VOLUME 12 ISSUE 1 2026

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 12, No. 1, 141-155 (2026)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Phytochemical and Pharmacological Studies of *Justicia betonica* against Human Pathogens

Muhammed Shariq K.[1], Farook M.A.[2]*, Zakirullah S.[2], Waheed D.[2] and Rohini J.[2]

[1]Department of Biochemistry, Islamiah College (Autonomous) (Affiliated to Thiruvalluvar University, Serkadu, Vellore 632115), Vaniyambadi 635 752, Tamil Nadu, India
[2]Department of Biotechnology, Islamiah College (Autonomous) (Affiliated to Thiruvalluvar University, Serkadu, Vellore 632115), Vaniyambadi 635 75, Tamil Nadu, India

**Corresponding Author*

Received: 14th July, 2025; **Accepted:** 22nd October, 2025; **Published online:** 24th January, 2026

https://doi.org/10.33745/ijzi.2026.v12i01.013

Abstract: *Justicia betonica* L., commonly known as Hill Justicia or White Shrimp Plant, is a medicinal shrub from the Acanthaceae family, traditionally used across Africa and the Indian subcontinent for its therapeutic properties. This study investigated the phytochemical profile, fluorescence characteristics, anti-oxidant potential, antimicrobial, and anthelmintic activities of various solvent extracts (aqueous, ethanol, acetone, and hexane) of *J. betonica* leaves. Fluorescence analysis under visible and UV light revealed distinct color variations upon treatment with different reagents, aiding in pharmacognostical evaluation. Phytochemical screening showed the presence of important bioactive compounds such as alkaloids, saponins, flavonoids, terpenoids, tannins, and glycosides, with variation across the solvents. Antioxidant activity, assessed by DPPH radical scavenging assay, was highest in ethanol extract, followed by aqueous, acetone, and hexane extracts. Antibacterial studies using the disc diffusion method revealed moderate activity of all extracts against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), while Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*) remained resistant. *Pheretima posthuma* (adult earthworms), due to their anatomical and physiological similarity to human intestinal parasitic helminthes, were selected as the experimental model for anti-helminthic activity. Additionally, ethanol extract demonstrated significant anti-helminthic activity against *Pheretima posthuma*, with a concentration-dependent effect comparable to the standard drug Albendazole. These findings affirm the ethnomedicinal value of *J. betonica* and highlight its potential as a source of natural bioactive compounds for pharmaceutical applications.

Keywords: *Justicia betonica*, Fluorescence, Phytochemical, Antioxidant, Antibacterial, Anti-helminthic, Albendazole, DPPH radical scavenging assay

Citation: Muhammed Shariq K., Farook M.A., Zakirullah S., Waheed D. and Rohini J.: Phytochemical and pharmacological studies of *Justicia betonica* against human pathogens. Intern. J. Zool. Invest. 12(1): 141-155, 2026.
https://doi.org/10.33745/ijzi.2026.v12i01.013

Introduction

Justicia betonica L., commonly known as Hill Justicia, Squirrel's Tail, White Shrimp Plant, or Paper Plume, is a flowering shrub belonging to the family Acanthaceae. Renowned for its ornamental

appeal, the plant features striking variegated bracts and delicate light purple flowers, making it a prominent addition to gardens and landscapes. It is native to the eastern and southern regions of Africa including Kenya, Mozambique, Lesotho, Namibia, South Africa, and Eswatini as well as the Indian subcontinent, specifically India and Sri Lanka (Gamble, 1956; Hooker, 1992). Beyond its aesthetic value, *J. betonica* holds significant medicinal importance and is widely used in traditional and folklore medicine systems. The plant exhibits a broad spectrum of pharmacological activities, including analgesic, anti-inflammatory, anti-malarial, and antimicrobial properties. It has been tested against various pathogenic bacteria, as listed in Table 1. Notably, Pascaline Jeruto *et al*. (2008) reported the plant's wound-healing and ulcer-curing potential. In traditional Indian and Sri Lankan medicine, poultices made from crushed leaves are applied to abscesses to alleviate pain and swelling. The aerial parts of the plant are used in the treatment of diarrhoea, inflammation, and localized swelling (Stangel *et al*., 2011). It is also employed in managing a variety of ailments such as orchitis, paralysis, headache, bruises, high cholesterol, gastric discomfort, urinary issues, and cough. Known locally in India as "Had Paata," all parts of the plant-- roots, leaves, and flowers are valued for their therapeutic benefits. Traditional healers in India and Sri Lanka utilize *J. betonica* in different forms: crushed leaf poultices for boils and abscesses, and inflorescences taken orally for treating vomiting and constipation (Stangel *et al*., 2011; Geone and Antônio, 2012; Godfrey *et al*., 2013). Additionally, the plant has demonstrated anti-*Plasmodium falciparum* activity, confirming its ethnobotanical use in treating malaria (Gangabhavani and Ravishankar, 2013).The present study aims to evaluate the phytochemical composition and antimicrobial activity of *Justicia betonica* against a range of human pathogenic bacteria.

Materials and Methods

Collection and preparation of J. betonica:

The plant *Justicia betonica* was collected from a rural area near Alangayam and taxonomically identified and authenticated by Dr. N. P. M. Mohamed Tariq, Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi, India. Following authentication, the collected plant material (Fig. 1) was processed for extraction. The samples were thoroughly washed with distilled water to eliminate surface contaminants and air-dried in the shade at ambient room temperature. Once dried, the plant material was ground into a coarse powder using a mechanical grinder. For extraction, 10 g of the powdered plant material were macerated in 100 ml of different solvents acetone, ethanol, hexane, and distilled water. The mixtures were intermittently stirred on an orbital shaker, following the procedure described by Kumar *et al*. (2016). After 48 h, the extracts were filtered, and the respective solvents were allowed to evaporate at room temperature for 18–24 h. The resulting crude extracts were collected as solid residues and stored at 4°C for further analysis (Fig. 1).

Fluorescence Analysis Test

A small amount of finely powdered *J. betonica* leaf sample was placed on a clean, grease-free microscopic glass slide. To this, 1–2 drops of freshly prepared chemical reagent were added and gently mixed by tilting the slide. The mixture was allowed to react for 1–2 min at room temperature. Subsequently, the slide was placed inside a UV viewing chamber and examined under both daylight and ultraviolet (UV) light at different wavelengths. The characteristic color changes produced by the interaction of the sample with the reagents under various lighting conditions were observed and recorded, following the methodologies described by Chang *et al*. (2002) and Mukherjee (2002).

