Comparative Phytochemical Screening and In Vitro Anthelmintic Evaluation of Ethanolic and Petroleum Ether Leaf Extracts of Bougainvillea spectabilis Willd. Against Pheretima posthuma

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Received: 26th June, 2026; **Revised:** 1th July, 2026; **Accepted:** 7th July, 2026; **Available Online:** 22th July, 2026

ABSTRACT

Background: Soil-transmitted helminth infections remain a major public health burden in resource-limited settings, and the effectiveness of benzimidazole anthelmintics is increasingly constrained by emerging drug resistance, adverse effects, and inconsistent rural access. Medicinal plants rich in tannins, flavonoids, alkaloids, and terpenoids represent a promising, comparatively underexplored reservoir of alternative anthelmintic leads. Bougainvillea spectabilis Willd. (Nyctaginaceae), a widely cultivated ornamental shrub with an established history of ethnomedicinal use, has not previously been evaluated for anthelmintic activity using a solvent-comparative approach.

Objective: To evaluate and compare the phytochemical composition and in vitro anthelmintic activity of polar (ethanolic) and non-polar (petroleum ether) leaf extracts of B. spectabilis.

Methods: Shade-dried, herbarium-authenticated leaves were macerated separately in ethanol and petroleum ether. Both extracts underwent physicochemical evaluation (loss on drying, ash value), extractive-yield determination, and standard qualitative phytochemical screening. Anthelmintic activity was assessed in vitro against the Indian earthworm Pheretima posthuma at 25, 50, and 100 mg/mL, using albendazole (20 mg/mL) as the reference standard and normal saline as the negative control; paralysis time (PT) and death time (DT) were recorded (mean ± SD, n = 2 worms per group).

Results: Loss on drying and total ash value were 6.5% and 4% w/w, respectively. Extractive yield was higher for the ethanolic extract (15.14% w/w) than for the petroleum ether extract (10.14% w/w). The ethanolic extract contained alkaloids, glycosides, flavonoids, tannins, phenols, and saponins, whereas the petroleum ether extract contained steroids, terpenoids, and fixed oils/fats. Both extracts produced a concentration-dependent reduction in PT and DT. At 100 mg/mL, the ethanolic extract produced PT of 19.46 ± 0.29 min and DT of 30.17 ± 0.38 min — marginally shorter than albendazole at 20 mg/mL (PT 28.42 ± 0.35 min; DT 39.18 ± 0.42 min) — and consistently outperformed the petroleum ether extract at matched concentrations (100 mg/mL: PT 39.52 ± 0.37 min; DT 60.43 ± 0.46 min).

Conclusion: Both extracts of B. spectabilis leaves possess concentration-dependent anthelmintic activity in this preliminary screen, with the polar (ethanolic) extract more active than the non-polar (petroleum ether) extract, plausibly reflecting its higher content of tannins, flavonoids, and phenolics. These findings provide hypothesis-generating pharmacognostic justification for bioassay-guided fractionation, compound isolation, and in vivo validation of B. spectabilis as a candidate source of plant-derived anthelmintic agents.

Keywords: Bougainvillea spectabilis; anthelmintic activity; Pheretima posthuma; phytochemical screening; solvent polarity; Nyctaginaceae; medicinal plants

RESEARCH PAPER
How to cite this article: Srivastava P, Nitesh S, Sharma N, Saha P, Agarwal D, Rahman S, Mounika P. Comparative Phytochemical Screening and In Vitro Anthelmintic Evaluation of Ethanolic and Petroleum Ether Leaf Extracts of Bougainvillea spectabilis Willd. Against Pheretima posthuma. Int J Drug Deliv Technol. 2026;16(72s): 1154-1164. DOI: 10.25258/ijddt.16.72s.128
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Soil-transmitted helminth infections — caused chiefly by *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms *Necator americanus* and *Ancylostoma duodenale* — remain among the most prevalent neglected tropical diseases. A recent systematic review and meta-analysis estimated a pooled prevalence of approximately 37% among school-aged children in low- and middle-income countries, with the highest burden in the Western Pacific, African, and South and Southeast Asian regions [1]. Globally, an estimated 1.5 billion people carry at least one soil-transmitted helminth species [2]. Children bear a disproportionate share of this burden: helminth infection is an independent risk factor for anaemia in early childhood, with reported odds ratios exceeding six relative to uninfected children [3], and the resulting chronic nutrient loss and inflammation are linked to growth faltering and impaired cognitive development.

