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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT OF BACOPA MONNIERI IN SWISS ALBINO MICE

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Abstract

Depression is a common symptom of mental illness. Typical symptoms include a low mood, loss of appetite, feelings of guilt or inadequacy, trouble sleeping or restlessness, and lack of energy. Mimosa pudica is traditionally used to treat a variety of ailments due to its numerous healing properties. The purpose of this study was to deepen our understanding of the antidepressant activity of Bacopa monnieri. Researchers investigated the potential of an ethanolic extract of Bacopa monnieri (EEBM) to alleviate depression in Swiss albino mice. Imipramine was used as the standard drug for comparison. Phytochemical analysis revealed the presence of glycosides, phenols, tannins, alkaloids, flavonoids, and carbohydrates in the extract. EEBM caused no mortality when administered orally at a dose of 2000 mg/kg, despite some notable behavioral effects. At a dose of 400 mg/kg, EEBM significantly reduced immobility time in both the Forced Swim Test (FST) and the Tail Suspension Test (TST). The decreased immobility time observed in these tests indicates that EEBM exhibits superior antidepressant activity, likely due to enhanced central 5-HT (serotonin) or catecholamine neurotransmission.

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Introduction

Medicinal plants have been used for thousands of years across cultures, making them the oldest form of medicine [1]. Modern pharmacotherapy often derives advanced drugs from traditional remedies, with natural substances remaining vital sources of therapeutic compounds. Isolating bioactive plant compounds is a key initial step [2-5], aided by modern extraction methods like ultrasound-assisted and supercritical fluid extraction [6]. Advanced analytical techniques such as HPLC and LC/MS have enhanced phytochemical analysis, alongside bioassay-guided methods and high-throughput screening [7-11]. Despite progress, comprehensive research on the phytochemical composition and health benefits of many species remains limited. Depression, a widespread condition marked by anxiety and restlessness, is a leading cause of disability and premature death globally, driving the search for

effective treatments including pharmaceuticals, psychotherapy, and ECT [12-13].

Aim and Objectives

A new era in the pharmacological evaluation of Bacopa monnieri's ethanolic extracts: Evaluation of antidepressant efficacy was the focus of this study.

1. Obtaining permission to collect the plant.
2. Preparation of ethanolic extracts from Bacopa monnieri leaves.
3. Assessment of the harmful effects these extracts may have on language function.
4. Examining upper body activity using the Forced Swim Test (FST) and evaluating the antidepressant potential using the Tail Suspension Test (TST).

Materials and Methods

The process plan involves a series of ordered steps to achieve specific goals according to established standards and guidelines. It covers the entire workflow-from field collection and identification of medicinal plants to ensuring measurement accuracy, standardizing protocols, preparing reagents, selecting solvents for extraction, defining procedures, and executing the standardized methods. Managing these materials and techniques requires a solid understanding and skilled hands. All pharmaceuticals and chemicals used in this study

were sourced from reputable Indian companies and met high-quality and safety standards.

Table 1: Drugs and Chemicals

S.No	Materials	Company Name
1	Imipramine	Nicholas Piramal Ltd

Instruments

Following instruments were required for the study:

Table 2: List of Instruments used for study

Thread	YVR medivision Pvt Ltd
Stop watch	ASGI®
Syringes	YVR medivision Pvt Ltd
Needles	YVR medivision Pvt Ltd
Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI®, Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
What mann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

Experimental Animals

Sixty male and female Swiss albino rats (20–30 g) were housed in groups of six in polypropylene cages under standard conditions (22 ± 1 °C, 12:12 hour light/dark cycle, lights on at 08:00) with free access to food and water. They were acclimated for one week prior to testing. Imipramine hydrochloride, supplied by Abbott Healthcare Pvt Ltd., was used as the reference drug. Wistar rats (150–200 g) and Swiss albino mice (18–22 g) were also used, housed under similar standard conditions (26 ± 1 °C, 12-hour light/dark cycle, 45–55% humidity), with ad libitum access to food and water. All procedures were approved by the Institutional Animal Ethics Committee (IAEC).

Getting Cloth Out of Plants

Everyone in the neighborhood is talking about the new Bacopa Monnieri. The plant fiber was washed, then chopped into smaller pieces, let to air-dry in the shade at room temperature. A coarse powder was then made using a blender. In the extraction process, the cloth that had been powdered was either kept or put to use.

Methods for Extracting Plant Matter

After washing the Bacopa Monnieri plants, the transparent portions were dried in the shade and ground into a fine powder to create an ethanolic extract. Roughly 200 grams of

dried Bacopa Monnieri leaf powder was extracted using 99.9% ethanol in a Soxhlet extractor for about 36 hours. The ethanol was removed by heating the liquid in a water shower within a measuring container. The extricate formed a brown glue and then took a 6-gram bulk. The resulting abdication was 3% w/w when applied to the dry powder.

