

Research Article



Phytochemical Composition and Biological Activities of Philippine *Premna serratifolia* L. Crude and Solvent-Partitioned Leaf Extracts

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ABSTRACT

Premna serratifolia L. is a medicinal plant widely used in traditional medicine, known for its diverse pharmacological properties. This study evaluated the phytochemical composition, antioxidant, antibacterial, and antiurolithiatic activities of the crude methanolic extract and solvent-partitioned fractions—hexane (HXE), ethyl acetate (EAE), and aqueous (AQE)—of *P. serratifolia* leaves. Phytochemical screening and FTIR revealed the presence of flavonoids, terpenoids, naphthoquinones, and saponins in varying amounts across fractions. For antioxidant activity, the AQE exhibited the highest DPPH-radical scavenging activity ($IC_{50} = 297.6$ ppm) and a total antioxidant activity of 1461.3 mg ascorbic acid equivalent (AAE)/g extract. Antibacterial activity evaluated using the Kirby–Bauer disk diffusion method showed all extracts inhibited *Bacillus cereus* and *Staphylococcus aureus*, with EAE producing the largest zones of inhibition (13–14 mm). EAE exhibited MIC values of 312.5 ± 0.0 μ g/mL against *Bacillus cereus* and 625 ± 0.0 μ g/mL against *Staphylococcus aureus*. TLC–agar-overlay bioautography localized the antibacterial activity of the EAE to relatively polar constituents. Antiurolithiatic activity was determined using an in vitro calcium oxalate nucleation assay. Among the extracts tested, only the HEXE significantly inhibited calcium oxalate crystal formation, exhibiting more than 80% inhibition during the initial stages of crystallization and reducing crystal density and size under microscopic examination. The results highlight the potential of *P. serratifolia* L. as a source of bioactive compounds for the development of antioxidant, antibacterial, and antiurolithiatic agents.



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1. Introduction

Premna serratifolia L., a member of the family Lamiaceae, is a shrub or a small tree widely distributed in tropical and subtropical regions of Asia, including the Philippines, India, Indonesia, Malaysia, and Vietnam (Dianita and Jantan 2017; Khairunnisa *et al.* 2022). In contrast, there are relatively few studies on *P. serratifolia*

in the Philippines. Instead, the closely related species *Premna odorata* Blanco ("alagaw") has received greater scientific attention because of its widespread use in Philippine folk medicine, particularly for the treatment of respiratory and inflammatory conditions (Philippine Traditional Knowledge Digital Library on Health 2015).

Previous phytochemical investigations have shown that *P. serratifolia* contains a wide array of secondary metabolites including flavonoids, saponins, tannins, steroids, terpenoids, glycosides, and phenolic compounds (Rajendran and Basha 2010). Several

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bioactive compounds isolated from the plant, such as oleanolic acid, diosmin, phytol, β -caryophyllene, and caryophyllene oxide, have been associated with antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, and antimicrobial activities. These findings suggest that *P. serratifolia* may serve as a promising source of natural therapeutic agents (Singh *et al.* 2011). The same studies also demonstrated the antimicrobial potential of *P. serratifolia* extracts and essential oils against various pathogenic microorganisms. Rajendran and Basha (2010) reported significant antimicrobial activity of crude extracts and fractions of *P. serratifolia* roots against several bacterial strains, suggesting its potential application as a phytomedicine. Similarly, Cruz *et al.* (2024) demonstrated the protective effects of *P. serratifolia* leaf extracts against gentamicin-induced nephrotoxicity in mice, showing its potential as a therapeutic agent for nephrotoxicity mitigation. Methanolic leaf extracts of *P. serratifolia* have also exhibited antibacterial, antioxidant, and anti-inflammatory activities in several *in vitro* studies (Febriyanti *et al.* 2025).

Despite the growing body of literature on the pharmacological properties of *P. serratifolia*, information remains limited on the relationship between its phytochemical composition and antimicrobial efficacy, particularly in locally occurring populations and under varying extraction conditions. Moreover, phytochemical profiles and bioactivities may vary depending on geographic origin, environmental conditions, extraction solvent, and plant part utilized. Therefore, further phytochemical characterization and antimicrobial evaluation are necessary to better understand the therapeutic potential of this species.

In this study, the phytochemical constituents of *P. serratifolia* leaf extracts were analyzed and its antimicrobial activity against selected bacterial pathogens was evaluated. The study aims to contribute additional scientific evidence supporting the medicinal potential of *P. serratifolia* and to explore its possible application as a source of natural antimicrobial compounds.

2. Materials and Methods

2.1. Plant Sample Collection

Leaf samples of *Premna serratifolia* L. were collected in the vicinity of Lake Danao Natural Park, Ormoc City, Leyte, Philippines. The verification of samples was done by Prof. Jay Torrefiel of the Natural History Museum and Herbarium of UP Tacloban. In the laboratory, the

leaf samples were thoroughly washed under running tap water and were sun-dried inside the greenhouse for almost 3 weeks. The dried leaf samples were powdered using a mechanical grinder.

2.2. Preparation of the Crude Extracts and Solvent-Partitioned Extracts

Dry leaf samples (11.7 g) were soaked with 150 mL methanol (MeOH) and were stored in a dark area overnight. After 12 h, the solids were separated from the supernatant using cheesecloth and concentrated in vacuo at 30°C. The CME (6.1 g) was reconstituted with 10% methanol and then partitioned consequently with other two solvents: (3 × 100 mL) n-hexane and ethyl acetate to fractionate the polar and non-polar compounds. The resulting solvent fractions were vacuum-dried to give hexane extracts (HXE, 86.2 mg), and ethyl acetate extracts (EAE, 409.4 mg) fractions. The remaining aqueous layer was also concentrated in vacuo at 30°C followed by flashing with N₂ gas until dry aqueous extracts (AQE, 871.8 mg). All extracts were stored in the dark before analysis.

2.3. Phytochemical Screening

The crude methanolic extract and solvent-partitioned extracts of *P. serratifolia* leaves were screened for the presence of major secondary metabolites, including flavonoids, alkaloids, terpenoids, tannins, steroids, naphthoquinones, and saponins, following the procedures with modifications described by Raaman (2006).

