

In Vitro Assessment of the Phytochemical Composition and Antibacterial Activity of *Pisonia alba* Leaf Extracts

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Submitted: 2026, Jul 06; Accepted: 2026, Aug 10; Published: 2026, Aug 18

Citation: Balamurugan, V., Ramachandran, L., Gothandapani, S. (2026). In Vitro Assessment of the Phytochemical Composition and Antibacterial Activity of *Pisonia alba* Leaf Extracts. *Int Nat Sci Int Res*, 1(2), 01- 09.

Abstract

Pisonia alba is a medicinally important plant that has been widely used in traditional systems of medicine, including Ayurveda and Siddha, for the treatment of various ailments. The present study was undertaken to investigate the phytochemical composition, antioxidant potential, and antibacterial activity of acetone, chloroform, and ethanolic leaf extracts of *Pisonia alba*. Preliminary phytochemical screening was carried out using standard qualitative methods to identify the major classes of secondary metabolites, while structural characterization of the isolated phytoconstituents was performed using proton (^1H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy. The antibacterial activity of the extracts was evaluated by the agar well diffusion method against *Pseudomonas aeruginosa* and *Bacillus subtilis*, using amoxicillin as the positive control. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and coumarins in the leaf extracts. The extracts also exhibited appreciable antioxidant activity. Among the tested solvents, the acetone extract showed the highest antibacterial activity, producing zones of inhibition of 30.8 ± 1.1 mm against *Pseudomonas aeruginosa* and 30.1 ± 0.8 mm against *Bacillus subtilis* at a concentration of 40 μL , whereas the ethanolic extract exhibited moderate activity and the chloroform extract showed comparatively lower antibacterial efficacy. These findings support the traditional medicinal use of *Pisonia alba* and suggest that its leaves are a promising source of bioactive phytochemicals with potential antioxidant and antibacterial applications.

Keywords: *Pisonia Alba*, Phytochemical Screening, Antibacterial Activity and NMR

1. Introduction

Medicinal plants are an important source of therapeutic compounds and have been widely used in traditional healthcare systems for centuries. In many parts of the world, including India and Nigeria, plant materials are commonly prepared as crude extracts, decoctions, infusions, or tinctures to treat various infectious and chronic diseases. According to the World Health Organization (WHO), more than 70% of the global population depends on medicinal plants for their primary healthcare needs. Ensuring the quality of herbal medicines is essential and involves evaluating their identity, purity, chemical composition, and biological properties, as well as maintaining standardized preparation methods. Quality control of herbal products combines traditional knowledge with modern scientific techniques to ensure their safety, efficacy, and consistency. The extraction of bioactive compounds is a crucial

step in herbal research, and the choice of solvent significantly influences the yield and activity of phytochemicals. With recent advances in science and technology, medicinal plants can now be systematically analysed using chromatographic and spectroscopic techniques, enabling the identification and characterization of their active constituents and supporting their potential therapeutic applications. Medicinal plants are valuable sources of bioactive compounds that contribute significantly to human health. These plants contain a wide range of phytochemicals, including alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids, and glycosides, which possess important biological and therapeutic properties.

Plant-derived compounds are widely used in traditional medicine, modern pharmaceuticals, nutraceuticals, and herbal formulations.

According to the World Health Organization (WHO), nearly 80% of the global population relies on medicinal plants and traditional herbal remedies for primary healthcare. This highlights the growing importance of medicinal plants as natural sources of safe and effective therapeutic agents [1-5].

Pisonia alba is a medicinal plant belonging to the family Nyctaginaceae and is widely distributed in tropical and subtropical regions. The leaves of *Pisonia alba* have long been used in traditional medicine for the treatment of various ailments. Previous studies have reported that the plant contains several phytochemicals, including flavonoids, alkaloids, tannins, saponins, phenolic compounds, steroids, and terpenoids, which are responsible for its diverse pharmacological activities [6-10]. These bioactive constituents are known to exhibit antioxidant, antibacterial, anti-inflammatory, analgesic, and wound-healing properties.