Phytochemical Analysis

The plant extracts of *J. betonica* obtained using aqueous, ethanol, acetone, and hexane solvents were subjected to preliminary phytochemical analysis to detect the presence of various bioactive

Table 1: Natural History and Medicinal value of *Justicia betonica*

Desirable Plant Features	Ornamental Flowers
Light Preference	Full Sun
Water Preference	Moderate Water
Mature Foliage Colour	Green
Size	*Justicia betonica* L. is a diffusely branched more or less under shrub or an erect shrub up to 2-2.5m height with terete and glabrous branches
Stem	The stem is striate and cylindrical with swollen nodes
Flower Colour	Purple
Flower	In terminal or subterminal spikes, sessile; white with purple lines outside and throat of deeper colour. Flowering throughout the year.
Fruit	A pubescent capsule contain four seeds; seeds spinulose. Fruiting throughout the year.
Seeds	Small, ovoid , slightly compressed, glabrous suborbicular, densely rugose, spinulose when wetted
Root	Root is taproot with numerous slender, curved rootlets , externally it is light brown in colour and inside is creamish white
Field tips	Bracts large, nerves conspicuously green.
Leaf Arrangement	Opposite-decussate
Leaf Type	Simple
Leaf Shape	Ovate-lanceolate, to obovate
Leaf Apex	Acuminate
Leaf Base	Acute-attenuate
Leaf Margin	Entire-crenate
Calyx and corolla	The corolla is gamopetalous, bilabiate, consisting of 5 petals, segments linear-lanceolate, pubescent
Corolla	1.25 cm long, pubescent outside, dull white, spotted with pink. 2-lipped; upper lip erect emarginated; lower lip deflexed
Stamens - 4	Filaments hairy at their base. The anther lobes are dorsifixed, separated by a broad connective
Ovary	2-celled, and each cell with 2 ovules
Style	Pubescent in nature
Medicinal Uses (Subbaraju *et al.* (2004), Katuura *et al.* (2007), Pascaline Jeruto *et al.* (2008), Stangel *et al.* (2011), Godfrey *et al.* (2013), Gangabhavani and Ravishankar (2013), Adia *et al.* (2014), Khan *et al.* (2017), Jollykutty Eapen *et al.* (2019) and Geone and Antônio (2023)	
✓ Analgesic	✓ Anti-Inflammatory
✓ Antimalarial	✓ Febrifuge
✓ Constipation	✓ Malaria
✓ Orchitis	✓ Pain
✓ Snake bite	✓ Stomach ache
✓ Swelling	✓ Vomiting
✓ Diarrhoea	✓ Scaly skin
✓ Asthma	✓ Dysentery
✓ Cough	✓ Leucorrhea
✓ Jaundice	✓ Paralysis

compounds. The screening was carried out to identify classes of phytoconstituents such as terpenoids, steroids, flavonoids, alkaloids, saponins, tannins, phlobatannins, phenols, and anthraquinones, following the standard procedures described by Harborne (1973).

Detection of Alkaloids

Wagner's Test:

To 1 ml of the plant extract, 1 ml of Wagner's reagent was added. The formation of a reddish-brown precipitate indicated the presence of

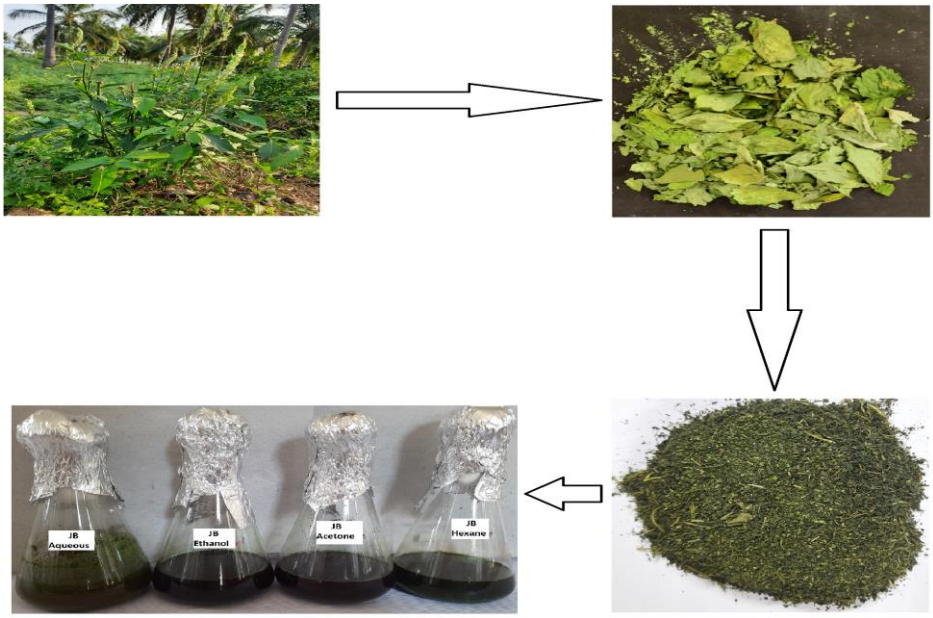

Fig. 1: Collection, preparation and extraction of *J. betonica.*

alkaloids.

Detection of Amino Acids

Xanthoprotein Test:

To 1 ml of the extract, 1 ml of concentrated nitric acid was added, resulting in the formation of a white precipitate. The mixture was then heated for 2–3 min, cooled, and followed by the addition of 1 ml of 20% sodium hydroxide. A yellow coloration indicated the presence of aromatic amino acids.

Detection of Carbohydrates

Benedict's test:

To 1 ml of the extract, 1 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2–3 min. The formation of a green, yellow, or red precipitate indicated the presence of reducing sugars.

Detection of Phenols

Ferric Chloride Test:

To 1 ml of the extract, 1 ml of 5% ferric chloride solution was added. The development of a dark green or bluish-black coloration confirmed the presence of phenolic compounds.

Detection of Flavonoids

Alkaline Reagent Test:

To 1 ml of the extract, 1 ml of 10% sodium hydroxide solution was added. The appearance of a yellow color, which turned colorless upon addition of dilute acid, indicated the presence of flavonoids.

Detection of Tannins

Ferric Chloride Test:

To 2 ml of the extract, 2 ml of 5% ferric chloride solution was added. A blue-black or greenish-black coloration indicated the presence of tannins.

Detection of Saponins

Foam Test:

To 2 ml of the extract, 2 ml of distilled water was added. The mixture was vigorously shaken, and the formation of stable, persistent foam indicated the presence of saponins.

Detection of Terpenoids

Salkowski's Test:

To 1 ml of the extract, 2 ml of chloroform was

added, followed by the careful addition of 3 ml of concentrated sulfuric acid. The development of a reddish-brown interface indicated the presence of terpenoids.

Detection of Phlobatannins

Hydrochloric Acid Test:

To 2 ml of the extract, 2 ml of 1% hydrochloric acid was added. The mixture was boiled in a water bath. A red precipitate confirmed the presence of phlobatannins.

Detection of Quinones

Hydrochloric Acid Test:

To 1 ml of the extract, 1 ml of concentrated hydrochloric acid was added. The appearance of a yellow or red color indicated the presence of quinones.

Detection of Coumarins

Sodium Hydroxide Test:

To 1 ml of the extract, 1 ml of 10% sodium hydroxide was added. The development of a yellow color indicated the presence of coumarins.

Detection of Oxalates

Glacial Acetic Acid Test:

To 3 ml of the extract, 1 ml of glacial acetic acid was added. The appearance of a white precipitate indicated the presence of oxalates.

Detection of Glycosides

Keller-Killiani Test:

To 2 ml of the extract, 2 ml of glacial acetic acid was added along with a few drops of 5% ferric chloride solution. Subsequently, concentrated sulfuric acid was carefully added along the sides of the test tube. The formation of a reddish-brown ring at the interface indicated the presence of cardiac glycosides.

