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PHYTOCHEMICAL ANALYSIS, EVALUATION OF THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF THE LEAF EXTRACTS OF *Premna schimperi*

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ABSTRACT. *Premna schimperi*, commonly known as chocho, is a medicinal plant in the Lamiaceae family, with its leaves traditionally used to treat external injuries, wounds, and common eye infections in cattle. The study focused on phytochemical analysis and evaluation of the antioxidant and antibacterial properties of the solvent extracts of the leaves. The leaves of *P. schimperi* (0.5 kg) were macerated successively with 1250 mL *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH), resulting in 1.1 % *n*-hexane, 2.6% ethyl acetate, and 5.02% methanol crude extracts. Phytochemical screening showed the presence of alkaloids, saponins, flavonoids, phenols, steroids, terpenoids, tannins, anthraquinones, and glycosides in the MeOH extract. The solvent extracts were tested against four bacteria using the disc diffusion method. Results showed greater inhibition at 200 mg/mL than at lower concentrations. The antioxidant activities of the crude extracts were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and H_2O_2 assays. Among the tested extracts, the MeOH extract demonstrated the most potent antioxidant activity in both assays, with the lowest IC_{50} values (35.35 µg/mL for DPPH and 51.4 µg/mL for H_2O_2 scavenging), performing closest to the ascorbic acid standard. Therefore, it is strongly recommended to isolate antioxidant components from the MeOH extract.

KEY WORDS: Maceration, Phytochemicals, Antibacterial, Antioxidant, *Premna schimperi*

INTRODUCTION

P. schimperi Engl. (Synonym: *Premna viburnoides* A. Rich) is used as traditional medical plant in Ethiopia [1]. It is a spreading shrub to tree up to 7-10 m high. The branches are pale brown to grey, and it grows in secondary forest and grassy meadows. It grows along paths in forests at an altitude of 1350-2500 m [2]. In Ethiopia, *P. schimperi* is found in Tigray, Gondar, Gojam, Welega, Shewa, Arsi, Harerge, Sidamo, Gamo Gofa, Kefa and Ilubabor, Wollo Administrative Regions. It also occurs in Sudan, Kenya, and Tanzania. The local names of *P. schimperi* Engl. are Chocho (Amharic); Urgeessa (Afaan Oromo); Tulangi (Sidama); Taitos (Gimira); Tumo (Kafa) [2].

In traditional medicine, leaf extracts of *P. schimperi* are used to treat external injuries and secondary infections [3]. It is also employed by farmers to treat common eye infections in cattle. In addition, it provides firewood, charcoal, medicine (from leaves), and fencing material (from cut branches) [4]. Various phytochemicals have been isolated and reported from the extracts of *P. schimperi* so far. The studies revealed the presence of diterpenoids, flavanones, and flavanoles [5]. Some of the representative examples of diterpenes and flavonoids that have been isolated and reported from *P. schimperi* include andrographolide, ent-12-oxolabda-8,13(16)-dien-15-oic acid, luteolin, quercetin, and kaempferide [6, 7].

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MATERIAL AND METHODS

Chemicals and reagents

All the chemicals, reagents, and solvents used in this study were analytical/HPLC grade and used as received without further purification. Methanol (> 99.7%, Sigma-Aldrich, USA), ethyl acetate (Loba Chemie Pvt. Ltd., India), *n*-hexane (99%, Labachemic Pvt. Ltd., India), DPPH (Alpha Chemika, India), acetic anhydride (Carlo Erba Reagents, Italy), NaOH pellets, sulfuric acid (98%), ferric chloride (99%), Chloramphenicol (98%), hydrochloric acid (35%), H_2O_2 (30%), and NaCl were used for the study.

Instruments and apparatus

Samples were ground and homogenized using an electrical grinder. All mass measurements were performed using a high-precision digital analytical balance. Extraction, fractionation, and concentration procedures utilized borosilicate round-bottom flasks and Erlenmeyer flasks of various sizes (Jlassco and Pyrex), separatory funnels, measuring cylinders, conical flasks, and a rotary evaporator. Sample filtration was carried out using Whatman No. 1 filter paper. General sterilization and incubation needs were managed using an autoclave, a laboratory oven, and an incubator.