Despite its traditional medicinal importance, further scientific investigation is required to identify and characterize the phytochemical constituents present in *Pisonia alba* leaves. Therefore, the present study aims to prepare leaf extracts of *Pisonia alba* using suitable solvents and perform preliminary phytochemical screening to identify the major classes of bioactive compounds. The findings of this study will provide valuable

information on the medicinal potential of *Pisonia alba* and support its future applications in pharmaceutical and healthcare research.

The escalating global incidence of antibacterial resistance has become a critical public health concern, rendering numerous conventional antibiotic regimens ineffective against pathogenic microorganisms [6,7]. The indiscriminate use of synthetic antimicrobial agents has precipitated multidrug resistance (MDR) in clinically important bacteria, underscoring the urgent necessity to identify and develop novel, plant-derived antimicrobial alternatives with favourable safety profiles [20-26].

Despite extensive ethnobotanical documentation, systematic scientific validation of the phytochemical composition and biological activities of *Pisonia alba* leaf extracts remains limited [8-19]. The present investigation, therefore, aimed to: (i) characterise the major classes of secondary metabolites present in aqueous and ethanolic leaf extracts of *Pisonia alba* using standard qualitative phytochemical protocols; (ii) elucidate the structural characteristics of the extract through ^1H and ^{13}C NMR spectroscopic analysis; and (iii) evaluate antibacterial efficacy against clinically relevant Gram-negative and Gram-positive bacterial strains using the agar well diffusion method.



Figure: 1: *Pisonia Alba* Leaves

2. Materials and Methods

2.1. Plant Material: Collection and Authentication

Healthy, mature leaves of *Pisonia alba* were collected from natural populations in Nadukuppam, Panruti Taluk, Cuddalore District, Tamil Nadu, India. The harvested material was washed thoroughly under running tap water, followed by two successive rinses with double-distilled water to remove surface contaminants. Botanical authentication of the plant was performed in accordance with the APG IV (Angiosperm Phylogeny Group IV) classification system. A voucher specimen was prepared and deposited at the institutional herbarium for future reference.

2.2. Preparation of Plant Extracts

Freshly collected *Pisonia alba* leaves were thoroughly washed with tap water followed by double-distilled water to remove dust and other impurities. The cleaned leaves were shade-dried, finely powdered, and used for extract preparation. For the aqueous extract, 10 g of the powdered leaf material was mixed with 100 mL of double-distilled water and heated at 60°C for 30 minutes in a water bath. The extract was allowed to cool to room temperature and then filtered through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was stored at 4°C until further use for phytochemical screening, antioxidant assays, and antibacterial studies. For the ethanolic extract, 10 g of the dried leaf powder

was soaked in 100 mL of 95% ethanol and macerated at room temperature for 72 hours with intermittent shaking. The mixture was subsequently filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator to obtain the crude extract. The dried extract was stored at 4°C in an airtight container until further analysis.

2.3. Preliminary Phytochemical Screening

The aqueous and ethanolic extracts of *Pisonia alba* leaves were subjected to qualitative phytochemical screening to detect the presence of major secondary metabolite classes, including alkaloids, phenolic compounds, saponins, tannins, flavonoids, terpenoids, anthraquinones, coumarins, carbohydrates, and betacyanins, following standard protocols [2,28].

2.3.1 Test for Alkaloids

- **Mayer's Reagent Test:** To 2 mL of each extract, 1 mL of Mayer's reagent (potassium mercuric iodide) was added dropwise along the inner wall of the test tube. Formation of a white or cream-coloured precipitate was interpreted as a positive indication for the presence of alkaloids.
- **Wagner's Test:** To 1 mL of the extract, 1 mL of Wagner's reagent (iodine in potassium iodide solution) was added. Development of a reddish-brown precipitate indicated alkaloid presence.
- **Dragendorff's Test:** To 0.5 mL of the filtrate, 2–3 drops of Dragendorff's reagent (bismuth nitrate and potassium iodide) were added. Formation of an orange-brown precipitate confirmed the presence of alkaloids.

