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## “Evaluation of anti-depressant activity of methanolic extract of *averrhoa bilimbi* using various animal models”

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### Abstract

**Aim of the study:** *Averrhoa bilimbi* is used to treat a variety of illnesses, including those that affect the central nervous system. In this study, albino rats were given methanolic extract of *Averrhoa bilimbi* leaves (MEAB) to investigate its effects using behavioral assays that are sensitive to antidepressants with clinical efficacy. **Methodology:** When mice were subjected to the Tail suspension test (TST) and Forced swim test (FST), the extract (125,250, and 500mg/kg) was able to reduce the immobility duration of the mice dose-dependently; the effects are comparable to those of standard medicines, i.e., imipramine (10mg/kg). **Results:** Our research revealed that MEAB, when administered at greater concentrations, significantly ( $p < 0.0001$ ) reduced immobility in tail suspension and forced swim model of depression comparable to Imipramine. These findings showed that AB exhibited in vivo effects that were selectively antidepressant. **Conclusion:** The results of this study, in summary, revealed that AB extracts may have antidepressant properties that make them potentially useful for treating patients with depressive disorders. However, more research is required to comprehend the mechanism of action and to pinpoint the key ingredient that produces antidepressant-like activity.

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### Introduction

A large percentage of the world's population, or several millions of people, suffer from depression. Two-thirds of depressive individuals have suicidal thoughts, and 10-15% of them make an attempt. The basic signs and symptoms of depression are brought on by functional deficits in the brain's dopamine, noradrenalin, and 5-hydroxytryptamine monoaminergic transmitter levels. The CNS levels of these neurotransmitters are raised by medications that have antidepressant properties [1]. New materials from natural herbal medicines are constantly sought out by society as a supplement and alternative medication due to the late offensive, weak reaction, and certain drawbacks of existing drugs [2]. *Averrhoa bilimbi* is a little tropical tree with a height of up to 10-15 metres that is endemic to Indonesia and Malaysia. Bilimbi leaves are alternating, pinnate, and range in length from 30 to 60 cm. Each leaf has 11-37 oval to oblong-shaped leaflets [3]. The study of *Averrhoa bilimbi* leaves (Family: Oxalidaceae, common name is Bilimbi), a common plant in Asia, is discussed in this paper. Traditional medicine has long used this plant to treat many diseases, including cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough, and hypertension. Additionally, *A. Bilimbi* contains a variety of ethnopharmacological effects that have been extensively documented, including anti-inflammatory,

anti-scorbutic, astringent, anti-bacterial, and postpartum protective properties. The phytochemical analysis of *Averrhoa bilimbi* leaves reveals the presence of secondary metabolites including flavonoid, cardiac glycoside, alkaloid, tannin, phenol, and coumarin as well as primary metabolites like, protein, sugar and aldehyde. In the literature, it has been noted that some secondary metabolites have pharmacological effects [4]. A preliminary study in our laboratory showed the Anti-depressant activity with extracts of *A. bilimbi* Leaves [5]. The purpose of this study is to learn more about the anti-depressant effects of methanolic extracts of AB Leaves (MEAB) on albino rats. The Tail suspension (TST) and Forced swim test (FST) are used to compare the effects of the methanolic extracts of AB Leaves on the immobility period [6].

### Materials and Methods

The leaves of *Averrhoa bilimbi* were obtained from a local market in Mangalore and authenticated by botanist Dr. H. S. Shenoy, MSc, M.Phil., Ph.D., Principal scientist and Head of Botany division. The current study was conducted at the Karavali College of Pharmacy's Department of Pharmacology.

## Animals

Albino rats weighing 150–250 g was acclimated to the experimental room, which had a regulated humidity level of 50–55% and a 12-hour cycle of light and darkness. They were kept in polypropylene cages with a maximum of two animals and given enough regular food pellets and water to drink. According to the directives of CPCSEA, Government of India (Reg. No. 117/1998/CPCSEA), all studies were authorized by the institutional animal ethical committee of Karavali college of Pharmacy, Mangalore, Karnataka.

## PLANT EXTRACT:

By macerating 500 gm. of leaf remnants with 80% methanol for 72 hours, crude Methanolic extracts were created. The extracted extract was filtered and concentrated at 45 °C with reduced pressure in a rotary evaporator. A greenish dark powder made up the finished crude extract. The extract was kept chilled at 4 to 8°C.

**Preliminary phytochemical Screening:** The methanolic extract of *A. Bilimbi* were screened for the presence of various phytoconstituents like Terpenoids, Cardiac glycosides, Anthocyanins, Flavonoids, Alkaloids, Phenols.

