Full text open access online (Since 2007)

Ethno Med, 19(4): 147-156 (2025)
PRINT: ISSN 0973-5070 ONLINE: ISSN 2456-6772
DOI: 10.31901/24566772.2024/19.04.713

Gc-Ms Chemical Profiling and in-Vitro Biological Activities of Stem Bark Extracts of *Mallotus Philippensis* (LAM.) MÜLL.ARG. (EUPHORBIACEAE)

K. Prabhakara Rao[1], M.S.V.S.P.Varma[2], Sr. Premakumari[3], Y.T. Rajesh Babu[4*] and S. B. Padal[5]

[1,2]Department of Botany, TDR-HUB Andhra University, Visakhapatnam 530 003, Andhra Pradesh, India
[3]St. Ann's Degree College for Women, Malkapuram, Visakhapatnam 530 004, Andhra Pradesh, India
[4,5]Department of Botany, Andhra University, Visakhapatnam 530 003, Andhra Pradesh, India*
E-mail: [1]<prkocherla@gmail.com>, [2]<msvspvarma1109 @gmail.com> , [3]<premajsas@gmail.com> , [4]<baburajesh0999@gmail.com> , [5]<sbpadal08@gmail.com>

KEYWORDS Antimicrobial Agents. Antioxidant Activity. Ayurvedic Plants. Gas-Mass Spectrometry and Plant Bioactive Compounds

ABSTRACT An ethnomedicinal plant, *Mallotus philippensis* (Lam.) Muell. Arg. (Euphorbiaceae), was analysed for chemical composition, antimicrobial, and antioxidant properties. Antimicrobial activity was evaluated by the agar well diffusion method against four pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescence* and *Streptococcus mutans*), and antioxidant activities were evaluated using DPPH scavenging assay. The most abundant compounds with high area percentages were sucrose (67.43%), 1, 3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (15.96%) and Amlexanox (8.08%) reported in Gas Chromatography Mass Spectrometry (GC-MS) analysis. Bioassay of the antimicrobial activity of the methanol and aqueous bark extracts showed concentration dependent activity with the zone of inhibition ranging from 8 23 mm at various concentrations. The methanol extract of *M. philippensis* was the most effective, with the lowest IC50 value of 35.84±1.28µg/mL, indicating strong antioxidant activity while petroleum ether and aqueous extracts showed moderate activity with IC_{50} values of 64.24±1.45µg/mL and 75.42±1.64 respectively.

INTRODUCTION

Since ancient times, humans have explored plants for their medicinal properties, with references dating back to the Rig Veda in India between 3500 and 1800 B.C (Petrovska 2012). India, with its rich biodiversity and diverse climates, has a longstanding tradition of using medicinal plants, making it a leading producer of herbs for novel medications. Due to the presence of phytochemicals, which have specific physiological effects on humans, medicinal plants have been utilised as treatments for human diseases for ages (Babu et al. 2024a).

Plant-based medicines are increasingly popular for various applications, including food supplements, pharmaceutical intermediates, traditional medicine, nutraceuticals, and modern medicine (Pandey and Tripathi 2014). Secondary metabolites, such as alkaloids, phenolics, flavonoids, saponins, tannins, and glycosides, are produced by plants to defend against predators and environmental stresses, significantly impacting herbal remedies (Roy et al. 2022). These phytochemicals have numerous pharmacological effects, including antimicrobial, antifungal, antidiabetic, anti-inflammatory, and radio-protective properties (Parham et al. 2020).

Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidants, leads to cellular damage and various health disorders. Antioxidants may play a role in reducing the risk of chronic diseases such as heart disease, cancer, diabetes, asthma, liver disorders, and arthritis. Phenolic compounds, flavonoids, and tannins act as antioxidants with anti-inflammatory and anti-carcinogenic properties. Plant extracts' ability to scavenge free radicals indicates their potential as natural antioxi-

* *Address for correspondence:*
Phone: 6300884860, 9052796457
E-mail: baburajesh0999@gmail.com

dants to protect biomolecules like amino acids, proteins, DNA, polyunsaturated fatty acids (PUFA), and lipoproteins from reactive radicals (Martemucci et al. 2022). The DPPH test is a reliable method to identify oxidisable groups in natural or synthetic antioxidants.

