drug discovery and underscore the importance of conserving medicinal plant resources for future therapeutic development.

Keywords: Antioxidant, dillapiole, GC-MS, methanol extract, *Peperomia pellucida*

INTRODUCTION

Peperomia pellucida (L.) Kunth, or "shining bush plant," is a small herb from the Piperaceae family with translucent stems and bright green heart-shaped leaves. It grows in wet, shady places like forest floors and gardens (Nasution et al. 2025). This plant is found in tropical areas, including Brazil, Indonesia, and Southeast Asia (Ifora et al. 2023). It is used in traditional medicine for its healing effects (Saensouk et al. 2025). In South America, it is employed to relieve inflammation, pain, skin irritation, and joint disorders. In the Philippines, it acts as a diuretic and treats gout. Ayurveda uses it for digestive and breathing problems (Kartika et al. 2020). Research supports these uses, showing plants' importance in different cultures' medicine. Its traditional use for various ailments underscores its pharmacological potential and reflects the interconnection between local knowledge and biodiversity conservation (Teodhora et al. 2025).

Phytochemicals are bioactive compounds derived from plants that provide health benefits and are widely used in herbal medicine (Hidayati et al. 2023; Muhtadi et al. 2025). Studying them could lead to new treatments. Some compounds may inhibit cancer growth or induce cancer cell death. Plant compounds offer safety and cost advantages over synthetic drugs (Triyanto et al. 2024). Recent

advances, including nanotechnology and metabolomic profiling such as UHPLC-MS/MS combined with molecular networking, have improved the identification of bioactive and novel compounds with potential pharmacological effects (Meinita et al. 2024). These findings support the reported antioxidant, anti-inflammatory, antimicrobial, antihypertensive, antinociceptive, antidiabetic, and osteogenic activities of many plant species, and provide a basis for further mechanistic and translational research (Gomes et al. 2022).

Antioxidants are essential for neutralizing harmful free radicals and reducing oxidative stress, a key factor linked to cancer, cardiovascular diseases, and diabetes (Islam et al. 2024; Tumilaar et al. 2024). Plant-derived natural antioxidants like flavonoids and carotenoids offer better bioavailability and fewer side effects than synthetic alternatives (Ouryemchi et al. 2024). Antioxidant-rich foods, including microgreens and algae, can enhance the body's defense against oxidative damage and help lower the risk of chronic diseases (Budzianowska et al. 2025; Monroy-García et al. 2025; Pourmontaseri et al. 2025; Rao et al. 2025).

Antibiotic resistance creates the need for new antibacterial treatments. Plant-based compounds are promising alternatives to conventional antibiotics (AlSheikh et al. 2020). Examples include *Ocimum basilicum* L. and plants from the Annonaceae family, which are effective against various bacteria, including drug-resistant strains (Azizah et al. 2023).

Methanol is commonly used to extract plant chemicals due to its solvent ability, cost-effectiveness, and quick evaporation (Backiam et al. 2023). It extracts various chemicals from plants like *Syzygium aqueum* (Burm.fil.) Alston and *Phyllanthus emblica* L. (Cui et al. 2020; Chen et al. 2022). *Peperomia pellucida* contains flavonoids, tannins, saponins, and alkaloids, giving it anti-inflammatory, antioxidant, and antimicrobial properties (Ashraf et al. 2024). To understand the active components and their effects on the human body, further investigation is essential.

This study aimed to safeguard biodiversity and promote sustainable medicinal plant use by evaluating their health benefits, product development potential, and bioactive compounds. It explored natural products for cancer prevention and treatment and assessed the antioxidant and antibacterial activities of *P. pellucida* leaf extracts. Research on medicinal plants aids in developing medicines, supplements, and cosmetics, preserving traditional knowledge, and advancing drug discovery. This study integrates Gas Chromatography-Mass Spectrometry (GC-MS), bioassay techniques, and molecular docking to validate the traditional uses of *P. pellucida*, providing an analysis of its bioactive compounds and mechanisms. This study combines traditional knowledge with modern science to explore *P. pellucida* as a source of bioactive compounds. This research sought to elucidate the bioactive constituents present in the methanolic extract of *P. pellucida* and to investigate their antioxidant and antibacterial mechanisms using both chemical analyses and molecular-level approaches. Dillapiole-target docking studies have shown the interactions between dillapiole and proteins, indicating its potential medical uses. This approach can identify drug targets, improve compounds, and accelerate drug discovery from *P. pellucida*. This study preserves traditional knowledge while advancing natural product research, leading to the development of new medicines, supplements, and cosmetics.

MATERIALS AND METHODS

Materials

Peperomia pellucida (L.) Kunth plants were collected from Bulukumba Village, South Sulawesi, Indonesia (5°31'20.2" S 120°11'36.2" E). The plant material was taxonomically identified and authenticated by a botanist at the Biology Laboratory, Universitas Negeri Makassar, South Sulawesi. A voucher specimen was prepared and deposited as reference.

Sample preparation

Fresh leaves of *P. pellucida* were separated, washed thoroughly under running water to remove dirt and debris, and then left to air-dry at room temperature. Once dried, the leaves were finely ground into powder using a mechanical grinder. Leaf powder (25 g) was macerated in 150 mL of methanol in a stoppered flask and left to soak overnight at room temperature. The extract underwent filtration with Whatman No.1 filter paper and was subsequently subjected to centrifugation at 2,500 rpm for a duration of 15 minutes. The supernatant was concentrated by rotary evaporation, and the resulting crude methanol extract was used for further analyses.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was conducted using a Shimadzu GCMS-QP2010 Ultra system, which was fitted with an Rtx-5 column measuring 30 meters in length, 0.25 mm in internal diameter, and 0.25 μm in film thickness, composed of diphenyl dimethyl polysiloxane. The temperature protocol was established as follows: it began at 60°C, where it was held steady for 2 minutes, and then it was increased to 280°C at a rate of 3°C per minute. The injection temperature was configured to 260°C using the split mode. Helium was utilized as the carrier gas, maintaining a steady flow rate of 1.0 mL/min. The entire process lasted for 50 minutes. Data collection and analysis were conducted using the LabSolutions DB/CS software. Quality control steps included baseline correction, peak deconvolution, and signal-to-noise ratio filtering to ensure reliable peak detection. Peak identification was carried out by comparing the acquired mass spectra against reference spectra from the NIST or Wiley libraries, using a match quality threshold typically above 85% to confirm compound identity (Mishra et al. 2023).

Fourier Transform-Infrared (FT-IR)

FT-IR analysis was conducted using a Shimadzu spectrometer using the KBr pellet method. Spectra were recorded in the range of 4000-399 cm^{-1} at a resolution of 4

cm^{-1}. The process of analyzing absorption infrared spectra was enhanced by automatically comparing the recorded spectra with the help of LabSolutions IR Version 2.27 (Gurning and Haryadi 2022).