2.3.2. Test for Phenolic Compounds

- **Ferric Chloride Test:** To 2 mL of extract, 2 mL of 2% ferric chloride (FeCl_3) solution was added. Development of a dark green or bluish-green colouration was indicative of phenolic compounds.
- **Lead Acetate Test:** To 2 mL of extract, 3 mL of 10% lead acetate solution was added. Formation of a bulky white precipitate was interpreted as a positive result.

2.3.3. Test for Saponins

Five millilitres of extract were diluted with distilled water to a total volume of 20 mL. The mixture was vigorously agitated for 15 minutes. Persistence of stable froth for a minimum of 10 minutes was considered indicative of saponin presence.

2.3.4. Test for Tannins

Two millilitres of extract were treated with 1 mL of FeCl_3 solution. Formation of a greenish colouration was indicative of condensed tannins.

2.3.5. Test for Flavonoids

- **Alkaline Reagent Test:** To 2 mL of extract, 10% sodium hydroxide (NaOH) solution was added. Development of an intense yellow colouration indicated the presence of flavonoids.
- **Lead Acetate Test:** Addition of lead acetate solution to the

extract; formation of a yellow precipitate was indicative of flavonoid constituents.

2.3.6. Test for Terpenoids (Salkowski Test)

Two millilitres of extract were mixed with 2 mL of acetic anhydride and concentrated sulphuric acid (H_2SO_4). Formation of a blue or green ring at the interface was indicative of the presence of terpenoids.

2.3.7. Test for Anthraquinones (Bornträger's Test)

Five millilitres of extract were hydrolysed with dilute H_2SO_4 , after which 1 mL of benzene and 1 mL of ammonia (NH_3) solution were added sequentially. Development of a rose-pink colouration in the ammoniacal layer was indicative of anthraquinone glycosides.

2.3.8. Test for Coumarins

A 10% NaOH solution was added to the plant extract. Appearance of a yellow colouration was taken as a positive indicator for coumarins.

2.3.9. Test for Carbohydrates

- **Molisch's Test:** Two drops of Molisch reagent (10% α -naphthol in ethanol) were added to 2 mL of the sample, followed by slow addition of 1 mL of concentrated H_2SO_4 along the wall of the tube. Formation of a purple/violet ring at the interface indicated the presence of carbohydrates.
- **Fehling's Test:** To 1 mL each of Fehling's solutions A and B, the sample was added and heated. Formation of a brick-red precipitate was indicative of reducing sugars.

2.3.10. Test for Betacyanins (Alkali Test)

Two millilitres of plant extract were combined with 2 N NaOH and heated at 100°C for 5 minutes. Formation of a yellow colouration was indicative of betacyanin pigments.

2.3.11. NMR Spectroscopic Analysis

Structural characterisation of isolated compounds from the *Pisonia alba* aqueous extract was performed using nuclear magnetic resonance (NMR) spectroscopy. The isolated fraction was dissolved in deuterated acetone (acetone-d_6). ^{13}C NMR spectra were acquired on a BRUKER spectrometer operating at 300.00 MHz at a controlled temperature of 300 K. ^1H NMR spectra were recorded at 400 MHz using CDCl_3 as the solvent at 298.5 K, with 64 transients accumulated to achieve adequate signal-to-noise ratio. Chemical shifts are reported in parts per million (δ , ppm) relative to the residual solvent signal as the internal reference.