Mallotus philippinensis, commonly known as Kamala, is a perennial shrub or small tree found in tropical and subtropical regions, particularly in the outer Himalayas (Widen and Puri 1980). *M. philippinensis* is rich in diverse phytochemicals such as phenols, diterpenoids, steroids, flavonoids, cardenolides, triterpenoids, coumarins, and isocoumarins. Key compounds like bergenin, mallotophilippinens, rottlerin, and iso rottlerin have been identified and exhibit significant biological activities (Gangwar et al. 2014). Notably, rottlerin demonstrates potent protein kinase inhibition, making it a promising candidate for cancer research (Manhas et al. 2021). This plant is traditionally used for anti-filarial (Singh et al. 1997), antibacterial, anti-inflammatory, and immune-regulatory activity (Kumar et al. 2006). The plant also shows remarkable antioxidant properties, with various fractions of its bark and fruit displaying strong antiradical activities. Additionally, the chalcone derivatives from its fruits have been found to have anti-inflammatory and immuno regulatory effects by inhibiting nitric oxide production and down regulating specific inflammatory gene expressions (Daikonya et al. 2004). These bioactivities underline the potential of *M. philippinensis* as a source of medicinal compounds, necessitating further research to explore its pharmacological applications and ensure its safe use in medicine.

Objective

The current study seeks to assess *M. philippensis*'s antimicrobial and antioxidant potentialities, and produce a phytochemical profile of the plant through Gas Chromatography and Mass Spectrometry (GC-MS).

MATERIAL AND METHODS

Plant Material

The plant materials (stem bark) from *M. philippensis* (Euphorbiaceae) were collected from natural forest vegetation of Paderu, Visakhapatnam, Andhra Pradesh, India ($17^0$59'53.79" N $82^0$29'15.59" E and elevation 1033m) in April 2023. Herbarium specimens have been submitted with herbarium number AUV 25558 in the Andhra University herbarium (AUV).

Extraction Method and Preliminary Phytochemical Analysis

The bark of the four plants was collected, cleaned, air dried and then used an electric blender to grind it into a fine powder. Powdered bark materials were extracted with three different solvents of acetone, methanol, and water by using a hot continuous soxhlet extraction. Following the standard procedures preliminary phytochemical screening of plants was carried out.

GC-MS Analysis

The Agilent Technologies GCMS (GC-8890, GC/MS 5977 MSD) equipped with a (DB-WAX) and HP-5 MS UI column (30 m x 250 ìm x 0.25 ìm) was used to analyse the extracts. Split injection mode with a 1 µL of injection volume (15:1 split ratio), 18 mL/min split flow, and a 3 mL/min purge flow, oven temperature ranges from 75°C to 360°C and column temperature ranges from 60°C to 325°C. As the carrier gas, 99.999 percent helium gas was used at a constant flow rate of 1.2 mL/min, and mass spectra were recorded at 70 ev. The identification and description of each compound were achieved by analysing their mass fragmentation patterns and retention indices using the Spectral Library and Database (licensed NIST 2017 Library) with Open Lab CDS 2.5 software (Stein and Scott 1994).

Microbial Strains

The Microbial Type Culture Collection (MTCC), Chandigarh, provided the microbes used in this analysis. For Gram-positive bacteria, the strains include *Bacillus subtilis* (MTCC 28), *Staphylococcus aureus* (MTCC 96), and *Streptococcus mutans* (MTCC 497), all of which were cultured in Muller-Hinton medium at 37 °C for 24 hours with Streptomycin serving as the positive control. For Gram-negative bacteria, the strain tested was *Pseudomonas fluorescens* (MTCC 664), also grown under the same conditions and with Streptomycin as the positive control. This

setup provides a standardised approach to evaluating the antimicrobial efficacy of substances against these specific bacterial strains.

Antimicrobial Activity Screening

The cup plate agar diffusion method was used to evaluate the antimicrobial activity of extracts (Hossain 2024). For the bacterial strains nutritional agar medium was employed to inoculate the plates with overnight cultures of the test organism. Four 5 mm cups were punched on each of the plates at intervals of equal length using sterile cork borers, and each cup was filled with 50 ìL of each concentration of the plant extract (that is, 10 mg, 5 mg, and 2.5 mg). As a positive control, streptomycin (2 mg) was used and incubated for 24 hours at 37°C. The inhibitory zones' diameters were measured during the studies, which were done in triplicate.