Collection of plant material

The Leaves of *P. schimperi* were collected in November 2022 from Minchu Kebele, South Wollo, Amhara Regional State, Ethiopia. The plant material was authenticated by a botanist at the Department of Biology, College of Natural Science, Wollo University (WU).

The fresh leaves of *P. schimperi* were collected, washed with tap water, air-dried at room temperature for 26 days, and then powdered using an electrical grinder to a uniform particle size. As a result, the powder sample was packed in plastic bags for extraction to prevent air ingress and mixing with surrounding material.

Solvent extraction of the leaves of P. schimperi

Leaf powder (500 g) of *P. schimperi* was first soaked in 1250 mL of *n*-hexane at room temperature for 72 h and then shaken. The mixture was filtered through a Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator, then weighed. The marc remaining in the extraction flask after exhaustive extraction with *n*-hexane was dried and extracted with ethyl acetate for 72 h at room temperature, shaken, filtered, and the EtOAc filtrate was concentrated using a rotary evaporator; the concentrate was weighed. The marc left was further extracted by the same procedure using MeOH. The dried methanol extract was measured. The three dried solvent extracts were transferred into beakers and kept in a refrigerator at 4 °C for further use [8].

Phytochemical screening of the crude solvent extracts

Preliminary phytochemical screening of secondary metabolites of *n*-hexane, EtOAc, and MeOH extracts of leaves of *P. schimperi* was carried out using standard tests [9]. The data were analyzed qualitatively, with the absence of secondary metabolites indicated by the symbol (-), a slight positive reaction for secondary metabolites indicated by (+), and a definitive positive reaction for secondary metabolites indicated by (++) [10].

Antibacterial activity by disc diffusion method

The antibacterial test was carried out at the Biology Laboratory, Bahir Dar University, Ethiopia. Antibacterial activity of the leaf extract of *P. schimperi* was tested against four bacteria (two Gram-positive, *S. aureus* and *L. monocytogenes*, and two Gram-negative, *E. coli* and *K. pneumoniae*) by the paper disk diffusion method on Mueller-Hinton agar.

Preparation of test solution

The stock extract solution (*n*-hexane, ethyl acetate, and methanol) of *P. schimperi* was prepared by dissolving 0.5 g of each extract in 1 mL of dimethyl sulfoxide (DMSO). Different concentrations (50, 100, and 200 mg/mL) of each extract were prepared from the stocks by dilution with DMSO. In Petri dishes, solutions of plant extracts with known concentrations (50, 100, and 200 mg/mL) were introduced using the paper disk method [11].

Collection and subculturing of test organisms

The selected human-pathogenic bacteria, *S. aureus, L. monocytogenes, K. pneumoniae, and E. coli*, were obtained from the Department of Biology, Bahir Dar University, Ethiopia. The bacteria were maintained on nutrient agar and stored in the refrigerator at 4 °C. All selected bacterial strains were cultured on nutrient agar plates and incubated for 24 h at 37 °C. The cultures were inoculated into Mueller-Hinton broth and incubated at 37 °C for 24 h [12].

Preparation of media

The nutrient media (Mueller-Hinton Agar (MHA) and broth) provided nutrients for growing bacteria under controlled conditions. The required amount of 3.9 g of nutrient broth was weighed, prepared in 100 mL of distilled water, and heated with frequent agitation until a clear solution was observed. Then it was poured into conical flasks, covered with non-absorbent cotton wool, wrapped with aluminum foil, and sterilized by autoclaving at 121 °C for 20 minutes. The Petri dishes containing the media were placed in a sterile environment until bacterial inoculum was spread on them.

Determination of zone of inhibition

The antibacterial activities of the crude leaf extracts of *n*-hexane, EtOAc, and MeOH of *P. schimperi* were determined by measuring the diameters of the zones of inhibition. The tested pathogens were cultured overnight (24 h) at 37 °C on nutrient agar to prepare cell suspensions using sterile diluents. Using a sterile pipette, 0.1 mL of the standard bacterial inoculum was added to the surface of solidified MHA. The suspensions were uniformly applied to the MHA surface using a sterile cotton swab. The plant extract-impregnated discs (50, 100, and 200 mg/L) were then applied to the inoculated MHA plates using sterile forceps at approximately equidistant positions and allowed to stand for 15 min at room temperature to permit proper diffusion of the extract. Finally, the plates were incubated for 24 h at 37 °C to allow maximum growth of the organisms. After incubation, the diameter of the zone of inhibition was measured in mm using a ruler for each extract against each pathogen. The experiments were done in triplicate, and all antibacterial activities were expressed as mean diameters (mm) of inhibition zones produced by the plant extract. The diameter of the inhibition zone was compared with that of the standard drug. The standard antibacterial, Chloramphenicol, was used as a positive control applied to the center of the agar plate, and DMSO as a negative control.

