

Phytochemical Screening & Evaluation of Antidepressant Activity of Ethanolic Extract of Leaves of *Ammania Baccifera*

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Abstract

The results of phytochemical analysis showed the presence of phytoconstituents other than Flavonoids like Alkaloids, Triterpenes, Tannins, saponins, glycosides etc., in EEAB. Previous research reports reveal that the presence of saponins and flavonoids [19] attributed to the anti-depressant activity of several plants. Depression is characterized by emotional symptoms such as hopelessness, apathy, and loss of self-confidence as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances and loss of appetite. The study was conducted to assess the anti-depressant activity of EEAB using FST and TST. Exposure to stress plays an important role in depression. FST and TST models of depression which provides a rapid and reliable behavior screening test for anti-depressants. In this study, treatment of EEAB decreased the duration of immobility in FST and in TST which reveals the antidepressant activity of EEAB. The presence of Phytoconstituents like saponins and flavonoids could attribute to the antidepressant activity of EEAB. However further studies like receptor binding assay interaction studies and neurotransmitter assay are required for elucidating the mechanism of action.

Keywords: Phytochemical Screening, Antidepressant Activity, Ethanolic Extract, Leaves, *Ammania Baccifera*

INTRODUCTION:

Depression is a mental disorder representing a significant and growing public health problem, with an estimated 300 million people afflicted worldwide [1]. The COVID-19 pandemic increased the number of anxiety and depression disorders by 25% during its first year, and the latest data from the World Health Organization estimate that 71% of people with depression do not receive mental health services [2,3]. In addition to the problem of depression itself, this disease brings with it medium- and long-term consequences, such as cognitive disorders, which include deficits in several domains (attention, executive functions, memory and processing speed), dementia and is an initial cause of Parkinson's disease [4,5]. Although drugs are available, access to pharmacological treatment for depression presents difficulties. The prescribed therapy is expensive, and only a small percentage of patients achieve remission with antidepressant monotherapy alone [6]. Another less discussed factor, a common problem affecting 30% to 50% of people with neurological diseases, is pharmacological refractoriness [7,8]. Refractory patients do not respond adequately to medications, even if administered correctly [9]. This reason is still not fully understood, but it is believed that some neurological diseases, because they are multifactorial, are involved in several biological aspects [10]. Thus, treatment may be effective for depression related to a specific etiological factor but not for depression secondary to another etiology [11]. The main classes of drugs available for the treatment of depression are selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenaline reuptake inhibitors (SNRI), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) [12]. The class used as the first choice for the treatment of depression is the SSRI, because, according to the pharmacological aspects, they are safe in overdose, have relative tolerability, have a generic form and have a broad spectrum of use compared to other classes [13]. Due to their lipophilic characteristics, SSRIs, especially fluoxetine, have a large volume of distribution

between 14 and 100 L/kg, which indicates extensive tissue accumulation, mainly in the lung [14,15]. For this reason, SSRIs can increase systole and diastole time (QT interval), which increases the chances of fatal arrhythmia [16]. Currently, the prescription of other classes of antidepressants is very restricted due to high toxicity and potentially lethal food interactions [17]. For example, the Class of MAOIs interacts with foods containing tyrosine, increasing adrenergic activity at high risk of hypertensive crisis [18]. The class of TCAs also has effects on cardiac function such as postural hypotension and high-dose cardiotoxicity [19]. Prescription occurs only in patients who do not respond to more tolerable medications or have refractory depression [20]. In recent years, the antidepressant effect of ketamine anesthetic has been reported in cases of treatment-resistant depression [20]. However, ketamine in clinical practice was limited over a period of time due to its side effects on the central nervous system and the characteristics of a drug of abuse [21].

The developments of the newly born chemical and pharmaceutical industry have brought about great social enthusiasm. The on-going discovery of more and more powerful new medicines, though not less toxic, seems to promise a bright future in which there is a specific pharmacological product to treat every disease. The rational and scientific use of plants, based on chemical and pharmacological research, is truly the only way to correctly use medicinal herbs. Furthermore the efficiency of medicinal herbs increases when they are used within the frame of natural revitalizing treatment.

The present study is to evaluate the phytochemical screening & anti-depressant activity of ethanolic leaf extract of *Ammania baccifera* in mice.

MATERIALS AND METHOD

Collection of Plant Materials:

Fresh leaves of *Ammania baccifera* were collected and dried. After drying they were again pulverized. The size is reduced. The dried plant leaves of *Ammania baccifera* powder was weighed about 200g. Extracted by maceration method using 90% ethanol as a solvent for 4 days. The yield of product was 3.10gms.

Authentication of Plant Material:

The fresh plants of *Ammania baccifera* were collected from the areas of Chittoor district, Andhra Pradesh, India, during the month of September 2023. The plant was taxonomically identified and authenticated by the Botanist Dr. Madhava Shetty.

Identification of Phytochemical Constituents:

Preliminary Phytochemical Tests:

Preliminary phytochemical tests were done by the methods described by usual procedures mentioned in Trease and Evans (1958) and also as specified in the book of Practical Pharmacognosy (Kokate, 2000). The details of the same are provided below. Ethanolic extract leaves of *Ammania baccifera* (EEAB) was subjected to qualitative tests for the identification of various active constituents.

Anti-Depressant Activity

Antidepressant activity of EEAB was assessed using Forced swimming test and Tail Suspension Test.

FORCED SWIMMING TEST

Assessment of anti depressant activity using forced swimming test in mice.