2.3.1. Flavonoids (Alkaline Reagent Test)

One milliliter (1 mL) of each extract was mixed with 2 mL of 10% sodium hydroxide (NaOH) solution. The formation of an intense yellow coloration indicated the possible presence of flavonoids. Subsequently, a few drops of 1% hydrochloric acid (HCl) were added. Disappearance of the yellow coloration upon acidification was considered a positive result for flavonoids.

2.3.2. Alkaloids (Hager's Test)

Each extract was dissolved in 2% hydrochloric acid and heated in a water bath, followed by filtration. The filtrate was then treated with 1% picric acid solution (Hager's reagent). The formation of a yellow precipitate was taken as evidence for the presence of alkaloids.

2.3.3. Naphthoquinones

One milliliter (1 mL) of each extract was treated with a few drops of 10% potassium hydroxide (KOH)

solution. The development of a brownish-red coloration indicated the presence of naphthoquinones.

2.3.4. Saponins (Froth Test)

One milliliter (1 mL) of each extract was mixed with 3 mL of distilled water and vigorously shaken for 2 min. The formation of a persistent froth was considered indicative of the presence of saponins.

2.3.5. Tannins

Two milliliters (2 mL) of each extract were treated with 1-2 drops of 1% ferric chloride (FeCl_3) solution. The appearance of a blue-black or greenish-black coloration was regarded as a positive result for tannins.

2.3.6. Steroids (Liebermann–Burchard Test)

One milliliter (1 mL) of each extract was mixed with 2-3 mL of chloroform (CHCl_3). Concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the test tube to form two distinct layers. The appearance of a reddish coloration in the chloroform layer and a yellowish-green coloration in the sulfuric acid layer indicated the presence of steroids.

2.3.7. Terpenoids (Salkowski Test)

One milliliter (1 mL) of each extract was mixed with 2 mL of chloroform (CHCl_3), followed by the careful addition of 2-3 mL of concentrated sulfuric acid (H_2SO_4) along the wall of the test tube to form a separate layer. The formation of a reddish-violet coloration at the interface was considered a positive indication of terpenoids.

2.4. FTIR Analysis

The functional groups present in the CME and solvent-partitioned extracts of *P. serratifolia* were determined from the spectra acquired using an Agilent Cary 630 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with a diamond attenuated total reflectance (ATR) accessory. A small amount of each dried extract was placed directly onto the ATR crystal and pressed to ensure contact with the crystal surface. Spectra were recorded over the range of $4000\text{--}650\text{ cm}^{-1}$ at a spectral resolution of 4 cm^{-1} , with 8 scans collected for each sample. Functional groups were assigned by comparing the observed absorption bands with standard FTIR reference data (Silverstein *et al.* 2014).

2.5. Test Organisms

The antibacterial screening assay used three bacterial test organisms, consisting of two Gram-positive species, *Bacillus cereus* (BIOTECH 1509) and *Staphylococcus*

aureus (BIOTECH 1582), and one Gram-negative species, *Pseudomonas aeruginosa* (BIOTECH 1335). All bacterial strains were obtained from the Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB). In addition, the phytopathogenic molds *Diaporthe phaseolorum* and *Neoscytalidium dimidiatum* were utilized in the antimicrobial screening.

2.6. Antimicrobial Activity using the Kirby–Bauer Disc Diffusion Method

The antimicrobial activity of the leaf crude and solvent-partitioned extracts were assessed using the Kirby–Bauer disc diffusion method following the protocol of Canlı *et al.* (2026) with slight modifications. Briefly, sterile 6-mm paper discs were impregnated with $20\text{ }\mu\text{L}$ of the extract solutions at a concentration of 100 mg/mL , corresponding to 2 mg of each extract per disc. The test bacterial inocula were then standardized according to the Clinical and Laboratory Standards Institute (CLSI) (2012a) guidelines using the direct colony suspension method. Bacterial colonies were suspended in sterile 0.85% saline solution and adjusted to a 0.5 McFarland standard (approximately $1.5 \times 10^8\text{ CFU/mL}$). The resulting suspensions were uniformly swabbed onto Mueller–Hinton agar (MHA) plates, after which the discs were aseptically placed on the agar surface. Methanol-impregnated discs served as the negative controls, whereas gentamicin discs ($10\text{ }\mu\text{g}$) served as the positive control. All assays were performed in triplicates. Following application of the discs, the plates were incubated at 37°C for 24 h, after which the diameters of the inhibition zones surrounding each disc were measured.

2.7. TLC - Agar - Overlay Bioautography Technique

The TLC - Agar - Overlay Bioautography Technique was adopted from Rahalison *et al.* (1991). Briefly, one milliliter of the bacterial strains with $1.5 \times 10^8\text{ CFU/mL}$ concentration was utilized for every 10 mL of Mueller - Hinton agar (MHA). The TLC plate was placed in a sterile Petri dish. A thin layer of the prepared MHA was poured over the TLC plate. Once solidified, the TLC plate was incubated for 24 hours at 35°C . The TLC - bioautography plates were sprayed with an aqueous solution of methylthiazol tetrazolium (Scientific Phygene). The clear zone of inhibition was observed against a purple background.

2.8. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the active extracts was determined to further evaluate their antimicrobial potential using the two-fold broth microdilution method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (2012b). The active partitioned extracts identified through the TLC–Agar Overlay Bioautography technique were reconstituted with methanol to a final concentration of 10 mg/mL. Two-fold serial dilutions were subsequently prepared in sterile 96-well microtiter plates, with each assay performed in triplicate for each test bacterium.

Briefly, the first well of each row was loaded with 100 µL of the prepared extract, while the succeeding wells each received 50 µL of Mueller–Hinton Broth (MHB). Two-fold serial dilutions were performed by transferring 50 µL from one well to the next, followed by thorough mixing to ensure homogeneity. This procedure was continued across the remaining wells, after which 50 µL was discarded from the final well to maintain equal volumes. Each well contained a final volume of 50 µL, resulting in extract concentrations ranging from 78 µg/mL to 10 mg/mL. The bacterial inocula were then standardized to a 0.5 McFarland turbidity standard and further diluted to achieve a final inoculum density of 5×10^5 CFU/mL in each well following the addition of 10 µL of the bacterial suspension.