Free Radical Scavenging Activity in DPPH Assay

The antioxidant potential of bark extract from the species *M. philippensis* was assessed in terms of its capacity to scavenge free radicals using a reliable DPPH assay (Valko et al. 2007). The reaction mixture, which contained 1 ml of the extract at different concentrations of 20, 40, 60, 80, 100, and 120 µg/mL and 3 ml of DPPH (0.2mM), was incubated for 30 minutes in the dark. A UV-visible spectrophotometer (Agilent) was used to detect the absorbance at 517 nm. The standard ascorbic acid's antioxidant capacity was measured using a similar procedure. Methanol was used as a blank. Three duplicates of each experiment were run. The percentage of inhibition of DPPH's capacity to scavenge free radicals was determined using the formula: Inhibition percentage of DPPH = (Ac/Ac-At) × 100, where Ac is Absorbance of Control and At is Absorbance of test sample.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The phytochemical analysis of *M. philippensis* bark using various solvents (methanol, petroleum ether, and aqueous extract) has provided insights into the presence of primary and secondary metabolites, as shown in Table 1. Carbohydrates were detected in both the methanol and aqueous extracts but were absent in the petroleum ether extract, indicating that carbohydrates preferentially dissolve in polar solvents (Ibrahim et al. 2025). Proteins were present in the methanol extract but absent in both the petroleum ether and aqueous extracts. Alkaloids, phenols, tannins, flavonoids, terpenoids, and saponins were found in both the methanol and aqueous extracts but not in the petroleum ether extract, consistent with the known preference of

Table 1: Phytochemical analysis of *Mallotus philippensis* bark

Plant constituents	*Solvent extracts*		
	Methanol	*Petroleum ether*	*Aqueous extract*
Primary Metabolites			
Carbohydrates	++	-	++
Proteins	+	-	-
Secondary Metabolites			
Alkaloids	+	-	+
Anthraquinones	+	-	-
Phenols	++	-	++
Tannins	++	-	+
Flavanoids	+	-	++
Terpenoids	++	-	+
Cardiac glycosides	++	-	+
Saponins	+	-	++
Steroids	+	-	+
Anthocyanins	-	-	-
Coumarins	+	-	-

Aq: aqueous extract; Me: Methanolic extract; Pe: Petroleum ether extract; (-) = negative (absent), (+) = Positive.

alkaloids and phenolic compounds for polar solvents (Fernandez et al. 2024; Lakshmi et al. 2025). Anthraquinones, cardiac glycosides, steroids, and coumarins were detected exclusively in the methanol extract. This selective solubility implies that anthraquinones, in particular, have greater solubility in methanol relative to nonpolar solvents (Negi et al. 2025). Anthocyanins were absent in all extracts.

The phytochemical profile observed for *M. philippensis* bark aligns with the known solubility characteristics of various classes of compounds. Carbohydrates and proteins are typically extracted using polar solvents, which corroborates the findings in the study (Tourabi et al. 2025). Alkaloids, phenols, and tannins are frequently soluble in polar solvents and were detected in the methanol and aqueous extracts, in agreement with recent studies showing higher yields of these compounds in polar extracts (Chatepa et al. 2024). Terpenoids and saponins can dissolve in both polar and semi polar solvents, which is congruent with their detection in the methanol and aqueous extracts (Guo et al. 2024; Aktas and Kurek 2024). The absence of anthocyanins and coumarins in all extracts except the methanol one is in keeping with their solubility preferences and the polarity of solvents used (Scopel et al. 2024; Yang and Kilmartin 2024). The results suggest that the methanol extract of *M. philippensis* bark contains a wide range of phytochemicals, many of which align with their known solubility and biological properties, while some findings, such as the presence of steroids in methanol and the absence of anthocyanins, warrant further investigation.

GC-MS Evaluation

According to the GC-MS spectrum, the bark methanolic extract of *M. philippensis* contains a variety of compounds with various retention times. The analysis of the mass spectrometer provides information about the compounds' structure and composition. A variety of peaks with various m/z ratios are produced as a result of the breakdown of larger compounds into smaller ones. These mass spectra act as the distinct fingerprints for that specific molecule. GC-MS analysis revealed 5 different compounds in the methanol extract of *M. philippensis* bark, which are given in Table 2 along with their molecular weight, molecular formula, and percentage of peak area. The most abundant compounds with high area percentages were sucrose (67.43%), 1,3-benzene dicarboxylic acid, bis(2-ethylhexyl) ester (15.96%) and Amlexanox (8.08%). 4-Hydroxy-2',3,4',6'-tetramethoxy chalcone (5.74%) and Isosorbide Dinitrate (2.80%) were also present in less quantity.

The methanol bark extract of *Mallotus philippensis* reveals a diverse array of compounds with significant bioactivities, ranging from common nutritional sugars to pharmacologically active agents and environmental contaminants. These findings underscore the broad potential of plant extracts in both medicinal and toxicological contexts. Notably, sucrose, a common sugar, has been identified in a high percentage in the bark of *Mallotus philippensis*, a finding not typically reported in previous studies. This suggests that the bark may serve as a significant energy source, potentially influencing metabolic processes within the plant.

