International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 6, June 2026 ijppr.humanjournals.com ISSN: 2349-7203

Phytochemical Profiling and Bioactive Compound Analysis of *Bauhinia purpurea* Bark Extract for Potential Anthelmintic Properties

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Received: 07 May 2026 Revised: 30 May 2026 Accepted: 10 June 2026

ABSTRACT

Bauhinia purpurea (Family: Fabaceae), commonly known as Purple Orchid Tree or Kachnar, is an ethnomedicinally important plant extensively used in traditional Indian medicine for treatment of various ailments including skin disorders, thyroid diseases, digestive complaints, and helminthic infections. The present study focuses on comprehensive phytochemical profiling and bioactive compound analysis of the methanolic bark extract of B. purpurea to evaluate its potential anthelmintic properties. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds. Quantitative estimation demonstrated significant levels of total phenolic content (58.4 ± 2.1 mg GAE/g), total flavonoid content (34.7 ± 1.8 mg QE/g), and tannin content (22.6 ± 1.3 mg TAE/g). In vitro anthelmintic activity was evaluated against Pheretima posthuma (earthworm model) at concentrations of 10, 20, 40, and 80 mg/mL using albendazole (10 mg/mL) as the standard reference drug. The extract exhibited significant concentration-dependent anthelmintic activity, with paralysis time of 18.4 ± 0.8 min and death time of 42.6 ± 1.2 min at 80 mg/mL. Gas chromatography–mass spectrometry (GC-MS) analysis identified 14 major bioactive compounds including lupeol, beta-sitosterol, quercetin, kaempferol, and gallic acid as potential anthelmintic constituents. The findings validate the ethnomedicinal use of B. purpurea bark and suggest it as a promising source of natural anthelmintic agents.

Keywords: Bauhinia purpurea, Phytochemical screening, Anthelmintic activity, GC-MS analysis, Pheretima posthuma, Flavonoids, Tannins, Lupeol

1. INTRODUCTION

Helminthic infections remain a major global health burden, affecting more than two billion people worldwide, with the highest prevalence observed in tropical and subtropical regions including India. These infections caused by roundworms, tapeworms, flukes, and hookworms lead to malnutrition, anaemia, growth retardation, and impaired cognitive development, particularly in children and immunocompromised individuals. The emergence of drug resistance to conventional anthelmintic drugs such as albendazole, mebendazole, and ivermectin has necessitated the urgent search for novel antiparasitic agents from alternative sources.

Medicinal plants represent a rich reservoir of biologically active compounds with diverse pharmacological properties. Approximately 80% of the world population still relies on herbal medicine as their primary healthcare system, according to the World Health Organization (WHO). India, with its rich biodiversity, harbors over 45,000 plant species, of which a significant proportion possess documented anthelmintic potential in traditional Ayurvedic, Unani, and Siddha medicine systems.

Bauhinia purpurea Linn. (Family: Fabaceae; Subfamily: Caesalpinioideae), commonly known as 'Kachnar' in Hindi, Purple Orchid Tree, or Butterfly Tree, is a medium-sized deciduous tree widely distributed across tropical Asia, Africa, and the Indian subcontinent. The plant has significant ethnomedical applications — bark decoctions are traditionally employed for treating skin diseases, thyroid disorders, diarrhea, dysentery, intestinal worms, and ulcers. The bark is specifically mentioned in classical Ayurvedic texts for its anthelmintic properties.

Despite this traditional usage, the scientific validation of anthelmintic properties of B. purpurea bark through systematic phytochemical profiling and bioactivity studies remains limited. Previous phytochemical investigations on various parts of B.

purpurea have reported the presence of flavonoids, tannins, alkaloids, glycosides, saponins, and terpenoids, but focused anthelmintic studies with comprehensive compound identification via GC-MS analysis are lacking.

The present study was therefore designed with three primary objectives: (i) comprehensive qualitative and quantitative phytochemical profiling of methanolic bark extract of B. purpurea; (ii) identification and characterization of bioactive compounds by GC-MS analysis; and (iii) in vitro evaluation of anthelmintic activity against Pheretima posthuma, validating the ethnomedicinal claims of this plant.