Antioxidant activity

DPPH radical scavenging assay

The standards used were ascorbic acid. A solution of ascorbic acid was prepared by dissolving 10 mg of ascorbic acid in 250 mL of methanol, and a solution of extract was prepared by dissolving 10 mg of extract in 250 mL of methanol. The standard addition curves were generated by preparing different concentrations, i.e., 2.5, 5, 10, 15, 20, and 25 µg/mL, of the standard solutions. Each measurement was conducted in triplicate.

The extract's antioxidant properties were assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. A 50 µM DPPH solution was prepared in methanol. The mixture, totaling 3.0 mL and comprising the extract and DPPH, was kept in the dark for 60 minutes to incubate. The absorbance was recorded at a wavelength of 517 nm using a methanol blank as a reference. The DPPH inhibition percentage was determined through the equation by Baliyan et al. (2022):

$$\text{Antioxidant activity (\%)} = 100 \times \frac{(\text{Ac-As})}{\text{Ac}}$$

Where :

Ac : Control reaction absorbance

As : Testing specimen absorbance

Determination of IC_{50} value

The test extract was dissolved in methanol to create a stock solution with a concentration of 1000 µg/mL. From this stock solution, a series of concentrations (5, 10, 15, 20, and 25 µg/mL) was prepared by serial dilution. For comparison, a standard ascorbic acid solution with identical concentrations was prepared. All the solutions were freshly prepared prior to use. The IC_{50} value was determined by plotting a graph of sample concentration (µg/mL) against the percentage of DPPH scavenging activity. The data obtained were analyzed using linear or non-linear regression (Microsoft Excel software) to ascertain the concentration that resulted in a 50% reduction in DPPH radicals. All tests were conducted in triplicate. Results are presented as the mean±Standard Deviation (SD). The statistical validity and data consistency were confirmed by calculating the correlation coefficient (R^2) from the regression curve. The IC_{50} value of the sample was compared with that of ascorbic acid to assess the relative antioxidant potential of the extract (Rhaman et al. 2019).

Tannin estimation

The tannin content was estimated using the Van Burden-Robinson method. Standard tannic acid solutions (20, 40, 60, and 80 µg/mL) were prepared. Each sample was mixed with 2 mL of 0.1 M $FeCl_3$ and 2 mL of 0.008 M potassium ferrocyanide, and absorbance was measured at 605 nm after 10 minutes. The concentration of tannins in the samples was determined by plotting a calibration curve derived from the standard curve (Gizaw et al. 2022).

Antibacterial activity

The antibacterial properties of the extract were assessed through the agar disc diffusion technique. The microorganisms tested included *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Shigella* sp. Identification and authentication of the bacterial strains were carried out in the microbiology laboratory, Panrita Husada Health College, Bulukumba.

Bacterial cultures were spread on Mueller-Hinton Agar (Oxoid) and incubated overnight. Sterile paper discs, each with a diameter of 6 mm, are impregnated with methanol extract of *P. pellucida* (10 µL), with chloramphenicol at a concentration of 50 µg per disc served as a positive control and distilled water as a negative control. These discs are placed on the medium surface, maintaining a minimum distance of 24 mm between them. Incubation is conducted at 37°C for 18-24 hours. Post-incubation, the inhibition zones surrounding the discs are observed, and their diameters are measured using a caliper or ruler. Data analysis involves calculating the average diameter of the inhibition zones from replicates and comparing the antibacterial efficacy among different extract concentrations as well as with the control. The results are interpreted by categorizing antibacterial activity based on the diameter of the inhibition zones (Rosyidah et al. 2025).

Docking studies

Basic validation of the PLANTS protocol

The YASARA software was utilized for the preparation of proteins and ref_ligands, which included the elimination of unnecessary ligands, cofactors, and protein segments. Using MarvinSketch at a pH of 7.4, the ligand was prepared and saved as ligand_2D.mrv. To explore different conformations of the ligand structure, reopen the ligand_2D.mrv file in MarvinSketch and utilize the "Conformers search" function. The resulting conformations were then stored in a .mol2 format ligand file.

The ligands and proteins that were prepared underwent docking using the PLANTS software. This process involved input files in the formats of protein.mol2 and ligand.mol2. The docking pose that achieved the highest score was considered to represent the predicted actual position of the ligand within the target protein structure. From this configuration, the Root Mean Square Deviation (RMSD) was determined using YASARA. The protocol was deemed valid if the docking pose resulted in an RMSD value of less than 2 Å (1 Å = 10^{-10} m) (Supanji et al. 2024).

Data analysis

The data were examined and analyzed by using Discovery Studio ver 21.1.1 to obtain 3D and 2D visualizations, as well as the binding regions of the ligand and target protein.

RESULTS AND DISCUSSION

Sample identification

The classification of *P. pellucida* was performed by botanists of the Biology Laboratory of Universitas Negeri

Makassar using the internal module reference key code 1b-2b-3b-4b-6b-7b-6a-41b-42b-43b-54b-59b-61b-62b-63a-64a-Fam. Piperaceae-Peperomia-*Peperomia pellucida* (L.) Kunth. Botanists at the Biology Laboratory of Universitas Negeri Makassar verified the species identification. A voucher specimen, numbered 001/SKAP/LAB.BIOLOGI/IX/2025, was kept in the same laboratory for future reference. Maceration of 25.00 g of *P. pellucida* leaf powder in methanol yielded 1.62 g of crude extract after rotary evaporation, yielding 6.48% (w/w, dry basis). The crude extract was then analyzed for its phytochemical properties and bioactivity.

GC-MS analysis

The GC-MS chromatogram of the methanol extract of *P. pellucida* leaves revealed the presence of 43 chemical constituents (Figure 1). Summary of the top 9 compounds with peak area percentage greater than 2%, shown in Figure 2 in the form of a bar chart.

The area percentage from the GC-MS instrument was used to analyze the relative quantities of the compound components in the extract. The major compounds (Figure 2) identified in the chromatogram were dillapiole (41.77%), followed by N,N'-diacridin-9-yl-hydrazine (7.44%), caryophyllene (6.94%), β-elemene (6.10%), 4-(4,5-bis(4-methoxyphenyl)-4H-imidazole-2-iyl)-2-methoxyphenol (4.79%), carotol (3.71%), isogermacrene D (2.92%), nerolidol (2.76%), 1,2-dimethoxy-4-(2-methoxyethenyl)benzene (2.41%), and geranyl linalool (2.13%). This study's findings align with numerous other studies that identified major compounds in *P. pellucida*, including dillapiole (Ngo et al. 2024), β-caryophyllene, and β-elemene (Ho et al. 2024).

