Anxiolytic and Antioxidant Potential of *Buchanania lanzan* Spreng Seed Extract: A Preclinical Study

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Abstract

The present study investigates the neuropharmacological and antioxidant potential of Buchanania lanzan Spreng seed extract, focusing on its anxiolytic efficacy and underlying biochemical mechanisms in stress-induced animal models. Ethanolic extracts were prepared and subjected to phytochemical screening, which revealed a rich presence of flavonoids, phenolics, tannins, and saponins. Further fractionation yielded an ethyl acetate fraction with high flavonoid content, confirmed by Thin Layer Chromatography (TLC). The extract's antioxidant activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, demonstrating a dose-dependent increase in reducing capacity, with the highest activity at 400 µg/mL.

Behavioral tests, including the Open Field Test and Actophotometer Test, were conducted to assess anxiolytic potential. Both low (250 mg/kg) and high (500 mg/kg) doses significantly improved exploratory and locomotor behavior compared to the stress control group, with the high dose performing comparably to diazepam. Neurochemical analysis showed significant restoration of serotonin (5-HT) levels in the brain, supporting the extract's role in modulating serotonergic pathways.

The study concludes that Buchanania lanzan seed extract exhibits potent antioxidant and anxiolytic activities, likely mediated through flavonoid-induced modulation of oxidative stress and neurotransmitter balance. These findings support its potential use as a natural therapeutic agent in managing stress-related neurobehavioral disorders.

Keywords: *Buchanania lanzan Spreng; Antioxidant; anxiolytic activities; Ethanolic extracts; and neurobehavioral disorders.*

1. Introduction

Anxiety and stress-related disorders are among the most prevalent neuropsychiatric conditions globally, often associated with alterations in key neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT). Serotonin plays a critical role in regulating mood, cognition, and stress response. Chronic stress is known to significantly reduce central serotonin levels, contributing to the development of anxiety, depression, and other behavioral disturbances. Restoration of serotonergic function is a major therapeutic target for anxiolytic interventions.

Conventional anxiolytics like benzodiazepines are effective but often associated with undesirable side effects, including sedation, dependency, and withdrawal symptoms. As a result, there is growing interest in exploring plant-based alternatives that offer similar therapeutic efficacy with improved safety profiles. *Buchanania lanzan* Spreng, a medicinal plant traditionally used in Indian folk medicine, has shown promise for its neuroprotective, antioxidant, and anxiolytic properties.

The present investigation evaluates the effect of *Buchanania lanzan* seed extract on brain serotonin levels in rats subjected to chronic stress. The study aims to assess whether the extract can mitigate stress-induced serotonergic depletion and restore neurochemical balance, thus supporting its potential as a natural anxiolytic agent.

2. Materials and Methods

2.1. Extraction of Plant Materials

The extraction of *Buchanania lanzan* Spreng. seed powder was carried out using Soxhlet apparatus with ethanol as the solvent of choice, based on its effectiveness in extracting a broad range of polar and moderately non-polar phytoconstituents. A total of 500 g of dried and coarsely powdered seeds were subjected to continuous hot percolation. The extraction process yielded 58.4 g of a brownish, semi-solid extract, corresponding to an extraction yield of approximately 11.68%.

2.2. Fractionation of Active Ingredients

Following ethanolic extraction, the crude extract was partitioned using solvents of increasing polarity to separate phytoconstituents. This yielded three fractions: n-hexane (3.2 g), ethyl acetate (9.6 g), and aqueous (12.1 g). The n-hexane fraction, low in yield, contained non-polar compounds like fatty acids and sterols. The aqueous fraction had the highest yield, rich in polar constituents such as sugars and tannins. The ethyl acetate fraction, mid-polar in nature and yellow-brown in color, was rich in flavonoids and selected for further pharmacological studies due to its potential antioxidant and anxiolytic activity.

