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Exploring the therapeutic efficacy of *Piper betle* and *Piper longum*: Focus on antimicrobial and antioxidant activities

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ABSTRACT

Background: Plants from the genus *Piper* have long been used in traditional medicine for their diverse pharmacological properties. Among them, *Piper betle* and *Piper longum* are known for their potential therapeutic effects, yet comparative studies on their antimicrobial and antioxidant activities remain limited. **Objective:** This study aimed to evaluate and compare the antimicrobial and antioxidant properties of various solvent extracts prepared from the leaf, stem, and root parts of *Piper betle* and *Piper longum*. **Materials and Methods:** Solvent extracts were prepared from different plant parts using appropriate solvents. Antimicrobial activity was tested against four bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*), and two fungal strains (*Aspergillus niger* and *Rhizopus* sp.) using the agar well diffusion method. Antioxidant activity was determined by the DPPH radical scavenging assay, and IC_{50} values were calculated. **Results:** *Piper betle* demonstrated a broader and stronger antimicrobial spectrum than *Piper longum*. The methanolic leaf extract of *P. betle* (A1MH) exhibited the highest inhibition zone (21 mm) against *Proteus vulgaris*, whereas *Pseudomonas aeruginosa* was resistant to all extracts. In antioxidant assays, A1MH showed the strongest activity with an IC_{50} value of 37.57 µg/ml, comparable to that of standard ascorbic acid (IC_{50} = –0.10 µg/ml). *Piper longum* extracts displayed comparatively lower antioxidant potential. **Conclusion:** The methanolic leaf extract of *Piper betle* exhibited significant antimicrobial and antioxidant activities, supporting its traditional use as a natural therapeutic agent. Further studies are warranted to isolate and characterize the bioactive compounds responsible for these effects.

Keywords: *Piper betle*, *Piper longum*, Antimicrobial, Antioxidant, Well diffusion, DPPH.

INTRODUCTION

The alarming rise in antimicrobial resistance (AMR) and the prevalence of oxidative stress-related disorders have intensified the global search for novel, effective, and safe therapeutic agents [1,2]. Medicinal plants, as natural reservoirs of structurally diverse phytochemicals, continue to offer promising pharmacological scaffolds, particularly for antimicrobial and antioxidant therapies [3,4]. Within this domain, the *Piper* genus (Family: *Piperaceae*) has garnered attention due to its rich ethnopharmacological history and diverse bioactive constituents [5].

Two notable species, *Piper betle* L. and *Piper longum* L., are widely used in Ayurvedic, Unani, and Southeast Asian traditional medicine systems [6,7]. *P. betle*, commonly known as betel vine, is reputed for a broad spectrum of pharmacological effects including analgesic, anticancer, anti-inflammatory, antimicrobial, antioxidant, antiseptic, and hepatoprotective activities, attributed to its rich array of phenolics, flavonoids, terpenoids, essential oils, and alkaloids [6]. Similarly, *P. longum* (long pepper or pippali) has been traditionally employed in the management of respiratory and gastrointestinal ailments, and exhibits various pharmacological properties such as anti-inflammatory, antioxidant, anticancer, immunomodulatory, and antimicrobial effects. Its major phytoconstituents include alkaloids like piperine and piperlongumine, along with flavonoids, lignans, and esters [8].

While the individual bioactivities of *P. betle* and *P. longum* have been documented in various preliminary studies, a comprehensive and comparative evaluation of their antioxidant capacity, and antimicrobial efficacy remains limited [9,10]. Given the interconnected nature of oxidative stress and microbial pathogenesis [11], simultaneous assessment of both bioactivities offers a more integrated perspective on their therapeutic value.

Therefore, the present study aims to systematically investigate and compare the *in vitro* antioxidant and antimicrobial activities of *Piper betle* and *Piper longum* through standard biochemical and microbiological assays. This integrative approach not only enhances understanding of their bioactive potential but also supports their pharmacognostic validation as candidates for drug development targeting oxidative stress and infection-mediated disorders.