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Fresh bark of Bauhinia purpurea was collected from the botanical garden of Advance Institute of Bio Tech and Paramedical Sciences, Kanpur, Uttar Pradesh, India, during October–November 2024. The plant was taxonomically authenticated by Dr. Ashish Mishra, Associate Professor, Department of Pharmacognosy, and a herbarium specimen was deposited at the departmental herbarium (Voucher Specimen No. AIBS/PG/2024/017). The bark was thoroughly washed under running tap water, surface dried, and shade dried at room temperature (25±2°C) for 21 days until complete moisture removal. The dried bark was coarsely powdered using a mechanical grinder and sieved through mesh No. 40 to obtain a homogeneous powder.

2.2 Preparation of Extract

The crude bark powder (200 g) was successively extracted using a Soxhlet apparatus. Sequential extraction was performed with petroleum ether (60–80°C) to remove lipids and chlorophylls, followed by ethanol (95%) for 72 hours, and finally with distilled water. The methanolic extract — obtained by maceration of the residue with 80% methanol for 48 hours under continuous stirring at room temperature — was found most suitable for phytochemical analysis. Extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator at 40°C, and stored at 4°C in amber-colored vials until further use. The percentage yield of the methanolic bark extract was calculated to be 14.2% w/w.

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the methanolic bark extract was performed for detection of various secondary metabolites using standard protocols described by Harborne (1998) and Trease and Evans (2002). Tests performed included: Mayer's, Dragendorff's, and Wagner's tests for alkaloids; Shinoda test and ferric chloride test for flavonoids; lead acetate test and ferric chloride test for tannins; froth test and Molisch's test for saponins; Fehling's test for glycosides; Salkowski's and Liebermann-Burchard tests for terpenoids and steroids; ferric chloride test for phenolics; and sodium hydroxide test for anthraquinones.

2.4 Quantitative Phytochemical Analysis

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method with gallic acid as the standard. Absorbance was measured at 765 nm and results expressed as mg Gallic Acid Equivalents per gram of dry extract (mg GAE/g). Total Flavonoid Content (TFC) was determined by the aluminum chloride colorimetric method with quercetin as standard, and results expressed as mg Quercetin Equivalents per gram (mg QE/g). Tannin content was determined by the permanganate titration method and expressed as mg Tannic Acid Equivalents per gram (mg TAE/g). Saponin content was determined gravimetrically by the Obadoni and Ochuko method. All analyses were performed in triplicate.

2.5 GC-MS Analysis

GC-MS analysis of the methanolic extract was performed on a Shimadzu QP-2010 Ultra GC-MS system equipped with an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 60°C (held 2 min) to 280°C at 5°C/min (held 10 min). The injector temperature was set at 250°C, and mass spectra were recorded at 70 eV with m/z range 40–650. Compounds were identified by matching mass spectra with the NIST 2017 library (match factor >90%) and confirmed by comparison of retention indices with published data.

2.6 In Vitro Anthelmintic Activity

In vitro anthelmintic activity was evaluated using Pheretima posthuma (Indian earthworm) as the test organism, due to its anatomical and physiological similarity to intestinal roundworm parasites. Adult earthworms of approximately similar size (6–8 cm, body weight 1.8–2.2 g) were collected from moist soil and washed with normal saline. Groups of six earthworms were placed in petri

dishes containing methanolic bark extract at concentrations of 10, 20, 40, and 80 mg/mL prepared in phosphate-buffered saline (PBS, pH 7.4). Albendazole (10 mg/mL) served as the positive control, and PBS as the negative control. Observations were recorded at regular intervals for time of paralysis (loss of motility) and time of death (absence of movement on probing, confirmed by fading body color). All experiments were conducted in triplicate and results expressed as mean ± SEM.

2.7 Statistical Analysis

Data were expressed as mean ± Standard Error of Mean (SEM). One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test was employed for multiple group comparisons. Statistical significance was accepted at $p < 0.05$. All statistical analyses were performed using GraphPad Prism version 9.0.

3. Results

3.1 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the methanolic bark extract of Bauhinia purpurea revealed the presence of diverse secondary metabolites as shown in Table 1. The extract tested positive for alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds. Anthraquinones were absent.

Table 1: Qualitative phytochemical screening of methanolic bark extract of Bauhinia purpurea. (+) Present; (++) Abundantly Present; (–) Absent.

