Panacea Journal of Pharmacy and Pharmaceutical Sciences
ISSN: 2349 7025

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Original Research Article **Volume 15 Issue 3** **July-Sept 2026**

QUALITATIVE AND QUANTITATIVE STUDY OF PHYTOCHEMICALS AND CNS ACTIVITY OF *BAUHINIA VAHLII* LEAF EXTRACT

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ABSTRACT

The present study investigated the phytochemical profile and anxiolytic-like activity of the hydroalcoholic extract of *Bauhinia vahlii*. The hydroalcoholic extraction yielded 14.5% w/w of crude extract, obtained as a dark-brown solid. Preliminary phytochemical screening revealed the presence of carbohydrates and proteins as primary metabolites, along with diterpenes, saponins, flavonoids, and tannins/phenolic compounds as secondary metabolites. Quantitative estimation demonstrated total phenolic content of 0.721 and total flavonoid content of 0.983. The anxiolytic-like activity of the extract was evaluated using the elevated plus-maze test in mice. Compared with the vehicle control, treatment with *B. vahlii* extract at doses of 100 and 200 mg/kg, p.o., increased the time spent in the open arms to 164.85 ± 3.25 and 174.28 ± 3.70 sec, respectively, while reducing the time spent in the closed arms to 112.36 ± 1.35 and 90.85 ± 1.20 sec. Diazepam (1 mg/kg, p.o.) produced a marked increase in open-arm exploration, with 180.62 ± 4.10 sec spent in the open arms. The higher dose of the extract produced a greater behavioral response, suggesting a dose-related anxiolytic-like effect. The observed activity may be associated with the flavonoids, phenolic compounds, saponins, and diterpenes identified in the extract. Overall, the findings indicate that the hydroalcoholic extract of *B. vahlii* possesses appreciable

phytochemical content and anxiolytic-like activity, supporting its potential for further pharmacological investigation.

Keywords: *Bauhinia vahlii*, hydroalcoholic extract, phytochemical screening, flavonoids, phenolic compounds, elevated plus-maze, anxiolytic activity.

Introduction

Skin diseases are among the most common health problems affecting individuals worldwide and include a wide range of inflammatory, infectious, allergic, and degenerative conditions (Olayemi and David, 2023). Disorders such as acne, dermatitis, eczema, fungal infections, and other inflammatory skin conditions can significantly affect the physical and psychological well-being of affected individuals (Basra and Shahrukh, 2009). Although conventional topical preparations are widely used for the management of skin disorders, prolonged use of certain synthetic agents may be associated with adverse effects such as irritation, dryness, hypersensitivity, and other local reactions (Akhavan and Bershad, 2003). Consequently, increasing attention has been directed toward the development of plant-based topical formulations that may provide therapeutic benefits with improved tolerability.

Medicinal plants have been traditionally used for the management of various skin ailments because of their diverse bioactive constituents, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, and saponins (Bittner Fialová et al., 2021). These phytoconstituents may exhibit antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties, making medicinal plants valuable candidates for the development of herbal dermatological preparations. The incorporation of plant extracts into suitable topical dosage forms can facilitate their application to the affected site and may enhance patient acceptability and therapeutic effectiveness (Bittner Fialová et al., 2021).

Uraria picta is a medicinal plant belonging to the family Fabaceae and has been traditionally employed in various systems of herbal medicine. Different parts of the plant have been reported to contain biologically active phytoconstituents that may contribute to its pharmacological properties (Gogoi and Rana, 2022). The aerial parts of *U. picta* are of particular interest as a potential source of phytochemicals with

antioxidant, antimicrobial, and anti-inflammatory activities. However, systematic formulation and characterization of its aerial-part extract into a suitable topical gel for skin-related applications remains comparatively limited (Saxena et al., 2014).

