WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
ISSN 2278 – 4357 Impact Factor 8.025
Volume 14, Issue 11, 589-597 Research Article Coden USA: WJPPAJ

PHYTOCHEMICAL SCREENING, ANTHELMINTIC, AND ANTIMITOTIC ACTIVITIES OF AQUEOUS AND ETHANOLIC EXTRACTS OF *PERESKIA BLEO* LEAVES

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Article Received on 01 Oct. 2025,
Article Revised on 21 October 2025,
Article Published on 01 Nov. 2025

https://doi.org/10.5281/zenodo.17473592

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How to cite this Article: Vennila V*, Deepa B and Senbaga Priya R (2025). Phytochemical Screening, Anthelmintic, And Antimitotic Activities Of Aqueous And Ethanolic Extracts Of Pereskia Bleo Leaves. World Journal of Pharmacy and Pharmaceutical Sciences, 14(11), 589–597.

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ABSTRACT

Background: The present research investigates the phytochemical composition and evaluates the *In-vitro* anthelmintic and antimitotic activities of aqueous and ethanolic extracts of *Pereskia bleo* leaves. Belonging to the family Cactaceae, *P. bleo* has long been used in traditional medicine for treating hypertension, inflammation, and various infections. **Materials and Methods:** In this study, qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, glycosides, saponins, and coumarins in both extracts, suggesting significant bioactive potential. The *In-vitro* anthelmintic activity was evaluated using *Pheretima posthuma* earthworms, with Albendazole as the standard reference drug. The antimitotic activity was assessed through a seed germination inhibition assay using Vigna radiata (green gram). **Results**: The results demonstrated that both aqueous and ethanolic extracts showed dose-dependent anthelmintic and antimitotic activity, with the ethanolic extract exhibiting stronger efficacy. The study validates the traditional use of *P. bleo* and indicates its potential as a natural source of anthelmintic and cytostatic agents.

KEYWORDS: *Pereskia bleo*, phytochemical screening, anthelmintic activity, antimitotic activity, *Pheretima posthuma*, Albendazole, *Vigna radiata*.

1. INTRODUCTION

Medicinal plants continue to be an indispensable source of therapeutic agents for humankind. The World Health Organization estimates that more than 80% of the global population depends on plant-based traditional medicine for primary health care. Plants are known to produce an enormous variety of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, glycosides, terpenoids, and saponins. These compounds often exert potent biological effects, including antimicrobial, antioxidant, anti-inflammatory, and antiparasitic activities.[1]

Helminth infections are among the most common infections affecting humans and animals, particularly in tropical and subtropical regions.[2] These infections lead to malnutrition, impaired physical growth, anemia, and poor cognitive development. Chemical anthelmintics such as Albendazole and Mebendazole are widely used, but frequent use has resulted in increasing resistance among parasites and adverse side effects in humans.[3] This has stimulated renewed interest in exploring safe, plant-derived anthelmintic agents that can be effective and affordable.

Cancer is one of the leading causes of mortality worldwide and remains a major public health challenge. It is characterized by uncontrolled cell division resulting from the failure of normal regulatory mechanisms of the cell cycle.[4] The search for novel anticancer agents that can selectively inhibit cell division without affecting normal cells is a key focus of current biomedical research. Mitotic division is a critical stage of the cell cycle during which chromosomes are evenly distributed to daughter cells. Disruption of this process can arrest cell proliferation, leading to apoptosis or cell death.[5] Agents that inhibit or delay mitosis are termed antimitotic compounds, and they play a vital role in cancer chemotherapy. Classical examples include vinca alkaloids and taxanes, which target microtubule dynamics and prevent spindle formation.[6]

In recent years, increasing attention has been given to plant-derived natural products as potential sources of antimitotic and anticancer compounds. Medicinal plants contain diverse bioactive phytochemicals, such as alkaloids, flavonoids, terpenoids, tannins, and phenolics, which can interfere with mitotic spindle formation or chromosomal segregation.[7] These compounds are considered safer, more biocompatible, and less toxic compared to synthetic chemotherapeutic agents.[8]

Pereskia bleo, a leafy cactus belonging to the family Cactaceae, is an ornamental and medicinal plant native to tropical America and cultivated widely across Southeast Asia. Traditionally, the leaves have been used as herbal remedies for hypertension, ulcers, inflammation, and detoxification of the body.[9] Reports indicate that the plant contains various phytochemicals such as carotenoids, ascorbic acid, alkaloids, and polyphenols, which could be responsible for its pharmacological actions.[10] However, limited data are available on its anthelmintic and mitotic inhibitory potential. The present study was designed to perform qualitative phytochemical screening of aqueous and ethanolic extracts of *P. bleo* leaves. Evaluate the *In-vitro* anthelmintic activity of these extracts using *Pheretima posthuma* as the test organism. Assess the *In-vitro* antimitotic activity using the green gram seed germination inhibition assay. This research aims to scientifically substantiate the traditional medicinal claims associated with *P. bleo* and identify its potential for pharmaceutical applications.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

Fresh, mature leaves of *P. bleo* were collected from a cultivated region in Thiruvananthapuram, Kerala. The plant was authenticated by a qualified botanist, and a voucher specimen was deposited in the herbarium for future reference. The leaves were washed thoroughly with tap water to remove dust and debris, then shade-dried for seven days until crisp. Dried leaves were ground to a fine powder using a laboratory mill and stored in airtight containers until extraction.