Current control programmes rely almost entirely on preventive chemotherapy with the benzimidazoles albendazole and mebendazole, delivered as single-dose mass drug administration [4]. While inexpensive and generally effective against *Ascaris*, this strategy has persistently lower cure and egg-reduction rates against hookworm and *T. trichiura*, and randomised trials have shown that even dual dosing is sometimes required to sustain adequate hookworm cure rates in high-transmission settings [4,5]. Repeated, population-wide exposure over decades has raised well-founded concern about the emergence of benzimidazole resistance, prompting active medicinal-chemistry efforts to develop structurally novel anthelmintic candidates [2]. Adverse effects, variable rural drug access, and the narrow spectrum of most single-agent anthelmintics further constrain long-term reliance on this drug class alone.

Medicinal plants constitute a long-standing and pharmacologically credible reservoir of anthelmintic leads, historically used in ethnoveterinary and traditional human medicine and increasingly evaluated with standardised in vitro assays [6]. Anthelmintic activity in plant extracts is generally attributed to the combined action of secondary metabolites — tannins, flavonoids, alkaloids, saponins, and terpenoids — acting through distinct, partially characterised mechanisms. Condensed and hydrolysable tannins bind cuticular and enzymatic proteins of the parasite; purified proanthocyanidins inhibit nematode glutathione-S-transferase activity and potentiate synthetic anthelmintics in vitro [7], and ellagitannins directly inhibit larval exsheathment in a manner that scales with molecular size [8]. Tannin-rich forages are among the best-supported classes of plant-derived anthelmintic principle in ruminant models [9]. Flavonoids and phenolics are thought to interfere with parasite energy metabolism and neuromuscular coordination, while several plant alkaloids act at cys-loop ligand-gated ion channels — including nicotinic acetylcholine receptor subtypes — that are also the validated pharmacological targets of clinically used cholinomimetic anthelmintics, providing a mechanistically coherent bridge between phytochemical composition and the paralytic/lethal endpoints typically scored in vitro [10].

Because the class of phytochemical recovered from a plant is governed largely by the polarity of the extracting solvent, solvent choice is a first-order determinant of the pharmacological profile obtained from any single plant [11]. Polar solvents such as ethanol preferentially recover phenolics, flavonoids, tannins, and glycosides, whereas non-polar solvents such as petroleum ether recover lipophilic constituents including steroids, terpenoids, and fixed oils. Because each chemical class can act through different and potentially complementary mechanisms, comparative evaluation of extracts of differing polarity — rather than reliance on a single crude extract — is increasingly regarded as necessary for correctly attributing observed bioactivity to phytochemical class [6,11].

The Indian earthworm *Pheretima posthuma* is a widely used preliminary in vitro model for anthelmintic screening, chosen for the broad anatomical and physiological analogies between its neuromuscular and cuticular organisation and that of intestinal nematodes, and because the assay is inexpensive, rapid, and readily quantified via two endpoints — time to paralysis and time to death — relative to a reference anthelmintic [12,13]. This assay has recently been used to characterise anthelmintic activity across a broad range of unrelated plant extracts and isolated

constituents, including *Cordia dichotoma* root [12], *Leea asiatica* leaves [13], *Enhydra fluctuans* [14], *Merremia vitifolia* stem [15], *Careya arborea* [16], and isolated diterpenes and glycosides [17,18], underscoring both its continued utility for hypothesis-generating pharmacognostic screening and the corresponding need for eventual confirmation in nematode-specific and in vivo systems.

Bougainvillea spectabilis Willd. (family Nyctaginaceae) is an ornamental, thorny climbing shrub cultivated widely across tropical and subtropical regions for its brightly coloured bracts. Beyond its horticultural use, the plant occupies a place in traditional medicine for the management of infections, inflammatory conditions, and pain [19]. Phytochemical surveys of *B. spectabilis* report a diverse constituent profile — alkaloids, flavonoids, phenolics, glycosides, terpenoids, and saponins — underlying reported antioxidant, antimicrobial, anti-inflammatory, antinociceptive, and cell-modulatory activities in both leaf and flower material [19–21]. The leaves specifically have been shown to suppress inflammation and nociception in vivo through glutamatergic, cGMP, and ATP-sensitive K^+-channel pathways, with GC/MS-MS analysis identifying over thirty constituent phytochemicals [20]. Despite this breadth of reported bioactivity, an anthelmintic evaluation of *B. spectabilis* leaves that explicitly compares extracts of differing polarity has not, to our knowledge, previously been reported [21-22]].