MATERIALS AND METHODS

Chemicals and Reagents

Culture media (HiMedia)- Nutrient broth, Sabouraud Dextrose broth, Mueller Hinton Agar, Sabouraud Dextrose Agar; Antibiotics-Streptomycin (25µg/disc), Ketoconazole (25µg/disc); Hexane, Methanol, Chloroform, Ethyl acetate, Ascorbic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl).

Collection and Identification of Plant Materials

Fresh and healthy parts of *Piper betle* and *Piper longum* were collected from Mulagumoodu (8° 16' 3.00" N, 77° 17' 28.20" E), Kanya Kumari District, Tamil Nadu, India. Live specimens of both the plants were identified and authenticated by Dr. R. Subitha Shajini, Department of Botany, Women's Christian College, Nagercoil, Kanya Kumari District, Tamil Nadu, India. The authentication of the plants was recorded under the authorization number A.C.NO: 7/Dept. of Bot./WCC/NGL.

Preparation of Plant Materials

The plant materials were thoroughly washed under running tap water to remove surface debris, followed by rinsing with distilled water. They were then shade-dried at room temperature until completely brittle. Once dried, the plant parts were ground into fine powder using an electric blender and immediately used for extraction [12].

Solvent Extraction of Plant Materials

About 20 gms of powdered plant materials from the leaf (A1), stem (A2), and root (A3) of *Piper betle*, and the leaf (C1), stem (C2), and root (C3) of *Piper longum*, were subjected to Soxhlet extraction using 200 mL of five different solvents: hexane (60°C), methanol (70°C), ethyl acetate (70°C), chloroform (60°C), and distilled water (100°C). Extraction was continued for 24 hours or until the siphon tube solvent turned colorless. The extracts were concentrated using a rotary evaporator at 40-60°C for 30 minutes. The extracts were coded as follows: hexane extracts (A1HX, A2HX, A3HX, C1HX, C2HX, C3HX), methanol extracts (A1MH, A2MH, A3MH, C1MH, C2MH, C3MH), ethyl acetate extracts (A1EA, A2EA, A3EA, C1EA, C2EA, C3EA), chloroform extracts (A1CH, A2CH, A3CH, C1CH, C2CH, C3CH), and aqueous extracts (A1AQ, A2AQ, A3AQ, C1AQ, C2AQ, C3AQ). All extracts were stored at 4°C in airtight containers until further use [13].

Inoculum Preparation

MTCC cultures of bacterial and fungal strains were used for this study. Bacterial inocula of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* were prepared in Nutrient Broth and incubated at 37°C for 24 hours. Fungal strains, *Aspergillus niger* and *Rhizopus* sp. were cultured in Sabouraud Dextrose Broth and incubated at 27-30°C for 48-72 hours.

Antimicrobial Activity of Plant Extracts

The antimicrobial activities of plant extracts (A1-A3 and C1-C3) were assessed using the agar well diffusion method. Standard drugs were tested by disc diffusion. Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were prepared by dissolving 38 g and 65 g of each respective medium (HiMedia) in 1000 mL distilled water, followed by autoclaving at 121 °C for 15 minutes at 15 lbs pressure.

Petri plates were poured with 25 mL of sterile medium. MHA plates were inoculated with bacterial strains and SDA plates with fungal strains. Wells were aseptically bored and filled with 50 µL of each extract. Streptomycin (25 µg/disc) and Ketoconazole (25 µg/disc) served as positive controls for bacterial and fungal strains, respectively, while sterile distilled water was used as the negative control. Plates were incubated at 37°C for 24 hours (bacteria) and 28°C for 48-72 hours (fungi). Zones of inhibition, including the diameter of wells or discs, were measured in millimetres. Absence of a zone indicated no antimicrobial activity. Activity was categorized as resistant (<7 mm), intermediate (8–10 mm), or sensitive (>11 mm) [14,15,16].