Antioxidant capacity assay

DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions [13]. The antioxidant activity of the extracts was measured using the scavenging activity of stable DPPH [14]. A 0.004% DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of MeOH. Stock solutions of ascorbic acid and crude extracts were prepared by dissolving 10 mg of ascorbic acid and crude extracts in a 10 mL volumetric flask separately, and methanol was added up to the mark to prepare 1000 μg/mL stock solutions. Then, from this stock solution of ascorbic acid and crude extracts, 62.5 μg/mL, 125 μg/mL, 250 μg/mL, 500 μg/mL, and 1000 μg/mL concentrations were prepared in separate test tubes, respectively. After shaking vigorously, the mixture was incubated in a dark place at room temperature for 30 minutes, and the absorbance was measured at 517 nm [15]. Ascorbic acid with a series of concentrations was used as a positive control, whereas methanol was used as a blank.

Hydrogen peroxide scavenging activity

The ability of the extract to scavenge hydrogen peroxide was determined using the method described by Rahmani *et al.* [16]. 0.04 mg/mL solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4) as the solvent. Stock solutions of *n*-hexane, ethyl acetate, and methanol extracts (20 mg) were prepared in 20 mL phosphate buffer (pH 7.4), and 5 mL was aliquoted for the following concentration range (0.0625, 0.125, 0.25, 0.5, and 1 mg/mL) in each test tube. Ascorbic acid was taken in 5 mL in the following concentration range (62.5, 125, 250, 500, and 1000 μg/mL) in each test tube, and 2 mL of 0.04 mg/mL H_2O_2 was added. The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate.

RESULTS AND DISCUSSION

Percentage yield of the extracts of P. schimperi leaves

The air-dried powdered leaves of *P. schimperi* (500 g) were extracted with *n*-hexane for 72 h to yield dark jelly extract (5.5 g, 1.1%). After the *n*-hexane extraction, the marc was weighed at 477.7 g and extracted with EtOAc for 72 h to yield a dark jelly extract (12.41 g, 2.6%). Again, after ethyl acetate extraction, the mass of marc was 462.9 g and was extracted with methanol for 72 h to yield a dark jelly extract (23.24 g, 5.02%). As a result, successive extraction of the leaves of *P. schimperi* gave the highest yield with methanol (23.24 g), followed by EtOAc (12.41 g), and *n*-hexane (5.5 g).

The results showed that the percentage yield increased with increased polarity. These results showed that more polar solvents extract higher yields. The increase in the extract obtained with solvent polarity likely implies that the plant contains more polar compounds than non-polar ones. As shown from the findings, the polarity of the solvent used for extraction is the primary factor dictating the yield. Since methanol is the most polar solvent, it yielded the highest amount of extract. The next highest yield was achieved by the solvent of intermediate polarity, ethyl acetate. The least amount of product was observed in the most non-polar solvent, *n*-hexane. These results showed that more polar solvents extract more polar compounds and give high yields, but non-polar solvents cannot extract polar compounds and, as a result, give low yields.

Phytochemical test results of the leaves extract of P. schimperi

Phytochemical studies revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenols, and terpenoids in the *n*-hexane extract of *P. schimperi* leaves [18]. Glycosides and anthraquinones were absent in the *n*-hexane crude extract of *P. schimperi* leaves. Alkaloids, flavonoids, terpenoids, saponins, steroids, phenols, and tannins were present in the ethyl acetate crude extract of *P. schimperi* leaves. Anthraquinones and glycosides were absent in the ethyl acetate crude extract of *P. schimperi* leaves. In the methanol crude extracts of *P. schimperi* leaves, alkaloids, flavonoids, phenols, terpenoids, steroids, saponins, anthraquinones, tannins, and glycosides were present.