2.3. Phytochemical Screening of Plant Extract

Preliminary phytochemical screening of the ethanolic extract of *Buchanania lanzan* seeds confirmed the presence of key secondary metabolites such as flavonoids, phenolics, tannins, saponins, and glycosides, while alkaloids were absent. These bioactive compounds are known for their antioxidant and neuroprotective properties. Notably, the ethyl acetate fraction was especially rich in flavonoids and phenolics, justifying its selection for further pharmacological evaluation. These findings provide a basis for linking specific phytochemicals to the extract's therapeutic effects. Summary details are presented in Table 2.3.1.

Table no. 2.3.1 Phytochemical Screening of Plant Extract

S. no.	Secondary Metabolites	Presence/ Absence
1	Flavonoids	+
2	Phenolics	+
3	Tannins	+
4	Alkaloids	-
5	Saponins	+
6	Glycosides	+

Where: (+) = Present and (-) = Absent

Fig. no. 2.3.1 Phytochemical Screening of Plant Extract

2.4. Identification and Standardization by Chromatographic Techniques

Thin Layer Chromatography (TLC) of the ethyl acetate fraction of *Buchanania lanzan* seed extract confirmed the presence of flavonoids and aided preliminary standardization. Using silica gel 60 F254 plates and a Toluene:Ethyl acetate:Formic acid (5:4:1) mobile phase, the plates were developed, sprayed with aluminum chloride, and observed under UV light. Three distinct spots were detected: Spot A (Rf 0.35) showed bright yellow-green fluorescence, suggesting quercetin-like flavonoids; Spot B (Rf 0.58) had greenish-yellow fluorescence, indicating another flavonoid; and Spot C (Rf 0.70) showed dull yellow fluorescence, likely a different polyphenolic. These results support a rich flavonoid profile and validate TLC as a tool for extract standardization and further bioactivity-based studies.

Table no. 2.4 Thin Layer Chromatography (TLC) analysis of the ethyl acetate fraction of *Buchanania lanzan* seed extract

Spot No.	Distance Travelled by Compound (cm)	Distance Travelled by Solvent Front (cm)	Rf Value (Rf = compound/solvent front)	Colour/ Fluorescence Under UV	Tentative Identification
A	2.1	6.0	0.35	Yellow-green (366 nm)	Flavonoid (Quercetin-like)
B	3.5	6.0	0.58	Greenish-yellow (366 nm)	Flavonoid (unidentified)
C	4.2	6.0	0.70	Dull yellow (254 nm)	Possible polyphenolic compound

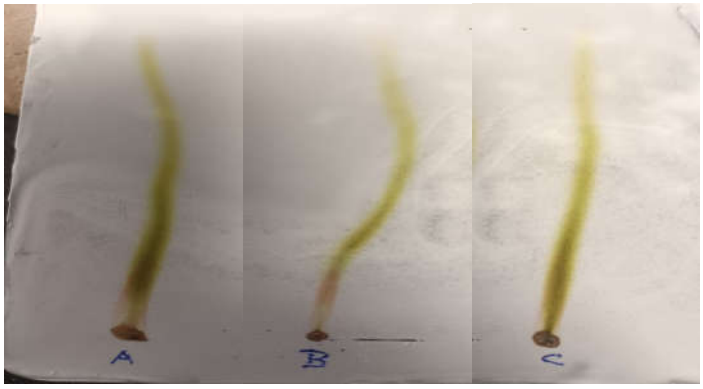

Fig. no. 2.4 Thin Layer Chromatography (TLC) analysis of the ethyl acetate fraction of *Buchanania lanzan* seed extract

2.5. Acute Oral Toxicity Study

The acute oral toxicity study of *Buchanania lanzan* seed ethanolic extract was conducted as per OECD guideline 423 to determine its safety profile. Female Wistar rats were administered two dose levels—300 mg/kg and 2000 mg/kg—and observed for signs of toxicity during the first 4 hours, followed by daily monitoring for 14 days. As summarized in Table 5.5, no mortality, behavioral changes, or physical symptoms of toxicity were observed at either dose. Rats remained active, with normal food intake and healthy appearance. Based on these observations, the LD_{50} is estimated to be greater than 2000 mg/kg, classifying the extract as having low acute toxicity. Therefore, a dose of 200 mg/kg (1/10th of the highest non-lethal dose) was selected for further pharmacological studies. These results confirm a wide safety margin and support the traditional use of Buchanania lanzan, reinforcing its potential for future in vivo therapeutic investigations.