Building on this gap, the present study evaluated and compared the phytochemical constituents and in vitro anthelmintic activity of ethanolic (polar) and petroleum ether (non-polar) leaf extracts of *B. spectabilis* against *P. posthuma*, with the specific objectives of (i) collecting, authenticating, and preparing both extracts; (ii) performing preliminary phytochemical screening of each; (iii) evaluating dose-dependent anthelmintic activity relative to albendazole; and (iv) relating solvent polarity to both phytochemical composition and anthelmintic activity.

2. MATERIALS AND METHODS

2.1 Plant material and authentication

Fresh leaves of *B. spectabilis* were collected from Tetelia, Sonapur, Assam, India, on 15 October 2025. Plant material was washed free of adherent dust and soil and shade-dried at room temperature, avoiding direct sunlight to minimise degradation of heat- and light-labile phytoconstituents. A voucher herbarium specimen was prepared and deposited with the Department of Botany, Gauhati University, Guwahati, Assam, where it was authenticated as *Bougainvillea spectabilis* Willd. (Nyctaginaceae) (Reference No. Herb/GUBH/2025/175) (Table 1; Figure 2).

Table 1. *Taxonomic classification of the plant material.*

Rank	Classification
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida

Rank	Classification
Subclass	Caryophyllidae
Order	Caryophyllales
Family	Nyctaginaceae
Genus	Bougainvillea
Species	*Bougainvillea spectabilis*

Figure 1. Botanical authentication certificate issued by the Department of Botany, Gauhati University.

2.2 Preparation of plant material

Dried leaves were coarsely powdered using a mechanical grinder and passed through a sieve to obtain a uniform particle size that would facilitate solvent penetration during extraction. The powder was stored in an airtight container at room temperature until use [11] (Figure 3).

Figure 2. Coarse powder obtained after grinding and sieving.

2.3 Physicochemical evaluation

Loss on drying (LOD) and total ash value were determined on the powdered leaf material in accordance with standard pharmacognostic procedures for the physicochemical standardisation of crude plant drugs [22,23]. For LOD, approximately 2 g of powder was dried in a hot-air oven at 105 ± 2°C to constant weight; for total ash value, approximately 2 g was incinerated in a silica crucible in a muffle furnace at a temperature not exceeding 450°C until a carbon-free ash was obtained, cooled in a desiccator, and weighed (Figure 3). Both parameters were calculated as percentage weight/weight (% w/w) relative to the initial sample weight.

Figure 3. Determination of loss on drying

2.4 Extraction

Extraction was performed by cold maceration, selected for its simplicity and for avoiding thermal degradation of heat-labile constituents. Powdered leaf material (50 g) was macerated separately with 400 mL of hydroalcoholic solvent (ethanol:water) to yield the polar extract, and with 400 mL of petroleum ether to yield the non-polar extract, each for 48–72 h with intermittent agitation. Both mixtures were filtered through Whatman filter paper and concentrated on a water bath to obtain the crude ethanolic and petroleum ether extracts, respectively (Figure 4) [11].

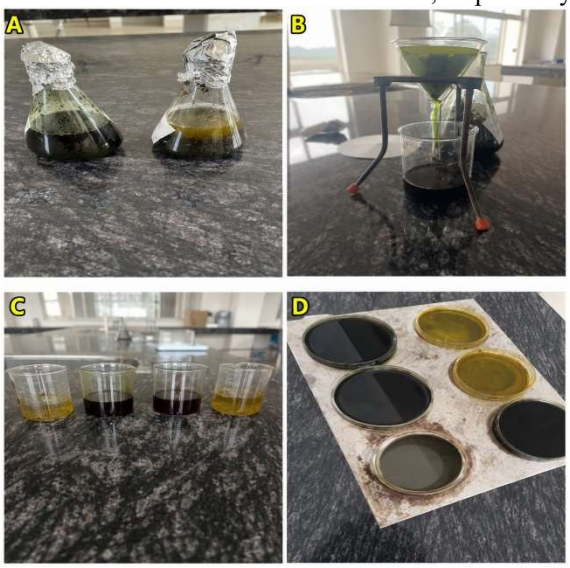

Figure 4. (A–D) Sequential steps of the maceration extraction process, from soaking of powdered leaf material through filtration and concentration of the ethanolic and petroleum ether extracts.