Antibacterial activities of P. schimperi leaf extracts

The antibacterial activities of *P. schimperi* leaf extracts against selected microorganisms were examined in the present study, and their potency *was* assessed by the presence or absence of inhibition zones compared with some antibiotics. Its antibacterial activity was evaluated in two Gram-positive (*S. aureus* and *L. monocytogenes*) and two Gram-negative (*E. coli* and *K. pneumoniae*) bacteria using the Disk diffusion method at different concentrations (50 mg/mL, 100 mg/mL, and 200 mg/mL). The commercial standard drug (Chloramphenicol) showed the greatest inhibitory effect against each tested bacterium at each concentration (50 mg/mL, 100 mg/mL, and 200 mg/mL) compared with the tested samples. The crude extracts are known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Therefore, leaves of *P. schimperi* extracts are valuable and could be a future target for replacing synthetic antibacterial agents. Results of the antibacterial activity by the disc diffusion method of *n*-hexane, ethyl acetate, and methanol extracts of *P. schimperi* along with the reference drug chloramphenicol are presented in Tables 1 and 2.

Table 1. Antibacterial activities of crude extracts against Gram-positive bacteria.

Tested bacteria	Solvent	Inhibition zone in (mm) at the following concentrations in mg/mL			Positive control (chloramphenicol 30 µg/mL)
		50	100	200	
S. aureus	Methanol	7.6 ± 0.43	8.47 ± 0.09	9.18± 0.038	17.24
	Ethyl acetate	6.5 ± 0.055	7.12± 0.013	7.43 ± 0.02	
	n-hexane	6.59 ± 0.01	7.21 ± 0.01	7.44 ± 0.01	
L. monocytogenes	Methanol	6.19 ± 0.01	7.5 ± 0.1	8.86± 0.005	22.33±0.1
	Ethyl acetate	6.73 ± 0.005	8.32± 0.005	9.41 ± 0.02	
	n-hexane	7.35 ± 0.15	8.14± 0.002	8.76 ± 0.01	

NB: Values are expressed as the mean ± STD of 3 individual experiments; positive control (chloramphenicol 30 µg/mL).

All crude extracts exhibit a greater inhibitory effect against the tested bacteria at 200 mg/mL and compared with other concentrations, 50 mg/mL and 100 mg/mL of the leaves of *P. schimperi*. The findings show that the antibacterial activity of different solvent extracts of *P. schimperi* leaf against *S. aureus* was found to display inhibition zones within the range between 6.5 mm and 7.7 mm at 50 mg/mL, 7.12 mm and 8.47 mm at 100 mg/mL, and 7.43 mm and 9.18 mm at 200 mg/mL. When the concentrations were increased from 50 to 200 mg/mL, increased zones of inhibition were observed in MeOH, EtOAc, and *n*-hexane solvent extracts. As a result, the crude methanol extract inhibited the Gram-positive (*S. aureus*) bacterial species across all concentration ranges, especially at 200 mg/mL, where the highest inhibition was observed, with a zone of 9.18 ± 0.038 mm. The crude *n*-hexane and ethyl acetate extracts showed weak inhibition at concentrations of 50 mg/mL, 100 mg/mL, and 200 mg/ms. Also, the ethyl acetate extract of leaves

showed the least antibacterial activity against the Gram-positive bacterium *S. aureus* at all concentrations (50 mg/mL, 100 mg/mL, and 200 mg/mL), as shown in Figure 1 (a-c). The commercial standard drug (chloramphenicol) (30 µg/mL) showed the greatest inhibition effect (17.24 mm) against *S. aureus* at 50 mg/mL, 100 mg/mL, and 200 mg/mL compared with the tested samples.

Different extracts of *P. schimperi* leaves showed antibacterial activity against *L. monocytogenes* at all concentrations; inhibition zones ranged from 6.19 mm to 7.35 mm at 50 mg/ml, 7.5 mm to 8.32 mm at 100 mg/mL and 8.76 mm to 9.41 mm at 200 mg/mL. Consequently, the highest inhibition was observed in ethyl acetate crude extracts (9.41 ± 0.02 mm) at 200 mg/mL against *L. monocytogenes*. Also, the methanol extract of leaves showed the least antibacterial activity against the Gram-positive bacterium *L. monocytogenes*, as shown in Figure 1 (d-f). However, all the solvent extracts inhibited the Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) at all tested concentrations. While the reference chloramphenicol (30 µg/mL) inhibited 22.33 mm.