2.3.12. Antibacterial Activity: Agar Well Diffusion Method

The antibacterial activity of leaf extracts from *Pisonia alba* was evaluated against two clinically significant bacterial strains — *Pseudomonas aeruginosa* (Gram-negative) and *Bacillus subtilis* (Gram-positive) — using the agar well diffusion method. Nutrient agar medium was prepared and sterilised by autoclaving at 121°C for 15 minutes and poured into sterile Petri dishes, which were then allowed to solidify under aseptic conditions. The bacterial strains were sub-cultured in nutrient broth and incubated at 37°C

for 24 hours to achieve log-phase growth. A standardised inoculum (approximately 1×10^5 CFU/mL) was prepared and uniformly spread on the solidified agar surface using a sterile cotton swab. Wells of 6 mm diameter were aseptically created in the agar using a sterile cork borer. Measured volumes of plant extract (10, 20, 30, and 40 μ L) were added to individual wells using a calibrated micropipette. Amoxicillin served as the positive control; DMSO served as the negative control. The plates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (ZOI) around each well in millimetres (mm) using a digital Vernier calliper. All assays were performed in triplicate to ensure reproducibility.

3. Results and Discussion

3.1. Phytochemical Screening

Qualitative phytochemical screening of the aqueous and ethanolic

leaf extracts of *Pisonia alba* was conducted to identify the major classes of secondary metabolites. The results are summarised in Table 1. Both extracts revealed the presence of alkaloids, phenolic compounds, saponins, tannins, flavonoids, terpenoids, and coumarins, while anthraquinones and betacyanins were absent in both fractions. The ethanolic extract showed a comparatively higher alkaloid content (++) , whereas the aqueous extract demonstrated a higher relative abundance of phenolic compounds (++) and terpenoids (++) .The presence of these bioactive secondary metabolites — particularly flavonoids, phenolics, and terpenoids — is widely recognised to confer significant antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties, thereby providing a plausible mechanistic basis for the pharmacological activities documented for this plant [22].



Figure 2: Qualitative Analysis of *Pisonia alba* leaves

S. No.	Phytochemical Constituent	Aqueous Extract	Ethanolic Extract
1	Alkaloids	++	++
2	Phenolic Compounds	++	++
3	Saponins	–	+
4	Tannins	+	++
5	Flavonoids	++	++
6	Terpenoids	+	++
7	Anthraquinones	–	–
8	Coumarins	++	+
9	Carbohydrates	+	+
10	Betacyanins	–	–

Table 1: Preliminary Phytochemical Screening of Aqueous and Ethanolic Leaf Extracts of *Pisonia alba*

++: Highly present; +: Moderately present; -: Absent

3.2. NMR Spectroscopic Analysis

Nuclear magnetic resonance spectroscopy was employed to

elucidate the structural features of the bioactive constituents present in the aqueous leaf extract of. The NMR *Pisonia alba* spectral data are presented in Tables 2 and 3.