Four control groups were included in the assay: (1) a positive control consisting of the vancomycin antibiotic (100 µg/mL) and the standardized bacterial inoculum; (2) a negative control containing MHB and the standardized bacterial inoculum; (3) a solvent control containing methanol, MHB, and the standardized bacterial inoculum; and (4) a sterility control containing MHB only. The microtiter plates were incubated at 35°C for 16–20 h and the MIC was defined as the lowest extract concentration that completely inhibited visible bacterial growth.

2.9. Food Poison Technique

The leaf extracts were tested for their fungitoxicity against *Diaporthe phaseolorum* and *Neoscytalidium dimidiatum* using the poisoned food technique (Grover and Moore 1962; Taguiam *et al.* 2020) with slight modifications. Two milliliters of the leaf extracts were separately amended to PDA medium. A PDA medium with mancozeb (1.25 µL/mL) and a PDA medium without chemical amendment were used as positive and negative controls, respectively. Afterwards, a 5-mm mycelial plug of each test mold was placed at the center of each

plate. The fungal radial growth was measured when the mycelium in the negative control reached the edge of the plate. All assays were performed in triplicates.

2.10. Nucleation Assay

The method presented by Saha and Verma (2013) for the inhibitory activity of the extracts on the nucleation of CaOx crystals was determined with modifications. In a 96-well plate, the following mixture was prepared 50 µL of synthetic urine (105.5 mM NaCl, 63.7 mM KCl, 17.0 mM Na₂SO₄, 3.2 mM tris-Sodium citrate, 32.3 mM Na₂HPO₄, 0.32 mM sodium oxalate, 3.85 mM MgSO₄·7H₂O, 4.50 mM CaCl₂·2H₂O), 50 µL of the sample (crude and solvent partitioned extracts of different concentration, malic acid (10 mg/mL) which served as the positive control and water which serves as the negative control) and 50 µL calcium chloride (10 mmol/L). Crystallization was initiated by adding 50 µL sodium oxalate (10 mmol/L) solutions to the mixture. The absorbance (optical density, OD) was recorded at 620 nm every 10, 30, 60 and 90 min. The percentage inhibition was calculated as

$$(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}} \times 100.$$

2.11. Aggregation Assay

The inhibitory activity of the HXE extracts on the aggregation of CaOx crystals was determined qualitatively according to Heirangkhongjam and Ngaseppam (2021) with the exemption of estimating the rate of aggregation. The CaOx crystals were prepared by mixing both 25 mL solutions of calcium chloride and sodium oxalate at 50 mM. The solutions were then equilibrated in a water-bath for 1 h at 60°C, cooled to 37°C, filtered and then dried. About 50 mg of the dried CaOx was dissolved in 50 mL synthetic urine at pH 6.5 to attain 1 mg/mL CaOx crystal suspension. Similarly, in a 96-well plate, the following mixture was prepared: 100 µL of CaOx crystal suspension and 100 µL of the HXE at 10 mg/mL and 5 mg/mL, and water. After 90 minutes the samples were observed under an Olympus CX23 microscope, then the pictures were taken using a smartphone (iPhone 13) at 40× and 100× magnification to visualize the size of the calcium oxalate crystals formed.

2.12. Data Analysis

All experiments were conducted in three repeats. Results were presented as mean ± standard deviation

(stdev). One-way ANOVA were used to analyze the difference and followed by Tukey's test to test significance between means using jamovi version 2.7 software (2025), and $p < 0.05$ was considered to be statistically significant. The IC_{50} was determined using AATBioquest (2026).

3. Results

A total of 6.7 g of methanolic crude extract was retrieved from 11.5 g of dry *P. serratifolia* leaf samples. Liquid-liquid extraction in increasing polarity yielded hexane extracts (HXE) (86.2 mg), ethyl acetate extracts (EAE) (409.3 mg) and aqueous extracts (AQE) (871.8 mg).

3.1. Phytochemical Screening of the Crude and Fractionated Solvent Extracts

Phytochemical screening of the crude methanolic extract (CME) and its solvent-partitioned fractions revealed the presence of flavonoids, terpenoids, naphthoquinones, and saponins in varying amounts (Table 1). The CME tested positive for flavonoids, terpenoids, naphthoquinones, and saponins, with saponins showing the strongest reaction. No alkaloids, tannins, or steroids were detected in any of the extracts.

Among the solvent-partitioned fractions, the HXE was positive only for terpenoids and saponins, suggesting that these metabolites constitute the major non-polar constituents of *P. serratifolia* leaves. The EAE fraction contained flavonoids, terpenoids, naphthoquinones, and saponins, with flavonoids and terpenoids exhibiting stronger reactions than those observed in the crude extract. Similarly, the AQE tested positive for flavonoids, terpenoids, naphthoquinones, and saponins.

3.2. Antioxidant Activity of the Crude and Solvent-Partitioned Extracts of *P. serratifolia*

The antioxidant activities of the crude methanolic extract and solvent-partitioned fractions of *P. serratifolia* were evaluated using the DPPH radical scavenging assay and total antioxidant activity expressed as ascorbic acid equivalents (AAE) (Table 2). Among the extracts tested, only the AQE exhibited an IC_{50} value below 400 $\mu\text{g/mL}$, with an IC_{50} of 297.6 $\mu\text{g/mL}$, indicating greater radical scavenging activity than the CME, HXE, and EAE, all of which showed IC_{50} values exceeding 400 $\mu\text{g/mL}$. As expected, ascorbic acid demonstrated the strongest antioxidant activity with an IC_{50} value of 82.5 $\mu\text{g/mL}$.

