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Evaluation of Acute Toxicity and DPPH Radical Scavenging Activity of *Bauhinia racemosa* Leaf Extract

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ABSTRACT

Medicinal plants have long been integral to traditional medicine, and *Bauhinia racemosa* is recognized for its therapeutic and antioxidant potential. This study evaluates the acute toxicity and antioxidant activity of the ethanolic extract from *B. racemosa* leaves to assess its safety and therapeutic potential. Acute toxicity was assessed following Organization for Economic Cooperation and Development (OECD) 423 guidelines, with female Wistar rats receiving oral doses of 5, 50, 300, and 2000 mg/kg. The rats were monitored for physiological and behavioral changes over 14 days. Antioxidant activity was evaluated by determining the total phenolic content (TPC) and total flavonoid content (TFC) using the Folin-Ciocalteu and aluminum chloride assays, respectively, and by measuring radical scavenging activity (RSA) through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. No adverse effects or mortality were observed at any dosage level, indicating the absence of acute toxicity. The TPC and TFC of the extract were 6.42 mg GAE/g and 7.39 mg QE/g, respectively. The DPPH assay revealed a dose-dependent increase in RSA, with an IC50 value of 206.56 µg/mL, confirming substantial antioxidant potential. The results suggest that *B. racemosa* ethanolic leaf extract is both safe and potent as a natural antioxidant, supporting its potential for therapeutic applications. Further studies on chronic toxicity and bioactive compound identification are needed to fully validate its efficacy in treating oxidative stress-related diseases.

Keywords: *B. racemosa mauritiana*, antioxidant, toxicology, phenolic content, flavonoid content, radical scavenging

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INTRODUCTION

Plants have been integral to traditional medicine for centuries, serving as primary resources for treating diverse illnesses. Many ethnopharmacologically significant plant species have contributed to early drug discovery and the development of medicinal compounds [1,2]. Plant-derived therapeutics are often well-tolerated and widely accepted in clinical practice, reinforcing their role in healthcare [1]. Notably, an estimated 35,000 to 70,000 plant species have been studied for their medicinal properties, highlighting their substantial therapeutic contributions [1].

Bauhinia racemosa (*B. racemosa*), commonly known as the Beedi leaf tree, is a small tropical tree native to Southeast Asia. Typically reaching a height of 3–5 meters with drooping branches, it blooms between February and May. Beyond its aesthetic appeal, *B. racemosa* has significant cultural and medicinal relevance. Traditionally, its leaves are used in beedi production (Indian cigarettes) and exchanged during festivals such as Dussehra and Vijayadashami, where they symbolize prosperity and are referred to as "Sonapatti" or "gold leaves" in Hindi [3]. Scientifically, its bark, leaves, flowers, roots, and seeds have been shown to contain bioactive compounds like flavonoids, lupeol, betulin, and stilbene (resveratrol), which contribute to its medicinal value. Extracts of the plant have demonstrated notable analgesic, antipyretic, and anti-spasmodic activities, and the plant's potential use in Ayurvedic medicine for early-stage cancer has also been documented [4].

The therapeutic potential of *B. racemosa* is primarily attributed to its phenolic and flavonoid compounds, which exhibit antioxidant properties crucial for mitigating oxidative stress. Oxidative stress is a key contributor to various diseases, including cancer and cardiovascular conditions, emphasizing the need for further exploration of the plant's medicinal applications [5].

Ensuring the safety of medicinal plants is essential, particularly in traditional medicine. Toxicological evaluations, guided by standardized protocols like those from the Organization for Economic Co-operation and Development (OECD), are critical for determining the safe use of herbal remedies. Such assessments are vital for *B. racemosa*, given its inclusion in traditional formulations [6-8].

Previous studies have demonstrated a wide range of medicinal properties for *B. racemosa* leaves, including analgesic, anti-inflammatory, antimicrobial, antipyretic, and antispasmodic activities. Additionally, its bioactive compounds, such as flavonoids and tannins, have been linked to antioxidant, antiulcer, antitumor, cytotoxic, hepatoprotective, and hypotensive effects. However, the safety and antioxidant efficacy of its ethanolic extract of *B. racemosa* remain underexplored [6-8].