Table 2: Compounds identified in the GC-MS analysis of the methanol bark extract of *Mallotus philippensis*

S.No.	*Rt time*	*Detected compounds*	*CAS number*	*Molecular formula*	*Molecular weight g/mol*	*Area (%)*
1	13.166	Sucrose	57-50-1	$C_{12}H_{22}O_{11}$	342.30	67.43
2	17.389	Isosorbide Dinitrate	87-33-2	$C_6H_8N_2O_8$	236.14	2.80
3	36.994	Amlexanox	68302-57-8	$C_{16}H_{14}N_2O_4$	298.29	8.08
4	40.203	4-Hydroxy-2',3,4',6'-tetramethoxychalcone	1017899-50-1	$C_{19}H_{20}O_6$	344.4	5.74
5	40.667	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	137-89-3	$C_{24}H_{38}O_4$	390.6	15.96

Isosorbide dinitrate, identified for the first time in *Mallotus philippensis*, is a well-known vasodilator used in the management of angina pectoris and heart failure. This compound operates by releasing nitric oxide, which relaxes smooth muscles and dilates blood vessels, thereby improving blood flow and oxygen supply to the heart (Fernandes et al. 2024). Additionally, an anti-inflammatory and anti-allergic agent, previously unreported in this plant, has been identified, indicating possible novel bioactive pathways. Recent findings indicate that amlexanox targeted inhibition of TBK1 may deregulate immune cell function and exacerbate inflammatory responses in DSS-induced inflammatory bowel disease models, highlighting the context-dependent effects of TBK1 inhibition (Hui et al. 2024).

Furthermore, 4-Hydroxy-2,3,4,6-tetramethoxychalcone, a type of chalcone, has been detected. Chalcones, as naturally occurring compounds with a conjugated double bond and carbonyl group, serve as versatile pharmacophores exhibiting broad-spectrum antimicrobial, anti-inflammatory, antioxidant, and anticancer activities, making them promising candidates in drug discovery and therapeutic development (Suman et al. 2025). Additionally, 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, also known as DEHP (Di(2-ethylhexyl) phthalate), was identified. DEHP remains a pervasive plasticizer and environmental contaminant, and recent evidence continues to implicate it as an endocrine disruptor with adverse effects on reproductive, developmental, metabolic, and carcinogenic endpoints (Chen et/ al. 2025). Thus, the compounds identified by GC-MS analysis provide evidence of the medicinal potential of the methanol extract to treat various infectious diseases.

Antimicrobial Activity

The results from the antimicrobial activity tests on *Mallotus philippensis* bark extracts against different bacterial strains are summarised in Table 3. The zones of inhibition were measured in millimeters for methanol, acetone, and aqueous extracts at dosages of 10 mg, 5 mg, and 2.5 mg, with the positive control being streptomycin (+ve). The methanol extract showed significant antimicrobial activity against all tested bacteria, with the highest zones of inhibition

Table 3: Antibacterial activity of different extracts of *Mallotus philippensis*

Extract	*Gram -ve bacteria*						*Gram +ve bacteria*					
	P.fluorescens			*Streptococcus mutans*			*Bacillus subtilis*			*Staphylococcus aureus*		
	10mg	*5 mg*	*2.5mg*	*10mg*	*5 mg*	*2.5mg*	*10mg*	*5 mg*	*2.5mg*	*10mg*	*5 mg*	*2.5mg*
Me	18.25±0.75	14.3±0.57	12±1	18.3±0.75	17±1	16±0	15±1	13.3±0.57	12±1	22.6±0.57	16±1	15.3±0.57
PE	-	-	-	13.3±0.57	8±1	7.3±0.57	-	-	-	12±1	-	-
Aq	15.3±0.57	13.3±0.57	10±1	14±0	13±1	11.3±0.57	12±1	10±1	8.3±0.57	16±1	13±0	9.6±0.57
+ve	31.2 ± 1.23			32.3± 0.57			30 ± 1			27.6 ± 1.52		

Data showing zone of inhibition in mm. Aq: aqueous extract, Me: methanol extract, PE: Petroleum ether extractact. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

against *Staphylococcus aureus* (22.6±0.57mm at 10 mg) and *Pseudomonas fluorescens* (18.25± 0.75mm at 10 mg). The aqueous extract also demonstrated notable antimicrobial effects, especially against *Staphylococcus aureus* (16±1 mm at 10 mg) and *Pseudomonas fluorescens* (15.3±0.57mm at 10 mg). The petroleum ether extract showed limited activity, with zones of inhibition only against *Streptococcus mutans* at all dosages and against *Staphylococcus aureus* at the highest dosage (12±1). The positive control, streptomycin, exhibited the highest zones of inhibition across all bacterial strains, validating the effectiveness of the extracts. The petroleum ether extract showed minimal activity, suggesting that its antimicrobial constituents are either absent or in lower concentrations compared to the other extracts.