The trend observed in the DPPH assay was further supported by the total antioxidant activity results. The AQE exhibited the highest antioxidant capacity at 1461.3 mg AAE/g extract, followed by the EAE (1148.4 mg AAE/g extract), crude methanolic extract (1073.7 mg AAE/g extract), and HXE extract (620.7 mg AAE/g extract). The high antioxidant activity of the AQE suggests that the major antioxidant constituents of *P. serratifolia* are predominantly polar compounds that were preferentially partitioned into the aqueous phase during solvent fractionation.

3.3. Antimicrobial activity of the Crude and Solvent-Partitioned Extracts of *P. serratifolia*

The preliminary antimicrobial activity of the crude methanolic extract and solvent-partitioned fractions of *P. serratifolia* was evaluated using the Kirby–Bauer disk diffusion assay (Figure 1). In this assay, the formation of a zone of inhibition indicates suppression of bacterial growth. As shown in Table 3, all extracts exhibited antibacterial activity against *B. cereus* and *S. aureus*,

Table 1. Phytochemical screening of the crude and solvent-partitioned extracts from the leaves of *Premna serratifolia*

	CME	HXE	EAE	AQE
Flavonoids				
Alkaline	+	-	++	++
Tannins	-	-	-	-
Alkaloids				
Wagner's	-	-	-	-
Hager's	-	-	-	-
Terpenoids	+	+	++	++
Steroids	-	-	-	-
Naphthoquinones	+	-	+	+
Saponins	+++	++	+++	++

(+) = slightly present; (++) = moderately present; (+++) = strongly present; (-) = not detected

Table 2. Antioxidant evaluation of the crude and solvent-fractionated extracts of *Premna serratifolia*

Extracts	DPPH-radical scavenging activity (IC_{50} , $\mu\text{g/mL}$)	Total antioxidant activity (AAE mg/ g extract)
Crude	>400	1073.7
Hexane	>400	620.7
Ethyl acetate	>400	1148.4
Aqueous	297.6	1461.3
Ascorbic acid	82.5	NA

Values are expressed as mean $\pm$ SD (n = 3). Means within the same column sharing different superscript letters are not significantly different according to Tukey's test following the one-way ANOVA ($p < 0.05$)

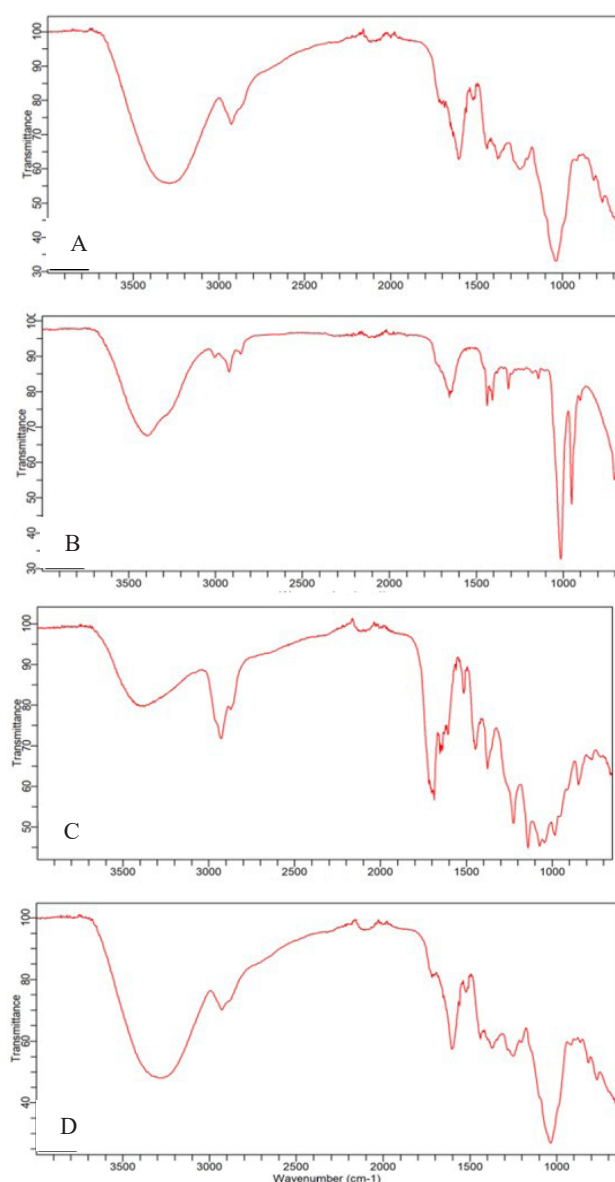


Figure 1. FTIR spectra of the CME and solvent-partitioned fractions. FTIR spectra of the crude extracts, CME (A) and the solvent partitioned fractions, HXE (B), EAE (C), and AQE (D) using an Agilent Cary 630 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA). Spectra were recorded over the range of 4000–650 cm^{-1} at a spectral resolution of 4 cm^{-1} , with 8 scans collected for each sample

Table 3. Zone of inhibition of the crude and solvent-partitioned extracts of *Premna serratifolia* against selected test organisms

Extracts	Zona of inhibition (mm)		
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
Crude	12	12	-
Hexane	11	13	-
Ethyl acetate	13	14	-
Aqueous	11	10	-
Gentamicin (10 µg/disk)	28	30	29
Negative (methanol)	-	-	-

The size of the disc is 6 mm

both of which are Gram-positive bacteria. In contrast, none of the extracts exhibited inhibitory activity against the Gram-negative bacterium *P. aeruginosa*. The positive control, gentamicin, exhibited strong antibacterial activity against all test organisms, producing inhibition zones of 28–30 mm, whereas the negative control showed no inhibition.

To identify the constituents responsible for the observed antibacterial activity, TLC-agar-overlay bioautography was performed against *S. aureus* and *B. cereus* (Figure 2). Among the extracts tested, only the EAE produced distinct zones of inhibition corresponding to separated chromatographic bands. Several inhibition zones were observed along the chromatogram, indicating the presence of multiple antibacterial constituents distributed across different Rf regions. Although all extracts exhibited antibacterial activity in the Kirby–Bauer disk diffusion assay, only the EAE showed detectable antibacterial zone of inhibition in the bioautography experiment. This discrepancy may be attributed to differences in the concentration and distribution of active compounds on the TLC plate. During chromatographic separation, the amount of bioactive constituent present in an individual spot may fall below the minimum concentration required to inhibit bacterial growth, thereby preventing the formation of visible inhibition zones. In contrast, the Kirby–Bauer assay evaluates the activity of the entire extract, where the combined or synergistic effects of multiple compounds may contribute to the observed antibacterial activity.