Evaluating the antioxidant activity of plant extracts is essential to establish their therapeutic value. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a widely accepted method for assessing the free radical scavenging ability of plant extracts, and it provides a basis for validating the antioxidant potential of *B. racemosa* [9].

This study aimed to evaluate the toxicological profile of the ethanolic extract of *B. racemosa* leaves in Wistar rats, following OECD 423 guidelines for acute oral toxicity. Additionally, we assessed the extract's antioxidant potential by determining its total phenolic and flavonoid content and performing a DPPH radical scavenging assay.

Thus, this study seeks to address the safety and antioxidant efficacy of *B. racemosa* leaves by integrating toxicological and antioxidant evaluations to provide insights into their pharmacological potential.

By combining toxicological and antioxidant evaluations, this research provides essential insights into the safety and pharmacological potential of *B. racemosa*. These findings may support the development of natural antioxidant therapies and guide future investigations into the plant's therapeutic applications.

MATERIALS AND METHODS

Plant Material and Extraction

Collection and Identification: Leaves of *B. racemosa* were collected from Hatlai Ghat, Dharashiv (Osmanabad), Maharashtra, India. The plant species was identified and authenticated by the Professor and Head of the Department of Botany at Venkatesh Mahajan Senior College, Dharashiv, affiliated with Dr.

Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhaji Nagar (Aurangabad), Maharashtra. A voucher specimen was deposited with herbarium no. 128(B) 2022-23.

Preparation of Extract: The collected leaves were rinsed with reverse osmosis-purified water and air-dried at room temperature for 21 days until dry and brittle. The dried leaves were powdered using an electric grinder. For extraction, 500 g of powdered material was macerated in 2 L of ethanol (absolute ethanol [99.5% ethanol]) in sealed glass containers under sterile conditions (four containers were used in total). The mixture was agitated 4–6 times daily for 3 days, then filtered using Whatman No. 1 filter paper. The ethanol was removed using a rotary evaporator, yielding a solvent-free extract.

Toxicological Evaluation

Animal Model: Fifteen female Wistar rats (6–8 weeks old) were selected for the study due to their physiological similarity to human metabolism. The rats were housed under standard laboratory conditions per OECD 423 guidelines, including appropriate bedding, cage size, and environmental enrichment. Ethical approval was granted by Scitesla Private Limited, Navi Mumbai, Maharashtra (approval no. SCI/IAEC/2024-25/107, dated July 17, 2024). The rats were provided unrestricted access to food and water during the study.

Organization for Economic Cooperation and Development 423 Guidelines: Acute oral toxicity was assessed using the stepwise procedure outlined in OECD 423 guidelines. Groups of three female rats each were administered the *B. racemosa* extract orally at doses of 5, 50, 300, and 2000 mg/kg body weight. A control group received an equivalent volume of distilled water. All animals were observed individually for toxicity signs, including changes in autonomic and central nervous systems, somatomotor activity, circulatory and respiratory systems, and physical characteristics such as eyes, fur, and mucous membranes. Specific symptoms like lethargy, tremors, convulsions, diarrhea, and salivation were noted. Observations were conducted within 30 minutes post-dosing, periodically during the first 24 hours, and daily for 14 days to detect any delayed effects [10].

Antioxidant Evaluation

Total Phenolic Content: The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with gallic acid as the standard. Dilutions of gallic acid (20–240 µg/mL) were prepared, and the ethanolic extract was tested at concentrations of 100 µg/mL and 200 µg/mL. A mixture of 0.5 mL extract/gallic acid, 3 mL distilled water, and 0.25 mL diluted Folin-Ciocalteu reagent was incubated for 5 minutes in the dark. Then, 1 mL of 7.5% sodium carbonate was added, and the solution was incubated for 60 minutes at room temperature. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Results were expressed as milligrams of gallic acid equivalents (mg GAE/g sample) based on the standard curve ($y = 0.0107x + 0.0277$, $R^2 = 0.9679$). All tests were conducted in triplicate for accuracy [11–13].