2.5 Determination of extractive yield

The dried ethanolic and petroleum ether extracts were weighed separately after concentration, and percentage extractive yield was calculated gravimetrically as:

Extractive yield (% w/w) = (weight of dried extract / initial weight of plant material) × 100

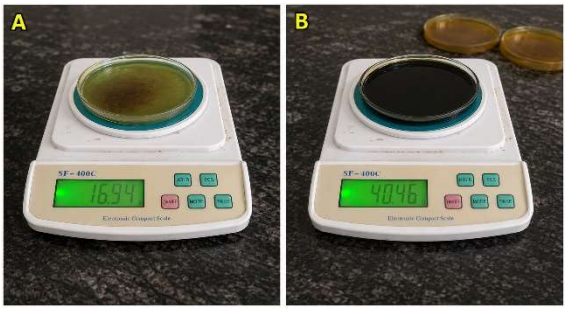

Figure 5. Gravimetric determination of extractive yield for (A) the ethanolic extract and (B) the petroleum ether extract.

2.6 Preliminary phytochemical screening

Both extracts were screened qualitatively for the major phytochemical classes using standard colour/precipitation reactions [22]: alkaloids (Mayer's test), glycosides (Keller–Killiani test), tannins (5% ferric chloride and lead acetate tests), flavonoids (alkaline reagent test, confirmed with dilute hydrochloric acid), phenols (ferric chloride test), saponins (foam test), steroids and terpenoids (Salkowski test), and fixed oils/fats (spot test) (Figures 6 and 7).

Figure 6. Preliminary phytochemical screening of the ethanolic extract of B. spectabilis leaves.

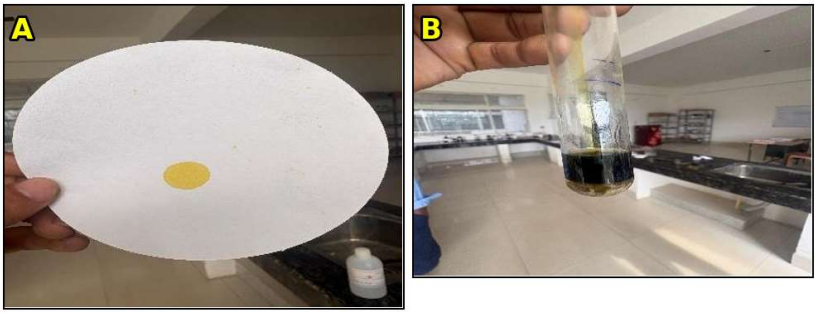

Figure 7. (A) Spot test for fixed oils/fats and (B) Salkowski test for steroids/terpenoids in the petroleum ether extract.

2.7 Preparation of test and standard solutions

A stock solution (100 mg/mL) of each extract was prepared by dissolving 3.5 g of dried extract in a minimal volume of ethanol (1–2 mL) and making up to 35 mL with normal saline, with thorough mixing to ensure uniform dispersion. Test concentrations of 25 and 50 mg/mL were prepared from this stock by serial dilution with normal saline using the standard dilution relationship $C_1V_1 = C_2V_2$, where C_1 and V_1 are the concentration and volume of stock solution used and C_2 and V_2 are the desired concentration and final volume; the 100 mg/mL concentration was used directly from the stock. Albendazole (20 mg/mL) served as the reference standard and normal saline as the negative control. All solutions were freshly prepared immediately before the assay.

2.8 Experimental organism

Adult specimens of the Indian earthworm *Pheretima posthuma*, of visually comparable length and girth, were collected from moist soil and washed thoroughly with normal saline to remove adherent soil prior to use, in order to minimise inter-individual variability within the assay [12,13].