Antioxidant Activity

The antioxidant activity of *J. betonica* solvent extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. A 0.135 mM DPPH solution was freshly prepared by dissolving 4 mg of DPPH in 100 ml of methanol. Various concentrations (12.5, 25, 50, 100, and 200 µg/ml) of *J. betonica* extracts prepared in water, ethanol, acetone, and hexane were used for the assay. For each concentration, 2 ml of the plant extract was mixed with 2 ml of the DPPH solution. A control was prepared by mixing 2 ml of DPPH solution with 2 ml of methanol without any plant extract. All samples were incubated in the dark at room temperature for 30 min. Following incubation, the absorbance of each solution was measured at 517 nm using a UV-Visible spectrophotometer. Methanol served as the blank for calibration. The percentage of DPPH radical scavenging activity (antioxidant activity) was calculated using the following formula:

$$\text{Initial Percentage} = \frac{\text{Control O.D.} - \text{Sample O.D.}}{\text{Control O.D.}} \times 100$$

Antibacterial Activity

The antibacterial activity of various solvent extracts of *J. betonica* was assessed using the disc diffusion method, following the procedure described by Sasikumar *et al*. (2007). Four bacterial strains, including two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*), were employed for the assay. All bacterial strains were cultured overnight in nutrient broth at 37 °C. Sterile Mueller-Hinton Agar (MHA) plates (HiMedia) were prepared, and 0.2 ml of the bacterial suspension was uniformly spread on each plate using a sterile L-shaped glass rod to ensure even distribution. Plant extracts were prepared at concentrations of 25 µg/ml, 50 µg/ml, and 100 mg/ml in dimethyl sulfoxide (DMSO). Sterile 6 mm discs were prepared from Whatman No.1 filter paper and autoclaved before use. Each MHA plate contained five discs: one impregnated with streptomycin (positive control), one with DMSO alone (negative control), and three with different concentrations (25 µg/ml, 50 µg/ml, and 100 mg/ml) of the same *J. betonica* extract. The discs were carefully placed on the surface of

the inoculated agar plates, and the plates were incubated at 37 °C for 24 h. Zones of inhibition were measured to evaluate the antibacterial efficacy of the extracts.

Anti-Helmintic Activity

The anthelmintic activity of different solvent extracts of *J. betonica* was evaluated following the method described by Ghosh and Bhattacharya (2015). *Pheretima posthuma* (adult earthworms) due to their anatomical and physiological similarity to human intestinal parasitic helminthes (Choudhary *et al.*, 2021; Manke *et al*., 2024), were selected as the experimental model. Few investigators have also used earthworms for testing anthelmintic activity of plant extracts (Shelke *et al*., 2020; Choudhary *et al.*, 2021; Mathias *et al.*, 2021; Manke *et al*., 2024; Saleh *et al.*, 2024; Bazán *et al.*, 2025; Sahu *et al.*, 2025; Kamsala *et al*., 2025; Janani *et al.*, 2025; Vinciya *et al.*, 2025).

Each extract was prepared at two concentrations (50 mg/ml and 100 mg/ml) using normal saline as the solvent. For the assay, individual earthworms (3–5 cm in length and 2–4 mm in width) were placed in separate Petri dishes containing 10 ml of the respective extract solution. Albendazole (50 mg/ml) served as the positive control, while normal saline alone was used as the negative control. The Petri dishes were observed at regular intervals, and the time taken for paralysis (defined as complete loss of movement, even upon vigorous shaking) and death (confirmed by transfer to a water bath maintained at 45 °C) was recorded for each worm. The anthelmintic efficacy of the extracts was compared based on these observations.

Results and Discussion

Fluorescence Analysis

The ultraviolet light produces fluorescence in several natural products, which do not noticeably fluoresce in daylight. If the substances themselves are not fluorescent, they may often be rehabilitated into fluorescent derivatives or decomposition products by applying diverse reagents. Therefore, some crude drugs are often assessed qualitatively in this technique and it is an imperative parameter of pharmacological evaluation. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluations. The fluorescence analysis of leaf powder of *J. betonica* was carried out after treating with several solvents. Fluorescence efficiency was observed using day light and UV light were carried out and represented with colour changes. The observations in the Table 2 and Figs. 2, 3 showed the variation in colour as observed by Gupta *et al.* (2006) and Malathi *et al*. (2018).

Phytochemical Screening

The phytochemical profiling of *J. betonica* was conducted to evaluate the presence of various bioactive compounds in different solvent extracts (aqueous, ethanol, acetone, and hexane). A total of 13 phytochemical groups were qualitatively tested using standardized protocols as described by Harborne (1973). The aim was to identify key secondary metabolites responsible for the plant's potential pharmacological activities. The phytochemical screening revealed the presence of diverse secondary metabolites, many of which play crucial roles in plant defense and possess significant therapeutic value (Usman and Osuji, 2017). These include alkaloids, terpenes, flavonoids, saponins, sterols, and others known for their antimicrobial, antioxidant, anti-inflammatory, and other medicinal properties. Results showed that carbohydrates were consistently present in all four solvent extracts. Saponins and glycosides were detected in the

Table 2: Fluorescence analysis of leaf powder of *J. betonica* in various reagents

Reagents	Colour under day light	Observation under UV
Powder + d. H_2O	Dark Green	Dark Green
Powder + 1 M H_2SO_4	Pale greenish yellow	Violet
Powder + 10 % $CuSO_4$	Green	Purple
Powder + 1 M HCL	Brown	Violet
Powder + Diluted HNO_3	Pale greenish yellow	Violet
Powder + 10 % NaOH	Fluorescent Green	Green
Powder + 1% Glacial Acetic Acid	Green	Pale Green
Powder + Concentrated HNO_3	Pale yellow	Brown
Powder + Conc. HNO_3 + Dil. HNO_3	Fluorescent yellow	Pale Brown
Powder + 1% Iodine	Pale Yellowish green	Purple
Powder + Ethanol	Green	Green