Antioxidant Activity of Plant Extracts

The antioxidant potential of plant extracts was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay [17]. A DPPH stock solution was diluted with methanol to yield a working solution with an absorbance of 0.98 ± 0.02 at 520 nm. Each test sample was prepared by dissolving 1 mg of extract in 1 mL methanol; ascorbic acid was used as the reference standard.

For the assay, 2 mL of the DPPH solution was added to test tubes containing 100 µL of plant extract or standard at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. The mixtures were incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 520 nm using a UV–Vis spectrophotometer.

Radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = [(\text{OD control} - \text{OD sample}) / \text{OD control}] \times 100$$

RESULTS AND DISCUSSION

Antimicrobial Activity of Plant Extracts:

The antimicrobial activity of hexane (HX), methanol (MH), ethyl acetate (EA), chloroform (CH), and aqueous (AQ) extracts from different parts: leaf (A1, C1), stem (A2, C2), and root (A3, C3) of *Piper betle* (A) and *Piper longum* (C) was evaluated against four bacterial strains (*Escherichia coli, Staphylococcus aureus, Proteus vulgaris,* and *Pseudomonas aeruginosa*) and two fungal strains (*Aspergillus niger* and *Rhizopus* sp.) (Table 1).

Among all the tested extracts, the methanolic extracts of *P. betle* exhibited the most pronounced antimicrobial activity. The leaf extract (A1MH) showed strong inhibition against *P. vulgaris* (21 mm, sensitive) and *S. aureus* (13 mm, sensitive), moderate activity against *A. niger* (8 mm, intermediate), and resistance to *E. coli* (7 mm). Additionally, it demonstrated antifungal sensitivity to *Rhizopus* sp. (12 mm). The stem extract (A2MH) followed a similar trend, showing sensitivity to *S. aureus* and *P. vulgaris* (12 mm each), *A. niger* (11 mm), and resistance to *E. coli* (7 mm). The root extract (A3MH) was comparatively less active, exhibiting sensitivity only to *S. aureus* (12 mm).

These results align with previous findings by Kaveti *et al*. (2011), who reported the absence of antimicrobial activity of aqueous *P. betle* leaf extract against *E. coli* and *P. aeruginosa*, [18] and are consistent with the present study where aqueous and methanolic extracts showed limited efficacy against *E. coli*. However, they contrast with Sutar (2018), who observed significant activity of methanolic *P. betle* leaf extract against *E. coli* (17 mm), *S. aureus* (22 mm), and *P. aeruginosa* (22 mm) [19], highlighting the influence of geographical variation, extraction method, and microbial strain susceptibility.

Similarly, Nidhinadas and Meiyalagan (2021) found chloroform extracts of *P. betle* to be inactive against *E. coli* and *P. aeruginosa* [20], whereas in the present study, chloroform extracts, particularly A2CH, showed intermediate activity against *E. coli* (8 mm), indicating variability in compound extraction and activity. Ethyl acetate extracts of *P. betle* demonstrated strong and broad-spectrum antimicrobial activity. The stem extract (A2EA) exhibited the highest inhibition against *P. vulgaris* (23 mm, sensitive), while the leaf (A1EA) and root (A3EA) extracts showed sensitivity against *S. aureus* (13 mm each) and intermediate activity against E. *coli* and *P. vulgaris*. Fungal

inhibition was also observed, particularly against *A. niger* (10-11 mm) and *Rhizopus* sp. (10 mm). These findings are stronger than those of Junairiah *et al*. (2017), who reported only mild inhibition zones (6-9 mm) with ethyl acetate extracts of *P. betle* against similar bacterial strains [21].

Chloroform extracts of *P. betle* also exhibited notable antimicrobial efficacy. The stem extract (A2CH) showed sensitivity to *S. aureus* and *P. vulgaris* (14 mm each), intermediate activity against *E. coli* (8 mm), and antifungal activity against *A. niger* (10 mm) and *Rhizopus* sp. (11 mm). The leaf extract (A1CH) was particularly effective against *P. vulgaris* (20 mm). These results, although more promising than earlier reports by Nidhinadas and Meiyalagan (2021), emphasize the variability in activity across different solvents and plant parts [20].