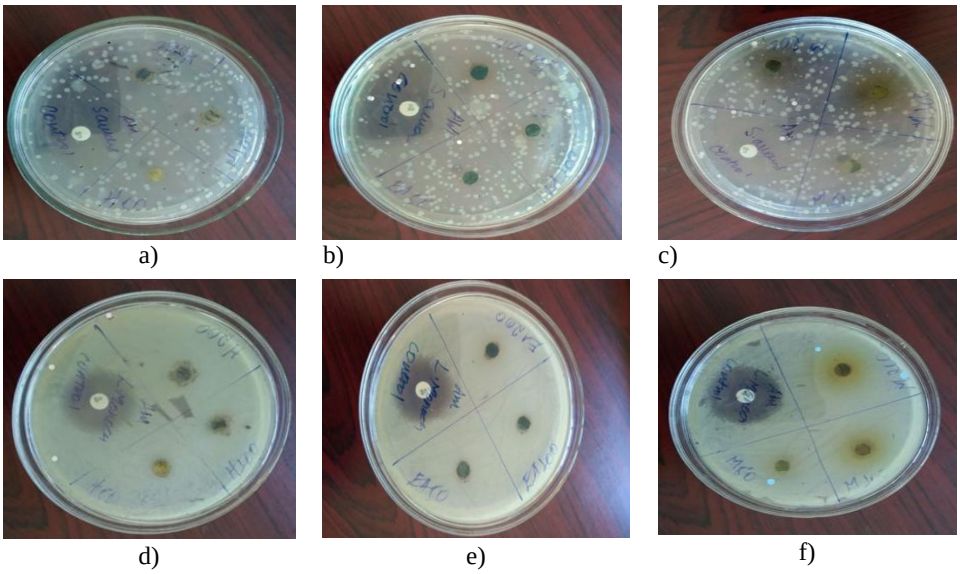


Figure 1. Antibacterial activities of *n*-hexane (a), ethyl acetate (b), and methanol (c) extracts of *P. schimperi* leaves against *S. aureus*; *n*-hexane (d), ethyl acetate (e), and methanol (f) extracts of *P. schimperi* leaves against *L. monocytogenes*.

Table 2. Antibacterial activities of crude extracts against Gram-negative bacteria.

Tested bacteria	Extraction solvent	Inhibition zone in (mm) at the following concentration in mg/mL			Positive control (chloramphenicol 30 µg/mL)
		50	100	200	
E. coli	Methanol	6.52 ± 0.02	7.74 ± 0.59	8.73 ± 0.05	18.19 ± 0.05
	Ethyl acetate	6.29 ± 0.01	7.2 ± 0.01	7.55 ± 0.06	
	n-hexane	8.23 ± 0.01	9.12 ± 0.01	10.16 ± 0.005	
K. pneumoniae	Methanol	10.37 ± 0.01	10.42 ± 0.04	12.27 ± 0.01	21.16 ± 0.01
	Ethyl acetate	6.86 ± 0.01	7.42 ± 0.01	8.82 ± 0.01	
	n-hexane	9.14 ± 0.04	10.33 ± 0.02	12.13 ± 0.01	

NB: Values are expressed as the mean ± STD of 3 individual experiments; positive control (chloramphenicol 30 µg/mL).

The above table shows different crude extracts of *P. schimperi* leaves for antibacterial activity against the Gram-negative (E. *Coli*). The extracts showed inhibition zones ranging from 6.29 mm to 8.23 mm at 50 mg/mL, 7.2 mm to 9.12 mm at 100 mg/mL, and 7.55 mm to 10.16 mm at 200 mg/mL. At a concentration of 200 mg/mL, the highest inhibition was observed in the *n*-hexane crude extract, with a 10.16 ± 0.5 mm diameter inhibition zone. Also, the ethyl acetate extract of leaves showed the least antibacterial activity against the Gram-negative bacterium, *E. coli*, at all concentrations (50 mg/mL, 100 mg/mL and 200 mg/mL), as shown in Figure 2 (a-c). The reference chloramphenicol (30 µg/mL) showed a zone of inhibition of 18.19 ± 0.06 mm.