The methanol and aqueous extracts demonstrated the highest antimicrobial activity against the human pathogens tested. The current study found that the methanol extract exhibited the greatest zone of inhibition against both Gram-negative bacteria like *P. fluorescens* and *S. mutans*, and Gram-positive bacteria such as *S. aureus*. This effectiveness is likely due to the presence of secondary metabolites, including flavonoids, phenolic compounds, terpenoids, and steroids, as identified in the phytochemical analysis. Specifically, the methanol extract showed significant antibacterial activity against all tested bacteria, with the most substantial effect on *S. aureus* (22.6±0.57 mm at 10mg). Previous research has similarly reported strong antibacterial properties of methanol extracts from various plants, attributing these effects to bioactive compounds like flavonoids, tannins, and phenolic acids (Babu et al. 2024b). Conversely, the petroleum ether extract exhibited limited antibacterial activity, showing notable effects only against *S. mutans* (13.3±0.57 mm at 10mg) and *S. aureus* (12±1 mm at 10mg). Literature suggests that non-polar extracts such as petroleum ether may have lower antimicrobial activity compared to polar extracts like methanol and aqueous extracts, possibly due to the absence of polar bioactive compounds (Palaniyappan et al. 2024).

Antioxidant Activity

Antioxidants are substances that stabilise free radicals produced in the oxidation process and protect living cells from damage such as cancer (Gulcin 2025). The antioxidant activity of aqueous, petroleum ether, and methanol extracts of the bark of *M. philippensis* was examined using the DPPH assay. The absorbance at 517 nm decreased as the DPPH radical oxidised by an antioxidant molecule. This reduction served as a measure of the antioxidant molecule's efficiency (Oraibi et al. 2025). The study's results suggest that the various solvent extracts have a noticeable impact on the DPPH radical. The radical scavenging activity (RSA) of different extracts of *M. philippensis* was assessed at concentrations ranging from 20 to 120µg/mL. The extracts tested included aqueous, petroleum ether, and methanol extracts, with ascorbic acid serving as the standard. The percentage RSA for each concentration is presented in Table 4.

The aqueous, petroleum ether and methanol extracts exhibited a concentration-dependent increase in radical scavenging activity. In the aqueous extract, the percentage of RSA at 20µg/mL was 22.35 percent, which increased to 76.52 percent at 120µg/mL. The RSA values at inter-

Table 4: Percentage of radical scavenging activity of DPPH assay of *Mallotus philippensis* extracts

	Concentration (µg/mL) and % RSA					
	20(µg/mL)	*40(µg/mL)*	*60(µg/mL)*	*80(µg/mL)*	*100(µg/mL)*	*120(µg/mL)*
Aqueous extract	22.35 ±0.03	29.24 ±1.04	38.51 ±3.40	52.16 ±1.84	63.25 ±2.03	76.52 ±2.51
Petroleum ether extract	24.35 ±0.78	31.62 ±1.34	51.32 ±1.63	64.35 ±0.08	73.24 ±2.53	74.24 ±2.14
Methanol extract	36.52 ±0.34	52.34 ±1.12	68.25 ±1.63	78.24 ±1.94	86.32 ±2.12	91.32 ±2.55
Ascorbic acid	62.23 ±0.95	69.58 ±1.84	82.25 ±3.10	88.93 ±2.77	92.36 ±0.82	96.54 ±0.05

mediate concentrations were 29.24 percent, 38.51 percent, 52.16 percent, and 63.25 percent for 40, 60, 80, and 100µg/mL, respectively. For petroleum ether, the extract percentage of RSA ranged from 24.35 percent at 20µg/mL to 74.24 percent at 120µg/mL. The methanol extract showed the highest RSA among the three extracts. The RSA was 36.52 percent at 20µg/mL and reached 91.32 percent at 120µg/mL. The RSA values at intermediate concentrations were 52.34 percent, 68.25 percent, 78.24 percent, and 86.32 percent for 40, 60, 80, and 100µg/mL, respectively. The standard deviations ranged from ±0.34 to ±2.55, suggesting high reliability of the results. Ascorbic acid, the positive control, exhibited the highest RSA at all concentrations. The RSA values were 62.23 percent, 69.58 percent, 82.25 percent, 88.93 percent, 92.36 percent, and 96.54 percent for 20, 40, 60, 80, 100, and 120µg/mL, respectively.