The ten components of this compound are shown in the chromatogram (Figure 3), which relates the correlation between signal intensity and m/z. Compounds were identified by matching their mass spectra with the NIST 17 standard library from the National Institute of Standards and Technology and Wiley 8 library by referencing standard compounds.

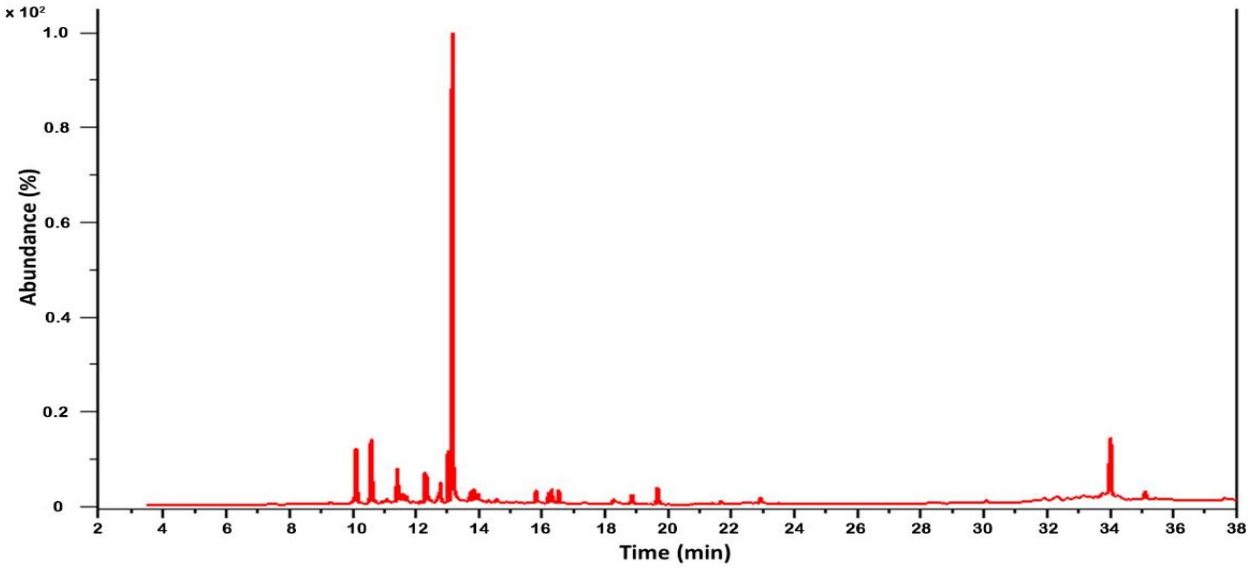

Figure 1. Total ion chromatograms of methanolic extract of *Peperomia pellucida*

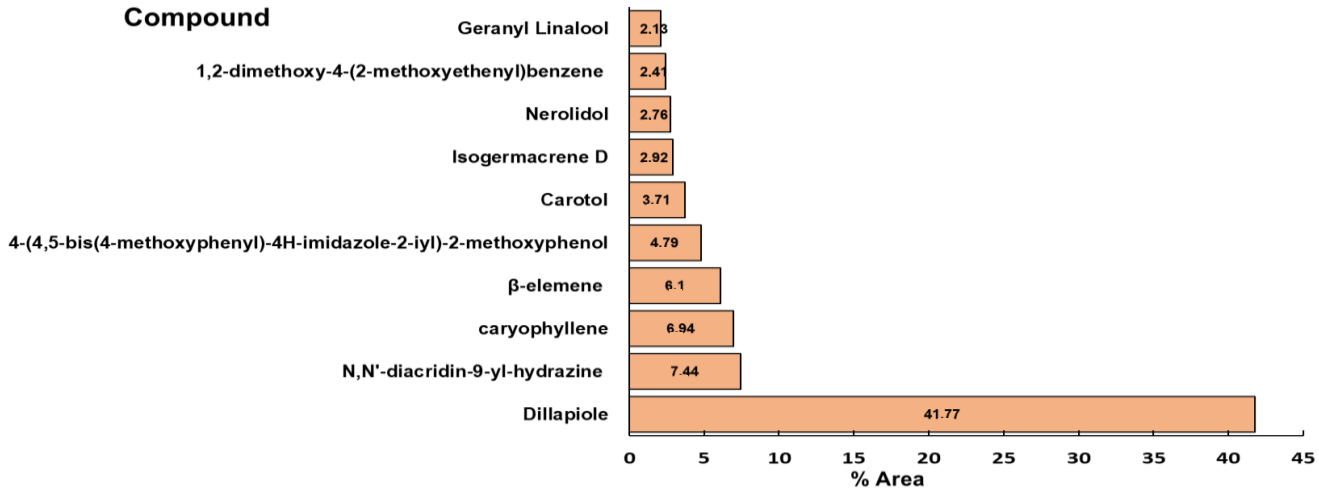

Figure 2. Summary of the top 10 compounds with % peak area greater than 2% from GC-MS analysis