TPC was calculated as follows: [14]

$$TPC = (C \times V) / M$$

Where:

C = concentration of phenolics in the extract solution, typically obtained from a standard calibration curve using gallic acid, expressed in mg/mL

V = volume of the extract solution used in the assay, in mL

M = mass of the extract used in the assay, in grams

Total Flavonoid Content: The total flavonoid content (TFC) was quantified using the aluminum chloride method, with quercetin as the standard (20–240 µg/mL). Extract concentrations of 100 µg/mL and 200 µg/mL were used. A mixture of 0.5 mL extract/quercetin, 2 mL distilled water, and 0.15 mL of 5% sodium nitrate solution was incubated for 5 minutes. After adding 0.15 mL of 10% aluminum chloride and 1 mL of 1 M

sodium hydroxide, the volume was adjusted to 5 mL with distilled water. Absorbance was measured at 510 nm. TFC was expressed as milligrams of quercetin equivalents (mg QE/g sample) based on the standard curve ($y = 0.0005x + 0.0062$, $R^2 = 0.9587$). All assays were performed in triplicate [11–13].

TFC was calculated as follows: [14]

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V = volume of the extract solution used in the ass
M = mass of the extract used in the assay, in gran

DPPH Radical Scavenging Assay: Antioxidant activity was evaluated using the DPPH radical scavenging assay following the method of Khorasani Esmaeili et al. [15]. A 0.1 mM DPPH solution was prepared in ethanol. For the assay, 2.4 mL DPPH solution was mixed with 1.6 mL of extract or standard (ascorbic acid) at varying concentrations (1–1000 µg/mL) and incubated in the dark for 30 minutes. Absorbance was recorded at 517 nm. The percentage of radical scavenging activity (RSA) was calculated as follows [11-16]:

$RSA\ (\%) = ((A_{control} - A_{sample}) / A_{control}) \times 100$
Where:
$A_{control}$ = absorbance of the control sample (DPI
(without the antioxidant)
A_{sample} = absorbance of the sample (*B. racemos*
with the antioxidant

The half-maximal inhibitory concentration (IC50) value, indicating the concentration required for 50% inhibition, was derived from the dose-response curve and expressed as milligrams of ascorbic acid equivalents (mg AAE/g sample). Each concentration of the extract and standard was tested in triplicate for reliability [11–15].

RESULTS

Toxicological Evaluation

Acute Oral Toxicity:

The acute oral toxicity of the ethanolic extract of *B. racemosa* leaves was assessed following OECD guideline 423. A total of 12 female Wistar rats were administered extract doses of 5, 50, 300, and 2000 mg/kg body weight, while 3 rats served as a control group, receiving distilled water.

Behavior, body weight, and mortality were monitored over 14 days. No significant signs of toxicity were observed at any dose level. The physiological responses of the rats, including fur condition, skin, eyes, mucous membranes, respiratory function, and circulatory system, remained normal. No abnormalities were detected in the central or autonomic nervous systems, and motor activities and behavioral patterns were consistent throughout the observation period.

Body weight measurements showed no significant deviations from baseline values. Additionally, necropsy at the study's conclusion revealed no gross pathological findings. The control group exhibited baseline parameters comparable to the experimental groups, indicating no vehicle-related effects. All doses were well tolerated, with no evidence of acute toxicity even at the highest dose of 2000 mg/kg.

Antioxidant Evaluation

Total Phenolic Content: The TPC of the ethanolic extract was measured using the Folin-Ciocalteu method, with gallic acid as the standard. Results, expressed as mg GAE/g, showed a concentration-dependent increase in phenolic content, with a maximum of 5.05 mg GAE/g at 200 µg/m

Table 1. Total Phenolic Content of Ethanolic Extract of *B. racemosa* Leaves

Concentration (µg/mL)	GAE (µg/mL)	TPC in mg GAE/g Extract Mean ± SD
100	60.49	3.02 ± 0.0012
200	101.20	5.05 ± 0.0017

Total Flavonoid Content: The TFC was determined using the aluminum chloride assay, with quercetin as the standard. Results, expressed as mg QE/g, indicated a concentration-dependent increase in flavonoid content, peaking at 6.70 mg QE/g at 200 µg/mL.