Preliminary phyto chemical screening

Following established protocols, we conducted an initial phytochemical screening of the plant extract to identify and quantify alkaloids, carbohydrates, tannins, saponins, steroids, phenols, and flavonoids.

1. Individual extracts were filtered do weak hydrochloric acid to find alkaloids.

- Mayer's Test:** Channels were treated with potassium mercuric iodide (Mayer's reagent). A yellow precipitate indicates the presence of alkaloids.
- Wagner's Test:** Filtrates were treated with Wagner's reagent (iodine in potassium iodide). A rapid brownish-red precipitate confirms alkaloids.
- Dragendorff's Test:** Filtrates were treated with potassium bismuth iodide (Dragendorff's reagent). A reddish precipitate indicates alkaloids.
- Hager's Test:** Filtrates were treated with Hager's reagent (picric acid). A yellow precipitate signifies alkaloids.

2. Carbohydrate Area

Extracts were filtered after being independently broken up in 5cc of distilled water. The proximity of carbs within the filtrates was examined.

- Molisch's Test:** Two drops of an alcoholic naphthol solution were added to a test tube containing filtrates. Carbohydrates are shown by the violet ring that forms at the crossing point.
- Filtrates were gently heated and treated with Benedict's reagent for the Benedict's Test. The proximity of decreasing sugars is shown by orange reddish quicken.
- The Fehling™s**
The test utilized diluted hydrochloric acid to hydrolyze the filtrates. Which were then heated using Fehling's A&B courses of action and neutralized by alkalization. The presence of reddish quicken indicates that sugar levels are dropping.

3. Enlightenment regarding spooning

Froth Test: Debilitated to 20 milliliters with purified water, the extracts were shaken for 15 minutes in a graduated barrel. The path of action of a one centimeter layer on an am. shows that saponins are quite close together. **Foam Test:** 0.5 grams of remove was mixed with 2 milliliters of water and shaken. The proximity of saponins is demonstrated if the foam remains for 10 minutes.

4. A discovery of steroids was made

- Salkowski's Test:** the extricates were filtered and treated with chloroform. A few drops of concentrated sulphuric acid were added to the filtrates, which were then agitated and left to stand. The proximity of triterpenes is indicated by the vibrant tone of the hue.
- The Libermann-Burchard test:** The extracted materials were filtered after being treated with chloroform. After bubbling and cooling, the filtrates

were treated with a few drops of acidic anhydride. There was a concentration of sulfuric acid that was toxic. The proximity of phytosterols is shown by the brown ring's activity at the crossing point.

5. How to Identify Phenols

To conduct the ferric chloride test, three or four drops of the chemical were applied to the extracted materials. The behavior of a bluish-black tint shows how near phenols are.

6. Tannins Analysis through Gelatin: 1% gelatin solution containing sodium chloride was added to the extract. The behavior of a white quicken indicates the proximity of tannins.

7. Flavonoid Dissolvable Reagent Test: Flavonoids were identified by adding sodium hydroxide (producing a yellow color that fades) and lead acetate (forming a yellow precipitate). For animal studies, *Bacopa monnieri* dosages were based on human equivalents (3–5 g/kg). Using conversion factors from Ghosh (1984), doses of 200 mg/kg for rats and 20 mg/kg for mice were established. Doses above 2000 mg/kg were considered highly toxic.

Pharmacological Evaluation Acute Oral Toxicity

Ethanollic extracts of *Bacopa monnieri* were tested for potential adverse effects in rats and mice, following OECD Test Guideline No. 425. After a 12-hour fast, a single dose up to 2000 mg/kg was administered. Animals were monitored for mortality and neurological or behavioral changes for 2 to 7 days [17-18].

Assessment of Upper Body Activity

The upper body activity related to the ethanollic extracts of *Bacopa monnieri* was evaluated using the Forced Swim Test (FST) and the Tail Suspension Test (TST).

Treatment

A total of 60 white-skinned Wistar rats were divided into 5 groups, each consisting of 6 rats. The medications were administered orally for 10 consecutive days following a 12-hour fasting period each day. The dosage was standardized and administered orally at 0.1 ml/10 g body weight.

- Group 1 received normal saline (10 ml/kg) as the control.
- Group 2 was given the standard antidepressant drug Imipramine at a dose of 15 mg/kg.
- Groups 3, 4, and 5 received ethanollic extracts of *Bacopa monnieri* at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively.
- For the acute study, 30 Wistar rats were divided into two subgroups:
- On Day 1, one group of 30 rats was tested using the Tail Suspension Test (TST), while the other group underwent the Forced Swim Test (FST). The second group received the test medications one hour prior to testing. For the sub-chronic evaluation, the same rats were re-tested on Day 10, one hour after receiving their respective treatments.