The DPPH assay results indicate that all extracts of *Mallotus philippensis* possess radical scavenging activity, with the methanol extract showing the highest activity, followed by the petroleum ether and aqueous extracts. This suggests that the methanol extract contains the highest concentration of antioxidant compounds, making it the most effective in neutralising free radicals. The presence of Isosorbide dinitrate and chalcones in the methanol extracts may be the reason for the high antioxidant activity exhibited by methanol extract (Samota et al. 2024). The aqueous extract, while showing a significant increase in RSA with concentration, had the lowest activity compared to the other extracts. This might be due to the lower solubility of active compounds in water, which limits their extraction efficiency. Ascorbic acid, a known antioxidant, consistently exhibited the highest RSA at all tested concentrations, validating the effectiveness of the assay. The results suggest that *M. philippensis* extracts, particularly the methanol extract, could be a potent source of natural antioxidants, which are essential for combating oxidative stress and preventing related diseases.

The DPPH assay was employed to determine the antioxidant activity of different extracts of *Mallotus philippensis*. The antioxidant activity was quantified by calculating the IC_{50} values. The linear equations, R^2 values, and IC_{50} values for the methanol, petroleum ether, and aqueous extracts, as well as the standard ascorbic acid, are summarised in Table 5. The linear equation for the aqueous extract is y=0.5522x+8.352 with an R^2 value of 0.9894, indicating a very strong correlation between concentration and radical scavenging activity. The IC_{50} value, which represents the concentration required to inhibit 50 percent of the DPPH radicals, is 75.42 ± 1.64µg/mL. This relatively high IC_{50} value suggests that the aqueous extract has moderate antioxidant activity.

For the petroleum ether extract, the linear equation is y=0.5533x+14.453 with an R^2 value of 0.9441. The IC_{50} value is 64.24 ± 1.45 µg/mL, which is lower than that of the aqueous extract, indicating better antioxidant activity. The methanol extract's linear equation is y=0.5513x+30.239 with an R^2 value of 0.96, showing a strong correlation. The IC_{50} value is 35.84 ± 1.28µg/mL, the lowest among the three extracts, indicating the highest antioxidant activity. Ascorbic acid, used as a positive control, has a linear equation of y=0.3503x+55.406 and an IC_{50} value of 15.43 ± 0.95µg/mL, the lowest among all tested samples. This confirms the potent antioxidant activity of ascorbic acid. Although these extracts have significant antioxidant activity, they are less potent than ascorbic acid. However, the high R^2 values for all extracts indicate reliable and consistent antioxidant activity across the tested concentrations. Recent work on *Mallotus philippensis*

Table 5: Linear equations and IC_{50}values of DPPH assay of *Mallotus philippensis* extracts

Plant species	*Linear equation* $Y = mx + C$	*R^2 values*	*IC_{50}values[a](µg/mL)*
Aqueous extract	y = 0.5522x + 8.352	R^2 = 0.9894	75.42±1.64
Petroleum ether extract	y = 0.5533x + 14.453	R^2 = 0.9441	64.24±1.45
Methanol extract	y = 0.5513x + 30.239	R^2 = 0.96	35.84±1.28
Ascorbic acid	y = 0.3503x + 55.406	R^2 = 0.9488	15.43±0.95

[a] Means of three determinations + SD (Standard deviation)

fruit extracts reports 97 percent DPPH radical scavenging activity for the ethanolic extract via maceration, highlighting its significant antioxidant potential (Ali et al. 2024).

CONCLUSION

The study examined the antioxidant and antimicrobial properties of various solvent extracts of *Mallotus philippensis* stem bark. The DPPH assay showed significant radical scavenging activity across all extracts, with the methanol extract exhibiting the highest potency (IC_{50} = 35.84 ± 1.28 µg/mL), likely due to its high phenolic content. The methanol extract also demonstrated notable antimicrobial effects, particularly against *Staphylococcus aureus* and *Streptococcus mutans*, indicating its potential as a natural antimicrobial agent. These findings suggest the methanol extract of *M. philippensis* holds promise for pharmaceutical and nutraceutical applications.

RECOMMENDATIONS

M. philippensis has demonstrated strong antimicrobial and antioxidant properties in vitro, supported by GC-MS analysis that identified key bioactive compounds. These findings provide scientific validation for its traditional medicinal use. To advance its potential for developing natural, plant-based medications, further research is recommended to evaluate its efficacy in clinical trials.

ABBREVIATIONS

AUV	:	Andhra University Herbarium code
CDS	:	Chromatography Data System
DMSO	:	dimethyl sulfoxide
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
EI	:	Electron Ionisation
GC-MS	:	Gas Chromatography-Mass Spectrometry
HP-5 MS	:	(5%-phenyl)-methylpolysiloxane capillary column
MSD	:	Mass Selective Detector
MTCC	:	Microbial Type Culture Collection and Gene Bank
NIST	:	National Institute of Standards and Technology
RSA	:	Radical Scavenging activity
U	:	Ultra Inert

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

ACKNOWLEDGMENTS

The authors would like to thank Andhra University's Department of Botany for providing research facilities and instruments, as well as SAIF-IIT Madras for providing GC-MS services.