Table 2. Total Flavonoid Content of Ethanolic Extract of *B. racemosa* Leaves

Concentration (µg/mL)	QE (µg/mL)	TFC in mg QE/g Extract Mean ± SD
100	27.80	1.39 ± 0.0100
200	134.00	6.70 ± 0.0100

The TPC and TFC values at various extract concentrations, demonstrate the concentration-dependent increase in phenolic and flavonoid content is illustrated in Figure 1.

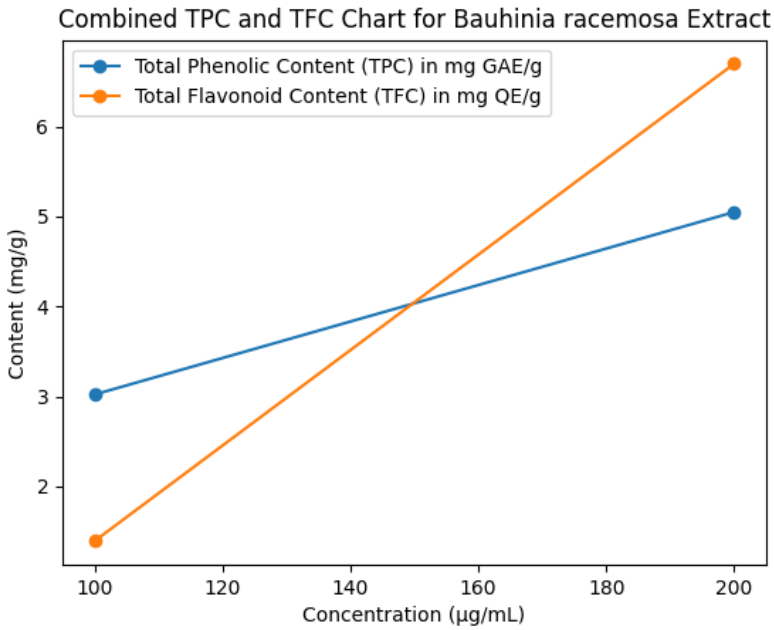

Figure 1. Total Phenolic Content and Total Flavonoid Content in Ethanolic Extract of *B. racemosa* Leaves

DPPH Radical Scavenging Activity: The antioxidant activity of the ethanolic extract was evaluated using the DPPH radical scavenging assay, with ascorbic acid as the standard. The %RSA and the IC50 value (the concentration required to inhibit 50% of DPPH radicals) were determined.

The ethanolic extract showed a concentration-dependent increase in DPPH scavenging activity, reaching 95.11% inhibition at 1000 µg/mL. The IC50 value of the extract was 206.56 µg/mL, indicating substantial antioxidant potential. In comparison, ascorbic acid demonstrated a significantly lower IC50 value of 24.96 µg/mL, reflecting higher efficacy at lower concentrations.

The % RSA values for both the ethanolic extract and ascorbic acid at different concentrations, along with their respective IC50 values, are presented in Table 3, offering a comparative overview of their antioxidant activities.

Table 3. DPPH Radical Scavenging Activity of Ethanolic Extract of *B. racemosa* Leaves

Concentration (µg/mL)	Ascorbic acid		*B. racemosa*	
	RSA (%)	IC50 (µg/mL)	RSA (%)	IC50 (µg/mL)
1	6.07	24.96	0.0640	206.56
10	27.87		10.3198	
25	37.94		24.9467	
50	60.73		42.4094	
100	94.95		75.3305	
250	96.08		83.8166	
500	96.64		90.9168	
1000	96.92		95.1173	

A comparison of the DPPH %RSA of the ethanolic extract with ascorbic acid across varying concentrations is shown in Figure 2, illustrating a dose-dependent increase in antioxidant activity as measured by the percentage of DPPH radical inhibition.