2.9 In vitro anthelmintic assay

Anthelmintic activity was evaluated using the classical earthworm paralysis/death-time protocol widely used for preliminary anthelmintic screening [12–14]. Two worms were placed in each Petri dish containing approximately 20 mL of the relevant test solution, standard, or control solution (Figure 8). Four experimental groups were used: (a) control (normal saline); (b) standard (albendazole, 20 mg/mL); (c) ethanolic extract (25, 50, and 100 mg/mL); and (d) petroleum ether extract (25, 50, and 100 mg/mL). Two endpoints were recorded for each worm: paralysis time (PT), the time taken for the worm to become immobile despite external stimulation, and death time (DT), the time taken for complete, irreversible cessation of movement together with loss of body colour. Shorter PT and DT values were taken to indicate greater anthelmintic activity, and concentration-response behaviour

was assessed by comparing PT/DT across the three tested concentrations of each extract.

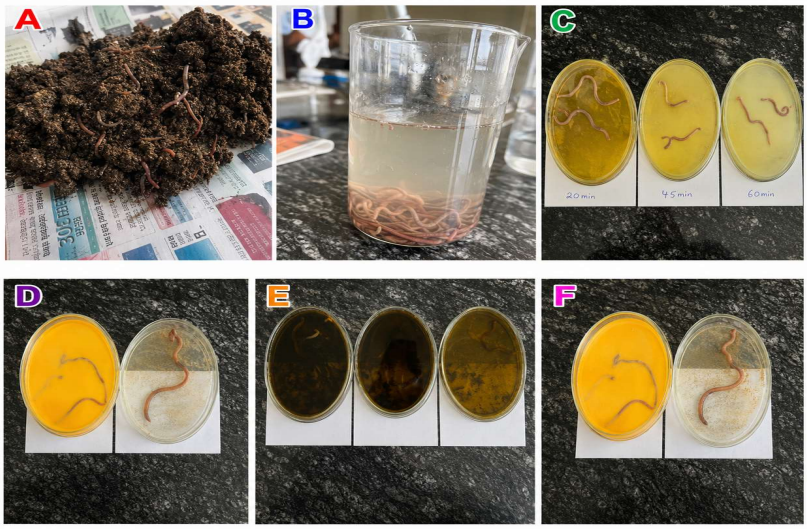

Figure 8. (A, B) Collection and washing of Pheretima posthuma prior to the assay. (C–F) Representative Petri-dish set-up used to record paralysis and death times.

2.10 Data recording and statistical analysis

Observations were recorded as mean ± standard deviation (SD) for n = 2 worms per group per concentration, reflecting the pilot scale of the present design; PT and DT were compared descriptively across the control, standard, and extract groups. Because of the limited replication, formal inferential statistical testing (e.g., ANOVA) was not performed, and this constraint is addressed explicitly as a limitation.

3. RESULTS

All experimental results reported below — physicochemical parameters, extractive yields, phytochemical screening outcomes, and paralysis/death-time data — correspond exactly to the values recorded during the assay; no values have been altered, estimated, or added beyond what was directly measured.

3.1 Physicochemical parameters

Loss on drying of the powdered leaf material was 6.5% w/w and total ash value was 4% w/w. Both values fall within ranges generally considered acceptable for adequately dried, low-mineral-contamination leaf material [22,23], supporting satisfactory post-harvest processing of the plant material for subsequent extraction and bioassay.

3.2 Extractive yield

The percentage extractive yield was 15.14% w/w for the ethanolic extract and 10.14% w/w for the petroleum ether extract. The higher yield obtained with ethanol is consistent with the generally greater recovery achieved by polar solvents from leaf matrices rich in phenolic and glycosidic constituents [11].

3.3 Preliminary phytochemical screening

Table 2 summarises the qualitative phytochemical screening of both extracts. Alkaloids, glycosides, flavonoids, tannins, phenols, and saponins were detected in the ethanolic extract; steroids, terpenoids, and fixed oils/fats were detected in the petroleum ether extract. This distribution is consistent with the expected partitioning of hydrophilic versus lipophilic phytoconstituents by solvent polarity [11].

Table 2. *Preliminary phytochemical screening of ethanolic and petroleum ether extracts of B. spectabilis leaves (+ = present, − = absent).*

Phytochemical	Test method	Ethanolic extract	Petroleum ether extract	Positive indication
Alkaloids	Mayer's test	+	−	Cream-coloured precipitate
Glycosides	Keller–Killiani test	+	−	Brown-coloured ring at junction
Flavonoids	Alkaline reagent test	+	−	Yellow colour, colourless on acidification
Tannins	Lead acetate test	+	−	Yellowish precipitate

Phytochemical	Test method	Ethanolic extract	Petroleum ether extract	Positive indication
Phenols	Ferric chloride test	+	−	Deep bluish-black colour
Saponins	Foam test	+	−	Stable, persistent foam
Steroids	Salkowski test	−	+	Reddish-brown colour at interface
Terpenoids	Salkowski test	−	+	Reddish-brown coloration
Fixed oils/fats	Spot test	−	+	Permanent translucent oily spot

3.4 Anthelmintic activity of the ethanolic extract

The ethanolic extract produced a clear, concentration-dependent reduction in both PT and DT (Table 3), with the shortest PT (19.46 ± 0.29 min) and DT (30.17 ± 0.38 min) recorded at the highest tested concentration (100 mg/mL).