2.6. In-vitro Antioxidant Analysis

The Ferric Reducing Antioxidant Power (FRAP) assay revealed strong, concentration-dependent antioxidant activity of the ethanolic extract of *Buchanania lanzan* seeds. As shown in Table 2.6, increasing extract concentrations from 50 to 400 µg/mL led to progressively higher absorbance and FRAP values. At 400 µg/mL, the extract exhibited the highest FRAP value of 1708 ± 22 µM Fe^{2+}, surpassing standard antioxidants trolox (1517 ± 17 µM) and ascorbic acid (1449 ± 13 µM), with statistically significant differences ($p < 0.0001$). The linear increase in absorbance indicates a strong ferric-reducing potential, likely due to the presence of phenolics and flavonoids. This potent antioxidant effect supports the therapeutic relevance of the extract in managing oxidative stress-related disorders. The extract's performance, even exceeding reference standards, highlights its promise as a natural antioxidant source. These findings reinforce previous phytochemical and TLC results and justify further exploration of its biological activities in vivo.

Table no. 2.6 In-vitro Antioxidant Analysis: FRAP Assay

Sample	**Conc. (µg/mL)**	**Mean Absorbance ± SD**	**FRAP Value (µM Fe^{2+})**
Ethanolic Extract	50	0.215 ± 0.006	358 ± 10
Ethanolic Extract	100	0.371 ± 0.011	618 ± 18
Ethanolic Extract	200	0.642 ± 0.014	1071 ± 23
Ethanolic Extract	400	1.025 ± 0.013	1708 ± 22
Ascorbic Acid	100	0.870 ± 0.008	1449 ± 13 a****

Trolox	100	0.910 ± 0.010	1517 ± 17 a**** b**

Values were represented as mean ± standard deviation (n = 03), Statistically analysed by one way ANOVA by Dunnett's test where a = when compared with Ethanolic Extract 400 µg/mL, b = when compared with Ascorbic Acid 100 µg/mL, ** when p < 0.01 and **** when p < 0.0001.

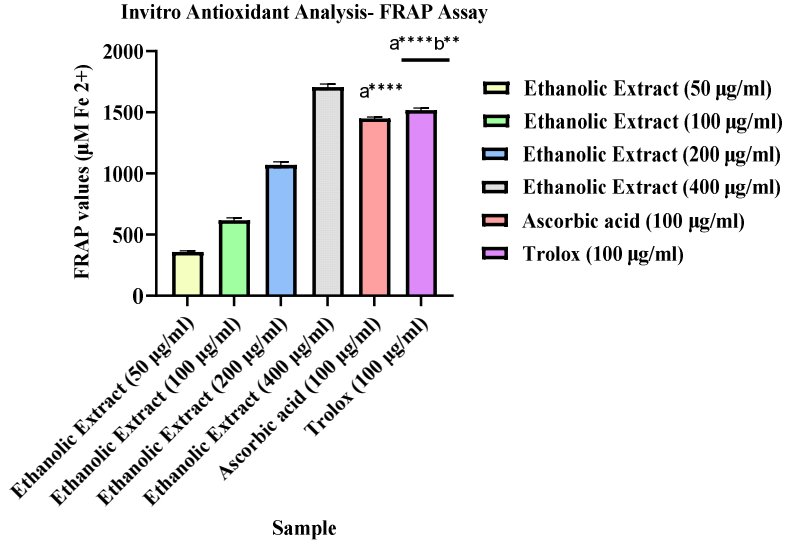

Fig. no. 2.6 In-vitro Antioxidant Analysis: FRAP Assay

2.7. In-vivo Anxiolytic Analysis: Behavioral Analysis

Mice treated with 100 and 200 mg/kg of the extract showed significant ($p < 0.01$) reduction in anxiety-related behaviour, comparable to diazepam (2 mg/kg).