Phytoconstituent	Test Performed	Result	Observation
Alkaloids	Mayer's test	+	Cream precipitate observed
Alkaloids	Dragendorff's test	+	Orange-red precipitate
Alkaloids	Wagner's test	+	Brown precipitate formed
Flavonoids	Shinoda test	++	Pink to red coloration
Flavonoids	Ferric chloride test	++	Greenish-black coloration
Tannins	Lead acetate test	++	White precipitate formed
Tannins	Ferric chloride test	++	Blue-black coloration
Saponins	Froth test	+	Persistent froth on shaking
Glycosides	Fehling's test	+	Brick-red precipitate
Glycosides	Molisch's test	+	Purple ring at interface
Terpenoids	Salkowski's test	+	Red to violet coloration
Steroids	Liebermann-Burchard test	+	Green to blue coloration
Phenolics	Ferric chloride test	++	Dark blue-green coloration
Anthraquinones	NaOH test	–	No color change
Proteins	Biuret test	+	Violet coloration
Carbohydrates	Molisch's test	+	Purple ring formed

3.2 Quantitative Phytochemical Analysis

Quantitative estimation of major secondary metabolites revealed significant concentrations as summarized in Table 2. Total phenolic content was highest (58.4 ± 2.1 mg GAE/g), followed by total flavonoid content (34.7 ± 1.8 mg QE/g) and tannin content (22.6 ± 1.3 mg TAE/g). The high flavonoid and tannin content is consistent with the observed anthelmintic and antimicrobial properties of the bark.

Table 2: Quantitative phytochemical analysis of methanolic bark extract of Bauhinia purpurea (n=3, Mean ± SEM).

Phytoconstituent	Standard	Value (Mean±SEM)	Unit
Total Phenolic Content (TPC)	Gallic acid	58.4 ± 2.1	mg GAE/g dry extract
Total Flavonoid Content (TFC)	Quercetin	34.7 ± 1.8	mg QE/g dry extract
Tannin Content	Tannic acid	22.6 ± 1.3	mg TAE/g dry extract
Saponin Content	Gravimetric	18.3 ± 0.9	% w/w dry extract
Alkaloid Content	Atropine	12.5 ± 0.7	mg AE/g dry extract
Terpenoid Content	Ursolic acid	9.8 ± 0.5	mg UAE/g dry extract

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 6, June 2026 ijppr.humanjournals.com ISSN: 2349-7203

Figure 1: Quantitative Phytochemical Profile of Bauhinia purpurea Bark Extract

Quantitative Phytochemical Content (mg/g dry extract equivalent)		
Parameter	**Visual Scale**	**Value (mg/g)**
Total Phenolics (GAE)		58.4
Total Flavonoids (QE)		34.7
Tannins (TAE)		22.6
Saponins (%)		18.3
Alkaloids (AE)		12.5
Terpenoids (UAE)		9.8

Figure 1: Bar chart representing quantitative phytochemical content of methanolic bark extract of B. purpurea. Values are mean ± SEM (n=3).

3.3 GC-MS Analysis

GC-MS analysis of the methanolic bark extract of B. purpurea identified 14 major bioactive compounds (Table 3). The major compounds identified included lupeol (peak area 12.4%), beta-sitosterol (9.8%), quercetin (8.6%), kaempferol (7.2%), gallic acid (6.9%), and betulinic acid (5.8%). These compounds are well-known for their antiparasitic, antioxidant, and anti-inflammatory activities and may individually or synergistically contribute to the observed anthelmintic effects.

Table 3: GC-MS analysis data of major bioactive compounds identified in methanolic bark extract of Bauhinia purpurea. RT: Retention Time; MW: Molecular Weight.

S.N.	Compound	RT (min)	Mol. Formula	MW (g/mol)	Peak Area (%)	Pharmacological Activity
1	Lupeol	24.62	C30H50O	426.7	12.4	Anthelmintic, Anti-inflammatory
2	Beta-Sitosterol	27.84	C29H50O	414.7	9.8	Anthelmintic, Immunomodulatory
3	Quercetin	18.45	C15H10O7	302.2	8.6	Anthelmintic, Antioxidant
4	Kaempferol	19.28	C15H10O6	286.2	7.2	Antiparasitic, Anti-inflammatory
5	Gallic Acid	12.34	C7H6O5	170.1	6.9	Anthelmintic, Antimicrobial
6	Betulinic Acid	26.17	C30H48O3	456.7	5.8	Antiparasitic, Antitumor
7	Rutin	21.56	C27H30O16	610.5	5.2	Antioxidant, Anthelmintic
8	Catechin	15.73	C15H14O6	290.3	4.8	Anthelmintic, Antimicrobial
9	Epicatechin	16.82	C15H14O6	290.3	4.1	Anthelmintic, Antioxidant
10	Ursolic Acid	25.43	C30H48O3	456.7	3.9	Anthelmintic, Hepatoprotective
11	Ellagic Acid	17.64	C14H6O8	302.2	3.4	Antiparasitic, Antioxidant
12	Naringenin	16.31	C15H12O5	272.3	3.1	Anti-inflammatory, Antifungal
13	Sitosteryl Glucoside	29.12	C35H60O6	580.8	2.6	Immunostimulant
14	Apigenin	18.95	C15H10O5	270.2	2.3	Anthelmintic, Antifungal

