J. Pharm. Res. 2026;25(4):246-251

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

ORIGINAL ARTICLE

Phytochemical Analysis and *In vitro* Evaluation of Anticoagulant and Anthelmintic Activities of Aqueous Extract of *Bougainvillea spectabilis* Willd. Flowers

Pasham Uma[1*], Cheruku Sowmya[2], Jatavath Srilatha[2], Guntala Bhavana[2], Guguloth Pavan Kalyan[2], Gujjula Varsha[2]

[1]*Assistant Professor, Department of Pharmacology, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District, Telangana, India*

[2]*Department of Pharmacology, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District, Telangana, India*

ARTICLE INFO

Article history:
Received 25-07-2026
Accepted 02-09-2026
Published 28-10-2026

** Corresponding author.*
Pasham Uma
pashamuma27@gmail.com

https://doi.org/10.18579/jopcr/v25.i4.161

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ABSTRACT

Medicinal plants are a very important source of bioactive chemicals that could be used to make safer alternatives to manufactured medications. *Bougainvillea spectabilis* Willd., commonly utilized in traditional medicine, has not been well investigated for its anticoagulant and anthelmintic effects. The current study aimed to analyse the phytochemical composition and examine the *Bougainvillea spectabilis* aqueous floral extract's (AEBSF) anthelmintic and anticoagulant qualities *in vitro*. Water maceration was used to extract the flowers after they were collected, checked, and shade-dried. The presence of pharmacologically relevant compounds, including flavonoids, tannins, saponins, alkaloids, glycosides, phenolic compounds, steroids, and sterols, was confirmed by preliminary phytochemical screening. By extending the prothrombin time to 303.01 ± 19.85 s, which was equivalent to the standard EDTA (323.00 ± 31.97 s), the aqueous flower extract of Bougainvillea spectabilis demonstrated strong anticoagulant action. At 20 and 40 mg/mL, the extract also demonstrated concentration-dependent anthelmintic action, causing Pheretima posthuma to become paralyzed and die, however this activity was marginally less than that of albendazole. These findings suggest that the extract possesses promising anticoagulant and anthelmintic properties due to its bioactive phytoconstituents.

Keywords: *Bougainvillea spectabilis* Willd; Anticoagulant activity; Anthelmintic activity; Phytochemical screening; Medicinal plants

INTRODUCTION

Medicinal plants have been essential to modern drug research efforts as well as traditional healthcare systems since ancient times. In order to prevent and treat a variety of disorders, traditional Chinese medicine, Ayurveda, Unani, Siddha, and other traditional medical systems mostly rely on medicinal plants as herbal medicines. About 80% of people in developing countries receive their primary medical care from traditional medicines, which are primarily made from plants, according to the World Health Organization (WHO)[1-4].

Among the important types of secondary metabolites present in plants with a range of pharmacological effects include alkaloids, flavonoids, tannins, saponins, glycosides,

terpenoids, phenolics, and steroids. These bioactive compounds have been demonstrated to have antioxidant, anti-inflammatory, antibacterial, antidiabetic, anticancer, anticoagulant, anthelmintic, and antiulcer properties. Rather than a single isolated molecule, the therapeutic efficacy of medicinal plants is frequently ascribed to the synergistic impact of many phytoconstituents[5-9].

The growing prevalence of parasitic infections and cardiovascular illnesses globally has drawn attention to the anticoagulant and anthelmintic properties of medicinal plants in recent years. Despite the effectiveness of synthetic anticoagulants and anthelmintic medications, their usage is frequently restricted by side effects, high cost, a limited therapeutic index, and the emergence of drug resistance. By preventing platelet aggregation, altering coagulation pathways, and lowering oxidative stress, plant-derived substances such flavonoids, coumarins, and tannins have demonstrated anticoagulant action. Similarly, anthelmintic activity is demonstrated by tannins and alkaloids, which cause paralysis and death by interfering with the energy metabolism and neuromuscular function of parasites[10-19].

MATERIALS AND METHODS

Collection of *Bougainvillea spectabilis* Willd. Flowers

Fresh flowers of *Bougainvillea spectabilis* Willd. were collected on 19 November 2025 from healthy, mature, disease-free plants growing in local areas of Hyderabad, Telangana, India. The collected plant material consisted of fresh flowers.

The plant material was authenticated by Dr. A. Vijaya Bhaskar Reddy, Assistant Professor, Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana, India, based on macroscopic examination followed by detailed scrutiny of the submitted plant specimen. The specimen was identified as *Bougainvillea spectabilis* Willd., belonging to the family Nyctaginaceae. The authentication was documented under Voucher No. OUAS-320, with the authentication certificate dated 21 November 2025. The authenticated voucher specimen was retained for future reference.