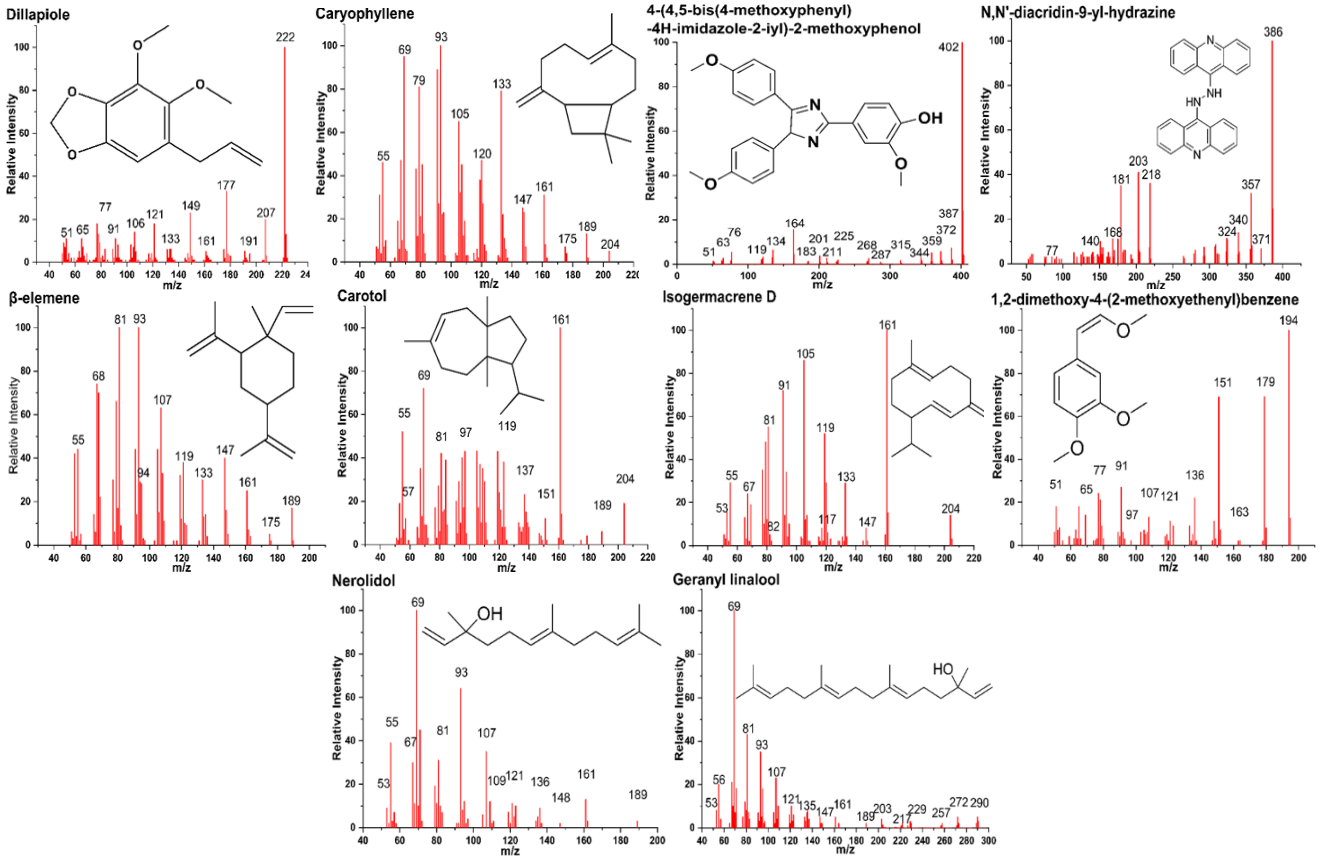

Figure 3. Resolved and standard mass spectra for the selected peak cluster of ten chemical compounds in methanol extract of *Peperomia pellucida*

In the analysis using Gas Chromatography-Mass Spectrometry (GC-MS), choosing a threshold, like a 2% peak area, is typically determined by the necessity to differentiate significant analyte signals from background noise and to ensure that the analytes of interest are properly quantified. Such a threshold helps to exclude minor peaks that may represent noise or insignificant compounds, thereby improving the reliability and clarity of the analysis. Utilizing a peak area threshold allows for a focused analysis of peaks that significantly contribute to the chemical profile of the sample, ensuring that the results are both meaningful and reproducible (El-Din et al. 2022; Luo and Reineccius 2022).

These compounds exhibit various bioactivities. For example, sesquiterpenes such as caryophyllene and β-elemene possess antimicrobial, anti-inflammatory, and anticancer properties. Similarly, phenylpropanoids like dillapiole have shown strong antioxidant and anti-inflammatory activities.

Gas Chromatography in tandem with Mass Spectrometry (GC-MS) is recommended for reliable qualitative and quantitative analyses. This method has been shown to have better selectivity and sensitivity, better detection limit, and much higher quantification limit for the continuous identification of multiple residues (Tran-Lam et al. 2021). In the methanol extract from the leaves of *P. pellucida* had been found to be rich in several compounds such as sesquiterpene, fatty acid, alkane, phenylpropene and phenolic/alcoholic. Sesquiterpene is found to have the activities of antibacterial, anti-tumor, anti-inflammatory, sedative, analgesic and fungicide (Narayanamoorthi et al. 2015). Fatty acids have been found to have antioxidant and antibacterial activity (de Azevedo et al. 2020), antifungal (Guimarães and Venâncio 2022). Alkanes exhibit antifungal activity (Bordoloi et al. 2017). Phenylpropene has anti-inflammatory, antimicrobial, anti-tumor, and antioxidant activity (Zhang et al. 2021). Alcoholic and phenolic compounds exhibit antimicrobial, antioxidant and cell-protective activities (Lin et al. 2022).

Extensive research has delineated the compound profile of *P. pellucida*, highlighting the presence of lupeol, palmitic acid (Kartika et al. 2022), ß-sitosterol, stigmasterol (Angelina et al. 2024), and apiol (Asiwe et al. 2024).

Overall, the chemical profile of the methanolic extract suggested a high potential for biological activity, which supports its traditional use in herbal medicine. A brief conclusion after the GC-MS results, highlighting the significance of the identified compounds before moving on to the FT-IR analysis, could smoothen the flow. For instance, the GC-MS analysis substantiates the rich phytochemical diversity of *P. pellucida*, setting a solid foundation for further exploring its biological activities through FT-IR analysis.

FT-IR analysis

FT-IR analysis provided additional evidence of key functional groups. Table 1 presents a summary of the principal peaks and their corresponding functional groupings, while Figure 4 provides a visual representation of these elements.

The broad absorption band detected at 3607 cm^{-1} is associated with the O-H stretching vibration, suggesting the presence of hydroxyl groups, which are likely sourced from polyphenolic compounds. The characteristic peaks at 2918 and 2850 cm^{-1} suggest the presence of aliphatic C-C and C-H bonds, respectively. The peak at 1681 cm^{-1} is attributed to C=O stretching, indicating the presence of conjugated carbonyl groups, likely from flavonoids or phenolic acids, in the sample. An additional peak at 1396 cm^{-1} was attributed to ring stretching, while the peak at 1265 cm^{-1} is associated to C-O stretching. The presence of a C-OH deformation vibration bond was revealed by an absorption band at 1030 cm^{-1}. Furthermore, the vibrational absorption of the C-H and C=C groups in the benzene ring was correlated with the peaks at 771 cm^{-1} and 648 cm^{-1}. These spectral features collectively suggest the presence of flavonoids, phenolic acids, and potentially terpenes in the sample. The combination of hydroxyl groups, carbonyl groups, and aromatic structures is particularly indicative of flavonoids and other polyphenolic compounds.

The findings corroborate the GC-MS results, underscoring the prevalence of hydroxylated and aromatic compounds within the extract, which may contribute to its antioxidant and antibacterial properties. These analyses furnish a comprehensive chemical profile of the extract, suggesting potential biological activities.

Antioxidant activity, IC_{50} and tannin estimation

The antioxidant activity of the *P. pellucida* extract was evaluated using the DPPH radical scavenging assay. A significant decrease in absorbance at 517 nm was observed as the concentration of the extract increased, indicating an effective radical scavenging activity (Table 2).

DPPH radical scavenging activity is presented in Figure 5 and Table 3, by comparing the antioxidant potential between the methanol extract of *P. pellucida* and standard ascorbic acid, interpreting the IC50 values and the dose-response relationship observed in the curves.