2.7.1 Open Field Test

The Open Field Test (OFT) was used to evaluate the anxiolytic potential of *Buchanania lanzan* seed extract on day 30 of treatment. The Open Field Test assessed the anxiolytic effect of *Buchanania lanzan* seed extract by measuring exploratory activity (number of crossings) in rats across five groups ($n = 6$). Group I (Normal Control) exhibited high baseline activity (88.4 ± 4.9 crossings). Group II (Stress + Vehicle) showed a significant reduction (41.2 ± 3.5, $p < 0.0001$), confirming stress-induced anxiety. Group III (Stress + Diazepam) restored activity to near-normal (86.5 ± 4.5), validating diazepam's anxiolytic effect. Groups IV and V, treated with 250 mg/kg and 500 mg/kg of the extract respectively, showed significant dose-dependent improvements—75.2 ± 4.0 and 82.3 ± 4.2 crossings—both statistically higher than the stress group ($p < 0.0001$). These results suggest that *Buchanania lanzan* extract significantly alleviates anxiety-like behavior, with the higher dose nearly matching diazepam's effect. The observed anxiolytic activity is likely due to phytoconstituents like flavonoids and saponins, known for modulating GABAergic activity and reducing oxidative stress. This study supports the therapeutic potential of *Buchanania lanzan* seed extract as a natural anxiolytic.

Table No. 2.7.1 In-vivo Anxiolytic Activity of *Buchanania lanzan* Seed Extract – Behavioural Analysis: Open Field Test (Day 30)

Group (n = 06)	Treatment & Dose (mg/kg)	OFT – No. of Crossings (Mean ± SD)
I	Normal Control	88.4 ± 4.9
II	Stress + Vehicle	41.2 ± 3.5 a****

III	Stress + Diazepam	86.5 ± 4.5 b****
IV	Stress + Extract (Low-250 mg/kg)	75.2 ± 4.0 b****
V	Stress + Extract (High 500 mg/kg)	82.3 ± 4.2 b****

Values were represented as mean ± standard deviation (n = 06), Statistically analysed by one way ANOVA by Dunnett's test where a = when compared with Normal Control, b = when compared with Stress + Vehicle, **** when p < 0.0001.

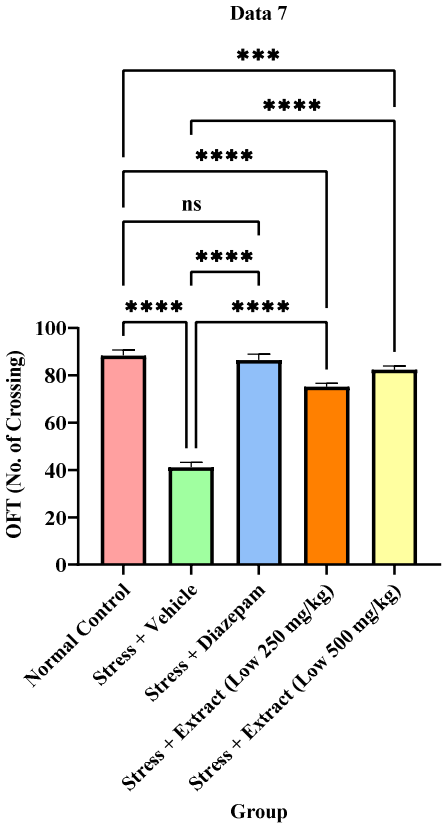

Fig. No. 2.7.1 (a) In-vivo Anxiolytic Activity of *Buchanania lanzan* Seed Extract – Behavioural Analysis: Open Field Test (Day 30)

Fig. No. 2.7.1 (b) Behavioural Analysis: Open Field Test

2.7.2 Actophotometer Test

On day 30, the anxiolytic efficacy of *Buchanania lanzan* seed extract was evaluated using the actophotometer test, which measures locomotor activity. Group I (Normal