Bougainvillea spectabilis Willd. aqueous flower extract preparation.

To get rid of dust and contaminants, fresh *Bougainvillea spectabilis* Willd. Flowers were thoroughly cleaned with tap water and then distilled water. To maintain its phytoconstituents, the cleaned plant material was shade-dried at room temperature until a stable weight was achieved. After drying, the material was ground into a coarse powder using a mechanical grinder and kept in an airtight container protected from moisture and light.

To extract the water-soluble phytoconstituents, a determined amount of the powdered material was combined with distilled water in a 1:10 (w/v) ratio and macerated for 24–48 hours, with occasional stirring. The extract was filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated on a water bath at a temperature below 50°C to obtain a semisolid extract. Before being used in further experiments, the concentrated extract was cooled and stored at 4°C in a tightly sealed container[20-22].

Percentage Yield of Plant Extract

The percentage yield of the aqueous plant extract was determined after concentration. Fifty grams (50 g) of dried plant material yielded 10.05 g of extract, corresponding to a percentage yield of 20.10%.

Preliminary phytochemical screening

In order to determine whether tannins, glycosides, saponins, flavonoids, terpenoids/steroids, and alkaloids were present in the aqueous flower extract of *Bougainvillea spectabilis* Willd., a preliminary phytochemical investigation was carried out using standard qualitative chemical tests.

In vitro anticoagulant activity

The in vitro anticoagulant activity was evaluated using a plasma recalcification (clotting-time) assay rather than a conventional Prothrombin Time (PT) assay. The assay was performed by recalcifying citrated platelet-poor plasma with calcium chloride, and the time required for visible clot formation was recorded.

Collection of blood sample

A healthy human volunteer was venipuncture under aseptic conditions to obtain fresh venous blood using sterile disposable syringes. To avoid spontaneous coagulation, the blood sample was promptly transferred into sterilized tubes containing 3.8% trisodium citrate as an anticoagulant in a blood-to-anticoagulant ratio of 9:1[23-26].

Preparation of platelet-poor plasma (PPP)

The citrated blood samples were centrifuged at 3000 rpm for 15 minutes at room temperature. Following centrifugation, the supernatant plasma layer was carefully separated using a micropipette to obtain platelet-poor plasma (PPP) for the subsequent plasma recalcification/clotting-time assay[27].

Storage of plasma samples

The separated plasma samples were transferred into clean, labelled tubes and maintained at room temperature. The samples were used within the recommended time frame to avoid deterioration of clotting factors.

Preparation of test mixture

For the evaluation of anticoagulant activity, 0.2 mL of platelet-poor plasma and 0.1 mL of the plant extract at various doses were added to clean test tubes. The mixture was gently shaken to ensure uniform distribution of the extract in the plasma.

Initiation of clotting reaction

The clotting reaction was initiated by recalcification of the citrated plasma. For this purpose, 0.1 mL of 25 mM calcium chloride ($CaCl_2$) solution was added to the plasma-extract mixture. The addition of calcium chloride restored the calcium required for the coagulation process and initiated clot formation.

Incubation: The reaction mixtures were incubated in a water bath maintained at 37°C to provide physiological temperature conditions during the clotting reaction.

Control test and standard preparation

For the control group, 0.2 mL of platelet-poor plasma was mixed with 0.1 mL of 0.9% normal saline, followed by the addition of 0.1 mL of 25 mM calcium chloride solution to initiate clot formation.

For the standard group, 0.2 mL of platelet-poor plasma was mixed with 0.1 mL of ethylenediaminetetraacetic acid (EDTA) as the reference anticoagulant, followed by the addition of 0.1 mL of calcium chloride solution.

For the test groups, 0.2 mL of platelet-poor plasma was mixed with 0.1 mL of aqueous floral extract of *Bougainvillea spectabilis* Willd. at the respective concentrations, followed by the addition of 0.1 mL of calcium chloride solution to initiate clot formation. All control, standard, and test mixtures were subjected to the same experimental conditions.

Observation and recording of clotting time

After the addition of calcium chloride, the reaction mixtures were maintained at 37°C. The test tubes were gently tilted at 5-second intervals to observe the formation of a visible clot. The time required for visible clot formation was recorded using a stopwatch and considered the clotting time.

The anticoagulant activity of the aqueous floral extract was assessed by comparing the clotting time of the extract-treated samples with that of the control. Prolongation of clotting time compared with the control was considered indicative of anticoagulant activity. Each experimental condition was evaluated in appropriate replicates.

***In vitro* anthelmintic activity**

Selection of test organism

Pheretima posthuma adult Indian earthworms were chosen as the model for the experiment because they resemble human intestinal helminths both anatomically and physiologically.

Collection and cleaning of earthworms

Healthy earthworms of approximately uniform size were collected and washed thoroughly with normal saline solution to remove soil particles and other impurities before experimentation.