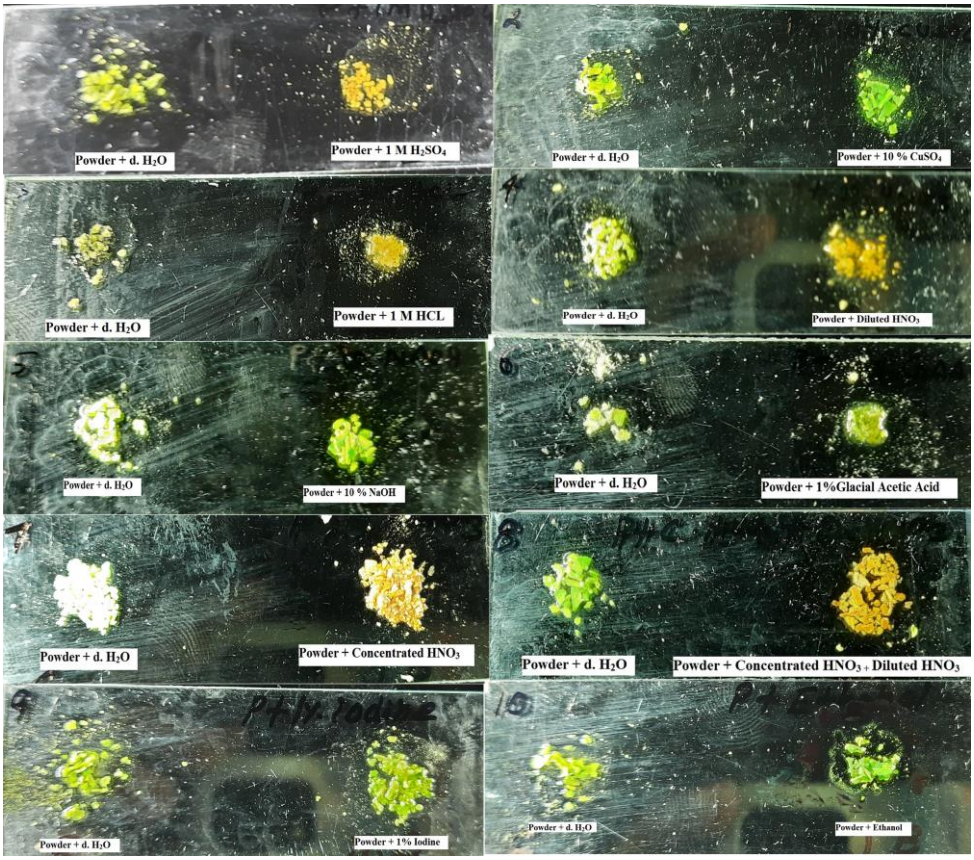

Fig. 2: Fluorescence analysis of leaf powder of *J. betonica* under day light.

aqueous, ethanol, and hexane extracts, while oxalates were present in ethanol, acetone, and hexane, but absent in the aqueous extract. Flavonoids and amino acids were identified in ethanol and acetone extracts, but not in aqueous or hexane. Alkaloids were observed in the aqueous and hexane extracts but were absent in ethanol and acetone. Phenolic compounds were found exclusively in the acetone extract, while terpenoids appeared only in the aqueous extract. Tannins were detected solely in the acetone extract, and quinones were observed only in the hexane extract. Notably, phlobatannins and coumarins were absent in all tested solvent extracts. These findings are summarized in Table 3 and visually represented in Figure 4. The

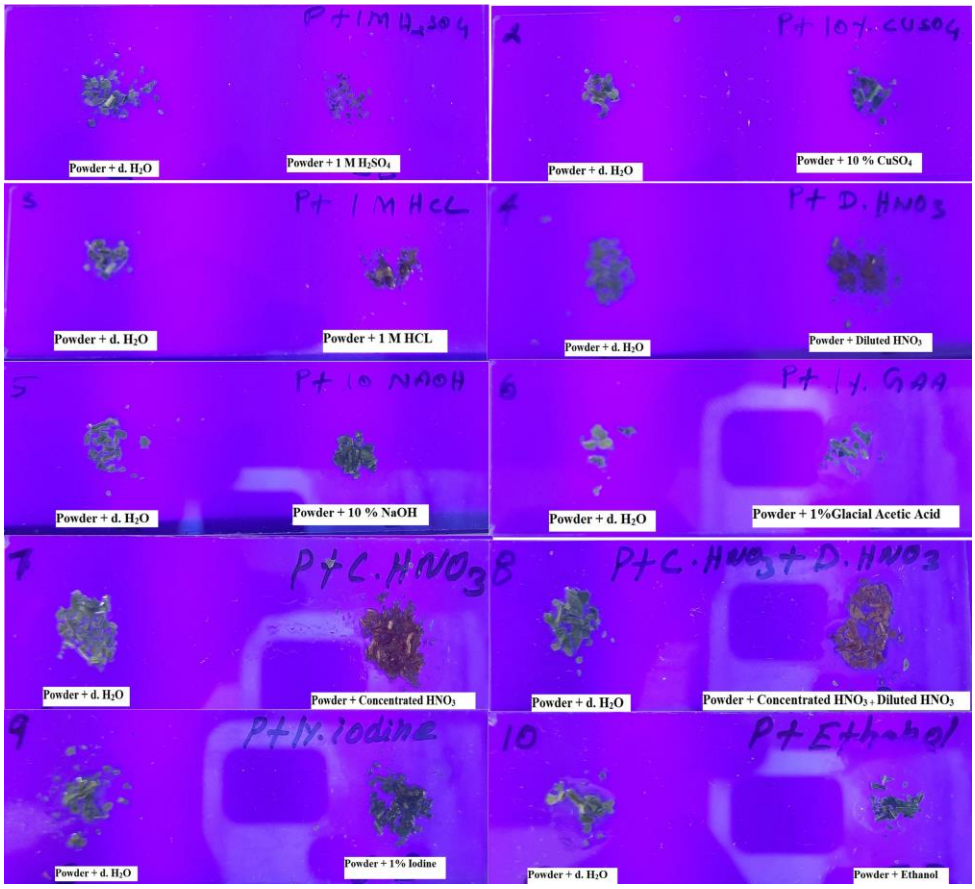

Fig. 3: Fluorescence analysis of leaf powder of *J. betonica* under UV light.

Table 3: The phytochemical analysis various organic extract of *J. betonica*

S. No.	Phytochemical Tests		Aqueous	Ethanol	Acetone	Hexane
1.	Alkaloids	**Wagners test**	+	-	-	+
2.	Amino Acid	**Xanthoprotein test**	-	+	+	-
3.	Carbohydrate	**Benedicts test.**	+	+	+	+
4.	Phenol	**$FeCl_3$ test.**	-	-	+	-
5.	Flavanoids	**Alkaline reagent test**	-	+	+	-
6.	Tannins	**$FeCl_3$ test.**	-	+	+	-
7.	Saponin	**Foam test.**	+	+	+	-
8.	Terpenoids	**Salkowskis test.**	+	-	-	-
9.	Phlobatanins	**1% HCL test**	-	-	-	-
10.	Quinones	**Hydrochloric Acid test**	-	-	-	+
11.	Coumarin	**Sodium hydroxide test**	-	-	-	-
12.	Oxalate	**Glacial acetic acid test**	-	+	+	+
13.	Glycoside	**Killarkillanis test**	+	+	+	-

qualitative phytochemical analysis confirms the presence of several pharmacologically relevant compounds such as terpenoids, alkaloids, and saponins, which is consistent with the findings reported by Godfrey *et al*. (2013).

Antioxidant Activity

The antioxidant potential of *J. betonica* extracts prepared using aqueous, ethanol, acetone, and hexane solvents was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The antioxidant activity of each extract was compared with that of the standard