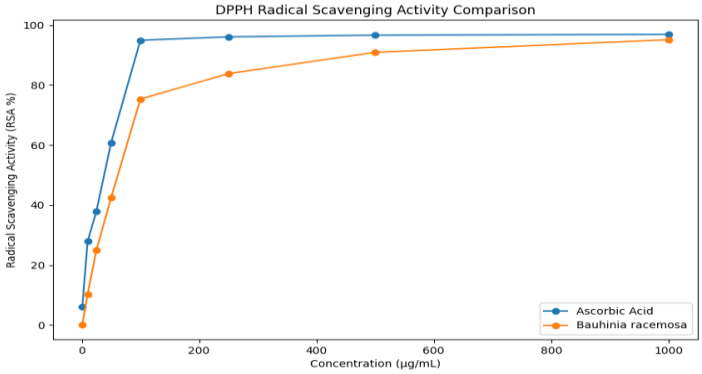

Figure 2. DPPH Radical Scavenging Activity of Ethanolic Extract of *B. racemosa* Leaves compared to Ascorbic Acid

DISCUSSION

The acute oral toxicity evaluation of the ethanolic extract of *B. racemosa* leaves demonstrated no signs of toxicity, even at the highest tested dose of 2000 mg/kg. Observations showed normal physiological and behavioral functions with no abnormalities, supporting the extract's safety and its suitability for further preclinical research. These findings align with earlier studies on *B. racemosa* and related species, such as *B. cheilantha* and *B. variegata*, which also demonstrated no toxicity at similar doses [17], reinforcing its safety margin. However, the lack of chronic toxicity data highlights the need for studies assessing long-term safety.

The extract exhibited significant antioxidant activity, as evidenced by its total phenolic (5.05 mg GAE/g) and flavonoid (6.70 mg QE/g) content, comparable to antioxidant-rich plants like green tea and grape seed, known for their protective effective against oxidative stress [18]. These findings align with previous studies on *B. racemosa*, which reported significant antioxidant activity and DPPH free radical scavenging in the leaves, flowers, and bark of the *B. racemosa* plant [8]. This bioactivity was confirmed by its IC50 value of 206.56 µg/mL in the DPPH assay, demonstrating effective free radical scavenging. While less potent than synthetic antioxidants like ascorbic acid (IC50 = 24.96 µg/mL), the extract holds value as a natural source, catering to demand for plant-derived antioxidants in therapeutic and nutraceutical applications.

The antioxidant potential and bioactive compound profile of *B. racemosa* suggest utility in managing oxidative stress-related conditions such as cardiovascular diseases, diabetes, and neurodegenerative disorders [19]. These properties align with its traditional use in addressing inflammation and metabolic dysfunctions. However, more targeted research is essential to confirm its efficacy in disease-specific models, elucidate its bioactive mechanisms, and establish safe therapeutic dosages.

CONCLUSION

This study established the safety and antioxidant potential of the ethanolic extract of *B. racemosa* leaves. Acute oral toxicity testing, adhering to OECD 423 guidelines, revealed no adverse effects at doses up to 2000 mg/kg, indicating a wide safety margin for preclinical investigations.

The extract demonstrated robust antioxidant activity, driven by significant phenolic and flavonoid content. These findings support its potential as a natural antioxidant, consistent with its traditional medicinal uses.

To fully explore its therapeutic applications, future studies should focus on long-term toxicity, bioactive compound identification, and efficacy evaluation in disease-specific models. These steps are vital for translating *B. racemosa*'s traditional applications into scientifically validated therapeutic and nutraceutical products.

In summary, the ethanolic extract of *B. racemosa* leaves is a promising candidate for natural antioxidant therapy, offering safety and efficacy for health enhancement and oxidative stress-related disease management.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

B. racemosa, *Bauhinia racemosa*; DPPH, 2,2-diphenyl-1-picrylhydrazyl; OECD, Organization for Economic Co-operation and Development; IC50, half-maximal inhibitory concentration; RSA, radical scavenging activity; TFC, total flavonoid content; TPC, total phenolic content; UV-Vis, Ultraviolet visible.

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