2.2. Preparation of Extracts

Approximately 50 g of powdered leaves were extracted separately using 500 ml of ethanol and distilled water through the maceration method. The mixtures were kept at room temperature for 48 hours with intermittent shaking. The extracts were filtered using Whatman No.1 filter paper, and the filtrates were concentrated using a rotary evaporator under reduced pressure at 45°C. The semisolid residues obtained were stored at 4°C until further use.

2.3. Phytochemical Screening

Preliminary phytochemical analysis was carried out using standard qualitative procedures described by Harborne (1998)[11] and Sofowora (1993)[12] to determine the presence of various classes of secondary metabolites.

2.4. *In-vitro* Anthelmintic Activity

The *In-vitro* anthelmintic activity was evaluated using adult *Pheretima posthuma* earthworms, as they share physiological resemblance to human intestinal nematodes. The worms were collected from moist soil and washed with normal saline to remove adhering debris. The assay was performed according to the method described by Ajaiyeoba *et al.* (2001).[13] Different concentrations (10, 20, 30, 40, and 50 mg/ml) of aqueous and ethanolic extracts were prepared in distilled water. Albendazole (10 mg/ml) served as the reference standard, while distilled water acted as the control. Six worms were introduced into each Petri dish containing 20 ml of the respective test solutions. Observations were made for the time taken for paralysis (no movement even after vigorous shaking) and death (no movement even after immersion in warm water at 50°C). Results were recorded as mean ± SD.

2.5. *In-vitro* Antimitotic Activity

The antimitotic activity was assessed by the green gram (*Vigna radiata*) seed germination inhibition assay. This method simulates the inhibition of mitosis in dividing cells.[14] Healthy seeds were surface-sterilized with 1% sodium hypochlorite and soaked in distilled water for uniform hydration. Then, seeds were transferred to Petri dishes lined with moist filter paper containing varying concentrations (10, 20, 30, 40, and 50 mg/ml) of aqueous and ethanolic extracts. Methotrexate (10 mg/ml) served as the positive control, and distilled water was used as the negative control. The Petri dishes were incubated at room temperature, and the sprout length of each seed was measured after 24, 48, and 72 hours. The inhibition percentage was calculated using:

Inhibition (%) = Lc – Lt / Lc X 100

where Lc is the mean length of control sprouts and Lt is the mean length of treated sprouts.

3. RESULTS

3.1. Phytochemical Screening

The results of the phytochemical screening (Table 1) revealed the presence of several important secondary metabolites in both extracts. Alkaloids, saponins, phenols, flavonoids, tannins, glycosides, coumarins, and proteins were present in both extracts, whereas phlobatannins were absent in both. Anthocyanins and quinones were detected in trace amounts in the ethanolic extract but absent in the aqueous extract. The presence of these compounds indicates potential antioxidant, antimicrobial, and antiparasitic properties.

Table 1: Screening of phytochemicals in aqueous and ethanolic extracts of *P. bleo* leaves.

S.No	Phytochemical	Test	Observation	Ethanolic extract	Aqueous extract
1	Alkaloids	Mayer's	Green colour	+	+
2	Steroids	Salkowski	Red colour	+	+
3	Saponins	Froth	Honeycomb	+	+
4	Phenols	Ferric chloride	Deep blue-black	+	+
5	Flavonoids	Lead acetate	White precipitate	+	+
6	Tannins	Braymer's	Blue-green	+	+
7	Phlobatannins	Phlobatannin	Red precipitate	–	–
8	Proteins	Xanthoprotein	Green colour	+	+
9	Glycosides	Liebermann's	Green colour	+	+
10	Coumarins	NaOH	Yellow colour	+	+
11	Anthocyanins	HCl	Red turns violet	+	–
12	Quinones	H_2 SO_4	Red colour	+	–

(+): Present; (–): Absent.

3.2. Anthelmintic Activity

Both aqueous and ethanolic extracts exhibited dose-dependent anthelmintic activity (Tables 2). The ethanolic extract displayed superior efficacy compared to the aqueous extract.