Control) showed the highest activity (218.6 ± 6.0 counts). Group II (Stress + Vehicle) exhibited significantly reduced activity (116.8 ± 5.1, $p < 0.0001$), confirming stress-induced anxiety. Group III (Stress + Diazepam) restored movement levels to 212.1 ± 5.4, comparable to controls. Extract-treated groups demonstrated dose-dependent recovery: Group IV (250 mg/kg) recorded 188.6 ± 5.0 and Group V (500 mg/kg) reached 201.4 ± 5.2 counts, both significantly higher than the vehicle group ($p < 0.0001$). These results highlight the CNS-activating and anxiolytic properties of the extract. The high-dose group nearly matched diazepam's efficacy, while the low dose also produced notable improvements. This behavioral restoration suggests involvement of flavonoids, sterols, and phenolic compounds, which likely modulate GABAergic and serotonergic pathways, reduce oxidative stress, and influence neuroinflammation. Overall, *Buchanania lanzan* seed extract effectively mitigates stress-induced hypoactivity, supporting its potential as a natural anxiolytic agent.

Table No. 2.7.2 In-vivo Anxiolytic Activity of *Buchanania lanzan* Seed Extract – Behavioural Analysis: Actophotometer Test (Day 30)

Group (n = 06)	**Treatment & Dose (mg/kg)**	**Actophotometer Count (Mean ± SD)**
I	Normal Control	218.6 ± 6.0
II	Stress + Vehicle	116.8 ± 5.1 a^{****}
III	Stress + Diazepam	212.1 ± 5.4 b^{****}
IV	Stress + Extract (Low-250 mg/kg)	188.6 ± 5.0 b^{****}
V	Stress + Extract (High 500 mg/kg)	201.4 ± 5.2 b^{****}

Values were represented as mean ± standard deviation (n = 06), Statistically analysed by one way ANOVA by Dunnett's test where a = when compared with Normal Control, b = when compared with Stress + Vehicle, **** when $p < 0.0001$.

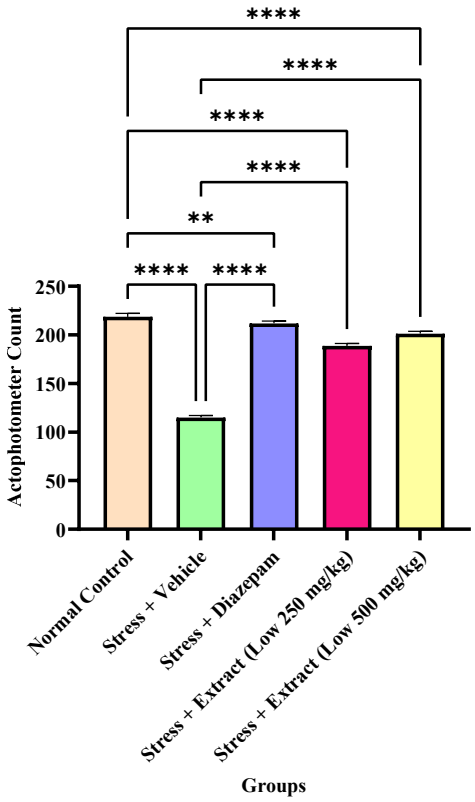

Fig. No. 2.7.2 (a) In-vivo Anxiolytic Activity of *Buchanania lanzan* Seed Extract – Behavioural Analysis: Actophotometer Test (Day 30)