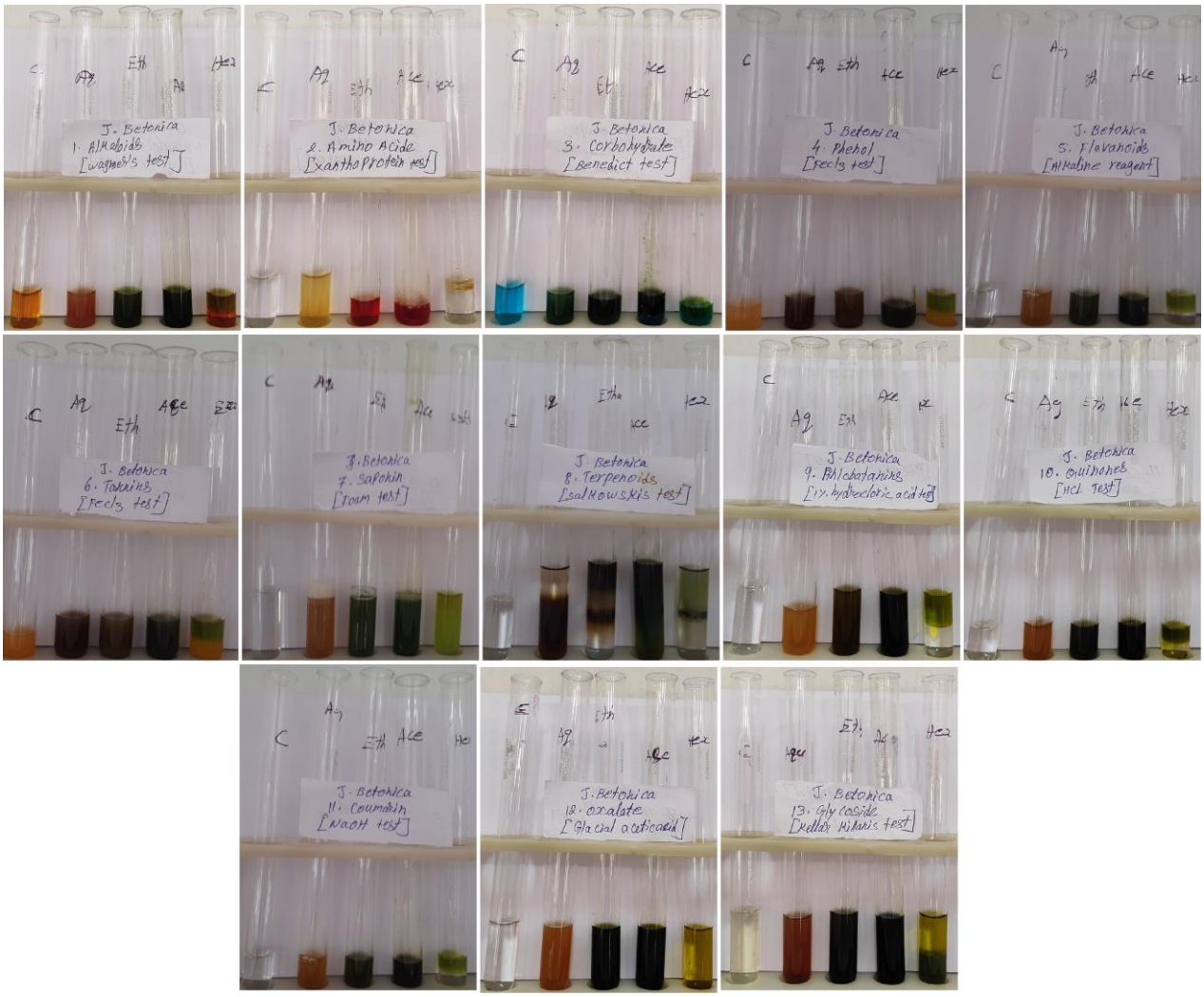

Fig. 4: The phytochemical analysis various organic extract of *J. betonica.*

antioxidant, ascorbic acid (Fig. 5). The DPPH assay is widely used to assess the free radical scavenging ability of biological substances, reflecting their overall antioxidant capacity. Recent studies have emphasized the role of free radicals and reactive oxygen species in contributing to oxidative stress, which is a known factor in the development of various diseases and disorders. This has driven growing interest in the identification of natural antioxidants derived from medicinal plants. The DPPH assay is not only a marker of antioxidant potential but also provides insight into the cell membrane-stabilizing ability of plant constituents, offering possible mechanisms through which phytomedicines may mitigate conditions related to oxidative stress, inflammation, and infection (Shahidi and Wanasundara, 1992). Among the tested extracts, the ethanol extract exhibited the highest antioxidant activity, followed by the aqueous, acetone, and hexane extracts. The absorbance (O.D.) values recorded for each concentration were used to calculate the percentage of inhibition and the standard error (SE). These results are presented in Figure 5, illustrating the comparative scavenging efficiency of the different extracts of *J. betonica*.

Antibacterial Activity

The antibacterial potential of *J. betonica* leaf extracts obtained using aqueous, ethanol, acetone, and hexane solvents was evaluated using the Disc Diffusion Method as described by Mostafa *et al.* (2018). The assay was conducted against four bacterial strains two Gram-positive (*B. subtilis* and

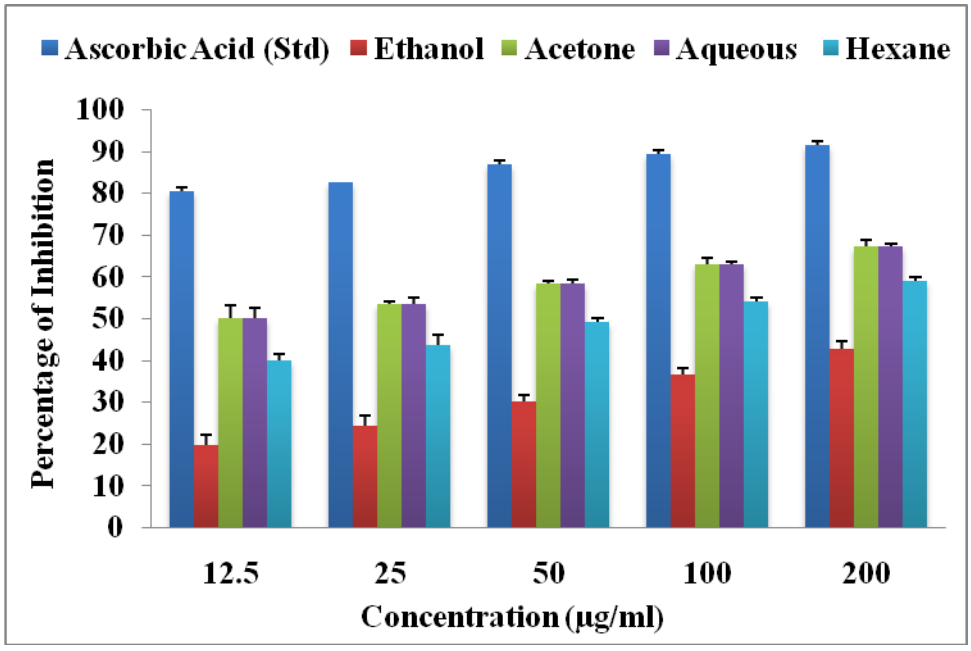

Fig. 5: DPPH radical scavenging activity of *J. betonica* leaves extracts.

S. aureus) and two Gram-negative (*E. coli* and *P. aeruginosa*). All bacterial cultures were incubated overnight at 37 °C in nutrient broth. Sterile Mueller-Hinton Agar (MHA) plates (HiMedia) were prepared, and 100 µl of each bacterial suspension was evenly spread across the agar surface using a sterile L-shaped spreader. Filter paper discs (6 mm diameter) were cut from Whatman No. 1 filter paper, sterilized by autoclaving, and impregnated with 10 µL of the respective extract concentrations (25 µg/ml, 50 µg/ml, and 100 µg/ml), prepared using dimethyl sulfoxide (DMSO) as the solvent. Each plate included five discs: one disc loaded with 10 µl of streptomycin (10 µg/ml) as the positive control, one with 10 µl of DMSO as the negative control, and three discs containing the varying concentrations of *J. betonica* extract. The plates were incubated at 37 °C for 24 h, after which zones of inhibition were measured to determine antibacterial efficacy.The results revealed that all solvent extracts exhibited moderate antibacterial activity against the Gram-positive bacteria (*B. subtilis* and *S. aureus*) at all tested concentrations. Notably, no inhibitory effect was observed against the Gram-negative bacteria (*P. aeruginosa* and *E. coli*), with the exception of *E. coli*, which showed a slight inhibition zone at 100 µg/ml of the aqueous extract. These findings are consistent with the observations of Sasikumar *et al*. (2007) and Abubakar *et al*. (2020), indicating that *J. betonica* demonstrates selective antibacterial activity, particularly against Gram-positive bacterial strains. The detailed results are summarized in Table 4 and illustrated in Figure 6.