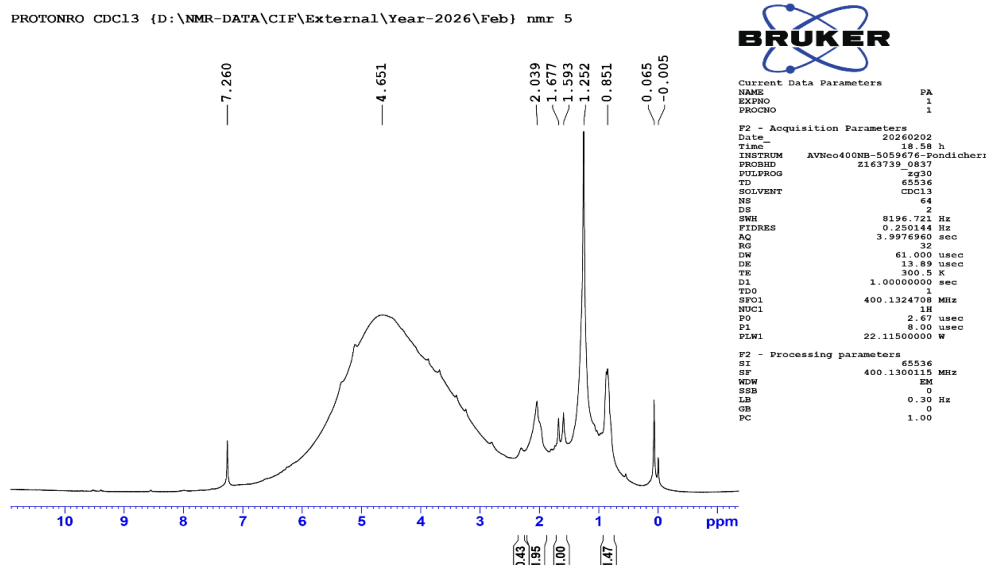


Figure 4: ¹H NMR Spectra of the *Pisonia alba* extract

The proton nuclear magnetic resonance (¹H NMR) spectrum of the *Pisonia alba* leaf extract was recorded using a 400 MHz Bruker NMR spectrometer with CDCl₃ as the solvent at 300.5 K. The spectrum provides valuable information regarding the hydrogen-containing functional groups present in the phytochemical constituents of the extract. A characteristic signal observed at δ 7.26 ppm corresponds to the residual proton of the deuterated chloroform (CDCl₃) solvent and is therefore not considered part of the plant extract. A resonance at δ 4.65 ppm indicates the presence of protons attached to oxygen-bearing carbons or exchangeable hydroxyl (–OH) protons, suggesting the occurrence of alcohols, glycosides, or other oxygenated phytochemicals. Signals appearing in the region of δ 2.04–1.59 ppm are attributed to methylene (–CH₂–) protons located adjacent to carbonyl groups or unsaturated carbon atoms, indicating the presence of fatty acids, terpenoids, and other aliphatic secondary metabolites. A prominent signal at

δ 1.25 ppm corresponds to methylene protons of long aliphatic hydrocarbon chains, while the resonance at δ 0.85 ppm is assigned to terminal methyl (–CH₃) groups, confirming the presence of long-chain lipid or hydrocarbon constituents.

Overall, the ¹H NMR spectrum demonstrates that the *Pisonia alba* leaf extract is rich in aliphatic and oxygenated organic compounds. The predominance of signals in the aliphatic region suggests the presence of fatty acids, terpenoids, sterols, and related metabolites, whereas the oxygenated proton signal supports the occurrence of alcohols, phenolic compounds, or glycosidic constituents. These classes of phytochemicals are widely recognized for their antibacterial and other biological activities. Therefore, the ¹H NMR analysis confirms the complex phytochemical composition of *Pisonia alba* leaf extract and supports its potential medicinal and pharmacological applications.

Chemical Shift (δ, ppm)	Proton Environment	Number of Protons
5.344	Olefinic (C=C–H)	2
2.800	α-Aminomethine (CH–NH ₂)	3
2.304	Methyl (CH ₃)	3
2.036	Acetyl methyl (CH ₃ CO)	6
1.675	Propargylic (C≡C–CH)	1
1.593	Methyl (CH ₃)	3
1.248	Methylene (CH ₂)	2
0.872	Terminal methyl (CH ₃)	6