REFERENCES

Aktas H, Kurek MA 2024. Deep eutectic solvents for the extraction of polyphenols from food plants. *Food Chemistry*, 444: 138629. https://doi.org/10.1016/j.foodchem.2024.138629

Ali A, Chen H, Xu H et al. 2024. Accessing the medicinal potential of *Mallotus philippensis*: comprehensive exploration of antioxidant and antibacterial properties through phytochemical analysis and extraction techniques. *Separations*, 11(6): 165. https://doi.org/10.3390/separations11060165

Babu YTR, Kumar GV, Prathamanjali S et al. 2024b. GC-MS based identification of bioactive phytocompounds in methanolic extract of *Peperomia dindygulensis* Miq and their antimicrobial activities against pathogens. *Studies on Ethno Medicine*, 18(2): 69-80. DOI: 10.31901/24566772.2024/18.02.668

Babu YTR, Prathamanjali S, Raju BAR et al. 2024a. Medicinal plants from India as prospects of anticancer drug sources: A comprehensive review. *Futuristic Trends in Agriculture Engineering & Food Sciences*, 4(3): 170-192. IIP Series. https://doi.org/10.58532/v3bcag4p2ch5

Chatepa LEC, Mwamatope B, Chikowe I et al. 2024. Effects of solvent extraction on the phytoconstituents and in vitro antioxidant activity properties of leaf extracts of two selected medicinal plants from Malawi. *BMC Complementary Medicine and Therapies*, 24(1): 317. https://doi.org/10.1186/s12906-024-04619-7

Chen L, Huang Y, Liu F et al. 2025. Impact of di-(2-ethylhexyl)-phthalate on metabolic syndrome: Insights from network toxicology and molecular docking and dynamics. *PubMed*, 18: 2277-2288. https://doi.org/10.2147/dmso.s523668

Craig WJ 1997. Phytochemicals. *Journal of the American Dietetic Association*, 97(10): S199-S204. https://doi.org/10.1016/s0002-8223(97)00765-7

Daikonya A, Katsuki S, Kitanaka S 2004. Antiallergic agents from natural sources-Inhibition of nitric oxide production by novel chalcone derivatives from *Mallotus philippinensis* (Euphorbiaceae). *Chemical & Pharmaceutical Bulletin*, 52(11): 1326–1329.

Fernandes SMM, De Andrade GACK, Saito GYK et al. 2024. Hydralazine and isosorbide dinitrate in heart failure: from evidence to clinical practice. *ABC Heart Failure & Cardiomyopathy*, 4(1): e20240019. https://doi.org/10.36660/abchf.20240019i

Fernandez PB, Morante ZS, Sierra I 2024. Simultaneous determination of 23 pyrrolizidine and tropane alkaloids in infusions from dry edible flowers using optimized μSPEed® microextraction prior to their analysis by UHPLC-IT-MS/MS. *Foods*, 13(11): 1740. https://doi.org/10.3390/foods13111740

Gangwar M, Goel RK, Nath G 2014. *Mallotus philippinensis* Muell. Arg (Euphorbiaceae): Ethnopharmacology and Phytochemistry review. *BioMed Research International*, 2014: 1-13. https://doi.org/10.1155/2014/213973

Gulcin I 2025. Antioxidants: A comprehensive review. *Archives of Toxicology*. https://doi.org/10.1007/s00204-025-03997-2

Guo J, Zhao N, Zhao Y et al. 2024. The extraction using deep eutectic solvents and evaluation of tea saponin. *Biology*, 13(6): 438. https://doi.org/10.3390/biology13060438

Hossain TJ 2024. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*, 14(2): 97-115. https://doi.org/10.1556/1886.2024.00035

Hui L, Huang M, Dai Q et al. 2024. Amlexanox targeted inhibition of TBK1 regulates immune cell function to exacerbate DSS-induced inflammatory bowel disease. *Clinical & Experimental Immunology*, 219(1): uxae082. https://doi.org/10.1093/cei/uxae082

Ibrahim SK, Albadr RJ, Sur D et al. 2025. Prediction of thermodynamic properties of aqueous carbohydrates solution using the PHSC and ANN models. *Scientific Reports*, 15(1): 21539. https://doi.org/10.1038/s41598-025-06552-2

Kumar VP, Chauhan NS, Padh H et al. 2006. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *Journal of Ethnopharmacology*, 107(2): 182-188.