The crude solvent extracts inhibited the Gram-negative (*K. Pneumoniae*) bacterial species at all concentration regions, which ranged from 6.86 mm to 10.37 mm at 50 mg/mL, 7.42 mm to 10.42 mm at 100 mg/mL, and 8.82 mm to 12.27 mm at 200 mg/mL. As a result, at a 200 mg/mL concentration, the highest inhibition was observed in the methanol crude extract, which was (12.27 ± 0.01) mm in diameter, but the *n*-hexane crude extract inhibition zone (12.13 ± 0.2 mm) was almost as large at the same concentration. Also, the ethyl acetate extract of leaves showed the least antibacterial activity against the Gram-negative bacterium, *K. pneumoniae*, at all concentrations (50 mg/mL, 100 mg/mL, and 200 mg/mL), as shown in Figure 2 (d-f). The reference chloramphenicol (30 µg/mL) gave a zone of inhibition of 21.16 ± 0.01 mm against *K. pneumonia*.

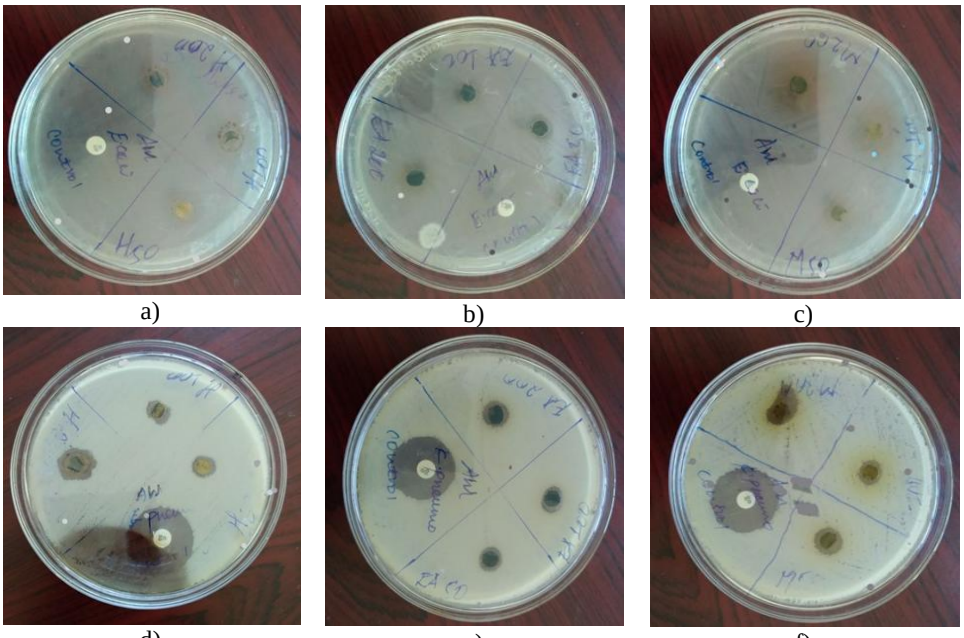


Figure 2. Antibacterial activities of *n*-hexane (a), ethyl acetate (b), and methanol (c) extracts of *P. schimperi* leaves against K. pneumoniae; n-hexane (d), ethyl acetate (e), and methanol (f) extracts of *P. schimperi* leaves against *E. coli.*

Antioxidant capacity assay

DPPH radical scavenging activity

DPPH radical scavenging assay was performed to determine the antioxidant activity of the extracts of the leaves of *P. schimperi* [19]. A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally fades when antioxidant molecules quench DPPH free radicals, converting them into a colorless/bleached product (i.e., 2,2-diphenyl-1-hydrazine or a substituted analogous hydrazine), resulting in a decrease in absorbance at the 517 nm band. This can be quantified spectrophotometrically to indicate the extent of DPPH scavenging activity by the plant extracts. Lower absorbance, followed by the degree of decoloration of the reaction mixture from purple to yellow, indicated the higher free radical-scavenging efficiency of the plant extract. The activities of the tested extracts in the DPPH scavenging assay can be expressed as a decrease in absorbance. As the absorbance decreased, the free radical scavenging activity became higher. Table 3 and Figure 3 show the results obtained for the antioxidant activities of the *n*-hexane, EtOAc, and MeOH extracts in relation to ascorbic acid standards.