The antioxidant activity of *P. pellucida* extract was evaluated using the DPPH radical scavenging assay. A concentration-dependent decrease in absorbance at 517 nm was observed, indicating effective radical scavenging activity. This was visually confirmed by a color change from purple to yellow, demonstrating the reduction of DPPH radicals. At a concentration of 50 µg/mL, the extract exhibited an average antioxidant activity of 54.23±0.81%.

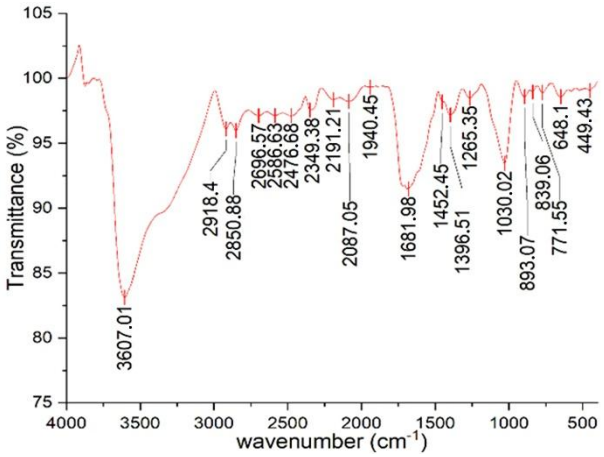

Figure 4. FTIR spectrum of methanol extract from *Peperomia pellucida* showing major functional groups indicating presence of polyphenols

Table 1. Theoretical exploration of FTIR spectral analysis and functional group determination

Vibration assignment (ν) (cm^{-1})	Absorption band
3607.01	O-H
2918.4	C-C stretching
2850.88	C-H stretching
1681.98	C=O stretching
1396.51	Ring stretching
1265.35	C-O stretching
1030.02	C-OH deformation vibration
771.55	C-H vibration of benzene ring
648.10	C=C vibration of benzene ring

Table 2. Antioxidant activity of *Peperomia pellucida* extract using DPPH

y = Concentration (µg/mL)	Sample	Absorbance at 517 nm	Antioxidant activity (%)	Mean	Standard deviation	Mean±SD
50	Control	0,981	-	-	-	-
50	SR1	0,452	53.92	54.23	0.81	54.23±0.81
50	SR2	0,44	55.15			
50	SR3	0,455	53.62			

Table 3. DPPH radical scavenging activity of the extracts and ascorbic acid as a comparator

Sample	% inhibition of DPPH radical						IC50 Value (µg/mL)
	2.5	5	10	15	20	25	
Ascorbic acid	16.31	29.46	50.76	63.40	74.11	79.90	12.71
Methanol extract	57.19	71.76	81.65	85.12	92.25	95.31	4.30

Table 4. Tannin concentration in 50 mg samples

Test tube	Absorbance at 605 nm	Dilution factor	Concentration (µg/mL)
Sample	1.436	50	49.57

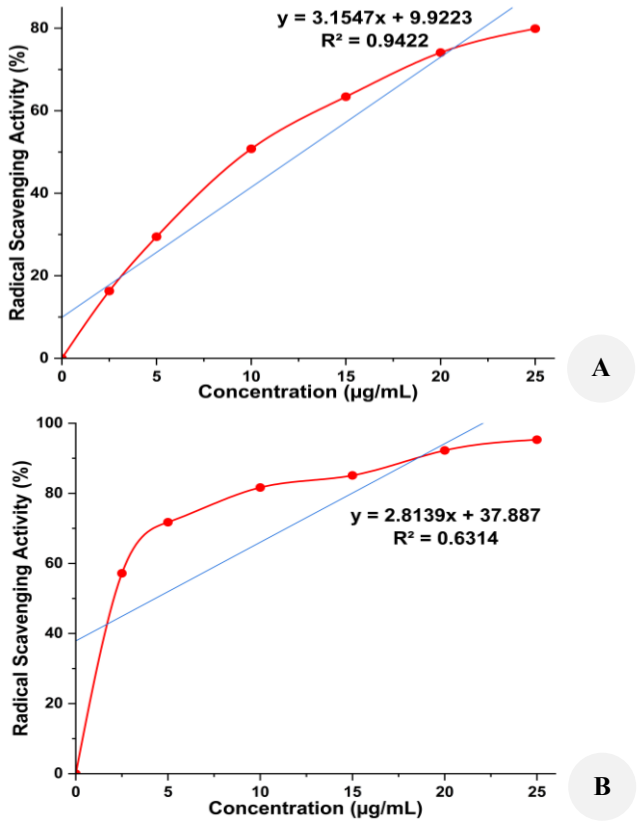

Figure 5. Curve of DPPH radical scavenging activity of: A. Standard ascorbic acid, B. Methanol extract of *Peperomia pellucida*

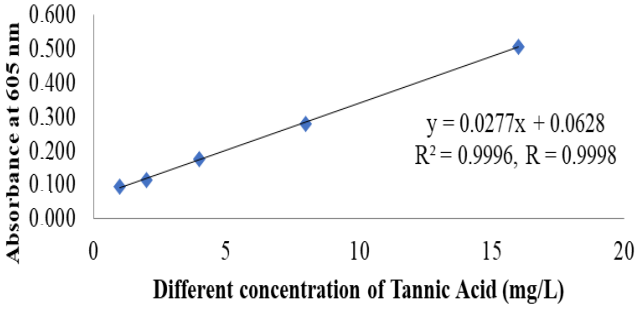

Figure 6. Standard curve of tannic acid

A comparison of DPPH radical scavenging activity between ascorbic acid (standard) and the methanol extract of *P. pellucida* revealed that the extract demonstrated superior antioxidant activity across all tested concentrations (2.5-25 µg/mL). The extract's IC_{50} value was determined to be 4.30 µg/mL, significantly lower than that of ascorbic acid (12.71 µg/mL), indicating a more potent antioxidant capacity. These findings suggest that *P. pellucida* extract possesses strong antioxidant properties, potentially attributable to its high tannin content and other bioactive compounds. Based on the sigmoid regression curve, the IC_{50} value was obtained as±4.30 µg/mL, which is the concentration required to produce 50% inhibition against DPPH free radicals. Several black pepper (*Piper nigrum* L.) extracts have reported DPPH IC_{50} values in the mid-tens to low-tens of µg/mL to a few dozen µg/mL (28-42 µg/mL) (Sharma et al. 2023).

Tannin content in the leaf samples was determined using the Van-Burden and Robinson method, which is based on the precipitation of tannins by proteins (in this case, casein), followed by the measurement of residual free phenolic compounds using the Folin-Ciocalteu reagent at a wavelength of 650 nm. The absorbance values of the samples were compared against a standard calibration curve prepared using tannic acid in the concentration range of 1-16 mg/L (Figure 6). A linear relationship was observed between the tannic acid concentration and absorbance (R^2>0.99), confirming the reliability of the calibration curve for quantification. The tannin concentration in a 50 mg sample was found to be 49.57 µg/mL, corresponding to an absorbance of 1.436 at 605 nm (Table 4).