The bioautography results further suggest that the antibacterial activity of *P. serratifolia* is associated primarily with compounds of moderate polarity, as the most prominent inhibition zones were observed at point of origin of the spot Rf values of 0.29.

Because the EAE was the only fraction that produced distinct antibacterial zones in the TLC–agar-overlay bioautography assay, this fraction was selected for MIC determination. The EAE exhibited MIC values of 312.5 ± 0.0 µg/mL against *B. cereus* and 625 ± 0.0 µg/mL against

S. aureus, demonstrating approximately two-fold greater inhibitory potency against *B. cereus*.

A separate food poisoning assay was conducted against the agricultural fungal pathogens *D. phaseolorum* and *N. dimidiatum*. However, none of the crude or solvent-partitioned extracts inhibited fungal growth under the conditions tested, indicating a lack of detectable antifungal activity against these organisms (data not shown).

3.4. Antiurolithiasis activity of the Crude and Solvent-Partitioned Extracts of *P. serratifolia*

The antiurolithiatic potential of the crude and solvent-partitioned extracts of *P. serratifolia* was evaluated using a calcium oxalate nucleation assay. Among the four extracts tested, only the HXE exhibited inhibitory activity against calcium oxalate crystal formation (Figure 3 and Figure 4). The positive control, malic acid (10 mg/mL), produced relatively low inhibition, with values ranging from approximately 18–22% throughout the 90-minute incubation period.

In contrast, the HXE demonstrated substantial inhibition of calcium oxalate nucleation at concentrations of 5 and 10 mg/mL. At 10 min of incubation, inhibition values of approximately 81% and 80% were observed for the 5 and 10 mg/mL treatments, respectively. Although the inhibitory effect gradually declined with increasing incubation time, both concentrations maintained inhibition levels greater than 50% after 90 minutes, indicating sustained suppression of crystal nucleation. The 1 mg/mL treatment exhibited considerably lower activity, with inhibition values remaining below 35% throughout the assay period.

Microscopic examination further supported the results of the nucleation assay (Figure 5). In the untreated control, numerous calcium oxalate crystals were observed, with crystals appearing larger and more densely distributed. In contrast, samples treated with the hexane extract exhibited markedly fewer crystals, which were generally smaller in size. These observations suggest that the hexane extract

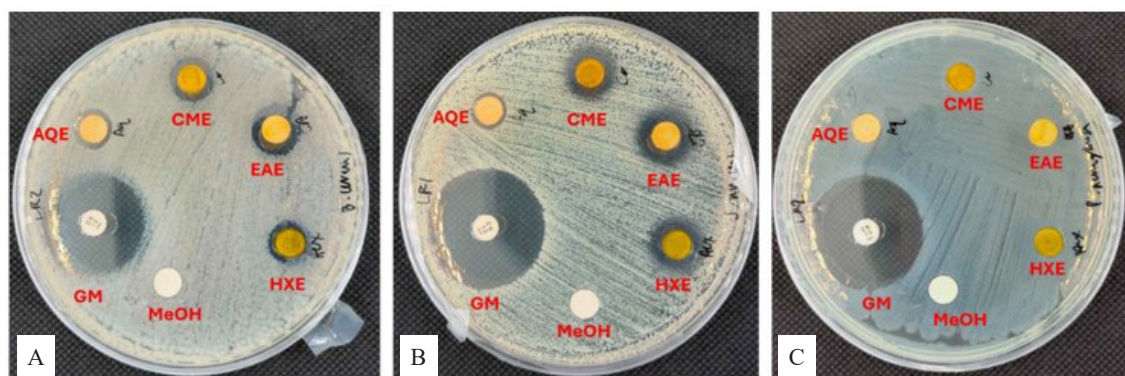


Figure 2. Zone of inhibition test using Kirby–Bauer disk diffusion assay against Gram- negative and Gram-positive organisms. The zones of inhibition around the disks containing either CME - crude extract, HXE - n-hexane extract, EAE-ethyl acetate extract, AQE - aqueous extract and GM - positive control (gentamicin, 10 µg) are clearly observed for both Gram-positive pathogens: A - *Bacillus cereus* and B - *Staphylococcus aureus*. Only GM - positive control (gentamicin, 10 µg) had a zone of inhibition for the Gram-negative bacterium: C - *Pseudomonas aeruginosa*. MeOH - negative control (methanol) did not show a zone of inhibition to the three pathogens. The size of the disk is 6 mm