Table 2: ¹H NMR Chemical Shift Data for the *Pisonia alba* Leaf Extract

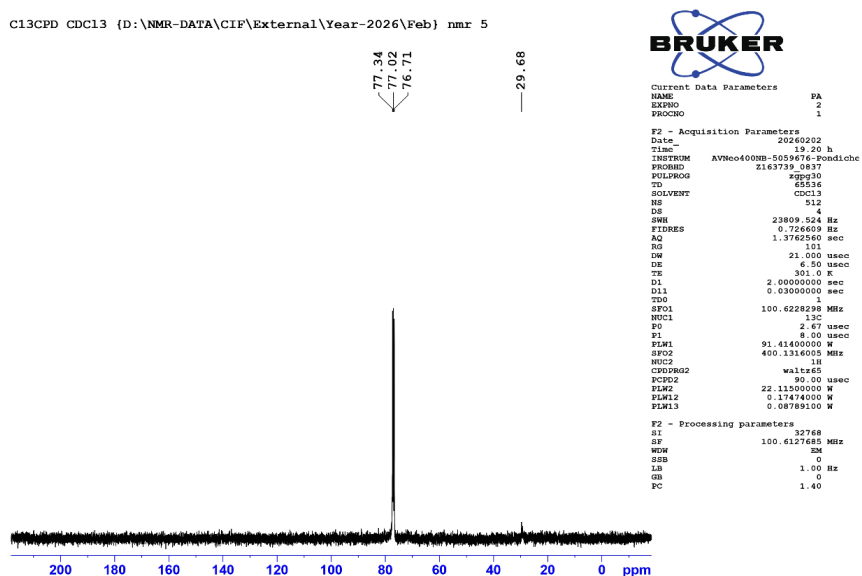


Figure 5: ^{13}C NMR Spectra of the Pisonia Alba Leaf Extract

Carbon Position	Chemical Shift (δ , ppm)
C1	76.71
C2	77.02
C3	77.34
C4	29.68

Table 3: ^{13}C NMR Chemical Shift Data for the Pisonia alba Leaf Extract

The ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectroscopy analysis was carried out to identify the carbon-containing phytochemical constituents present in the Pisonia alba leaf extract. The spectrum exhibited a distinct resonance signal at δ 29.68 ppm, indicating the presence of aliphatic carbon atoms, which are commonly associated with methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2-$) groups found in fatty acids, terpenoids, steroids, and other bioactive plant metabolites. The peaks observed at δ 76.71, 77.02, and 77.34 ppm correspond to the deuterated chloroform (CDCl_3) solvent and are not attributed to the plant extract. The predominance of the aliphatic carbon signal suggests that the extract contains saturated hydrocarbon chains and lipophilic phytochemicals. These compounds may contribute to the biological activities of Pisonia alba, including its antioxidant and antimicrobial properties. Thus, the ^{13}C NMR analysis supports the presence of bioactive organic constituents in the leaf extract and provides valuable structural information that complements other phytochemical characterization techniques used in the study.

3.3. Antibacterial Activity

The antibacterial efficacy of acetone, chloroform, and ethanolic extracts of Pisonia alba leaves was evaluated against two test organisms using the agar well diffusion method, and results were compared with amoxicillin as the positive control.

3.4. Activity Against Pseudomonas Aeruginosa

Among the three solvent extracts of Pisonia alba leaves tested against Pseudomonas aeruginosa, the acetone extract exhibited the highest antibacterial activity, producing zones of inhibition of 27.6 ± 1.2 mm and 30.8 ± 1.1 mm at concentrations of 30 μL and 40 μL , respectively, which were comparable to the positive control, amoxicillin (32.5 ± 1.0 mm). The ethanolic extract showed moderate antibacterial activity, with a maximum zone of inhibition of 27.2 ± 0.9 mm at 40 μL , while the chloroform extract demonstrated comparatively lower antibacterial efficacy. A concentration-dependent increase in antibacterial activity was observed for all solvent extracts, indicating that increasing the extract concentration enhanced bacterial growth inhibition. Similar dose-dependent antibacterial activity of medicinal plant extracts has been reported by Dahiya and Purkayastha [27]. The superior antibacterial activity of the acetone extract may be attributed to its greater efficiency in extracting bioactive phytochemicals such as flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids from Pisonia alba leaves. Differences in antibacterial efficacy among the solvent extracts are likely due to variations in solvent polarity, which influence the extraction and solubility of antimicrobial phytochemicals.



Figure 3: Plates Showing Antibacterial Activity of *Pisonia alba* Against *Pseudomonas Aureus*

3.5. Activity Against *Bacillus Subtilis*

Against *Bacillus subtilis*, the acetone extract again exhibited the strongest antibacterial activity, producing zones of inhibition of 28.1 ± 1.0 mm and 30.1 ± 0.8 mm at 30 μ L and 40 μ L, respectively, while amoxicillin produced a zone of inhibition of 31.6 ± 0.9 mm. The ethanolic extract was the second most effective, producing a maximum inhibition zone of 28.0 ± 0.8 mm at 40 μ L, whereas the chloroform extract showed comparatively lower antibacterial activity. The antibacterial activity increased with increasing extract concentration, demonstrating a clear dose-dependent response. Reported enhanced antibacterial activity with increasing concentrations of medicinal plant extracts.