Anti-Helminthic Activity

The anthelmintic activity of various solvent extracts of *J. betonica* was evaluated *in vitro* using the Indian earthworm *Pheretima posthuma* as a model organism. *P. posthuma*, along with *Eisenia fetida*, is widely employed in anthelmintic assays due to its physiological and anatomical similarity to gastrointestinal helminths that affect humans and livestock (Kushwaha *et al*., 2011; Adate *et al*., 2012; Jayaramu and Prathibha, 2016; Shelke *et al*., 2020; Choudhary *et al.*, 2021; Mathias *et al.*, 2021; Manke *et al*., 2024; Saleh *et al.*, 2024; Bazán *et al.*, 2025; Sahu *et al.*, 2025; Kamsala *et al*., 2025; Janani *et al.*, 2025; Vinciya *et al.*, 2025). Among the tested extracts, the ethanol extract demonstrated the most significant anthelmintic activity. At a

Table 4: Antibacterial activity of *J. betonica* against various bacterial pathogens

Bacteria	Solvents	Zone of Inhibition in mm				
		Negative Control (DMSO)	Positive Control (10 µg)	25 µg	50 µg	100 µg
B. subtilis	Aqueous	-	15	0	0	0
	Ethanol	-	14	0	0	0
	Acetone	-	13	4	5	4
	Hexane	-	13	5	5	8
S. aureus	Aqueous	-	6	5	6	4
	Ethanol	-	7	0	0	0
	Acetone	-	7	3	3	3
	Hexane	-	7	5	3	3
E. coli	Aqueous	-	6	0	0	5
	Ethanol	-	6	0	0	0
	Acetone	-	5	0	0	0
	Hexane	-	6	0	0	0
P. aeruginosa	Aqueous	-	6	0	0	0
	Ethanol	-	5	0	0	0
	Acetone	-	8	0	0	0
	Hexane	-	0	0	0	0

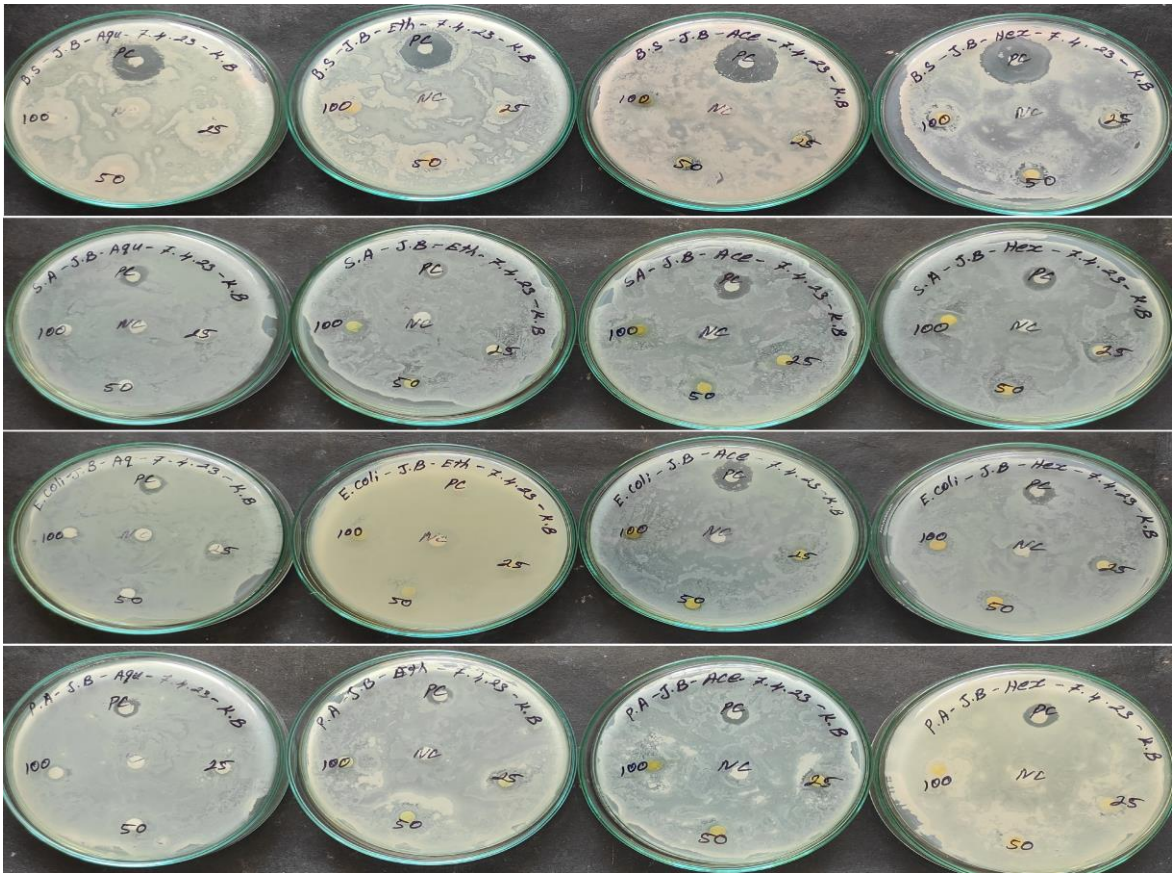

Fig. 6: Antibacterial activity of *J. betonica* against various bacterial pathogens.

Table 5: Anthelmintic activity of leaf extracts of *J. betonica*

Group	Treatment	Concentration used (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	NC - Normal Saline	-	-	-
2	PC - Albendazole	50	8.00± 0.58	13.33± 0.33
2	PC - Albendazole	100	5.33± 0.33	8.33± 0.33
3	Aqueous	50	609.67± 1.45	640.67± 1.85
4		100	517.00± 6.02	561.00± 8.08
5	Ethanol	50	115.33 ± 3.17	127.33 ± 2.60
6		100	78.00 ± 1.73	88.67 ± 1.20
7	Acetone	50	713.67± 3.28	773.67± 3.28
8		100	673.00± 3.51	708.33± 5.92
9	Hexane	50	433.00± 6.08	459.33± 2.96
10		100	394.00± 7.02	427.00± 8.02