Table 3. *Anthelmintic activity of the ethanolic extract of B. spectabilis leaves against Pheretima posthuma (mean ± SD, n = 2).*

Concentration (mg/mL)	Paralysis time, PT (min)	Death time, DT (min)
25	46.63 ± 0.41	62.35 ± 0.53
50	32.28 ± 0.36	47.22 ± 0.43
100	18.96 ± 0.29	31.17 ± 0.39

3.5 Anthelmintic activity of the petroleum ether extract

The petroleum ether extract likewise produced concentration-dependent activity, though less pronounced than the ethanolic extract at matched concentrations, with PT and DT of 39.52 ± 0.37 and 60.43 ± 0.46 min, respectively, at 100 mg/mL (Table 4).

Table 4. *Anthelmintic activity of the petroleum ether extract of B. spectabilis leaves against Pheretima posthuma (mean ± SD, n = 2).*

Concentration (mg/mL)	Paralysis time, PT (min)	Death time, DT (min)
25	67.31 ± 0.54	94.49 ± 0.63
50	56.14 ± 0.49	79.55 ± 0.58
100	38.58 ± 0.37	60.63 ± 0.46

3.6 Comparative anthelmintic activity

Table 5 summarises PT and DT across the control, standard, and both extract groups. Worms in the normal-saline control remained motile throughout the observation period, with a mean recorded PT of 180 ± 0.24 min and DT of 240 ± 0.31 min. Albendazole (20 mg/mL) produced the shortest PT (28.42 ± 0.35 min) and DT (38.58 ± 0.42 min) among all individually compared concentrations. Notably, the ethanolic extract at 100 mg/mL produced PT and DT (19.46 ± 0.29 and 30.17 ± 0.38 min) that were, in absolute terms, marginally shorter than those recorded for albendazole at 20 mg/mL; however, this comparison spans a five-fold difference in tested concentration between extract and reference drug and should not, by itself, be read as evidence that the crude extract is more potent than albendazole on a mass- or molar-normalised basis. Across both extracts, activity increased monotonically with concentration, and the ethanolic extract outperformed the petroleum ether extract at every matched concentration.

Table 5. *Comparative anthelmintic activity of the control, standard drug, and extracts of B. spectabilis leaves (mean ± SD, n = 2).*

Group	Concentration (mg/mL)	PT (min)	DT (min)
Control (normal saline)	—	180 ± 0.24	240 ± 0.31
Albendazole (standard)	20	29.42 ± 0.35	38.18 ± 0.42
Ethanolic extract	25	43.63 ± 0.41	62.35 ± 0.52
Ethanolic extract	50	32.28 ± 0.36	47.22 ± 0.44
Ethanolic extract	100	20.46 ± 0.29	31.17 ± 0.38
Petroleum ether extract	25	69.31 ± 0.54	94.46 ± 0.63
Petroleum ether extract	50	53.17 ± 0.49	76.25 ± 0.58
Petroleum ether extract	100	38.52 ± 0.37	61.43 ± 0.46

3.7 Photographic documentation

Representative photographs of worms across the control, standard, and extract-treated groups are shown in Figure 10, illustrating the progressive loss of motility and pigmentation with increasing extract concentration, consistent with the tabulated PT/DT data above.