Antibacterial activity

The methanol extract of *P. pellucida* demonstrated notable antibacterial activity against all the tested organisms, as shown in Table 5 and Figure 7. Differences in inhibition zone diameters among bacterial species were analyzed using one-way ANOVA, followed by Tukey's HSD post-hoc test. Normality was assessed with the Shapiro-Wilk test and homogeneity of variance with Levene's test. Statistical significance was set at $p<0.05$. The largest inhibition zone was observed against *S. aureus* (14 mm), followed by *S. typhi* (12 mm), *Shigella* sp. (12 mm), and *E. coli* (11 mm). The smallest inhibition zone was recorded for *K. pneumoniae* (10 mm), indicating a moderate susceptibility.

These findings are comparable to previous studies. Alves et al. (2019) reported that a methanolic extract of *P. pellucida* leaves showed inhibition zones ranging from 8 to 13 mm against various bacterial strains, including *S. aureus* and *E. coli*. Another study by Narayanamoorthi et al. (2015) observed inhibition zones of 10-15 mm for similar bacterial strains, with the highest activity also recorded against *Salmonella* species.

Furthermore, aqueous extract of *P. pellucida* reported inhibition zones of 13 mm against *E. coli*, 15 mm against *K. pneumoniae*, 11 mm against *Shigella flexneri* and 15 mm against *S. aureus*, which aligns with the results of the current study (Nivetha 2023). The variation in antibacterial efficacy across studies may result from differences in plant origin, extraction solvents, and bacterial strain sensitivity. These comparative data validate the antimicrobial potential of *P. pellucida* methanolic extract and reinforce its traditional use.

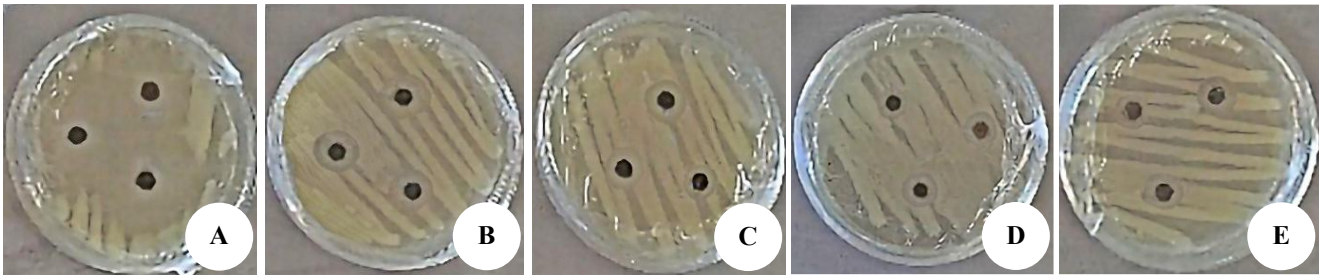

Figure 7. The Result of disc diffusion test on: A. *Staphylococcus aureus*, B. *Escherichia coli*, C. *Salmonella typhi*, D. *Klebsiella pneumoniae*, E. *Shigella* sp.

Table 5. Zone of inhibition of *Peperomia pellucida* leave extract against tested microorganisms

Bacterial	Minimum inhibition zone (Mean±SD)
Staphylococcus aureus	14.01±0.09
Salmonella typhi	12.07±0.15
Shigella sp.	12.00±0.09
Escherichia coli	11.11±0.17
Klebsiella pneumoniae	10.07±0.15

The observed bioactivity is likely attributed to the presence of major sesquiterpenes, such as caryophyllene, β-elemene, and germacrene D, which have been documented to exhibit broad-spectrum antibacterial effects through mechanisms including membrane disruption and inhibition of DNA synthesis (Santos et al. 2021; Gao et al. 2024). Building on the antibacterial activities observed, molecular docking studies were conducted to explore the potential mechanisms at the molecular level.

The study demonstrated that the methanolic extract derived from *P. pellucida* leaves exhibited inhibitory effects on the growth of pathogenic bacteria, including *S. aureus*, *E. coli*, *S. typhi*, and *Shigella* sp. This finding is noteworthy as it corroborates the traditional use of this plant for treating infections and suggests its potential as a novel source of antibacterial agents. The extract was particularly effective against *S. aureus*, which was attributed to a compound named dillapiole, which binds efficiently to bacterial proteins. This study employed molecular docking to investigate the interaction of dillapiole with bacteria at the molecular level, providing insights into the efficacy of the extract. Expanding this study to elucidate how these interactions impair bacterial functions, such as by inhibiting enzyme activity or disrupting cell wall synthesis, would be advantageous. Comparing the binding affinity of dillapiole with other antibacterial agents could determine whether it is superior or can be used synergistically. Furthermore, understanding whether dillapiole preferentially targets bacterial proteins over human proteins is essential for its therapeutic application in humans. This study currently lacks data on toxicity and selectivity, which should be addressed in future research. This would aid in validating the potential of *P. pellucida* extracts as a treatment, including in vivo testing and safety assessment.

Molecular docking

The compound dillapiole exhibits binding modes at the active sites of six target proteins from different pathogenic bacteria, namely *E. coli*, *K. pneumoniae*, *S. typhi*, *Shigella* sp., and *S. aureus* (Figure 8). The compound dillapiole demonstrates potential as an antibacterial agent through its interactions with the active sites of six target proteins from different pathogenic bacteria, namely *E. coli*, *K. pneumoniae*, *S. typhi*, *Shigella* sp., and *S. aureus*. Each interaction exhibits unique characteristics and binding affinities, as reflected by the docking score values (Table 6). In *E. coli*, dillapiole interacts with residues VAL A:43, VAL A:71, and PHE A:169 through alkyl and pi-alkyl interactions, supported by carbon-hydrogen bonds and van der Waals forces. Despite the presence of some unfavorable bumps, this interaction maintains a relatively strong affinity with a docking score of -63.52 kJ/mol, primarily driven by hydrophobic forces. The dominant interactions at hydrophobic residues (valine, phenylalanine) indicate that dillapiole may interfere with enzyme function through blockage of the substrate channel or allosteric inhibition, preventing binding of the natural substrate. In *K. pneumoniae*, dillapiole forms more complex interactions involving residues HIS A:114, GLY A:78, and ILE A:76 through pi-alkyl, alkyl, π-π stacking, carbon-hydrogen bonds, and van der Waals interactions, resulting in the strongest binding affinity with a docking score of -70.79 kJ/mol. The strong interaction with histidine suggests that dillapiole may interfere with the catalytic logic or function of metal cofactors (if present), thereby inhibiting the activity of vital enzymes such as beta-lactamases or virulence enzymes. For *S. typhi*, the interaction is dominated by π-π stacking and pi-anion interactions with residues PHE A:41 and ASP A:166, along with hydrogen bonds with ASP A:116 and SER A:117, leading to the lowest binding affinity with a docking score of -48.97 kJ/mol. The presence of interactions with charged residues such as aspartate and serine indicates potential inhibition of serine protease or transferase enzymes, but weak binding strength may result in suboptimal antimicrobial effects on these bacteria. In *Shigella* sp., dillapiole binds via T-shaped π-π interactions with TYR A:160 and pi-alkyl interactions with ILE A:248 and LEU A:211, in addition to carbon-hydrogen bonds and van der Waals forces, with a docking score of -57.93 kJ/mol. Dillapiole disrupts the active site structure through aromatic and hydrophobic interactions,