Herbal gels offer several advantages as topical drug delivery systems, including ease of application, good spreadability, non-greasy characteristics, rapid drug release, and improved patient compliance (Sabry et al., 2025). A gel formulation containing *U. picta* aerial-part extract may therefore provide a convenient approach for delivering its bioactive constituents directly to the skin. Therefore, the present study was undertaken to prepare and characterize a herbal gel containing the hydroalcoholic extract of the aerial parts of *Uraria picta*. The prepared extract was subjected to preliminary phytochemical evaluation, and the formulated herbal gel was evaluated for physicochemical characteristics, including appearance, homogeneity, pH, viscosity, spreadability, extrudability, drug/extract content, and in-vitro performance. The study was aimed at exploring the potential of *U. picta* aerial parts as a plant-based topical preparation for the management of skin diseases.

Material and Methods

Methods

Extraction using microwave assisted extraction technique

The shade dried leaves of *Bauhinia vahlii* were coarsely powdered and subjected to extraction. Plant material extracted by hydroalcoholic solvent (Ethanol: water; 75:25v/v) was used. Followed by drying, powders of plant material were prepared using mixer grinder and then 70 gram powder for extraction was carried out by microwave assisted extraction technique [79]. Then extract were centrifuged at 7000 rpm for 10min. Supernatant was collected in petriplates and solvent was allowed to evaporate room temperature. Powder was scrapped from the petriplates from which the solvent has been evaporated (Harborne; 1998).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater

amount of product after extraction (Mukherjee; 2007). The % yield was calculated by using formula:

% yield = [(weight of dried extract) / (weight of dried plant sample)] x 100

Phytochemical screening

Plants generate compounds known as phytochemicals. These are created by the primary and secondary metabolisms of the plant. These phytochemicals are necessary for plants to survive or to fend off other plants, animals, insects, microbial pests, and pathogens. They also protect plants from illness and damage induced by environmental threats such as pollution, UV, stress, and drought. They have been employed as traditional medicine and as poisons since ancient times (Kokate; 1994).

Quantitative estimation of phenols and flavonoids

Estimation of total phenolic content

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample (Parkhe et al., 2019).

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared[82]. 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A

standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance (Parkhe et al., 2019).

Central nervous system activity of leaves extract of *Bauhinia vahlii*

Animals

Swiss albino mice were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Mice were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. A separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Toxicity studies were carried out by OECD guidelines, acute oral toxicity study of *Bauhinia vahlii* extract. Acute toxicity study was performed based on OECD guideline no. 423. The mice were assessed for signs of toxicity throughout the next 14 days. *Bauhinia vahlii* extract was given orally by the safe dose. Clinical symptoms like behavioural alterations, changes in the eyes, body weight, skin and fur were noted (Gilani et al., 2022; Kazmi et al., 2023)

Experiential design

Group 1: Received Normal control saline

Group 2: Received 1 mg/kg diazepam orally (Standard)

Group 3: Received 100 mg/kg of *Bauhinia vahlii* extract

Group 4: Received 200 mg/kg of *Bauhinia vahlii* extract

Elevated plus-maze test

The Elevated plus-maze comprised two open (25cm × 5 cm) and two enclosed (25cm × 5 cm×16 cm) arms that radiated from the central plate form (5cm × 5 cm) to form a plus sign. The maze was constructed of black acrylic sheet. The plus maze was elevated to a

height of 50 cm above from the floor level by a single central support. All the four arms consist of infra-red beams fitted at regular distance. The experiment was conducted during the dark phase of the light cycle (9:00 – 14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. During the 5 min experiment, the behavior of mice was recorded as (i) preference of the mice for its first entry into the open and closed arms, (ii) the numbers of entries into the open or closed arms, and (iii) time spent by the mice in each of the arms. The mice was considered to have entered an arm when and four paws were on the arm. The apparatus was cleaned thoroughly between trails with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment of the mice had received (Yoshizaki et al., 2020).