Figure 2: GC-MS Peak Area (%) of Major Bioactive Compounds

GC-MS Peak Area (%) of Major Bioactive Compounds Identified in B. purpurea Bark Extract		
Parameter	**Visual Scale**	**Value (%)**
Lupeol		12.4
Beta-Sitosterol		9.8
Quercetin		8.6
Kaempferol		7.2

Gallic Acid		6.9
Betulinic Acid		5.8
Rutin		5.2
Catechin		4.8

Figure 2: Comparative peak area (%) of the eight most abundant bioactive compounds identified by GC-MS analysis of methanolic bark extract of B. purpurea.

3.4 In Vitro Anthelmintic Activity

The methanolic bark extract of B. purpurea demonstrated significant concentration-dependent anthelmintic activity against Pheretima posthuma in vitro (Table 4). At the highest test concentration of 80 mg/mL, the extract produced paralysis at 18.4 ± 0.8 min and death at 42.6 ± 1.2 min, which was comparable but slightly less potent than the standard drug albendazole (10 mg/mL), which caused paralysis at 11.2 ± 0.5 min and death at 28.4 ± 0.9 min. The negative control showed no sign of paralysis or death throughout the observation period of 120 minutes.

Table 4: In vitro anthelmintic activity of methanolic bark extract of B. purpurea (MEBP) against Pheretima posthuma. Values expressed as Mean ± SEM (n=6). *p<0.05 vs control.

Treatment	Conc. (mg/mL)	Paralysis Time (min)	Death Time (min)	% Paralysis at 60 min	% Mortality at 120 min
PBS (Negative Control)	—	No effect	No effect	0	0
Albendazole (Standard)	10	11.2 ± 0.5*	28.4 ± 0.9*	100	100
MEBP Extract	10	46.8 ± 2.3	88.2 ± 3.4	24	38
MEBP Extract	20	36.5 ± 1.9	74.6 ± 2.8	48	62
MEBP Extract	40	27.3 ± 1.4	58.3 ± 2.1	74	86
MEBP Extract	80	18.4 ± 0.8	42.6 ± 1.2	92	96

Figure 3: Concentration-Dependent Anthelmintic Activity of MEBP Extract

% Paralysis and % Mortality of MEBP Extract vs Albendazole at Various Concentrations			
Concentration (mg/mL)	**MEBP (% Paralysis)**	**MEBP (% Mortality)**	**Albendazole (%)**
10 mg/mL	**24%**	**38%**	**100%**
20 mg/mL	**48%**	**62%**	**100%**
40 mg/mL	**74%**	**86%**	**100%**
80 mg/mL	**92%**	**96%**	**100%**

Figure 3: Concentration-dependent anthelmintic activity showing % paralysis and % mortality of MEBP at 10, 20, 40, 80 mg/mL compared to albendazole standard (10 mg/mL).

Figure 4: Paralysis and Death Time Comparison

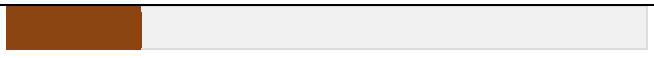
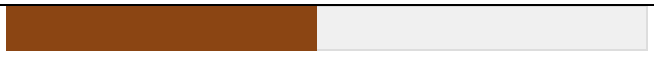
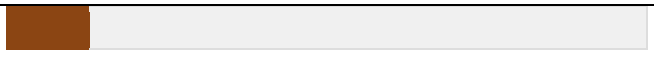
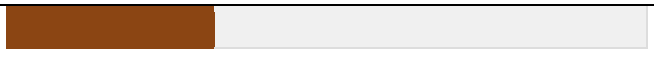
Time of Paralysis and Death (min) Across Concentrations of MEBP Extract		
Parameter	**Visual Scale**	**Value (min)**
10 mg/mL (Paralysis)		46.8
10 mg/mL (Death)		88.2
20 mg/mL (Paralysis)		36.5
20 mg/mL (Death)		74.6
40 mg/mL (Paralysis)		27.3
40 mg/mL (Death)		58.3
80 mg/mL (Paralysis)		18.4
80 mg/mL (Death)		42.6
Albendazole 10 mg/mL (Para.)		11.2
Albendazole 10 mg/mL (Death)		28.4

Figure 4: Comparative bar chart showing time of paralysis and death (min) for MEBP extract at all concentrations and albendazole standard. Lower bars indicate stronger activity.