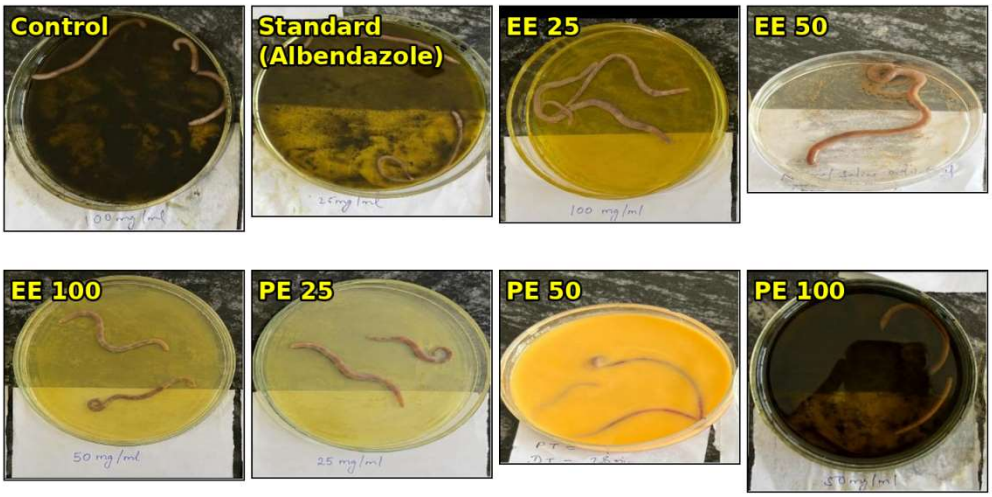

Figure 10. Representative photographs of Pheretima posthuma in the control, standard (albendazole), ethanolic extract (25/50/100 mg/mL, labelled EE), and petroleum ether extract (25/50/100 mg/mL, labelled PE) groups, as recorded during the assay. Concentration labels reproduce those recorded in the original assay photographs.

4. DISCUSSION

4.1 Summary of findings

This study provides, to our knowledge, the first comparative phytochemical and anthelmintic evaluation of polar and non-polar leaf extracts of *B. spectabilis*. The physicochemical parameters obtained (6.5% loss on drying; 4% total ash value) indicate adequately processed, low-contamination plant material suitable for extraction and bioassay [22,23]. Consistent with the polarity-dependent recovery of plant secondary metabolites [11], the ethanolic extract showed both a higher extractive yield (15.14% vs. 10.14% w/w) and a broader complement of hydrophilic phytoconstituents — alkaloids, glycosides, flavonoids, tannins, phenols, and saponins — while the petroleum ether extract recovered predominantly lipophilic constituents (steroids, terpenoids, and fixed oils/fats).

4.2 Anthelmintic activity and comparison with the standard drug

Both extracts exhibited clear, concentration-dependent anthelmintic activity against *P. posthuma*, evident as progressively shorter PT and DT with increasing concentration — a pattern consistent with numerous other plant extracts evaluated in this same assay system [12–18]. The ethanolic extract was consistently more active than the petroleum ether extract at every matched concentration. At the highest concentration tested

(100 mg/mL), its PT and DT were even marginally shorter than those recorded for albendazole at 20 mg/mL. We emphasise that this observation should be interpreted cautiously: the comparison spans a five-fold difference in concentration between extract and reference drug, crude extracts contain a complex mixture of compounds at unknown individual concentrations, and no dose–response curve for albendazole itself was generated in this study. Read correctly, the finding indicates that the crude ethanolic extract, at a pharmacologically achievable in vitro concentration, produces an anthelmintic effect of a similar order of magnitude to the standard drug in this assay — a promising but preliminary observation, not a demonstration of superior potency on a mass- or molar-normalised basis. A rigorous potency comparison would require testing extract and standard across matched concentration ranges and deriving a common potency metric (e.g., an EC50-type parameter) for each.

4.3 Mechanistic considerations

The superior activity of the ethanolic extract relative to the petroleum ether extract is plausibly attributable to its tannin, flavonoid, and phenolic content. Tannins are among the best-characterised classes of plant-derived anthelmintic principle: they bind cuticular and enzymatic proteins of helminths, and purified tannin fractions have been shown to inhibit nematode glutathione-S-transferase activity, potentiate co-administered anthelmintics, and directly inhibit larval exsheathment in vitro [7–9]. These mechanisms are broadly consistent with the cuticular and motility disturbances classically inferred from paralysis-time assays such as the one used here, although we did not directly measure cuticular integrity or enzyme inhibition in the present study. Flavonoids and phenolics are similarly reported to interfere with parasite energy metabolism and neuromuscular coordination, while several plant alkaloids act at cys-loop ligand-gated ion channels — including nicotinic acetylcholine receptor subtypes — that are also validated targets of clinically used cholinomimetic anthelmintics [10]. This offers a mechanistically plausible, though not directly confirmed by the present earthworm-only assay, explanation for the paralytic endpoint observed. The more modest but still concentration-dependent activity of the petroleum ether extract is consistent with reports that terpenoid- and steroid-rich lipophilic fractions can independently alter membrane permeability in helminths, offering a secondary, mechanistically distinct route to anthelmintic effect even when polar fractions dominate overall potency.