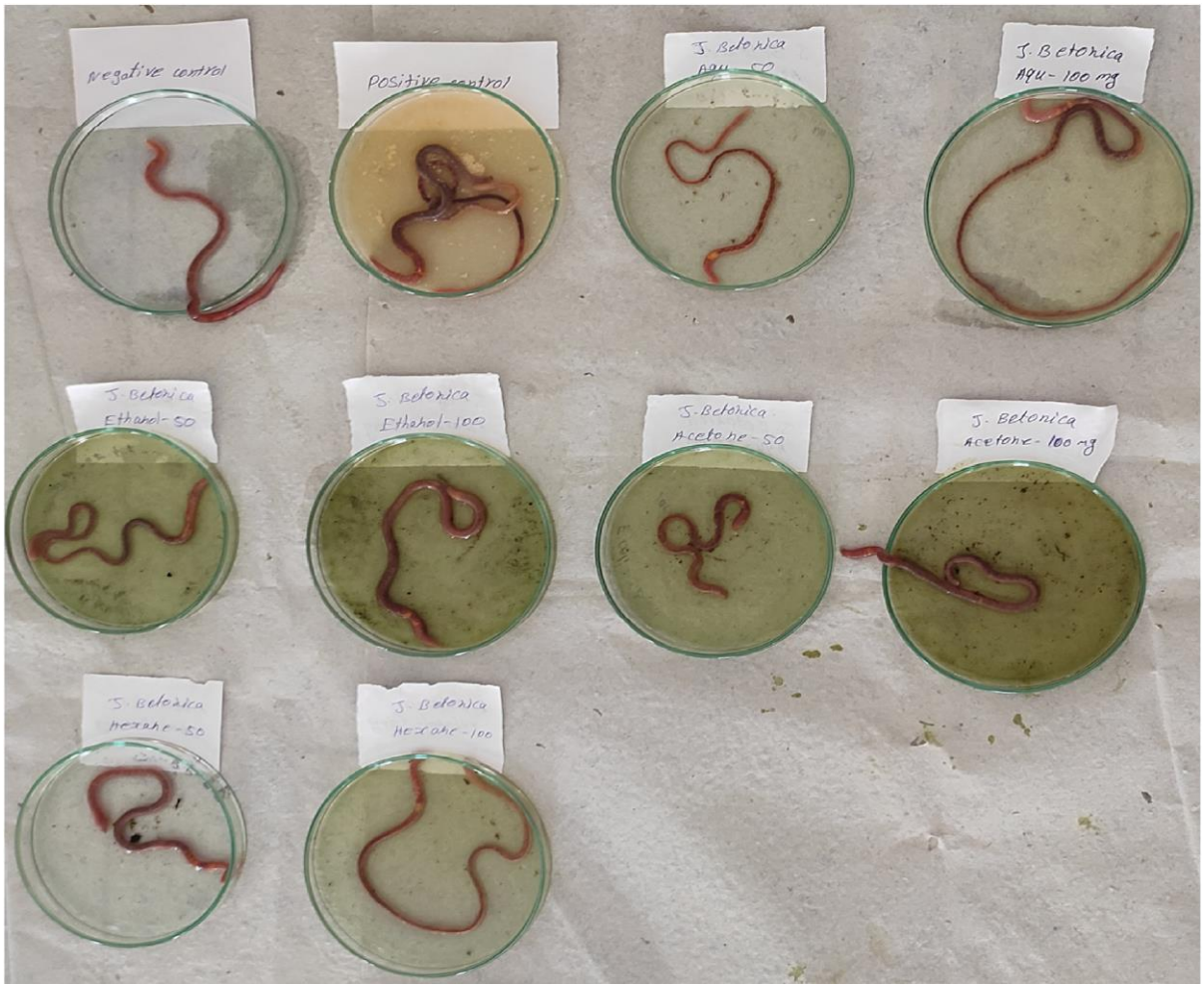

Fig. 7: Anthelmintic activity of leaf extracts of *J. betonica.*

concentration of 50 mg/ml, the ethanol extract caused paralysis of the worm at 59 min, while increasing the concentration to 100 mg/ml reduced the paralysis time to 10 minutes, indicating a dose-dependent response (Table 5, Fig. 7). In comparison, the standard drug Albendazole (100 mg/ml) induced paralysis and death in *P. posthuma* at 8.33 min and *5.33* min, respectively.These findings are in line with previous studies where methanolic extracts of

Euphorbia helioscopia and *Euphorbia heterophylla* demonstrated superior anthelmintic efficacy against *P. posthuma* compared to other solvent extracts (Majeed *et al*., 2011; Lone *et al*., 2013; Prathibha and Jayaramu, 2019).

Conclusion

The present study highlights the significant ethno pharmacological potential of *Justicia betonica* L., a traditionally valued medicinal plant widely used in African and South Asian regions. The fluorescence analysis confirmed the presence of diverse chemical constituents in the leaf powder, aiding its pharmacognostic identification. Phytochemical screening of aqueous, ethanol, acetone, and hexane extracts revealed a broad range of bioactive secondary metabolites, including alkaloids, flavonoids, terpenoids, saponins, and glycosides, each known for their therapeutic significance. The antioxidant activity, measured through DPPH radical scavenging assay, was most potent in the ethanol extract, reinforcing its potential role in combating oxidative stress-related disorders. Antimicrobial assays demonstrated moderate activity of the extracts particularly against Gram-positive bacteria (*B. subtilis* and *S. aureus*) while showing limited or no effect on Gram-negative strains (*E. coli* and *P. aeruginosa*). Furthermore, the ethanol extract exhibited notable anthelmintic activity against *Pheretima posthuma*, supporting its traditional use against parasitic infections. These findings collectively validate the traditional applications of *J. betonica* and suggest that its bioactive constituents warrant further exploration for potential development into natural therapeutic agents. Future studies focusing on isolation, structural elucidation, and *in vivo* testing of the active compounds may provide deeper insights into its pharmacological efficacy and safety.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Biotechnology, Islamiah College (Autonomous) (Affiliated to Thiruvalluvar University, Serkadu, Vellore), Vaniyambadi, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors extend sincere thanks to the Management and the Principal, Islamiah College (Autonomous), Vaniyambadi for providing the facilities to carry out this research work.

Conflict of Interest

The authors declare no conflicts of interest.

References

Abubakar MA, Etonihu AC, Kigbu PE, Owuna JE and Audu SI. (2020) Phytochemical and antimicrobial analyses of leaf extracts of *Cerathoteca sesamoides* and *Chromolaena odorata*. Int J Res Granthaalayah 8(8): 65-74.

Adate PS, Parmesawaran S and Yamani C. (2012) In vitro anthelmintic activity of stem extracts of *Piper betle* Linn. against *Pheritima posthuma.* Pharmacogn J. 4: 61-69.

Adia MM, Anywar G, Byamukama R, Kamatenesi-Mugisha M, Sekagya Y, Kakudidi EK and Kiremire BT. (2014) Medicinal plants used in malaria treatment by Prometra herbalists in Uganda. J Ethnopharmacol. 155(1): 580-588.

Bazán D, Alvarenga N, Gayoso E, Burgos-Edwards A and Rojas GD. (2025) Chemical composition, *in vitro* anthelmintic activity against *Eisenia fetida*, and *in silico* analysis of methanolic extracts from three *Campomanesia* species of Paraguayan flora. J Appl Biol Biotech. 13(4):146-156.

Chang CC, Yang MH, Wen HM and Chern JC. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 10(3): 178-182.

Choudhary N, Khatik GL, Choudhary S, Singh G and Suttee A. (2021) In vitro anthelmintic activity of *Chenopodium album* and in-silico prediction of mechanistic role on *Eisenia foetida*. Heliyon 7(1): e05917.

Gamble JS. (1956) Flora of the presidency of Madras. Part 4, Government of India.