Forced Swim Test (FST) – Upper Body Activity Assessment

In the Forced Swim Test, rats were placed in a 25°C water-filled cylinder (18 cm × 50 cm) after 15 minutes of habituation. They were observed for six minutes, with the last four minutes

timed for immobility. Afterward, rats were dried and placed under low light. Water was changed between tests.

Point Tail Suspension Test

Based on Steru et al., the Tail Suspension Test measured rat immobility while suspended 50 cm above a table. After two unrecorded minutes, immobility was timed over six minutes. Data were analyzed using one-way ANOVA with Dunnett's post-hoc test ($p < 0.05$) and reported as SEM.

Results

Phytochemical Screening Test

The precise *Bacopa Monnieri* leaf extract was subjected to phytochemical screening procedures in order to identify a number of active components. Alkaloids, tannins, steroids, phenolic chemicals, flavonoids, carbohydrates, and glycosides are all included in the crude *Bacopa Monnieri* extract, as indicated in Table 04. Testing the chemical groups of *Bacopa Monnieri* leaf ethanollic extract yielded the results shown in Table 03.

Table 03: chemical groups of *Bacopa Monnieri* leaf ethanollic extract yielded

Phytochemical	Presence
Carbohydrates	++
Tannins	+
Flavonoids	++
Saponins	++
Phenols	+++
Steroids	+
Alkaloids	++
Glycosides	++

Anti Depressant activity of *Bacopa Monnieri*

On Day 1 of the acute study, the experimental groups showed significantly reduced immobility times in both the Forced Swim Test (FST) and the Tail Suspension Test (TST) compared to the control group (see Table 1, Figure 1, and Figure 2). This reduction was observed in animals treated with either the standard antidepressant Imipramine (15 mg/kg) or the test compound, ethanollic extract of *Bacopa monnieri* (EEBM), at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. On Day 10, during the subacute study, a continued reduction in immobility time was observed in both the FST and TST in groups treated with Imipramine (15 mg/kg) and EEBM at 100 mg/kg and 400 mg/kg, as compared to the control group.

Table 04: Day 1: Behavioral Test Results

Group	Tail Suspension Test (seconds)	Forced Swim Test (seconds)
Normal Saline (Control)	241.2 (±05.02)	149.2 (±1.03)
Imipramine 15 mg/kg	179.03 (±13.04)*	110.12 (±2.10)*
EEBM 100 mg/kg	170.2 (±10.2)*	102.15 (±2.10)*
EEBM 200 mg/kg	192.3 (±2.12)*	119.14 (±1.31)*
EEBM 400 mg/kg	130.2 (±1.02)*	118.10 (±0.03)*

A statistically significant value is indicated by an asterisk (*), whereas a value that is not statistically significant is denoted by a hash (#). Immobility time is displayed in seconds as the mean (\pm SEM).

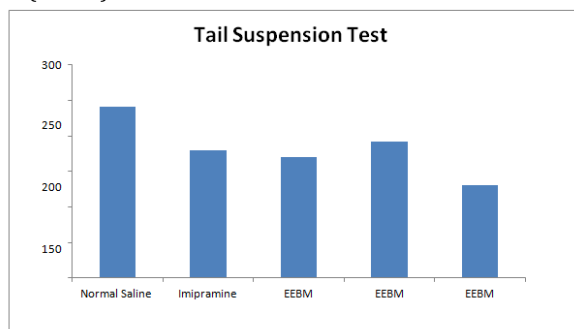


Fig 1: Tail Suspension Test – Day 1

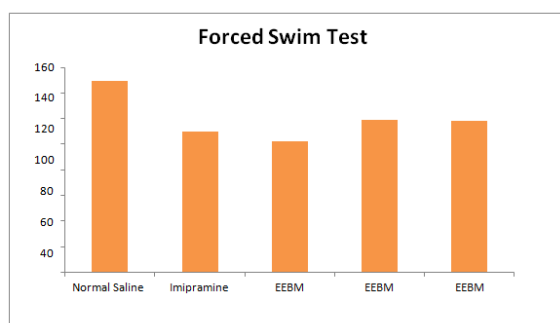


Fig 2: Forced Swim Test Day-1

Table 3: Day 10: Behavioral Test Results

Group	Tail Suspension Test (seconds)	Forced Swim Test (seconds)
Normal Saline (Control)	235.01 (\pm 2.10)	150 (\pm 1.05)
Imipramine 15 mg/kg	150.08 (\pm 1.41)*	95.1 (\pm 2.03)*
EEBM 100 mg/kg	95.08 (\pm 2.10)*	76.10 (\pm 2.01)*
EEBM 200 mg/kg	214.02 (\pm 10.12)#	95.501 (\pm 12.01)*
EEBM 400 mg/kg	109 (\pm 12.01)*	93.150 (\pm 1.15)*

The immobility time is displayed in seconds, with # denoting a statistically non-significant result and SEM (standard error of the mean) indicating the measure of variability.