**Acute toxicity:** Methanolic extract of *A. Bilimbi* (was studied for acute oral toxicity as per revised OECD (2002) guideline no.423. Animals were observed for four hours hourly for behavior changes and daily for fourteen days. The extract was devoid of any toxicity in mice when given in dose up to 2000mg/kg by oral route. Hence, for further studies 125,250 and 500mg/kg doses of extract were used.

## EXPERIMENTAL DESIGN:

**Tail suspension Method:** A total number of 30 mice were divided into five groups of six mice each.

**Group I:** control (2% Saline solution, 20ml/kg).

**Group II:** standard (Imipramine 10mg/kg).

**Group III:** Test (Methanolic extract of *A. bilimbi* (125mg/kg B. Wt.).

**Group IV:** Test (Methanolic extract of *A. bilimbi* (250mg/kg B. Wt.).

**Group V:** Test (Methanolic extract of *A. bilimbi* (500mg/kg B. Wt.).

As a control, Group 1 received 20 ml/kg of saline solution, Group 2 received Imipramine and Groups 3,4 and 5 received 125,250 and 500 mg/kg of *A. bilimbi* methanolic extract, respectively. The medication was administered orally one hour prior to the trial. The animal was inserted into the model to begin the test.

Adhesive tape was used to hold each mouse in situ 150 cm above the ground, about 1 cm from the tail tip. Testing will be carried out in a darkened room with minimal background noise. The immobility time was recorded for a total of 6 minutes. [7] The animals attempted to flee at first by moving frantically, but when they were unable to do so, they became immobile. When an animal hung passively without making any body movements, it was said to be immobile. After that, Drugs were administered to the mice for 7 days. On the eight day the duration of immobility was recorded for a period of 6 mins.

**Forced swim test:** A total number of 30 mice were divided into five groups of six mice each

**Group I:** control (2% Saline solution, 20ml/kg).

**Group II:** standard (Imipramine 10mg/kg).

**Group III:** Test (Methanolic extract of *A. bilimbi* (125mg/kg B. Wt.).

**Group IV:** Test (Methanolic extract of *A. bilimbi* (250mg/kg B. Wt.).

**Group V:** Test (Methanolic extract of *A. bilimbi* (500mg/kg B. Wt.).

As a control, Group 1 received 20 ml/kg of saline solution, Group 2 received Imipramine and Groups 3,4 and 5 received 125, 250 and 500 mg/kg of *A. bilimbi* methanolic extract, respectively. The medication was administered orally one hour prior to the trial. The animal was inserted into the model to begin the test.

Individual mice were made to swim for 15 minutes in a glass beaker with a diameter of 11 cm and a height of 15 cm filled with fresh water to a height of 6 cm and maintained at a temperature of 27 + 2 C. [8] The "Pretest" session ended here. Each mouse was once more made to swim in the same habitat for 6 minutes in a "Test session" 24 hours later. Before and after the drug therapy, the test session was held. When a mouse floats still or barely moves enough to maintain its head above the water's surface, it is regarded as static. The final four minutes of the six-minute exam were spent immobile on average.

## Result and Observation

**Preliminary phytochemical Screening:** On preliminary phytochemical analysis of MEAB showed the presence of phytoconstituents like Terpenoids, Cardiac glycosides, Anthocyanins, Flavonoids, Alkaloids, phenols.

**Acute toxicity:** Methanolic extract of *A. Bilimbi* showed no behavioral changes nor mortality at dose 2000mg/kg.

By examining the variations in the period of inactivity in the two models, the anti-depressant effects of the methanolic extract of *A. Bilimbi* (125, 250, and 500mg/kg) and Standard medication Imipramine were investigated:

1) Forced swim test.

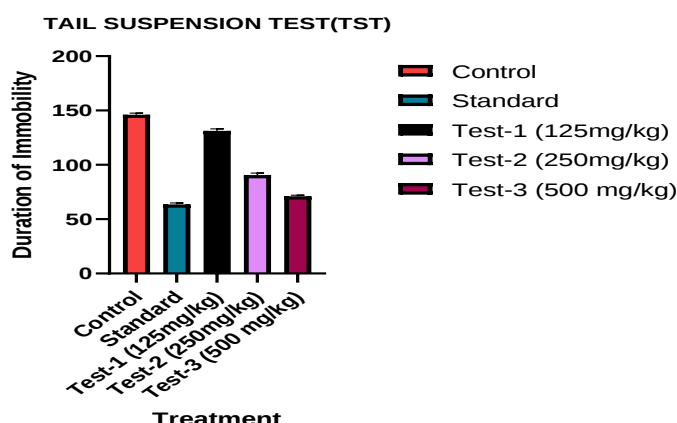
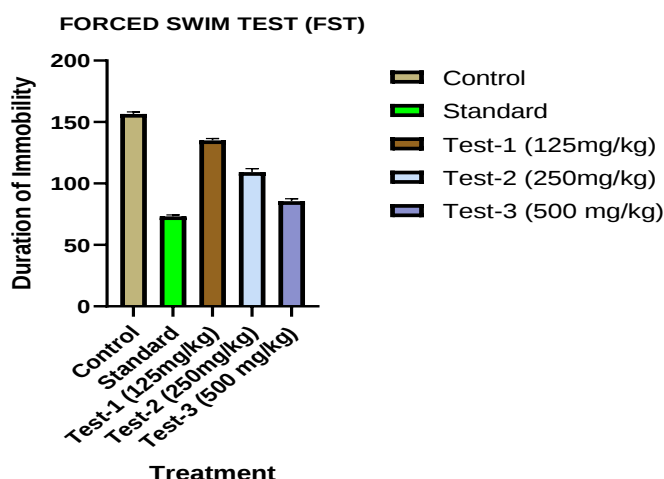
2) Tail suspension test.