Table 2: *Invitro* anthelmintic activity of aqueous and ethanolic extracts of *P.bleo* leaves against *Pheretima posthuma.*

Test substance	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control	distilled water	-	-
Standard (Albendazole)	10	24.6± 2.16	34.6± 2.48
Aqueous extract of *P.bleo*	10	21.3± 1.18	31.6± 2.68
	20	23.6± 1.78	34.3± 1.77
	30	25.6± 1.90	29.6± 2.48
	40	29.6± 1.64	32.6± 2.16
	50	33.7± 1.40	35.3± 2.13
Ethanolic extract of *P.bleo*	10	25.3± 2.94	16.6± 6.97
	20	24.6± 3.34	32.3± 2.48
	30	27.3± 2.02	31.3± 1.78
	40	32.6± 2.48	35.6± 1.35
	50	35.3± 2.13	37.3± 1.23

Values expressed as Mean±S.D

At 50 mg/ml concentration, the ethanolic extract caused paralysis at 35.3 ± 2.13 minutes and death at 37.31 ± 1.23 minutes, which was comparable to Albendazole (34.6 ± 2.48 min). The aqueous extract also showed significant activity but required slightly longer times for paralysis and death. The observed anthelmintic effect may be attributed to the presence of tannins and alkaloids, which interfere with the worm's energy metabolism and disrupt neuromuscular coordination.

3.3. Antimitotic Activity

Both extracts inhibited seed germination in a concentration-dependent manner. The ethanolic extract showed higher inhibition compared to the aqueous extract, indicating a stronger effect on cell division. At 10 mg/ml, the ethanolic extract exhibited 100% inhibition in sprout growth, while the aqueous extract showed 100% inhibition at the concentration of 30 mg/ml relative to the control(Table 3). Length of sprout measured in mm.

Table 3: *Invitro* antimitotic activity of aqueous and ethanolic extracts of *P.bleo* leaves.

Test substance	Concentration (mg/ml)	Length of Sprout (mm)		
		Day 1	Day 2	Day 3
Control	distilled water	5.6± 3.45	15.9± 9.75	57..6± 2.27
Standard (Methotrexate)	10	-	-	-
Aqueous extract of *P.bleo*	10	-	9.6± 2.48	32.33± 16.86
	20	-	6.3± 3.56	25.6± 9.41
	30	-	-	-
	40	-	-	-
	50	-	-	-
Ethanolic extract of *P.bleo*	10	-	-	-
	20	-	-	-
	30	-	-	-
	40	-	-	-
	50	-	-	-

Values expressed as Mean±S.D

This inhibition may be due to alkaloids and flavonoids interfering with spindle formation and chromosomal segregation during mitosis. Such effects are characteristic of compounds possessing cytostatic or anticancer potential.

4. DISCUSSION

The phytochemical analysis of *P. bleo* reveals a rich composition of secondary metabolites, which are likely responsible for its biological activities. The presence of flavonoids, alkaloids, and tannins suggests that the extracts possess strong antioxidant, antimicrobial, and antiparasitic effects. Flavonoids are known to inhibit oxidative stress and disrupt metabolic enzymes in parasites, while tannins bind to cuticular glycoproteins, leading to structural damage in helminthes.[15]

The *In-vitro* anthelmintic results confirm that both extracts are effective against *Pheretima posthuma*, with potency increasing in a concentration-dependent manner. The ethanolic extract's enhanced performance could be due to the better extraction of non-polar bioactive compounds. Similar results have been observed with other medicinal plants like *Azadirachta indica* and *Mimosa pudica*, which also contain tannins and flavonoids contributing to anthelmintic properties.[16]

The antimitotic assay demonstrated that *P. bleo* extracts can inhibit seed sprouting, reflecting their capacity to suppress cell division. The ethanolic extract again showed superior inhibition, indicating the presence of compounds that may disrupt microtubule polymerization or DNA synthesis. These findings align with previous studies that reported cytotoxic and antiproliferative activity of *P. bleo* against various cancer cell lines.[17]

Overall, the study provides scientific support for the ethnomedicinal use of *P. bleo* and establishes a foundation for further phytochemical isolation, *in-vivo* pharmacological testing, and mechanistic studies.

5. CONCLUSION

The present study concludes that both aqueous and ethanolic extracts of *P. bleo* leaves possess significant phytochemical constituents responsible for their pharmacological potential. The ethanolic extract exhibited better anthelmintic and antimitotic activity than the aqueous extract, suggesting that ethanol is a more efficient solvent for extracting active compounds. The results validate the traditional use of *P. bleo* in the treatment of parasitic infections and support its potential role in anticancer drug discovery. Further isolation and structural elucidation of active constituents are recommended to determine their specific mechanisms of action and therapeutic safety.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the PG and Research Department of Biochemistry, D.G. Govt. Arts College for Women, Mayiladuthurai, for providing the necessary facilities, guidance, and support to carry out this research work successfully. The authors also express their gratitude to the Principal and all faculty members of the Department for their constant encouragement and valuable assistance throughout the study.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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