which play a role in stabilizing the substrate or cofactor, thereby inhibiting enzymatic catalysis. The strongest and most stable interaction is observed with *S. aureus*, with a docking score of -70.53 kJ/mol, involving hydrophobic residues such as VAL, ILE, and MET, reinforced by pi-alkyl and π-π stacking interactions with residues PHE A:100, MET A:218, and A:226. Interactions with important hydrophobic and aromatic residues such as methionine and phenylalanine suggest that dillapiole may inhibit biosynthetic pathways, such as cell wall synthesis (e.g., PBPs - Penicillin-Binding Proteins), or redox enzymes critical in bacterial homeostasis. The diversity and abundance of interaction types suggest that dillapiole occupies a highly stable position within the active site of the *S. aureus* protein. Overall, while dillapiole exhibits favorable binding to all target proteins through hydrophobic and aromatic interactions, the interaction with *S. aureus* appears to be the most dominant in terms of strength and stability. This highlights dillapiole as a promising candidate for further development as an antibacterial agent, particularly against *S. aureus* infections.

In general, the compound dillapiole exhibits high affinity for all proteins through hydrophobic and aromatic interaction mechanisms, with specific variations for each protein. However, the interactions with the *S. aureus* protein appear to be the most dominant in terms of both number and types of interactions. The involvement of numerous hydrophobic residues (VAL, ILE, MET) and reinforcement from pi-alkyl and pi-π stacked interactions indicate that dillapiole is in a highly stable position at the active site of the *S. aureus* protein. Therefore, it can be concluded that dillapiole is most likely to inhibit the activity of the *S. aureus* protein compared to the five other bacterial proteins, as it demonstrates the strongest binding. This potential makes dillapiole a promising candidate for application as an antibacterial agent against infections caused by *S. aureus*.

The in vitro antibacterial activity of *P. pellucida* methanol extract correlated well with the in silico molecular docking results for dillapiole, particularly for *S. aureus*, which showed the strongest activity in both approaches. The extract demonstrated moderate activity against *E. coli*, *S. typhi*, and *Shigella* sp., aligning with intermediate docking scores, while *K. pneumoniae* exhibited a discrepancy between in vitro and in silico results. Molecular docking revealed hydrophobic and aromatic interactions as key binding mechanisms, consistent with the antibacterial activity of sesquiterpenes, such as caryophyllene, found in the extract. This correlation suggests that dillapiole may be a key active compound that contributes to the observed antibacterial effects, especially against *S. aureus*. However, the relationship is not perfect for all bacteria tested, indicating other extract components or factors likely influence the overall activity.

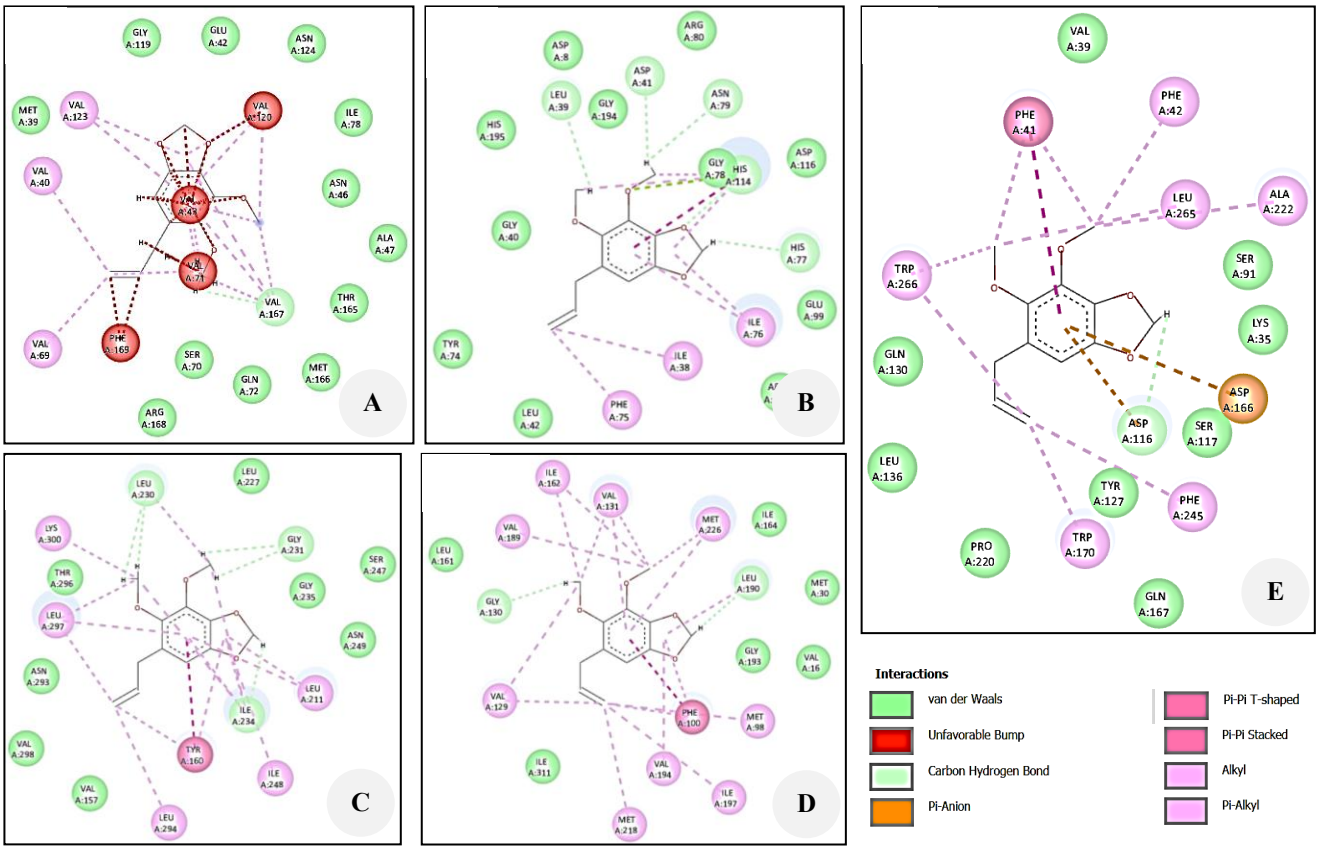

Figure 8. Binding modes of dillapiole in the active site of protein: A. DNA gyrase *Escherichia coli* (PDB ID: 1KZN), B. Essential lipid A *Klebsiella pneumoniae* (PDB ID: 6PIB), C. *Salmonella typhimurium* (PDB ID: 5GTA), D. *Shigella* sp. (PDB ID: 3R9V), and E. *Staphylococcus aureus* (PDB ID: 4DXD)