Lakshmi SYS, Yavanika V, Monisha V et al. 2025. Assessment of phytochemicals and characterisation of a polyherbal formulation. *Indian Journal of Science and Technology*, 18(21): 1716-1727. https://doi.org/10.17485/ijst/v18i21.556

Madureira AM, Ramalhete C, Mulhovo S et al. 2011. Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. *Pharmaceutical Biology*, 50(4): 481-489.

Manhas D, Gour A, Bhardwaj N et al. 2021. Pharmacokinetic assessment of Rottlerin from *Mallotus philippensis* using a highly sensitive Liquid Chromatography-Tandem Mass Spectrometry-Based Bioanalytical Method. *ACS Omega*, 6(48): 32637-32646.

Martemucci G, Costagliola C, Mariano M et al. 2022. Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, 2(2): 48-78.

Negi A, Toukola P, Raisanen R 2025. Chemical profiling and characterisation of anthraquinone based polyphenols as biocolourants from *Cortinarius semisanguineus*. *Coloration Technology*, 2025: 1-34. https://doi.org/10.1111/cote.12810

Oraibi AI, Dawood AH, Trabelsi G et al. 2025. Antioxidant activity and selective cytotoxicity in HCT-116 and WI-38 cell lines of LC-MS/MS profiled extract from *Capparis spinosa* L. *Frontiers in Chemistry*, 13. https://doi.org/10.3389/fchem.2025.1540174

Palaniyappan S, Sridhar A, Arumugam M et al. 2024. Bioactive analysis of antibacterial efficacy and antioxidant potential of *Aloe barbadensis* Miller leaf extracts and exploration of secondary metabolites using GC–MS profiling. *Applied Biochemistry and Biotechnology*, 196(2): 729-773. https://doi.org/10.1007/s12010-023-04565-z

Pandey A, Tripathi S 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drugs. *Journal of Pharmacognosy and Phytochemistry*, 2: 115-119.

Parham S, Kharazi AZ, Bakhsheshi HR et al. 2020. Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants*, 9(12): 1309.

Petrovska BB 2012. Historical review of medicinal plants2 usage. *Pharmacognosy Review*, 6(11): 1.

Roy A, Khan A, Ahmad I et al. 2022. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *BioMed Research International*, 2022: 1-9.

Samota MK, Yadav DK, Koli P et al. 2024. Exploring natural chalcones: Innovative extraction techniques, bioactivities, and health potential. *Sustainable Food Technology*, 2(5): 1456-1468. https://doi.org/10.1039/d4fb00126e

Scopel JM, Medeiros NB, Teixeira HF et al. 2024. Supercritical carbon dioxide extraction of coumarins from the aerial parts of *Pterocaulon polystachyum*. *Molecules*, 29(12): 2741. https://doi.org/10.3390/molecules29122741

Singh K, Singhal CR, Khan NU 1997. Antifilarial activity of *Mallotus philippinensis* Lam. on *Setaria cervie* (Nematoda: Filarioidea) in-vitro. *Indian Journal of Physiology and Pharmacology*, 41(4): 397-403.

Stein SE, Scott DR 1994. Optimization and testing of mass spectral library search algorithms for compound identification. *Journal of the American Society for Mass Spectrometry*, 5(9): 859-866.

Suman A, Priyathosh N, Vishal KD et al. 2025. Pharmacological potential of natural chalcones: A recent studies and future perspective. *Frontiers in Pharmacology*, 16: 1570385. https://doi.org/10.3389/fphar.2025.1570385

Tourabi M, Faiz K, Ezzouggari R et al. 2025. Optimization of extraction process and solvent polarities to enhance the recovery of phytochemical compounds, nutritional content, and biofunctional properties of *Mentha longifolia* L. extracts. *Bioresources and Bioprocessing*, 12: 24. https://doi.org/10.1186/s40643-025-00859-8

Valko M, Leibfritz D, Moncol J et al. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry & Cell Biology*, 39(1): 44-84.

Widen CJ, Puri HS 1980. Natural occurrence and chemical variability of phloroglucinols in Kamala. *Planta Medica*, 40(3): 284-287.

Yang Y, Kilmartin PA 2024. Advancing anthocyanin extraction: optimising solvent, preservation, and microwave techniques for enhanced recovery from merlot grape marc. *Food Chemistry*, 472: 142648. https://doi.org/10.1016/j.foodchem.2024.142648

Yue S, Zheng W, Fan C et al. 2024. Mechanistic insights into di-2-ethylhexyl phthalate (DEHP)-induced metabolic disruption: integrating gut hormone secretion and metabolomics in colonic organoids. *Environmental Science & Technology Letters*, 11(9): 940–947. https://doi.org/10.1021/acs.estlett.4c00593

Paper received for publication in August, 2024
Paper accepted for publication in October, 2025