Overall, *Pisonia alba* leaf extracts exhibited greater antibacterial activity against *Bacillus subtilis* than against *Pseudomonas aeruginosa*. This difference may be attributed to the structural characteristics of the bacterial cell wall. As a Gram-positive bacterium, *B. subtilis* lacks the outer lipopolysaccharide membrane present in Gram-negative bacteria such as *P. aeruginosa*, allowing phytochemical constituents to penetrate the bacterial cell more readily. The observed antibacterial activity is likely associated with the presence of flavonoids, phenolic compounds, tannins, alkaloids, and terpenoids, which are known to disrupt bacterial cell membranes, inhibit cell wall synthesis, interfere with nucleic acid replication, and ultimately suppress bacterial growth [29].



Figure 4: Plate showing antibacterial activity of *Pisonia alba* against *Bacillus Subtilis*

Extract / Control	10 μ L	20 μ L	30 μ L	40 μ L
Acetone (<i>P. aeruginosa</i>)	23.5	25.9	27.6 $\pm$ 1.2	30.8 $\pm$ 1.1
Chloroform (<i>P. aeruginosa</i>)	18.9	21.4	23.8	25.6
Ethanol (<i>P. aeruginosa</i>)	19.7	22.3	24.6	27.2
Acetone (<i>B. subtilis</i>)	24.1	26.3	28.1 $\pm$ 1.0	30.1 $\pm$ 0.8
Chloroform (<i>B. subtilis</i>)	18.5	20.9	23.7	25.4
Ethanol (<i>B. subtilis</i>)	20.8	—	25.9	28.0 $\pm$ 0.8
Amoxicillin (<i>P. aeruginosa</i>)	32.5 $\pm$ 1.0	32.5 $\pm$ 1.0	32.5 $\pm$ 1.0	32.5 $\pm$ 1.0
Amoxicillin (<i>B. subtilis</i>)	31.6	31.6	31.6	31.6

Values represent mean zone of inhibition (mm) $\pm$ standard deviation from triplicate experiments.

Table 4: Antibacterial Activity (Zone of Inhibition, mm) of *Pisonia alba* Leaf Extracts Against Test Bacteria

4. Conclusion

The present study provides a comprehensive in vitro evaluation of the phytochemical constituents and antibacterial activity of *Pisonia alba* leaf extracts, thereby providing scientific support for its traditional medicinal use. Qualitative phytochemical screening confirmed the presence of several biologically active secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and coumarins in the investigated extracts. Structural characterization using ^1H and ^{13}C NMR spectroscopy revealed characteristic aliphatic and olefinic proton signals, indicating the presence of terpenoid- and fatty acid-derived compounds.

Among the tested extracts, the acetone extract exhibited the strongest antibacterial activity against both *Pseudomonas aeruginosa* and *Bacillus subtilis*, producing zones of inhibition of 31.1 ± 1.2 mm and 29.8 ± 0.8 mm, respectively, at a concentration of 40 μL , which were comparable to the standard antibiotic amoxicillin. The ethanolic extract showed moderate antibacterial activity, whereas the chloroform extract exhibited comparatively lower inhibitory effects. The antibacterial activity of all extracts increased with increasing concentration, demonstrating a clear dose-dependent response.

Overall, the findings highlight the therapeutic potential of *Pisonia alba* leaves as a natural source of antioxidant and antibacterial compounds. The observed biological activities are likely associated with the synergistic action of flavonoids, phenolic compounds, tannins, terpenoids, and alkaloids, which are known to possess antioxidant, antimicrobial, and anti-inflammatory properties. Further studies involving bioassay-guided fractionation, isolation and characterization of active phytoconstituents, investigation of their molecular mechanisms, in vivo pharmacological evaluation, and toxicological assessment are recommended to facilitate the development of safe and effective plant-derived therapeutic agents.

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