Table 6. Docking score, binding energies, hydrogen bonds, and key interactions of dillapiole with bacterial target proteins

Protein target of bacterial	Docking score (kJ/mol)	Number of Hydrogen bonds	Key residues involved (Chain:Position)	Dominant interaction types
Escherichia coli	-63.52	1	VAL A:43, VAL A:71, PHE A:169	Alkyl, π-alkyl, C-H bonds, van der Waals
Klebsiella pneumoniae	-70.79	1	HIS A:114, GLY A:78, ILE A:76	π-π stacking, π-alkyl, alkyl, C-H bonds, van der Waals
Salmonella typhi	-48.97	1	PHE A:41, ASP A:166, ASP A:116, SER A:117	π-π stacking, π-anion, hydrogen bonds, van der Waals
Shigella sp.	-57.93	1	TYR A:160, ILE A:248, LEU A:211	T-shaped π-π, π-alkyl, C-H bonds, van der Waals
Staphylococcus aureus	-70.53	2	PHE A:100, MET A:218, VAL, ILE, MET A:226	π-π stacking, π-alkyl, alkyl, hydrophobic interactions

Dillapiole is a phenylpropene compound synthesised via the phenylpropanoid pathway, which is initiated by phenylalanine through the action of the enzyme Phenylalanine Ammonia Lyase (PAL) to produce coniferyl alcohol. Subsequent enzymatic modifications involving coniferyl alcohol acetyltransferase and O-methyltransferase result in the formation of the characteristic methoxy aromatic structure of dillapiole (Liu et al. 2024). Its antibacterial efficacy is predominantly attributed to cell membrane disruption, wherein its lipophilic properties enhance membrane permeability, leading to the leakage of intracellular constituents and eventual bacterial cell lysis (Abers et al. 2021). Additionally, dillapiole may inhibit specific metabolic enzymes, induce oxidative stress, and reduce biofilm formation (Cáceres et al. 2020). However, empirical evidence suggests that pure dillapiole exhibits reduced activity compared to dillapiole-rich essential oils, indicating a synergistic interaction with other constituents that enhances its antibacterial effect. The observed antibacterial activity of *P. pellucida* is generally attributed to a combination of secondary metabolites, including flavonoids, tannins, saponins, and phenylpropanoids, with dillapiole contributing when present. In contrast to *Piper aduncum* L., whose essential oil is predominantly composed of dillapiole, extracts from *P. pellucida* demonstrate more multifaceted antimicrobial activity, with dillapiole being only one of several contributors to its bioactivity (Parise-Filho et al. 2011). This multifactorial composition accounts for the superior efficacy of *P. pellucida* extracts compared to the anticipated activity of dillapiole alone, as the collective effect of various secondary compounds synergistically supports the antibacterial mechanism.

Peperomia pellucida holds a significant position among tropical ethnobotanical species because of its adaptability to moist, shaded, and often disturbed habitats, comparable to *Centella asiatica* (L.) Urb. and *Moringa oleifera* Lam., which also possess notable traditional medicinal value and bioprospecting potential (Anzano et al. 2021; Witkowska et al. 2024). The presence of primary and secondary metabolites, such as phytol, a diterpenoid alcohol derived from chlorophyll degradation that serves as a biosynthetic precursor for tocopherols and plastoquinones, and hexadecanoic acid (palmitic acid), a major product of the fatty acid synthase pathway, contributes not only to plant physiology and structural integrity but also to ecological functions as defensive or chemical signaling compounds (Bobe et al. 2020). The reported antimicrobial and anti-inflammatory activities of these metabolites support the traditional use of *P. pellucida* in various local medicinal systems, while highlighting their relevance to biodiversity conservation and genetic resource utilization frameworks governed by the Convention on Biological Diversity and Nagoya Protocol. Owing to its ease of cultivation and non-threatened status, *P. pellucida* can serve as an ideal model for ethically conducted preliminary bioactivity studies that integrate access, benefit-sharing mechanisms, and community empowerment, thereby ensuring that its use supports the preservation of traditional knowledge while fostering sustainable pharmaceutical innovation.

In conclusion, the comprehensive analysis of *P. pellucida* leaves reveals a promising profile of bioactive compounds with significant antimicrobial and antioxidant properties. GC-MS and FT-IR analyses identified 43 bioactive compounds, predominantly sesquiterpenoids, and confirmed the presence of polyphenolic compounds. The major components included dillapiole (41.77%), N,N'-diacridin-9-yl-hydrazine (7.44%), and caryophyllene (6.94%). The extract exhibited significant antioxidant activity in the DPPH radical scavenging assay, achieving an IC_{50} value of 4.30 µg/mL. This value is lower than that of ascorbic acid, which is 12.71 µg/mL, yet it still reflects a strong antioxidant capability. The extract exhibited significant antibacterial activity against multiple pathogens, with the largest inhibition zone observed against *S. aureus* (14 mm), followed by *S. typhi* and *Shigella* sp. (12 mm each). The molecular docking studies reveal that the compound dillapiole demonstrates varying binding affinities and interaction patterns with proteins from different pathogenic bacteria. While the compound exhibits interactions with all six target proteins, it shows the strongest and most stable binding with the *S. aureus* protein. This binding is characterized by numerous hydrophobic interactions, pi-alkyl, and pi-π stacked interactions, suggesting a high potential for inhibiting the activity of this protein. The significant binding affinity of dillapiole (-70.53 kJ/mol) suggests its potential as a targeted therapeutic agent against *S. aureus*. These findings indicate that dillapiole could be a promising candidate for further development as an antibacterial agent, particularly

against *S. aureus* infections. However, further in vivo studies, toxicity profiling, and formulation development are necessary to fully evaluate the extract's efficacy, safety, and practical application in pharmaceutical and nutraceutical fields. These findings provide a solid foundation for future research into the therapeutic potential of *P. pellucida* and its constituents.

The methanolic extract of *P. pellucida* demonstrates strong antioxidant and antibacterial activities, largely associated with its sesquiterpenoid constituents, particularly dillapiole. These results support the ethnopharmacological applications of this species and highlight its relevance as a promising source for natural product-derived therapeutics and for efforts in biodiversity conservation.

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