Table 3. The DPPH scavenging activities of crude extracts.

Conc. µg/mL	Hexane		EtOAc		MeOH		AA	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
62.5	0.896192	62.43	0.56106	76.48	0.27664	88.4	0.0916	96.16
125	0.779634	67.31	0.43324	81.84	0.21948	90.8	0.0845	96.46
250	0.698139	70.73	0.42187	82.31	0.19017	92.03	0.0825	96.54
500	0.646604	72.89	0.32191	86.5	0.147271	93.83	0.0632	97.35
1000	0.495438	79.23	0.20566	91.38	0.109657	95.4	0.0452	98.1
IC_{50}	-	50.06	-	40.86	-	35.35	-	32.5

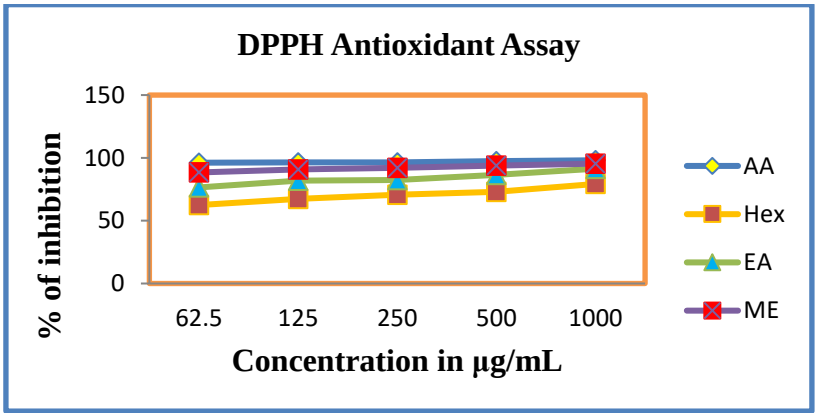


Figure 3. DPPH radical scavenging activities of ascorbic acid (AA), *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) extracts.

For the DPPH radical scavenging assay, different concentrations of ascorbic acid, *n*-hexane, ethyl acetate, and methanol (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL) were used to construct the calibration curve. The calibration curve was constructed as a function of % inhibition versus the concentrations of ascorbic acid, *n*-hexane, ethyl acetate, and methanol

extracts, as shown in Figure 3. For the DPPH antioxidant activity study, different concentrations of *n*-hexane, ethyl acetate, and methanol extracts of *P. schimperi* (62.5 µg/mL, 125 µg/mL, 250 µg/mL, 5mL, and 1000 µg/mL) were screened and showed radical-scavenging activity. The change in absorbance of DPPH radicals caused by antioxidants is due to the reaction between the antioxidant molecules of the plant extract and the DPPH radicals. The data in Table 3 describe the minimum and maximum percentage inhibition of DPPH at the concentrations of 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL as 88.4%-95.4%, 76.48%-91.38 %, and 62.43%-79.23 % for MeOH, EtOAc, and *n*-hexane extracts, respectively. The standard ascorbic acid showed DPPH inhibition of 96.16%, 96.46%, 96.54%, 97.35%, and 98.10% at the same concentration, respectively. The *n*-hexane, ethyl acetate, and MeOH extracts reduced the stable DPPH radical by 79.23%, 91.38%, and 95.40%, respectively, relative to the ascorbic acid standard (98.1%) at 1000, suggesting that they are potential radical scavengers. The crude extracts and standard exhibited IC_{50} values of 50.06 µg/mL (*n*-hexane), 40.86 µg/mL (ethyl acetate), 35.35 µg/mL (methanol), and 32.5 µg/mL (ascorbic acid). This result showed that the methanol extract has stronger free radical-scavenging activity than the ethyl acetate and *n*-hexane extracts. Therefore, the results showed that the plant extracts have medicinal value and may be utilized for their antioxidant properties.