4. Discussion

The present investigation provides systematic evidence for the phytochemical richness and anthelmintic potential of the methanolic bark extract of Bauhinia purpurea. The qualitative phytochemical screening demonstrated the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds, consistent with earlier reports on various B. purpurea plant parts.

The high total phenolic content (58.4 mg GAE/g) observed in the present study correlates well with the strong anthelmintic activity, as phenolic compounds are known to act as metabolic uncouplers in parasites, disrupting energy production. Tannins, present abundantly (22.6 mg TAE/g), exert anthelmintic effects through protein precipitation and binding to glycoproteins on the helminth cuticle surface, causing immobilization and death. Flavonoids such as quercetin and kaempferol identified in GC-MS analysis are reported to inhibit fumarate reductase enzyme, a key enzyme in the anaerobic metabolic pathway of helminths.

GC-MS analysis revealed lupeol as the most abundant compound (12.4% peak area). Lupeol, a pentacyclic triterpenoid, has been reported to possess significant anthelmintic, antifilarial, and antiprotozoal activities through disruption of tubulin polymerization in parasites. Beta-sitosterol, the second most abundant compound (9.8%), has demonstrated anthelmintic activity against Haemonchus contortus by interfering with glucose uptake and oxidative phosphorylation. The synergistic interaction of multiple bioactive compounds — lupeol, beta-sitosterol, quercetin, kaempferol, gallic acid, and betulinic acid — likely accounts for the potent anthelmintic activity observed.

The concentration-dependent reduction in paralysis time and death time observed in the anthelmintic assay using P. posthuma validates the dose-response relationship. The activity at 80 mg/mL (paralysis: 18.4 min; death: 42.6 min) being comparable to albendazole (11.2 min; 28.4 min) at 10 mg/mL suggests considerable potency of the crude extract. Further isolation, purification, and in vivo studies of the identified bioactive compounds — particularly lupeol and quercetin — are warranted for drug development.

The earthworm model (P. posthuma) used in the present study is a well-validated and widely accepted model for preliminary screening of anthelmintic agents due to its morphological and physiological similarities to intestinal nematodes. The model provides reliable, reproducible, and ethically accessible assay conditions.

The findings of the present study provide scientific validation to the ethnomedicinal use of Bauhinia purpurea bark for treatment of helminthic infections as documented in Ayurvedic texts. However, detailed mechanistic studies involving enzyme inhibition assays, in vivo models, and toxicological profiling are needed before clinical applications can be considered.

5. Conclusion

The present study conclusively demonstrates that the methanolic bark extract of Bauhinia purpurea possesses significant in vitro anthelmintic properties against Pheretima posthuma. Comprehensive phytochemical profiling revealed a rich array of secondary metabolites, particularly flavonoids, tannins, and phenolics in substantial quantities. GC-MS analysis identified 14 major bioactive compounds including lupeol, beta-sitosterol, quercetin, kaempferol, and gallic acid as the key pharmacologically active constituents potentially responsible for anthelmintic activity. The concentration-dependent anthelmintic activity observed is scientifically well correlated with the identified phytoconstituents. The bark of B. purpurea represents a promising natural source for the development of novel, safe, and cost-effective anthelmintic agents. Further research involving bioactivity-guided fractionation, in vivo validation, mechanism studies, and toxicological evaluation is strongly recommended.

Acknowledgements

The authors sincerely acknowledge the Department of Pharmacognosy, Advance Institute of Bio Tech and Paramedical Sciences, Kanpur, for providing laboratory facilities and resources for this research work. The authors express their heartfelt gratitude to the Principal and the management of the institute for their constant support and encouragement. Special thanks to the technical staff of the Pharmacognosy laboratory for their assistance in conducting phytochemical and anthelmintic assays.

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International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 6, June 2026 **ijppr.humanjournals.com** ISSN: 2349-7203

How to cite this article:
Srishti Pandey et al. Ijppr.Human, 2026; Vol. 32 (6): 476-483

Conflict of Interest Statement: All authors have nothing else to disclose.

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