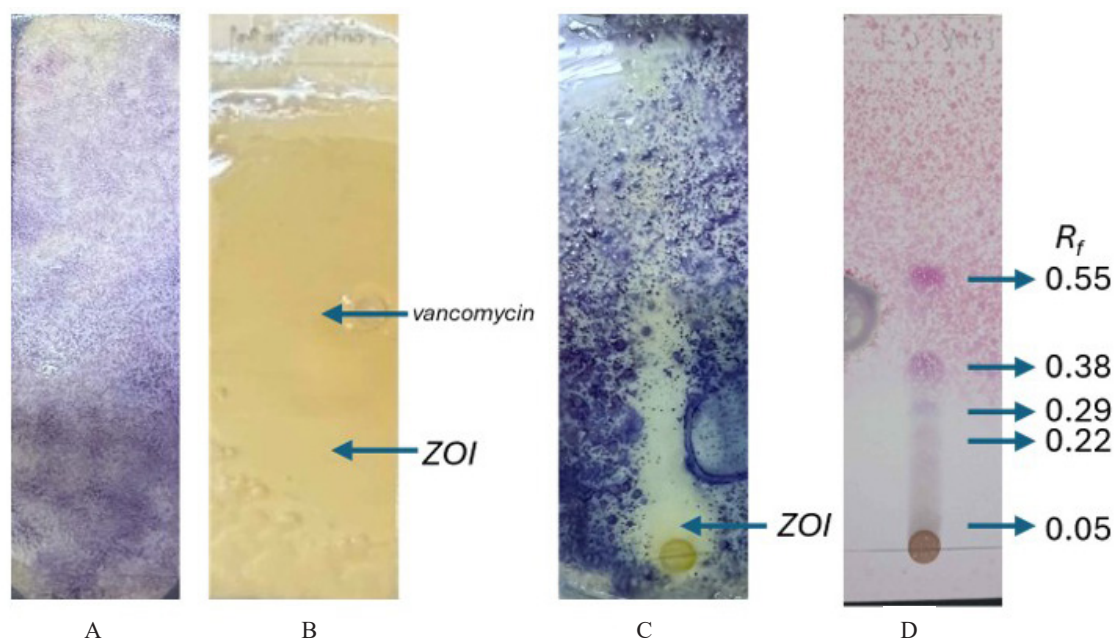


Figure 3. TLC-agar-overlay bioautography of the ethyl acetate extract of *Premna serratifolia* against *Staphylococcus aureus*. Bioautograms of the negative control (A), gentamicin positive control (B), and ethyl acetate extract (C) are shown. Viable bacteria are stained in purple using methylthiazol tetrazolium (MTT). The corresponding TLC chromatogram of the extract (D) displays the separated constituents and their respective R_f values. The extract was developed on silica gel TLC plates using hexane acetate (3:2, v/v) as the mobile phase. Separated compounds were visualized by spraying with vanillin–sulfuric acid reagent followed by heating

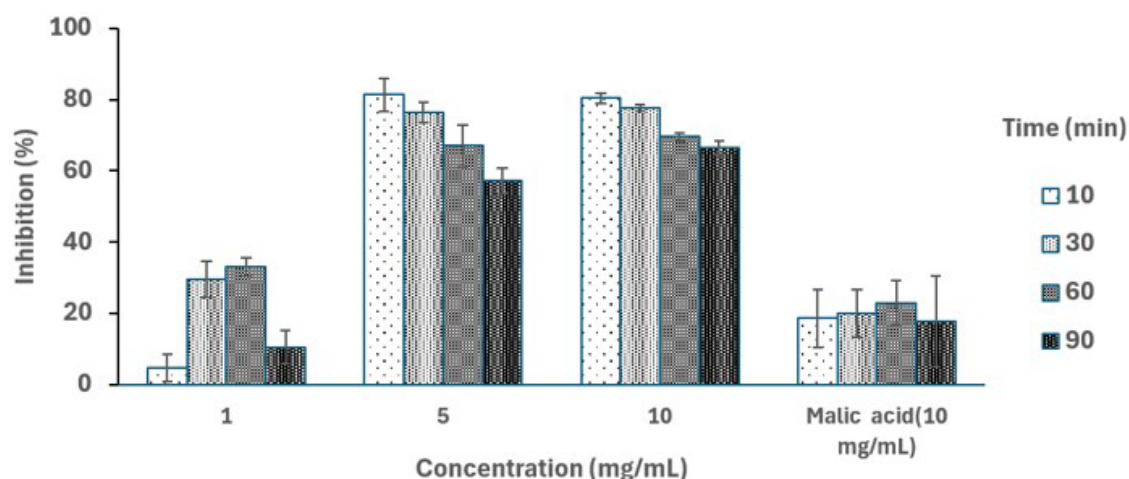


Figure 4. Time- and concentration-dependent inhibitory effects of the hexane extract of *Premna serratifolia* on calcium oxalate crystal nucleation. The hexane extract was evaluated at concentrations of 1, 5, and 10 mg/mL and compared with malic acid (10 mg/mL) as the positive control. Percent inhibition of calcium oxalate crystal formation was determined after 10, 30, 60, and 90 min of incubation. Values are presented as mean $\pm$ standard deviation ($n = 3$)

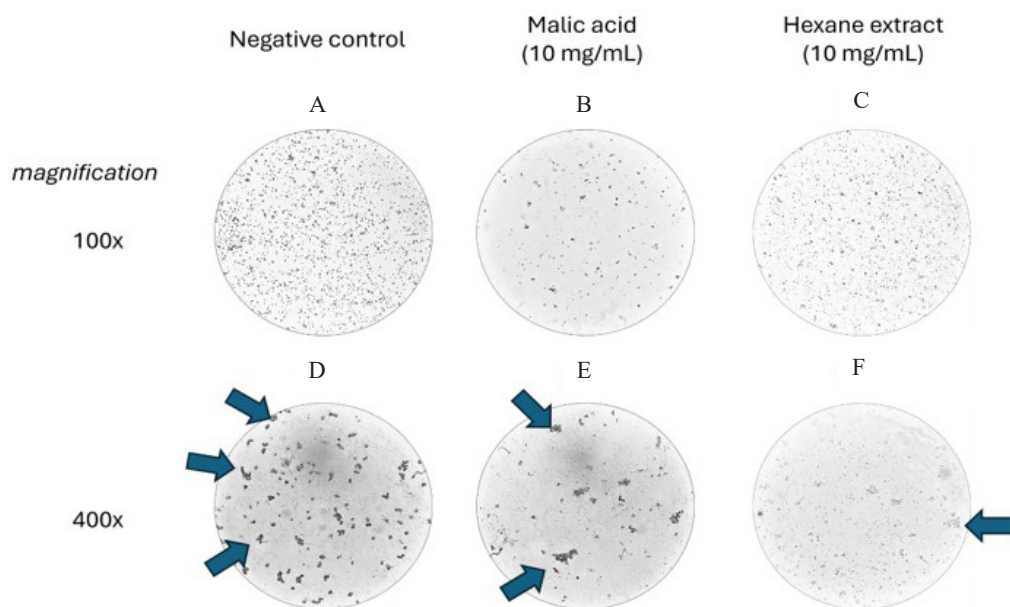


Figure 5. Representative photomicrographs showing the effects of malic acid and the hexane extract of *Premna serratifolia* on calcium oxalate (CaC_2O_4) crystal formation in the nucleation assay. Images correspond to the negative control (A, D), malic acid (10 mg/mL) (B, E), and hexane extract (10 mg/mL) (C, F) observed at 100 $\times$ (A-C) and 400 $\times$ (D-F) magnifications. The blue arrows indicate representative calcium oxalate crystals and crystal aggregates. Compared with the negative control, treatment with malic acid and the hexane extract reduced crystal density and aggregation, with the hexane extract exhibiting the greatest suppression of calcium oxalate crystal formation. These microscopic observations corroborate the results of the nucleation assay, demonstrating the inhibitory effect of the hexane extract on calcium oxalate crystallization