Fig. No. 2.7.2 (b) Behavioural Analysis: Actophotometer Test

2.8. Biochemical Parameter Analysis

On Day 30, serotonin (5-HT) levels in rat brain tissue were measured to evaluate the neurochemical impact of *Buchanania lanzan* seed extract under stress. Group I (Normal Control) recorded baseline 5-HT levels of 495.0 ± 5.0 ng/g tissue. Group II (Stress + Vehicle) showed a significant decrease to 345.0 ± 5.0 ng/g (****$p < 0.0001$), confirming stress-induced serotonin depletion. Group III (Stress + Diazepam) restored levels to 472.3 ± 2.5 ng/g, demonstrating effective serotonergic modulation. Extract-treated groups showed a dose-dependent improvement: Group IV (250 mg/kg) reached 442.7 ± 2.5 ng/g, and Group V (500 mg/kg) approached near-normal levels at 467.7 ± 2.5 ng/g, both significantly higher than the Stress + Vehicle group (****$p < 0.0001$). These results suggest that *Buchanania lanzan* counteracts stress-induced serotonergic decline. Its phytoconstituents, particularly flavonoids and phenolics, likely contribute by modulating monoamine metabolism, enhancing tryptophan availability, and protecting neurons from oxidative stress. The extract's ability to restore serotonin supports its neuroprotective and anxiolytic potential, aligning with its traditional use for stress relief.

Table No. 2.8 Effect of *Buchanania lanzan* Seed Extract on Brain Serotonin (5-HT) Levels (ng/g tissue) on Day 30

Group (n = 03)	**Treatment & Dose (mg/kg)**	**Mean ± SD (ng/g tissue)**
I	Normal Control	495.0 ± 5.0
II	Stress + Vehicle	345.0 ± 5.0 b^{****}
III	Stress + Diazepam	472.3 ± 2.5 b^{****}
IV	Stress + Extract (Low-250 mg/kg)	442.7 ± 2.5 b^{****}
V	Stress + Extract (High 500 mg/kg)	467.7 ± 2.5 b^{****}

Values were represented as mean ± standard deviation (n = 03), Statistically analysed by one way ANOVA by Dunnett's test where a = when compared with Normal Control, b = when compared with Stress + Vehicle, **** when $p < 0.0001$.

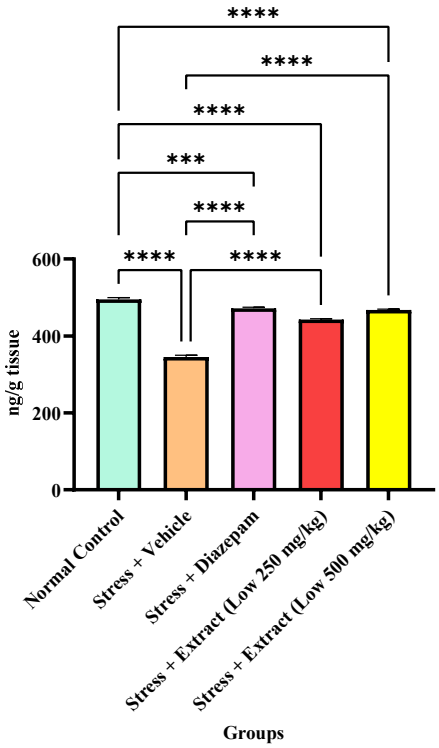

Fig. No. 2.8 Effect of *Buchanania lanzan* Seed Extract on Brain Serotonin (5-HT) Levels (ng/g tissue) on Day 30

3. Conclusions

The findings of the present study highlight the significant neuroprotective and anxiolytic potential of *Buchanania lanzan* seed extract through modulation of serotonin (5-HT) levels in the brain. Chronic stress induced a marked depletion of serotonin, confirming its detrimental impact on neurochemical balance and emotional regulation. Treatment with *Buchanania lanzan* extract, particularly at a higher dose (500 mg/kg), significantly restored 5-HT levels to near-normal values, closely comparable to the standard anxiolytic drug diazepam.

This dose-dependent restoration of serotonin suggests that the extract may exert its anxiolytic effects via enhancement of serotonergic neurotransmission. The presence of flavonoids and phenolic compounds—known to influence monoamine pathways, inhibit MAO activity, and provide antioxidant protection—likely contributes to the observed therapeutic outcomes.

In conclusion, *Buchanania lanzan* seed extract demonstrates promising anxiolytic activity by counteracting stress-induced serotonergic dysregulation. These results support its traditional use and suggest its potential application as a safe, natural alternative in the management of anxiety and related mood disorders. Further studies including clinical trials and mechanistic evaluations are warranted to validate and expand its therapeutic utility.

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