Hexane extracts, being non-polar, were less effective overall. The leaf extract (A1HX) inhibited *P. vulgaris* (18 mm, sensitive) and *Rhizopus* sp. (11 mm), but only showed resistant-level activity against *S. aureus* (7 mm). The root extract (A3HX) demonstrated intermediate activity against *S. aureus* (10 mm), while all other hexane extracts of *P. betle* were inactive. These findings are comparable to Junairiah *et al*. (2017), who observed only weak activity from hexane extracts, reporting inhibition zones of 6-8 mm for *E. coli* and 6 mm for *S. aureus* [21].

Aqueous extracts of *P. betle* were the least effective. Only A1AQ (leaf extract) showed moderate activity against *P. vulgaris* (15 mm, sensitive) and intermediate activity against *S. aureus* (10 mm), while all other aqueous extracts were inactive against both bacteria and fungi. This outcome is consistent with previous reports (Kaveti *et al*., 2011), confirming that water poorly extracts key antimicrobial phytochemicals [18].

In contrast, *P. longum* extracts displayed limited antimicrobial activity. The methanolic extracts (C1MH–C3MH) were inactive against all tested bacterial strains but showed sensitivity to *Rhizopus* sp. in the stem and root extracts (C2MH: 11 mm; C3MH: 10 mm), suggesting selective antifungal potential. Ethyl acetate and chloroform extracts (C1EA–C3EA and C2CH–C3CH) were similarly inactive against bacteria but inhibited *Rhizopus* sp. to varying degrees. Notably, chloroform extracts showed mild antifungal effects (12 mm and 9 mm) but no antibacterial activity, which contrasts with findings by Usha and Sakthi (2021), who reported significant inhibition of *S. aureus*, *E. coli*, and *P. aeruginosa* by *P. longum* chloroform extracts [22]. This discrepancy highlights the impact of experimental conditions and the importance of microbial strain selection.

Hexane and aqueous extracts of *P. longum* were largely ineffective. Only the stem hexane extract (C2HX) showed intermediate antifungal activity against *Rhizopus* sp. (8 mm), while all other extracts remained inactive. These results suggest that *P. longum* contains fewer extractable antibacterial compounds under the tested conditions.

Importantly, there is a noticeable gap in the literature concerning comprehensive studies that evaluate all five solvents across different organs (leaf, stem, and root) of *P. longum* against filamentous fungi such as *A. niger* and *Rhizopus* sp. The current study addresses this gap and emphasizes the selective antifungal potential of *P. longum*, particularly in methanolic, ethyl acetate, and chloroform stem/root extracts.

Overall, antimicrobial activity varied with plant species, solvent polarity, and microbial strain. *P. betle* exhibited broader-spectrum and stronger antimicrobial activity than *P. longum*, particularly in methanol, ethyl acetate, and chloroform extracts. Among bacteria, *P. vulgaris* was the most susceptible, whereas *P. aeruginosa* was completely resistant to all extracts. *Rhizopus* sp. was more sensitive than *A. niger*, especially to *P. longum* extracts. Methanol and ethyl acetate emerged as the most effective solvents, likely due to their capacity to extract diverse bioactive compounds such as alkaloids, flavonoids, and phenolics.

These findings support the ethnopharmacological applications of *P. betle*, particularly its leaf and stem, in the treatment of microbial infections. While *P. longum* demonstrated limited antibacterial activity, its selective antifungal potential warrants further phytochemical and pharmacological investigation.

Antioxidant Activity of Methanol Extracts:

The antioxidant potential of methanolic extracts from the leaf (A1MH, C1MH), stem (A2MH, C2MH), and root (A3MH, C3MH) of *Piper betle* and *Piper longum* was assessed using the DPPH free radical scavenging assay. The percentage inhibition across varying concentrations and the corresponding IC_{50} values are detailed in Tables 2-7 and Figures 1-6. Ascorbic acid was used as the reference standard (Table 8, Figure 7).