not only reduces the extent of calcium oxalate crystal formation but may also interfere with crystal growth and aggregation. The pronounced activity of the hexane fraction indicates that the compounds responsible for the antiurolithiatic activity of *P. serratifolia* are likely non-polar in nature and were preferentially partitioned into the hexane solvent during extraction.

4. Discussion

P. serratifolia is a very well-studied plant species for its biological activities and pharmacological importance. Studies have reported this plant species to possess antioxidant, anti-glucosidase, antiarthritis, antiparasitic, and anticancer activities (Dianita and Jantan 2017; Febriyanti *et al.* 2025; Hadiarti *et al.* 2026). Although in the Philippines, the most studied *Premna* species being studied is *P. odorata* because of its known medicinal purposes. Studying *P. serratifolia* sampled here in the Philippines may provide additional knowledge on the pharmacological importance of this plant species. The study of Hadiarti *et al.* (2026) demonstrated that *P. serratifolia* samples collected from different areas in Indonesia exhibited different activities.

In this study, only the aqueous extract exhibited antioxidant activity, as evidenced by its DPPH IC_{50} value of 297.6 $\mu\text{g/mL}$. This activity is lower than the reported DPPH-scavenging activity as reported by other studies which reported DPPH IC_{50} value of $\sim 7 \mu\text{g/mL}$ (Timotius *et al.* 2018) and $\sim 66 \mu\text{g/mL}$ (Simamora *et al.* 2020). Their study reported strong antioxidant activity in aqueous preparations of *P. serratifolia* leaves and attributed the activity to water-soluble phenolic compounds and flavonoids. Similarly, Simamora *et al.* (2020) found that both water and ethanolic extracts of *P. serratifolia* exhibited substantial antioxidant activities, with the aqueous extract generally containing higher levels of antioxidant phytochemicals. The high antioxidant activity observed in the aqueous fraction suggests that the antioxidant constituents of *P. serratifolia* are predominantly highly polar compounds that remained in the aqueous phase following partitioning with hexane and ethyl acetate. Hien *et al.* (2019) and Simamora *et al.* (2020) reported that polar extracts of *P. serratifolia* contain iridoid glycosides and phenylethanoid glycoside such as isoacetoside. The FTIR spectrum exhibited strong absorptions at approximately $3400\text{--}3200 \text{ cm}^{-1}$ and $1150\text{--}1000 \text{ cm}^{-1}$, corresponding to O-H stretching and C-O-C and C-O stretching vibrations, respectively (Figure 6D). These peaks are characteristic of carbohydrate moieties

and glycosidic linkages (Morikawa *et al.* 2010). The FTIR data may suggest the occurrence of such polar metabolites in the aqueous fraction, although definitive identification requires more chromatographic and spectroscopic confirmation.

On the other hand, all extracts have shown antibacterial activity against Gram-positive *Bacillus cereus* and *Staphylococcus aureus* in the Kirby-Bauer assay. However, only the EAE consistently exhibited this activity on the TLC-agar-overlay bioautography. As mentioned above, the discrepancy may be attributed to differences in the concentration and distribution of active compounds on the TLC plate. The antibacterial activity observed in the lower-R_f region of the ethyl acetate fraction suggests that the active constituents are relatively polar compounds. The study of Hadiarti *et al.* (2025) reported the isolation of the methoxylated flavonoids from *P. serratifolia* leaf ethyl acetate fraction which includes centaureidin, pectolarigenin, syringetin, kaempferide, and casticin. These compounds have been reported to possess antibacterial activity against various Gram-positive bacteria (Amin *et al.* 2017; Dimkić *et al.* 2020; Abbas *et al.* 2025).

To date, antimicrobial studies on *P. serratifolia* have predominantly employed agar diffusion assays, with relatively few investigations reporting MIC values. Rajendran and Basha (2010) evaluated root extracts and fractions against a range of bacterial and fungal pathogens and included MIC determination as part of their antimicrobial assessment. In contrast, broth microdilution MIC studies are more commonly reported for related *Premna* species, such as *Premna resinosa*, whose CME exhibited MIC values of 0.06 mg/mL against *Staphylococcus aureus* ATCC 29312 and 0.25 mg/mL against both *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* NCTC 10400 (Albadawi *et al.* 2017). Although the EAE evaluated in the present study exhibited higher MIC values than those reported for *P. resinosa*, direct comparison is limited because the studies involved different *Premna* species, test bacterial strains, and experimental conditions. Nevertheless, these findings highlight the novelty of the present work, as MIC data for *P. serratifolia* remain scarce, thereby providing baseline quantitative evidence of the antibacterial activity of this species.