Preparation of test solutions

• *Bougainvillea spectabilis* aqueous extract was dissolved in distilled water to produce concentrations of 10, 20, and 40 mg/ml.

• Albendazole (20 mg/mL) served as the standard reference medication.

• The control was normal saline.

Grouping and exposure

Each group of earthworms was made up of six worms. Separate Petri dishes with 20 mL of the corresponding treatment solutions were used for each group.

Adult *Pheretima posthuma* earthworms were randomly assigned to five groups of six earthworms each for the assessment of anthelmintic activity.

- **Group I (Control):** Received 0.9% sodium chloride (normal saline).
- **Group II (Standard):** Treated with Albendazole at a concentration of 10 mg/ml.
- **Group III (Test I):** Treated with aqueous extract of *Bougainvillea spectabilis* flowers (AEBSF) at 10 mg/ml.
- **Group IV (Test II):** Treated with 20 mg/ml of Bougainvillea spectabilis flower aqueous extract (AEBSF).
- **Group V (Test III):** Treated with aqueous extract of *Bougainvillea spectabilis* flowers (AEBSF) at 40 mg/ml.

Assessment of paralysis and death time

The time required for paralysis was recorded when worms ceased movement even upon stimulation with normal saline. The time required for death was recorded when worms showed complete immobility accompanied by fading of body colour[28].

Evaluation of anthelmintic activity

Anthelmintic activity was assessed by comparing the paralysis and death times of treated groups with those of

control and standard groups. A decrease in paralysis and death time relative to control was considered indicative of significant anthelmintic activity.

RESULTS AND DISCUSSION

Table 1: Phytochemical Analysis of the Aqueous Flower Extract of *Bougainvillea spectabilis* Willd.

S. No	Phytochemical constituents	Result
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Carbohydrates	+
5	Tannins	+
6	Phenolic compounds	+
7	Proteins & amino acids	+
8	Sterols	+
9	Saponins	–

(+/Present), (-/Absent)

Preliminary phytochemical screening of the aqueous flower extract of *Bougainvillea spectabilis* Willd. revealed the presence of alkaloids, flavonoids, glycosides, carbohydrates, phenolic compounds, tannins, proteins and amino acids, and sterols, whereas saponins were not detected (Table. 1). These findings indicate the presence of diverse classes of phytoconstituents in the aqueous extract. However, the screening was qualitative and preliminary; therefore, the individual compounds responsible for the biological activities observed in the present study cannot be established. Further chromatographic and spectroscopic characterization would be required to identify and quantify the constituents of the extract.

Table 2: *In Vitro* Anticoagulant Activity of the Aqueous Flower Extract of *Bougainvillea spectabilis* Willd. on Human Platelet-Poor Plasma (Clotting Time Method)

S. No	Group	Prothrombin Time test (Sec)
1	I (Control)	0±
2	II (Standard EDTA)	323.0 ± 31.97
3	III (Test AEBSF)	303.01 ± 19.85

Data are shown as mean ± SEM (n=3), AEBSF- Aqueous extract of *Bougainvillea spectabilis* flowers.

The aqueous flower extract of *Bougainvillea spectabilis* Willd. produced an increase in clotting time in the *in-vitro* plasma recalcification/clotting-time assay (Table. 2). The EDTA-treated group showed a clotting time of 323.0 ± 31.97 s, whereas the extract-treated group (AEBSF) showed a clotting time of 303.01 ± 19.85 s. Compared with the control group, the prolonged clotting time observed with the extract indicates preliminary anticoagulant activity under the experimental conditions employed (mean ± SEM, n = 3).

The present assay demonstrates an effect of the extract on plasma clot formation but does not determine the precise coagulation factor or pathway responsible for the observed effect. Therefore, the findings should not be interpreted as evidence of a specific molecular anticoagulant mechanism. Further studies using standardized coagulation assays, including pathway-specific tests and individual clotting-factor investigations, are necessary to clarify the basis of the observed activity.

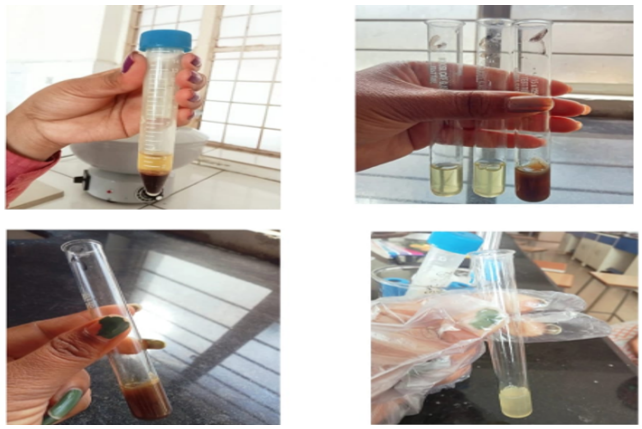


Fig. 1: Photographic Representation of the Anticoagulant Effect in Human Plasma

Table 3: *In Vitro* Anthelmintic Activity of the Aqueous Flower Extract of *Bougainvillea spectabilis* Willd. Based on Paralysis Time