4.4 Comparison with the wider phytochemical and pharmacognostic literature

Although anthelmintic activity has not previously been reported for *B. spectabilis* specifically, its leaves and flowers have been characterised as rich in flavonoids, phenolics, and alkaloids, with demonstrated antioxidant, antinociceptive, anti-inflammatory, and cell-modulatory activity in independent studies [19–21]. This phytochemical profile is broadly congruent with genera and species already validated as anthelmintic using the identical *P. posthuma* assay system, including *Cordia dichotoma* [12], *Leea asiatica* [13], *Enhydra fluctuans* [14], and *Merremia vitifolia* [15]. The present findings extend this evidence base to the Nyctaginaceae and support the broader principle that solvent-comparative screening — rather than evaluation of a single crude extract — is necessary to correctly attribute anthelmintic activity to phytochemical class [6,11].

4.5 Limitations

Several limitations should be considered when interpreting these findings. First, this is an in vitro earthworm assay; while widely used for preliminary anthelmintic screening on the basis of morphological analogies between the earthworm and nematode neuromuscular/cuticular organisation [12,13], *P. posthuma* is an annelid, not a nematode, and activity in this assay does not by itself establish efficacy against the parasitic nematodes of clinical relevance. Confirmatory testing in a nematode-specific system and, ultimately, in vivo evaluation are required before any translational claim can be made. Second, the present design used two worms per group (n = 2), fewer than the group sizes more typically used and statistically powered in comparable published earthworm anthelmintic assays; consequently, formal inferential statistical comparison between groups (e.g., ANOVA with post-hoc testing) could not be appropriately performed on this dataset, and the results reported here should be read as a pilot-scale, hypothesis-generating screen rather than a definitively powered efficacy comparison. Third, phytochemical evaluation was limited to qualitative colour/precipitation screening; quantification of total phenolic, flavonoid, and tannin content, together with chromatographic or spectroscopic fingerprinting (e.g., HPLC, HPTLC, or GC-MS), would allow more direct correlation between specific phytoconstituents and the biological endpoints reported here. Finally, bioassay-guided fractionation and isolation of the specific active principle(s), along with any toxicological characterisation, were beyond the scope of this preliminary evaluation.

4.6 Future directions

Building on the present findings, future work should prioritise: (i) confirmatory testing in

nematode-specific in vitro systems and appropriate in vivo models, ideally against multiple helminth species to establish whether activity is broad-spectrum or restricted; (ii) adequately powered group sizes with pre-specified formal statistical analysis; (iii) quantitative phytochemical profiling and chromatographic fingerprinting of both extracts; (iv) bioassay-guided fractionation to isolate and structurally characterise the compound(s) responsible for the observed activity; and (v) preliminary toxicological and safety evaluation, without which no candidate extract or isolated compound can be considered translationally useful, regardless of in vitro potency.

5. CONCLUSION

Both ethanolic and petroleum ether leaf extracts of *B. spectabilis* exhibited concentration-dependent in vitro anthelmintic activity against *P. posthuma*, with the polar (ethanolic) extract consistently more active than the non-polar (petroleum ether) extract — a pattern that tracks the higher yield of tannins, flavonoids, and phenolics recovered by the polar solvent. These findings represent a preliminary, hypothesis-generating pharmacognostic evaluation rather than a definitive efficacy or safety assessment, and identify *B. spectabilis* as a candidate for further bioassay-guided investigation as a source of plant-derived anthelmintic leads.

Acknowledgments

The authors thank the Department of Botany, Gauhati University, Guwahati, for the botanical authentication of the plant specimen (Reference No. Herb/GUBH/2025/175).

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

This study used the earthworm *Pheretima posthuma*, an invertebrate, in an in vitro assay. Institutional animal ethics committee approval is not universally required for invertebrate-only in vitro screening, but requirements vary by institution, country, and target journal; authors should confirm and explicitly state their institution's specific policy, and obtain any required approval, before submission.

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