Staircase test

The testing apparatus is a white polyvinyl chloride (PVC) enclosure containing a five-step staircase with uniform dimensions (2.5 cm x 10 cm x 7.5 cm). The enclosure maintains a consistent internal height throughout the staircase. Importantly, each animal undergoes the test only once. The test substance is administered either 60 or 30 minutes before behavioral evaluation. The animal is placed in the arena facing away from the staircase, and the number of times it climbs the steps and the number of times it rears on its hind legs are recorded during a 3-minute observation period. To ensure an accurate assessment of climbing behavior, a stringent criterion is employed. For a step to be considered ascended, the subject must place all four paws on the designated step surface. Data are normalized to the control group, with step count and rearing (Simiand et al., 1984).

Results and Discussion

The hydroalcoholic extraction of *Bauhinia vahlii* yielded 14.5% w/w of crude extract, which was obtained as a dark-brown solid. The observed yield indicates satisfactory extraction of the soluble phytoconstituents from the plant material using the hydroalcoholic solvent system (Table 1). Hydroalcoholic solvents are capable of extracting a broad range of polar and moderately polar phytoconstituents, which may contribute to the pharmacological activity of the extract.

Preliminary phytochemical screening revealed the presence of several primary and secondary metabolites in the hydroalcoholic extract of *B. vahlii* (Table 2). Carbohydrates and proteins were detected among the primary metabolites, whereas diterpenes, saponins, flavonoids, and tannins/phenolic compounds were detected among the secondary metabolites. Amino acids, fats and oils, steroids, glycosides, and alkaloids were absent. The presence of flavonoids, tannins, phenolic compounds, saponins, and diterpenes suggests that the extract contains a diverse group of bioactive constituents that may contribute to its biological activities.

Quantitative estimation of bioactive constituents showed that the hydroalcoholic extract contained 0.721 total phenolic content and 0.983 total flavonoid content (Table 3). The presence of phenolic and flavonoid compounds is particularly important because these groups of phytoconstituents are commonly associated with antioxidant and other pharmacological properties. Thus, the phytochemical profile and quantitative constituent content support the potential biological activity of the *B. vahlii* extract.

The anxiolytic-like activity of *B. vahlii* extract was evaluated using the elevated plus-maze test in mice. The normal control group showed 68.25 ± 3.05 sec spent in the open arms and 212.45 ± 4.60 sec in the closed arms. Treatment with diazepam (1 mg/kg, p.o.), used as the reference standard, markedly increased the time spent in the open arms to 180.62 ± 4.10 sec. The *B. vahlii* extract also increased open-arm exploration in a dose-dependent manner, with values of 164.85 ± 3.25 sec at 100 mg/kg and 174.28 ± 3.70 sec at 200 mg/kg. A corresponding reduction in time spent in the closed arms was observed, with values of 112.36 ± 1.35 sec and 90.85 ± 1.20 sec, respectively (Table 4; Figure 1). The effects were statistically significant compared with the vehicle control, indicating increased exploration of the open arms following extract treatment.

The higher dose of *B. vahlii* extract (200 mg/kg) produced a greater increase in open-arm time than the 100 mg/kg dose, suggesting a dose-related behavioral response. The observed activity may be associated with the presence of flavonoids, phenolic compounds, saponins, and diterpenes detected during phytochemical screening (Table 2). These constituents may act individually or synergistically to contribute to the observed pharmacological effect. The findings from the elevated plus-maze experiment

therefore indicate that the hydroalcoholic extract of *B. vahlii* possesses measurable biological activity and warrants further investigation.