**Table 1: effect of MEAB on duration of immobility in tail suspension test (TST).**

| SL.No. | TREATMENT                                                                      | DOSE     | DURATION OF IMMOBILITY IN TST (IN SECONDS) |
|--------|--------------------------------------------------------------------------------|----------|--------------------------------------------|
| 1      | CONTROL(Saline)                                                                | 0.42 ml  | 146.00±1.528                               |
| 2      | STANDARD (imipramine)                                                          | 10 mg/kg | 63.66±1.174                                |
| 3      | TEST 1(2% saline suspension of methanolic extract of <i>Averrhoa bilimbi</i> ) | 125mg/kg | 131.33±1.745                               |
| 4      | TEST 2(2% saline suspension of methanolic extract of <i>Averrhoa bilimbi</i> ) | 250mg/kg | 90.50±1.945                                |
| 5      | TEST 3(2% saline suspension of methanolic extract of <i>Averrhoa bilimbi</i> ) | 500mg/kg | 71.00±1.065                                |

**Table 2: effect of MEAB on duration of immobility in forced swim test (FST).**

| SL.No. | TREATMENT                                                                      | DOSE     | DURATION OF IMMOBILITY IN FST (IN SECONDS) |
|--------|--------------------------------------------------------------------------------|----------|--------------------------------------------|
| 1      | CONTROL(Saline)                                                                | 0.42 ml  | 156.50±1.784                               |
| 2      | STANDARD (imipramine)                                                          | 10 mg/kg | 73.33±1.054                                |
| 3      | TEST 1(2% saline suspension of methanolic extract of <i>Averrhoa bilimbi</i> ) | 125mg/kg | 135.00±1.506                               |
| 4      | TEST 2(2% saline suspension of methanolic extract of <i>Averrhoa bilimbi</i> ) | 250mg/kg | 109.33±2.813                               |
| 5      | TEST 3(2% saline suspension of methanolic extract of <i>Averrhoa bilimbi</i> ) | 500mg/kg | 85.50±2.012                                |

**Figure 1. Comparative profile of duration of immobility in TST (in seconds) after oral administration 125,250,500mg/kg of MEAB.****Figure 2. Comparative profile of duration of immobility in FST (in seconds) after oral administration 125,250,500mg/kg of MEAB.**

MEAB 125,250 and 500mg/kg resulted in a noticeable reduction in both the FST and the TST ( $p < 0.0001$ ). When compared to the animal from the Control group's

## Discussion [9,10]

Depression is an important psychiatric disorder that affects individuals' quality of life and social relation directly. Depression is characterized by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness, and amotivation, as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances and loss of appetite. When the symptoms are very severe major depression is considered.

The goal of current study was to assess the anti-depressant property of an MEAB. In the current investigation, the TST and FST were used as unconditioned behavioral models. These tests don't need the animal to undergo any special training because they are based on unconditioned behavior and rely on their normal behavioral responses. These depressant models are also referred as the "ethologically based" models because they rely on species specific reaction (such as social contact). The TST and FST are most common animal models used for screening potential Anti-depressant agent which induce a state of immobility in animals facing an inescapable situation. In FST showed that administering a high dose of MEAB (500mg/kg) produce a marked reduction in immobility time at dose of 250 and 500mg/kg in the mice. TST and FST, with a comparable to that observed for the classical anti-depressant drug Imipramine. Phytochemical screening shows the presence of Flavonoids and Phenolic compounds. The Flavonoids such as Umbelliferon and resveratrol are responsible for the Anti-depressant activity of the Methanolic extract of *Averrhoa Bilimbi*.

## Conclusion

The present study provides the first evidence indicating that methanolic extract of *Averrhoa bilimbi* showed significant anti-depressant activity in TST and FST models of depression. further research is required to know the mechanism of its action.

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