Hydrogen peroxide scavenging assay of P. schimperi

The hydrogen peroxide scavenging power (antioxidant capacity) of the methanol, ethyl acetate, and *n*-hexane extracts of *P. schimperi* was determined, and the results are shown in Figure 4. The highest percentage hydrogen peroxide scavenging activity at 62.5, 125, 250, and 1000 µg/mL were obtained in methanol crude extracts (60.82, 66.67, 73.07, 74.87 and 79.70%, respectively) which is comparable to that of ascorbic acid standard that exhibited a 72.28, 78.38, 80.63, 84.86, 95.38% H_2O_2 inhibition at the same concentration (Table 4). The lowest hydrogen peroxide scavenging activity was observed in *n*-hexane crude extracts at 62.5, 125, 250, 500, and 1000 µg/mL (3.89, 10.92, 11.23, 14.99, and 14.997%, respectively). In all tests, as the concentration increased, the percentage scavenging also increased. Therefore, the antioxidant results showed that 79.70% free radical inhibition was achieved with the MeOH extract; moderate free radical inhibition was obtained in the EtOAc extract, which was 55.56%, and the *n*-hexane extract also showed the lowest antioxidant activity (14.997%), as compared to the standard (95.38%) inhibition at 1000 µg/mL, respectively.

Among the extracts, the polar methanol extract (MeOH) demonstrated the strongest H_2O_2 scavenging potency (IC_{50} = 51.4 µg/mL), performing nearly as effectively as the ascorbic acid standard (IC_{50} = 43.23 µg/mL), whereas non-polar hexane showed minimal activity. Control and higher-concentration absorbance readings approaching or exceeding 1.5-2.0 operate near the upper limit of Beer-Lambert linearity and serve primarily as relative indicators of scavenging activity. The present study revealed that better antioxidant inhibitory activity was observed in the DPPH assay than in the H_2O_2 assay for each solvent extract at comparable concentrations.

Table 4. The H_2O_2 scavenging activities of crude extracts.

Conc., µg/mL	Hexane		EtOAc		MeOH		AA	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
62.5	1.92222	3.89	1.5874	20.63	0.78360	60.82	0.5543	72.28
125	1.781555	10.92	1.49006	25.5	0.6666	66.67	0.4325	78.38
250	1.775459	11.23	1.26908	36.55	0.5386	73.07	0.3875	80.63
500	1.700148	14.99	0.99933	50.03	0.5026	74.87	0.3028	84.86
1000	1.700039	14.997	0.88878	55.56	0.40601	79.7	0.0924	95.38
IC_{50}	-	803.3	-	151.48	-	51.4	-	43.23

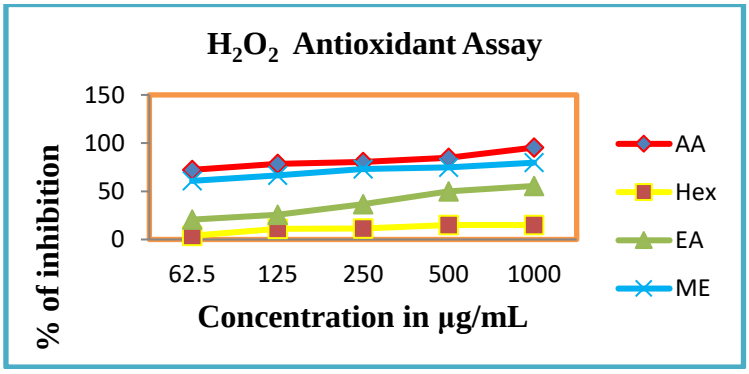


Figure 4. The H_2O_2 scavenging activities of *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) extracts.

CONCLUSION

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. The results of this study revealed a wide range of phytochemicals in *P. schimperi* leaf extracts, with potential antioxidant and antibacterial activities. The phytochemical screening tests of the *n*-hexane, EtOAc, and MeOH crude extracts showed the presence of flavonoids, alkaloids, saponins, terpenoids, phenols, steroids, and tannins, with the absence of anthraquinones and glycosides in the *n*-hexane and ethyl acetate crude extracts. In disc diffusion assays, all extracts exhibited activity against both Gram-negative and Gram-positive bacteria. Thus, the extracts exhibit good activity against the tested bacteria. All three extracts were also found to exhibit free radical-scavenging activity in a concentration-dependent manner in DPPH and H_2O_2 assays. MeOH exhibited the strongest antioxidant activity, followed by EtOAc and *n*-hexane extracts in both assays. MeOH was a better solvent in this study for the extraction of antioxidant substances from the plant compared to the other solvents by providing slightly higher yields and antioxidant activities. The antioxidant and antibacterial activities of solvent extracts increased with increasing their concentrations.

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