S. No	*Groups*	Time of Paralysis (min)
1	Group I (Control)	0
2	Group II (Albendazole 10mg/ml)	87.66±1.45
3	Group III (AEBSF-10mg/ml)	0
4	Group IV (AEBSF 20mg/ml)	119.00±7.8
5	Group v (AEBSF 40mg/ml)	95.01±2.86

Above data are shown as mean ± SEM (n = 6), AEBSF-Aqueous extract of *Bougainvillea spectabilis* flowers.

The *in-vitro* anthelmintic activity of the aqueous flower extract of *Bougainvillea spectabilis* Willd. was assessed using *Pheretima posthuma* by recording the time required to induce paralysis (Table. 3). No paralysis was observed in the control group during the experimental observation period. Albendazole (10 mg/mL), used as the reference drug, produced paralysis at 87.66 ± 1.45 min.

The extract did not produce paralysis at 10 mg/mL. At 20 mg/mL, paralysis occurred at 119.00 ± 7.80 min, while at 40 mg/mL, paralysis was observed at 95.01 ± 2.86 min. The

observed activity at 20 and 40 mg/mL indicates that the aqueous extract affected the motility of *P. posthuma* under the experimental conditions. However, the response was not strictly proportional to concentration, as the paralysis time at 40 mg/mL was shorter than that observed at 20 mg/mL. This variation may reflect differences in the response of the test organisms or the composition of the crude extract and should therefore be interpreted cautiously.

Table 4: *In Vitro* Anthelmintic Activity of the Aqueous Flower Extract of *Bougainvillea spectabilis* Willd. Based on Death Time

S. No.	Groups	Time of Death (min)
1	Group I (Control)	0
2	Group II (Albendazole 10mg/ml)	145.08±2.88
3	Group III (AEBSF-10mg/ml)	0
4	Group IV (AEBSF 20mg/ml)	134.09±7.02
5	Group v (AEBSF 40mg/ml)	1.6±2.10.92

The AEBSF-Aqueous extract of Bougainvillea spectabilis flowers is displayed above as mean ± SEM (n = 6).



Fig. 2: Photographic Representation of Paralysis and Mortality in Earthworms During the *In-Vitro* Anthelmintic Assay

The anthelmintic effect of the aqueous flower extract was further evaluated by recording the time required to produce mortality in *Pheretima posthuma* (Table. 4). No mortality was observed in the control group during the observation period. Albendazole (10 mg/mL) produced mortality at 145.08 ± 2.88 min.

The extract at 10 mg/mL did not produce mortality, whereas the 20 mg/mL concentration produced mortality at 134.09 ± 7.02 min. For the 40 mg/mL group, the recorded death-time value was reported as 1.6 ± 2.10.92 min in the experimental data. This value appears to contain a typographical or transcription error and should be verified against the original experimental record before publication.

Overall, the findings demonstrate preliminary *in-vitro* anthelmintic activity of the aqueous flower extract at the tested concentrations. Because the study was performed using a crude extract and an *in-vitro* earthworm model, the results cannot by themselves establish efficacy against parasitic helminths in biological systems.

CONCLUSION

The present study provides preliminary *in-vitro* evidence of anticoagulant and anthelmintic activities of the aqueous flower extract of *Bougainvillea spectabilis* Willd. The extract increased clotting time in the human platelet-poor plasma clotting assay and produced paralysis and mortality in *Pheretima posthuma* at selected concentrations.

Preliminary phytochemical screening demonstrated the presence of several classes of phytoconstituents, including alkaloids, flavonoids, glycosides, phenolic compounds, tannins, proteins and amino acids, and sterols. However, the present findings do not establish which specific constituents are responsible for the observed biological effects, nor do they confirm the underlying mechanisms of action.

The results should therefore be interpreted as preliminary findings obtained under specific *in-vitro* experimental conditions rather than as evidence of therapeutic efficacy. Further investigations involving detailed phytochemical characterization, isolation and identification of active constituents, standardized and pathway-specific coagulation assays, mechanistic studies, appropriate toxicity assessment, and *in-vivo* evaluation are warranted to determine the reproducibility, biological relevance, and potential applications of the observed activities.

Acknowledgments

We express our sincere gratitude to the principal and staff of Malla Reddy College of Pharmacy for their constant support, valuable guidance, and cooperation throughout this work.

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