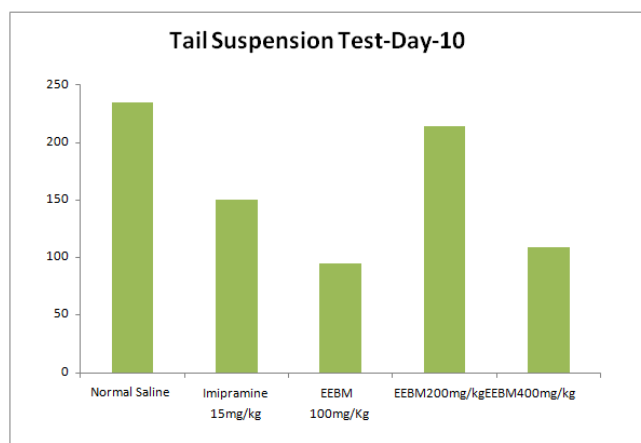


Fig 3: Tail Suspension Test Day-10

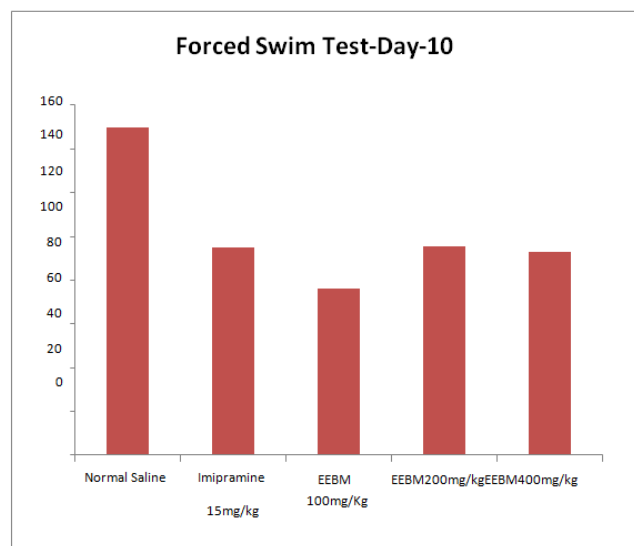


Fig 4: Forced Swim Test Day-10

Discussion

The ethanolic extract of *Bacopa monnieri* leaves showed significant effects in the Forced Swim Test (FST) and Tail Suspension Test (TST), consistent with previous studies. Both Imipramine and *Bacopa monnieri* significantly reduced immobility times in the acute and subacute phases of the FST and TST [23]. Notably, the shortest immobility durations were observed at a *Bacopa monnieri* dosage of 100 mg/kg. In some cases, *Bacopa monnieri* was more effective than Imipramine in reducing immobility times during both acute and subacute stages. Imipramine alleviates depression by increasing the availability of serotonin and norepinephrine transporters in the synaptic cleft [24]. Similarly, the antidepressant-like effects of *Bacopa monnieri* observed in these behavioral models suggest comparable mechanisms of action. Phytochemical analysis of the extract revealed the presence of tannins, alkaloids, and flavonoids, which are likely to contribute to the observed antidepressant effects of *Bacopa monnieri* [25, 26].

Summary and Conclusion

This study focused on evaluating specific therapeutic effects of *Bacopa monnieri*, though not all of its potential uses were explored. Ethanol was used to extract compounds from the dried leaves, and the extract underwent acute toxicity testing in white mice. Behavioral assessments using the Forced Swim Test (FST) and Tail Suspension Test (TST) showed that a 100 mg/kg dose significantly reduced immobility time, indicating antidepressant-like activity. Traditionally used for various ailments, *Bacopa monnieri* is known for its neuropharmacological benefits, and prior studies have shown its effects to be comparable to standard antidepressants. In this study, mice exhibiting depressive-like behavior showed increased immobility, pain sensitivity, and cognitive impairment. Phytochemical screening identified the presence of carbohydrates, alkaloids, tannins, flavonoids, glycosides, and phenols. The extract showed antidepressant-like effects similar to Imipramine, supporting its traditional use as a neuroactive agent and suggesting its potential as a source of novel antidepressant compounds. However, further studies are

required to understand the exact mechanisms involved before clinical application. Overall, the ethanolic extract of *Bacopa monnieri* appears promising as a potential treatment for depressive disorders.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Both are contributed equally

Financial Support

None

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