Among the tested samples, the methanolic root extract of *P. betle* (A3MH) displayed the most potent antioxidant activity (Table 2), exhibiting a strong dose-dependent response from 34.76% inhibition at 6.25 µg/mL to 89.42% at 100 µg/mL (Figure 1), with a notably low IC_{50} value of –13.38 µg/mL. This indicates a high concentration of potent antioxidant compounds. Notably, no previous reports were found in the literature for antioxidant DPPH activity of methanolic root or stem extracts of *P. betle*, highlighting the novelty of these findings.

The leaf extract of *P. betle* (A1MH) also demonstrated strong activity (Table 3), reaching 77.17% inhibition at the highest concentration tested (Figure 2) and an IC_{50} of 37.57 µg/mL. These results are supported by earlier findings from Ukey and Gogle (2024), who reported a comparable IC_{50} value of 29.79±0.973 µg/mL, reaffirming the leaf's strong antioxidant potential [23].

In contrast, the stem extract (A2MH) showed only moderate antioxidant activity (Table 4) with a maximum inhibition of 15.58% at 100 µg/mL (Figure 3), and a high IC_{50} value of 335.92 µg/mL, with no previous reports available for direct comparison.

The methanolic extracts of *P. longum* were generally less potent. Among these, the stem extract (C2MH) exhibited slightly higher activity (14.62% at 100 µg/mL) (Figure 4) with an IC_{50} of 473.90 µg/mL (Table 5). Interestingly, Biswas *et al*. (2024) previously reported an IC_{50} of 166.2±1.12 µg/mL with a corresponding antioxidant activity of 59.59±0.57% for *P. longum* stem methanol extract [24], indicating better performance under different conditions or sample sources. This discrepancy could stem from variations in environmental factors, extraction methods, or plant chemotypes.

The leaf extract of *P. longum* (C1MH) achieved 13.41% inhibition (Figure 5) with an IC_{50} of 486.01 µg/mL (Table 6) in the current study, which is consistent with earlier findings by Sultana *et al*. (2019), who reported an IC_{50} of 149.42 µg/mL for the same extract [25]. The observed differences could again be attributed to regional or methodological factors. The root extract (C3MH) showed the lowest activity, with only 6.32% inhibition (Figure 6) and a very high IC_{50} value of 852.52 µg/mL (Table 7), highlighting its limited antioxidant efficacy.

As expected, ascorbic acid outperformed all test samples with 96.65% inhibition at 100 µg/mL (Figure 7), and an IC_{50} of –0.10 µg/mL (Table 8), confirming the assay's reliability.

Overall, the results clearly demonstrate species- and part-specific antioxidant behavior among the *Piper* extracts. The superior performance of *P. betle*, particularly its root and leaf extract, suggests a rich phytochemical composition with significant redox potential. These findings align with traditional uses of *P. betle* and highlight the promising antioxidant value of its lesser-studied root. Conversely, *P. longum* extracts, though traditionally significant, showed comparatively weaker antioxidant properties in the methanolic extracts analyzed here.

This study emphasizes the need for further phytochemical profiling and bioactivity-guided fractionation, particularly of *P. betle* root and leaf, to isolate and characterize the specific compounds responsible for the observed effects, thereby open avenues for their development as natural antioxidant agents in therapeutic formulations.