Table 1: % Yield of crude extract

Extracts	Colour	Consistency	Yield (% w/w)
Bauhinia vahlii			
Hydroalcoholic	Dark brown	Solid	14.5%

Table 2: Preliminary qualitative phytochemical tests for *Bauhinia vahlii* extract

Phytoconstituents	***Bauhinia vahlii* extract**
i) Primary Metabolites	
Carbohydrates	**+**ve
Amino acids	-ve
Proteins	**+**ve
Fats and oils	-ve
ii) Secondary metabolites	
Steroids	-ve
Diterpenes	**+**ve
Glycosides	-ve
Saponins	**+**ve
Flavonoids	**+**ve
Tannins & Phenol	**+**ve
Alkaloids	-ve
HE = Hydroalcoholic extract; '**+**ve' = Present; '-ve' = Absent	

Table 3: Total bioactive constituents content of *Bauhinia vahlii*

S. No.	Extract	Total phenol content	Total Flavonoid content
1	Hydroalcoholic extract	0.721	0.983

Table 4: Effects of *Bauhinia vahlii* extract in the elevated plus-maze test in mice

Group	Treatment	Time Spent in Open Arm (sec)	Time Spent in Close Arm (sec)
1	Normal Control (Saline)	68.25 ± 3.05	212.45 ± 4.60
2	Diazepam (1 mg/kg, p.o.)	180.62 ± 4.10***	72.15 ± 1.85
3	*B. vahlii* Extract (100 mg/kg, p.o.)	164.85 ± 3.25**	112.36 ± 1.35
4	*B. vahlii* Extract (200 mg/kg, p.o.)	174.28 ± 3.70***	90.85 ± 1.20

Values represent means±S.E.M. (n = 6). $^{**}P < 0.01$, $^{***}P < 0.0001$ compared with vehicle (One-way ANOVA followed by Tukey's post hoc test).

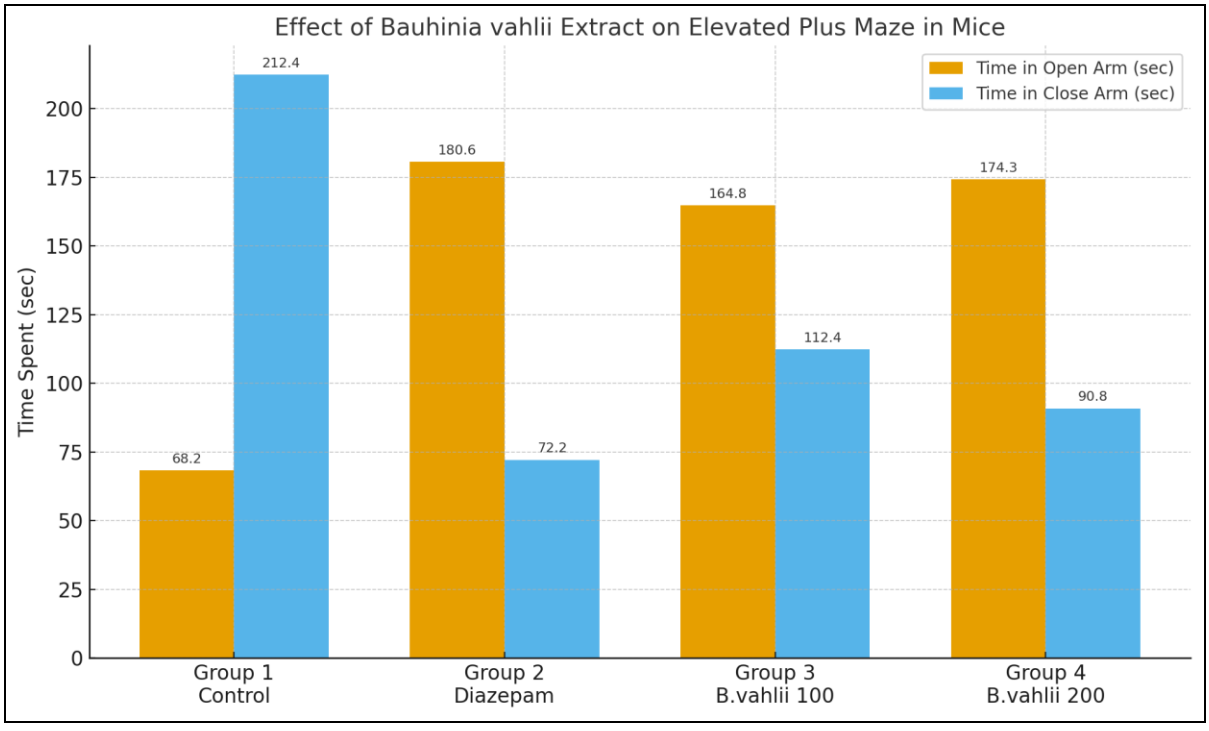

Figure 1: Effects of *Bauhinia vahlii* extract in the elevated plus-maze test in mice