Furthermore, the presence of methoxylated flavonoids may also explain the relatively weak DPPH radical-scavenging activity ($IC_{50} > 400 \text{ ppm}$) of the EAE compared with the AQE. Methoxylated flavonoids has a decrease number of free phenolic hydroxyl groups available for

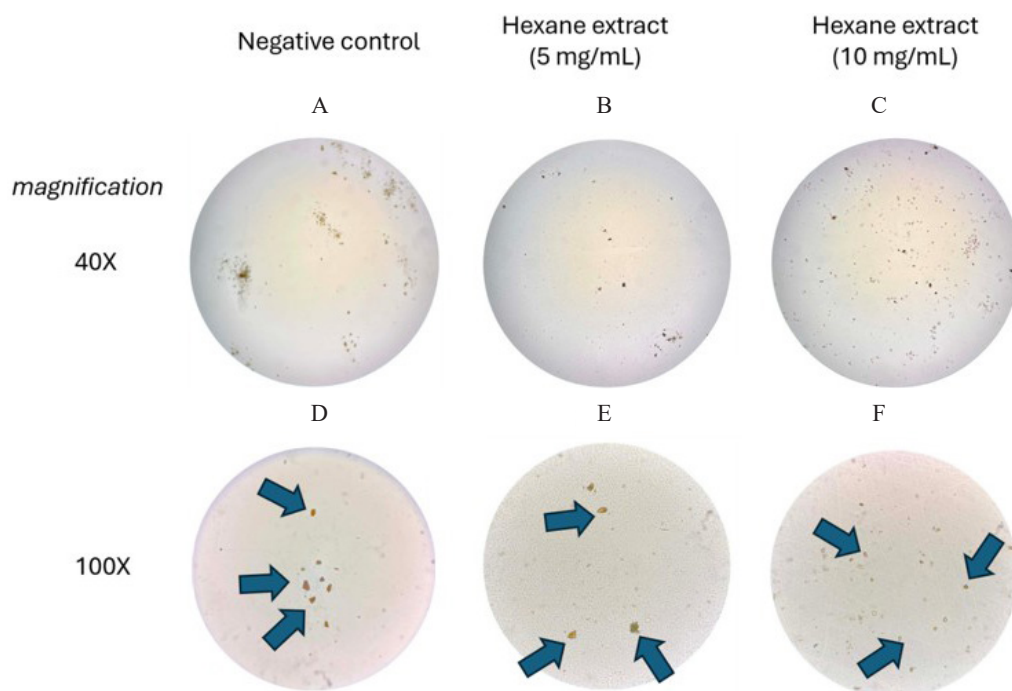


Figure 6. Representative photomicrographs showing the effects of the HXE of *Premna serratifolia* on calcium oxalate (CaC_2O_4) crystal aggregation assay. Images correspond to the negative control (A, D), HXE (5 mg/mL) (B, E), and HXE (10 mg/mL) (C, F) observed at 40X (A-C) and 100X (D-F) magnifications. The blue arrows indicate representative calcium oxalate crystal aggregates. Compared with the negative control, HXE at 10 mg/mL produced smaller crystals suggesting inhibition of calcium oxalate crystal aggregation

hydrogen donation, thereby reducing radical-scavenging efficiency relative to highly hydroxylated phenolics and phenylethanoid glycosides (Rice-Evans *et al.* 1997; Heim *et al.* 2002). For example, casticin, centaureidin, tricetin, and pectolinarigenin contain multiple methoxy substituents that generally enhance lipophilicity and membrane interactions but may diminish antioxidant capacity compared with polyhydroxylated flavonoids.

Among all extracts tested, only the hexane fraction significantly inhibited calcium oxalate nucleation. At concentrations of 5 and 10 mg/mL, inhibition exceeded 75% during the early stages of crystal formation and remained above 50% after 90 min of incubation. Microscopic observations further demonstrated substantial reductions in crystal density and crystal size relative to the negative control, corroborating the nucleation assay results. Direct comparisons with other *Premna* species studies are difficult because no previous antiurolithiatic studies on the species were found. In the Philippines, the herbal plant *Blumea balsamifera* (Sambong) is widely used for the management of urolithiasis. The study of Montealegre *et al.* (2017) reported that high concentration of *B. balsamifera*, prepared as tea, decreases induction time and increases

nucleation rate. In this action, nucleation is not really inhibited but a large number of smaller crystals are formed. Formation of smaller stones are easier to eliminate by urination. Possibly, this mechanism also occurs for the HXE as microscopic analysis also shows crystal formations but as not as large as with the negative control. These findings indicate that the compounds responsible are concentrated predominantly in the non-polar fraction. Phytochemical screening revealed the presence of terpenoids and saponins in the HXE. Previous phytochemical investigations of *P. serratifolia* have demonstrated that non-polar and n-hexane fractions are enriched in lipophilic metabolites, including terpenoid derivatives, phytol, and fatty acids such as palmitic, stearic, and oleic acids (Rahman *et al.* 2023; Bhayye *et al.* 2026). The FTIR spectrum of HXE was characterized by intense aliphatic C–H stretching bands at approximately 2950–2850 cm^{-1} together with a broad hydroxyl absorption around 3400 cm^{-1} , indicating the presence of lipophilic oxygenated metabolites (Hadiarti *et al.* 2025) (Figure 6D). The 2950–2850 cm^{-1} peak cannot be attributed to flavonoid glycosides as phytochemical screening for HXE did not detect flavonoids. In addition, a strong absorption observed in the 1100–1000 cm^{-1} region

corresponds to C–O stretching vibrations of alcohols and ethers, suggesting the occurrence of oxygenated terpenoid-type constituents. For the HXE, it is expected that it contains nonpolar compounds. Thus, the peaks are more likely attributable to compounds such as phytol, triterpenoids, and related lipophilic metabolites rather than glycosidic constituents which supports previous studies (Rahman *et al.* 2023; Bhayye *et al.* 2026).

In conclusion, the crude methanolic extract and solvent-partitioned fractions of *P. serratifolia* leaves exhibited distinct biological activities that were influenced by their phytochemical composition. The AQE demonstrated the strongest antioxidant activity while the EAE fraction exhibited the highest antibacterial activity against *B. cereus* and *S. aureus*. Notably, only the HXE significantly inhibited calcium oxalate nucleation (Figure 6) and reduced crystal density and size, providing the first evidence of the antiurolithiatic potential of *P. serratifolia*. These findings demonstrate that *P. serratifolia* is a promising source of bioactive compounds with antioxidant, antibacterial, and antiurolithiatic properties and highlight the importance of solvent partitioning in concentrating compounds with specific biological activities. Further bioassay-guided isolation and structural characterization studies are necessary to identify the active constituents responsible for these pharmacological effects.

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