Table 1: Antimicrobial activity of plant extracts

Plant Extract	Diameter of Zone of Inhibition (in mm) against Test Microbes					
	Bacteria				Fungi	
	Escherichia coli	*Staphylococcus aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Rhizopus* sp.
A1MH	07	13	21	-	08	12
A2MH	07	12	12	-	11	-
A3MH	07	12	-	-	-	-
C1MH	-	-	-	-	-	-
C2MH	-	-	-	-	-	11
C3MH	-	-	-	-	-	10
A1HX	-	07	18	-	-	11
A2HX	-	-	-	-	-	-
A3HX	-	10	-	-	-	-
C1HX	-	-	-	-	-	-
C2HX	-	-	-	-	-	08
C3HX	-	-	-	-	-	-
A1EA	08	13	15	-	07	10
A2EA	-	11	23	-	10	-
A3EA	07	13	-	-	11	-
C1EA	-	-	-	-	-	08
C2EA	-	-	-	-	-	09
C3EA	-	-	-	-	-	11
A1CH	07	08	20	-	10	10
A2CH	08	14	14	-	10	11
A3CH	-	-	-	-	-	-
C1CH	-	-	-	-	-	-
C2CH	-	-	-	-	-	12
C3CH	-	-	-	-	-	09
A1AQ	-	10	15	-	-	-
A2AQ	-	-	-	-	-	-
A3AQ	-	-	-	-	-	-
C1AQ	-	-	-	-	-	-
C2AQ	-	-	-	-	-	-
C3AQ	-	-	-	-	-	-
PC	20	20	12	19	24	21
NC	-	-	-	-	-	-

Table 2: DPPH scavenging activity of sample A3MH

Concentration (mcg/mL)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.642	0.647	35.02	34.51	34.76
12.5	0.310	0.312	68.62	68.42	68.52
25	0.208	0.201	78.94	79.65	79.30
50	0.122	0.125	87.65	87.34	87.50
100	0.102	0.107	89.67	89.17	89.42
IC 50	**-13.38**				

Table 3: DPPH scavenging activity of sample A1MH

Concentration (mcg/mL)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.888	0.860	10.12	12.95	11.53
12.5	0.737	0.741	25.40	25.00	25.20
25	0.348	0.351	64.77	64.47	64.62
50	0.246	0.241	75.10	75.60	75.35
100	0.227	0.224	77.02	77.32	77.17
IC 50	**37.57**				

Table 4: DPPH scavenging activity of sample A2MH

Concentration (mcg/mL)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.973	0.977	1.51	1.11	1.31
12.5	0.940	0.938	4.85	5.06	4.95
25	0.897	0.891	9.21	9.81	9.51
50	0.951	0.955	13.86	13.46	13.66
100	0.872	0.876	15.78	15.38	15.58
IC 50	**335.92**				

Table 5: DPPH scavenging activity of sample C2MH

Concentration (mcg/mL)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.953	0.951	3.54	2.73	3.13
12.5	0.939	0.937	4.95	5.16	5.06
25	0.902	0.905	8.70	8.40	8.55
50	0.878	0.881	11.13	10.82	10.98
100	0.842	0.845	14.77	14.47	14.62
IC 50	**473.90**				

Table 6: DPPH scavenging activity of sample C1MH

Concentration (mcg/mL)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.948	0.951	4.04	3.74	3.89
12.5	0.901	0.905	8.80	8.40	8.60
25	0.898	0.894	9.10	9.51	9.31
50	0.863	0.861	18.72	12.85	15.78
100	0.858	0.853	13.15	13.66	13.41
IC 50	**486.01**				

Table 7: DPPH scavenging activity of sample C3MH

Concentration (mcg/ml)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.982	0.984	0.60	0.40	0.50
12.5	0.962	0.967	2.63	2.12	2.37
25	0.961	0.954	2.73	3.44	3.08
50	0.930	0.932	5.87	5.66	6.32
100	0.927	0.924	6.17	6.47	6.32
IC50	**852.52**				

Table 8: DPPH scavenging activity of standard ascorbic acid

Concentration (mcg/ml)	Absorbance 1	Absorbance 2	Percentage of Inhibition 1	Percentage of Inhibition 2	Mean Percentage of Inhibition
6.25	0.738	0.735	25.30	25.60	25.45
12.5	0.345	0.341	65.08	65.48	65.28
25	0.183	0.187	81.47	81.07	81.27
50	0.085	0.088	91.39	91.09	91.24
100	0.031	0.035	96.86	96.45	96.65
IC50	**-0.10**				