Gangabhavani K and Ravishankar K. (2013) Evaluation of analgesic and anti-inflammatory activities of ethanolic extract of whole plant *Justicia betonica*. World J Pharma Pharmaceut Sci. 2(6): 5218-5228.

Geone MC and Antônio F de CA. (2012) Chemical constituents and biological activities of species of Justicia – a review. Rev Bras Farmacogn. 22(1): 220-238.

Ghosh K and Bhattacharya TK. (2015) Chemical constituents of *Piper betle* Linn. (Piperaceae) roots. Molecules 10: 798-802.

Godfrey SB, David BK, Aloysius L, Nathan M, Jasper OO and Olwa O. (2013) Anti-*Plasmodium falciparum* activity of *Aloe dawei* and *Justicia betonica*. African J Pharma Pharmacol. 7(31): 2258-2263.

Gupta MK, Sharma PK, Ansari SH and Lagarkha R. (2006) Pharmacognostical evaluation of *Grewia asiatica* fruits. Int J Plant Sci. 1(2): 249-261.

Harbone JB. (1973) Phytochemical methods. Chapman and Hall Ltd., London.

Hooker JD. (1992) Flora of British India. The Secretary of State for India in Council, Reeve & Co. Ltd.

Janani G, Bharathi T, Saravanan M and Udayakumar R. (2025) Anthelmintic and phytochemical studies of ethanolic extract of stem bark of *Alangium salvifolium.* Intern J Zool Invest. 11(2): 1194-1203.

Jayaramu SC and Prathibha M. (2016) Evaluation of in-vitro antihelmintic properties of the extract of *Russelia equisetiformis* (Schlecht. and Cham.) Scrophulariaceae. Int J Pharma Pharmaceut Sci. 8(1): 459-461.

Jollykutty E, Ansary PY, Shahul HA, Indulekha VC and Resny AR. (2019) Pharmacognosy of leaf stem and root of *Justicia betonica* L. Int J Res Ayurveda Pharma. 10(1): 44-48.

Kamsala A, Rayadurgam N, Sandeep Raj G, Sireesha B, Sowmya N, Sreenath R, Sudhakar Reddy S and Keerthana N. (2025) Evaluation of anthelmintic activity of *Quillaja saponaria* bark of saponins on indian earthworms**.** Cuestiones De Fisioterapia 54(5). https://doi.org/10.48047/1n2aba38.

Katuura E, Waako P, Ogwal-Okeng J and Bukenya-Ziraba R. (2007) Traditional treatment of malaria in Mbarara District, western Uganda. African J Ecol. 45: 48-51.

Khan I, Jan SA and Shinwari ZK. (2017) Ethnobotany and medicinal uses of folklore medicinal plants belonging to family Acanthaceae: An updated review. MOJ Biol Med. 1(2): 34-38.

Kumar M, Tamanna T, Jain D, Dhuria RK, Goswami A and Sharma T. (2016) Comparative evaluation of in vitro antibacterial activity of several extracts of *Achyranthes aspera*, *Azolla pinnata*, *Cissus quadrangularis* and *Tinospora cordifolia*. Int J Chem Stud. 5(1): 154-157.

Kushwaha R, Swati P and Nilambari P. (2011) Anthelmintic activity of combination of *Piper betle* and *Moringa oleifera*, inventi rapid: Planta Activa 68: 11.

Lone BA, Bandh SA, Chisti MZ, Bhat FA, Tak H and Nisa H. (2013) Anthelmintic and antimicrobial activity of methanolic and aqueous extracts of *Euphorbia helioscopia* L. Trop Anim Hlth Prod. 45: 743-749.

Majeed AS, Menaka H and Basha KAN. (2011) Investigation of in vitro anthelmintic activity of *Euphorbia heterophylla* against *Pheretima posthuma*, J Pharmacol Toxicol. 1: 48-52.

Malathi R, Kaviyarasan D and Chandrasekar S. (2018) Preliminary phytochemical analysis of *Justicia adhatoda* leaves extract using different solvents. Int J Pharmaceut Drug Analysis 6(2): 186-190.

Manke MB, Vinayaksing SS, Surwase SS, Tachale VD, Surwase SJ, Tile Pravin S and Giram Padmaja S. (2024) In vitro evaluation of anthelmintic activity of *Lactuca sativa* leaf extract against *Pheretima posthuma*. IP Int J Comprehensive Adv Pharmacol. 11(3): 202-205.

Mathias S, Mshelia E, Danbala B and Biambo A. (2021) In *vitro* anthelmintic activity of leaf extracts of *Celosia laxa* Schum. & Thonn. Open J Appl Sci. 11: 1277-1286.

Mostafa AA, Al-Askar AA Almaary KS, Dawoud TM and Bakri MM. (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci. 25(2): 361-366.

Mukherjee KP. (2002) Quality control of herbal drugs – An approach to evaluation of botanicals. New Delhi: Business Horizons. 2002: 426-483.

Pascaline Jeruto A, Catherine Lukhoba C and George Ouma A. (2008) An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. J Ethno Pharmacol. 2008: 370-376.

Prathibha M and Jayaramu SC. (2019) Phytochemical screening and in vitro antihelmintic properties of *Justicia betonica* L. Int J Pharma Biol Sci. 9(1): 1426-1430.

Saleh MN, Rewaida AG, Afaf A, Sarah AA, Saleh Al-Q and Esam MA. (2024) Anthelmintic activity of *Artemisia monosperma* methanol extracts against *Eisenia fetida* in vitro study. Indian J Anim Res. 58(1): 115-121.

Sahu PM, Gayatri T, Padhy R and Das M. (2025) Phytochemicals screening and anthelmintic activity of some therapeutic plants against *Pheretima posthuma*. Int J Plant Environ. 11(2): 259-264.

Sasikumar JM,Thayumanavan T, Subashkumar R, Janardhanan K and Lakshmanaperumalsamy P. (2007) Antibacterial activity of some ethnomedicinal plants from the Nilgiris, Tamil Nadu, India. Natural Product Radiance 6: 34-39.

Shahidi F, Janitha PK and Wanasundara PD. Phenolic antioxidants. Critical Rev Food Sci Nutrit. 32(1): 67-103.

Shelke PS, Pradnya NJ and Prajakta RT. (2020) In-vitro anthelmintic activity of *Boswellia serrata* and *Aloe barbadensis extracts* on *Pheretima posthuma*: Indian earthworm. Int J Res Med Sci. 8(5): 1843-1847.

Stangel T, Alele PE, Katuura E and Lye KA. (2011) Plants used to treat malaria in Nyakayojo sub-county, western Uganda. J Ethnopharmacol. 137(1): 154-166.

Subbaraju GV, Kavitha J, Rajasekhar D and Jimenez JI. (2004) Jusbetonin, the first indolo [3,2-] quinoline alkaloid glycoside from *Justicia betonica*. J Natural Products 67: 461-462.

Usman H and Osuji JC. (2017) Phytochemical and in vitro antimicrobial assay of the leaf extract of *Newbouldia laevis*. African J Traditional, Complem Altern Med. 4(4): 476- 480.

Vinciya T, Sudha M, Noorul Alam I, Harikrishnan N and Abdul Sameer S. (2025) In vitro evaluation of anthelmintic activity of ethanolic leaf extract of *Thespesia populnae* on Indian earthworms *Eiseniella fetida*. Int J Biol Pharma Allied Sci. 14(1): 418-427.