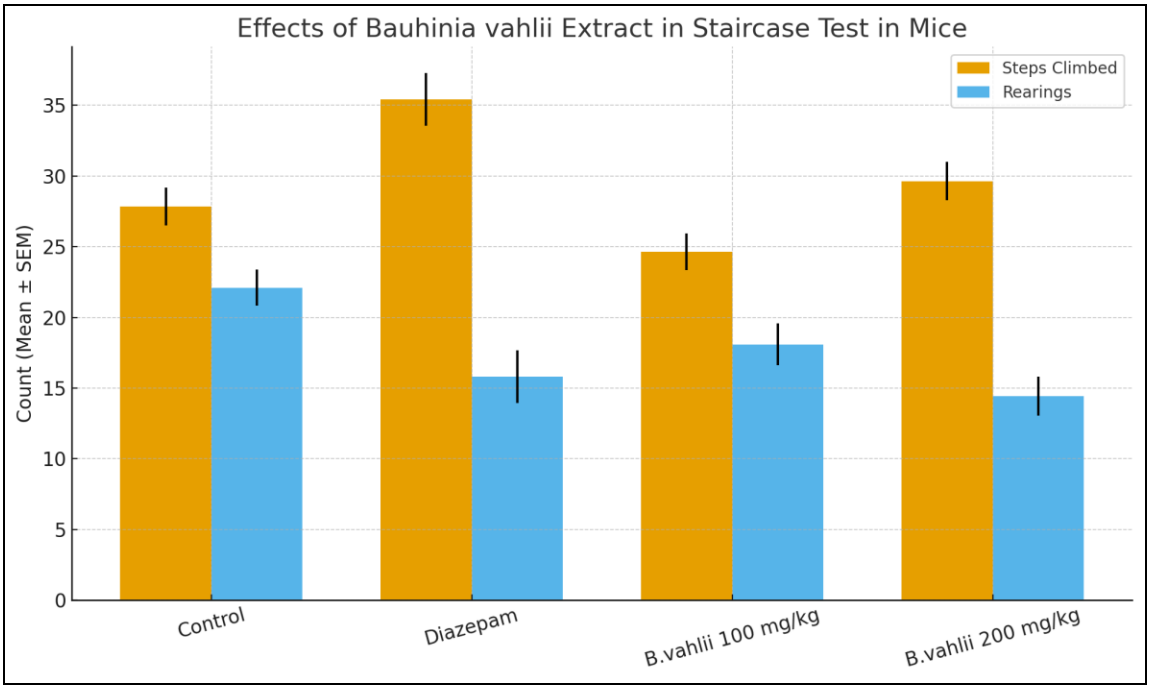

Figure 2: Effects of *Bauhinia vahlii* extract in the staircase test in mice

Conclusion

The hydroalcoholic extract of *Bauhinia vahlii* showed a satisfactory extraction yield and diverse phytochemical profile. The extract contained flavonoids, phenolic compounds, saponins, and diterpenes along with carbohydrates and proteins. Quantitative analysis confirmed the presence of appreciable phenolic and flavonoid contents. The extract demonstrated anxiolytic-like activity in the elevated plus-maze test. Both tested doses increased the time spent in the open arms compared with the control group. The 200 mg/kg dose produced a greater response than the 100 mg/kg dose. The extract also reduced the time spent in the closed arms in a dose-related manner. These findings suggest that *B. vahlii* may possess constituents capable of producing anxiolytic-like effects. The observed activity may result from the individual or synergistic effects of its bioactive phytoconstituents.

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