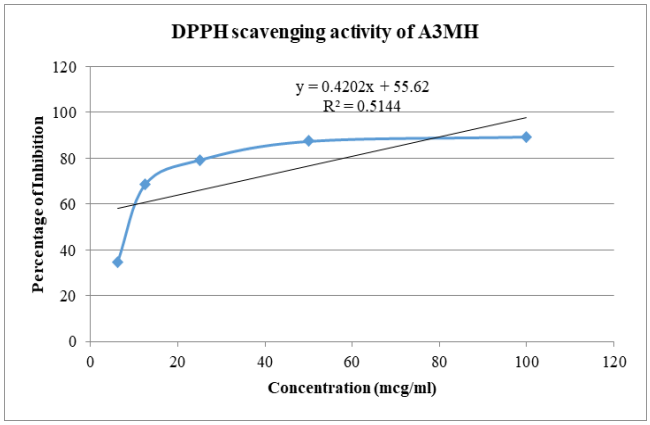

Figure 1: DPPH scavenging activity of sample A3MH

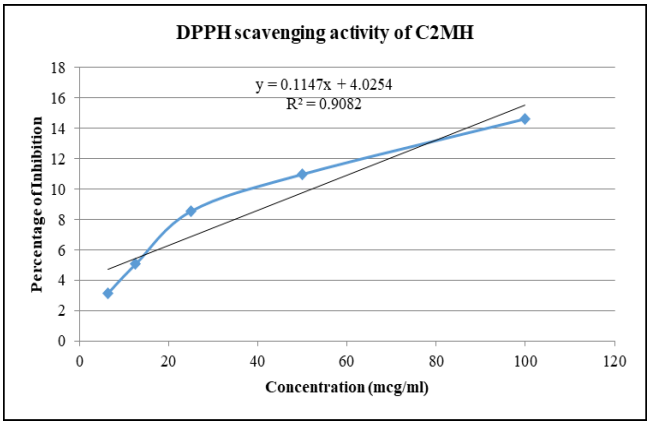

Figure 4: DPPH scavenging activity of sample C2MH

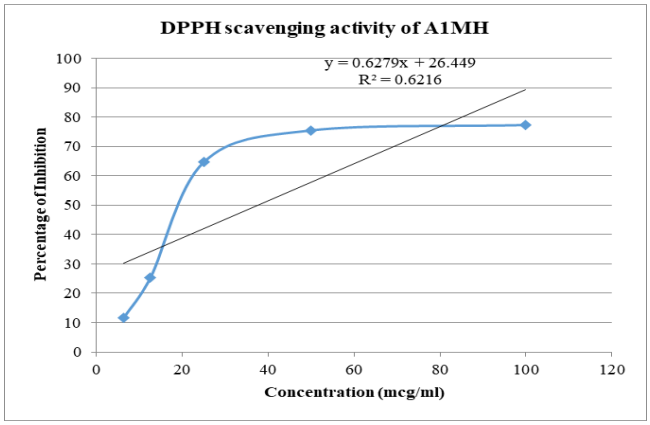

Figure 2: DPPH scavenging activity of sample A1MH

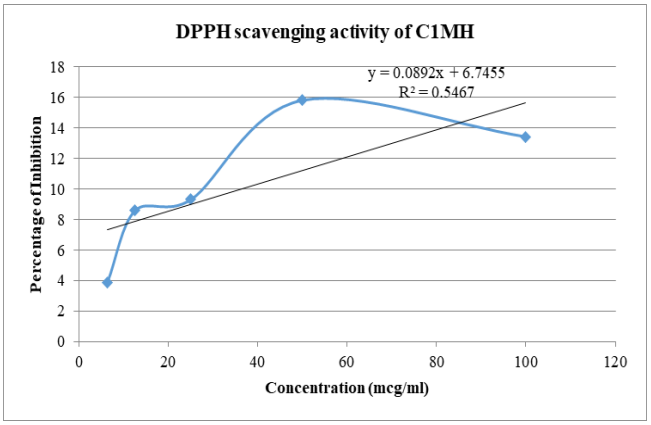

Figure 5: DPPH scavenging activity of sample C1MH

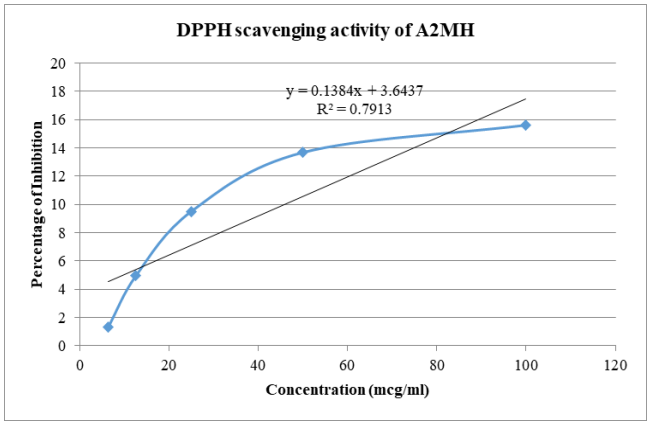

Figure 3: DPPH scavenging activity of sample A2MH

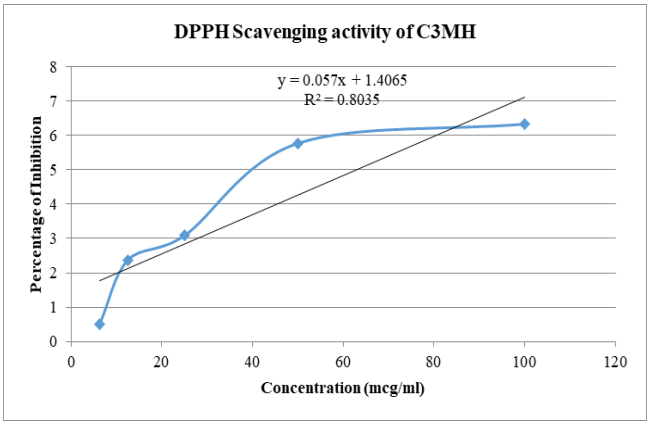

Figure 6: DPPH scavenging activity of sample C3MH

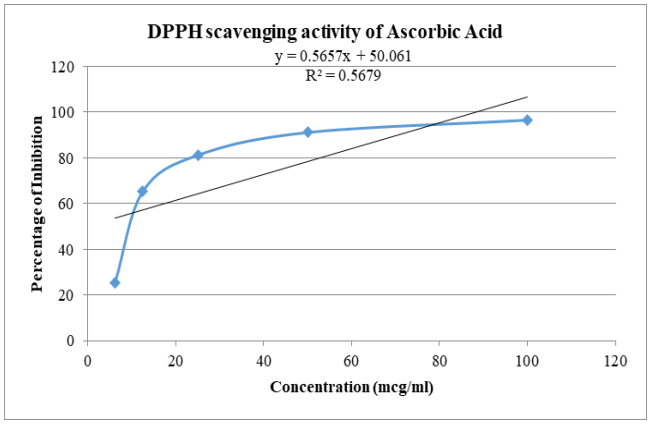

Figure 7: DPPH scavenging activity of sample ascorbic acid

CONCLUSION

The present study highlights the dual bioactive potential of *Piper betle* and *Piper longum* methanolic extracts, demonstrating both antimicrobial and antioxidant activities in a part- and species-specific manner. Among the tested samples, *P. betle* leaf extract (A1MH) showed the most potent broad-spectrum antimicrobial efficacy, particularly against *Proteus vulgaris*, and also exhibited strong antioxidant activity with an IC_{50} value of 37.57 µg/ml, closely approaching that of standard ascorbic acid. In contrast, *P. longum* extracts displayed selective antifungal activity and comparatively weaker antioxidant potential. The results affirm the significance of solvent choice in phytochemical extraction, with methanol proving most effective. Overall, the findings validate the ethnomedicinal relevance of *P. betle*, especially its leaf part, and warrant further isolation and characterization of the active compounds for potential therapeutic applications.

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Conflict of interest

The authors declared no conflict of interest.

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