Experimental Design

Swiss Albino mice weighing around 20 to 25 gram were used for this study. They were divided into four groups each group consist of 6 animals (n=6).

Group I: Served as control group which received Propylene glycol (5ml/kg P.o)

Group II: Served as standard drug treatment group which received only Fluoxetine (20mg/kg, P.o)

Group III: Received EEAB 200mg/kg. P.o

Group IV: Received EEAB 400mg/kg. P.o

Procedure

Forced Swim Test:

The method described by *Porsolt, et. al.* was used in our study (Porsolt RD, et.al., 1977) ^[15]. Each animal was placed individually in a 5 liter glass beakers, filled with water upto a height of 15 cm and were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test. A decrease in the duration of immobility is indicative of an antidepressant like effect.

1. **GROUP 1 RECEIVED** - Propylene glycol (5ml/kg P.o)
2. **GROUP 2 RECEIVED** - Fluoxetine (20mg/kg P.o) (standard)
3. **GROUP 3 RECEIVED** - EEAB 200mg/kg (t1)
4. **GROUP 4 RECEIVED** - EEAB 400mg/kg (t2)

TAIL SUSPENSION TEST

Assessment of anti depressant activity using tail suspension test in mice.

Experimental Design

Swiss Albino mice weighing around 20 to 25 gram were used for this study. They were divided into four groups each group consist of 6 animals (n=6).

Group I: Served as control group which received Propylene glycol (5ml/kg P.o)

Group II: Served as standard drug treatment group which received only Fluoxetine (20mg/kg, P.o)

Group III: Received EEAB 200mg/kg. P.o

Group IV: Received EEAB 400mg/kg. P.o

Procedure

Tail Suspension Test:

The method described by *Steru, et. al.* was used in our study (Steru L, et.al., 1985) ^[16]. The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an

adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless. A decrease in the duration of immobility is indicative of an antidepressant effect.

1. **GROUP 1 RECEIVED** – Propylene glycol (5ml/kg P.o)
2. **GROUP 2 RECEIVED** - Fluoxetine (20mg/kg P.o) (standard)
3. **GROUP 3 RECEIVED** - EEAB 200mg/kg (t1)
4. **GROUP 4 RECEIVED** - EEAB 400mg/kg (t2)

Statistical Analysis

The collected data was subjected to appropriate statistical tests including one-way ANOVA (Analysis of Variance), followed by Dunnett's test. P values of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using Graph pad prism software of version 4.

RESULT

Ethanollic leaf extract of *Ammania baccifera* leaves were subjected to qualitative phytochemical tests for different phytochemical constituents. From the Phytochemical analysis, the plant extract shows the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenes.

Table no.1 - Phytochemical Analysis of Ethanolic Extract of *Ammania baccifera* Leaves

S.NO	PHYTOCONSTITUENTS	ETHANOLIC EXTRACT
1.	Carbohydrates	-
2.	Amino Acids	-
3.	Lipids	-
4.	Alkaloids	+
5.	Triterpenes	+
6.	Tannins	+
7.	Saponins	+
8.	Flavonoids	+
9.	Glycosides	+

(+) Present, (-) Absent

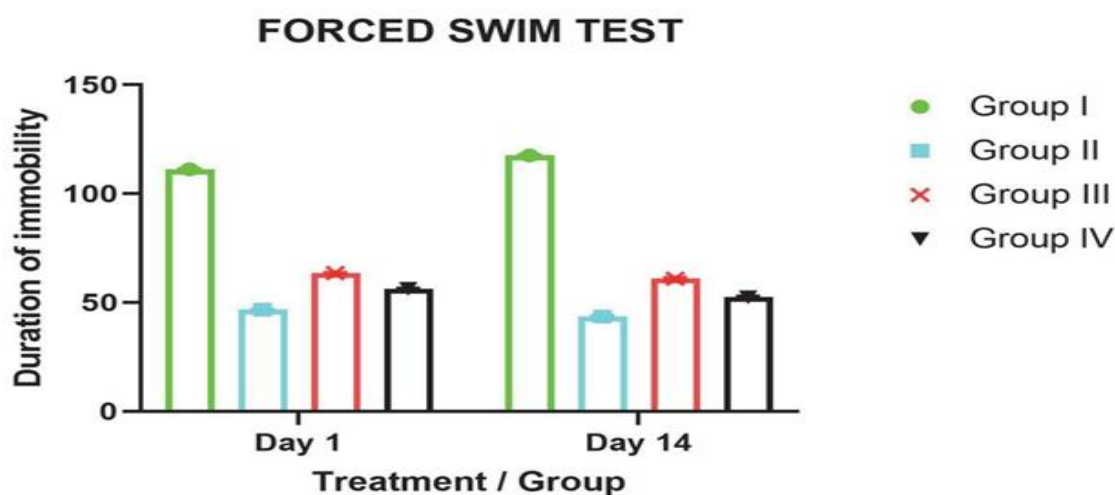
EVALUATION OF ANTI-DEPRESSANT ACTIVITY FORCED SWIMMING TEST

Group I animals showed duration of immobility for 111.2 ± 0.21 seconds on day 1 and on day 14, duration of immobility was slightly increased to 117.5 ± 0.32 seconds. On day 1, Group II animals treated with fluoxetine showed lesser duration of immobility for about 46.7 ± 0.19 seconds and on day 14, they showed further reduction in the duration of immobility for about 43.5 ± 0.56 seconds. Group III and Group IV (100 mg/kg and 200mg/kg of extract) also showed reduction in the duration of immobility.

A significant decrease in duration of immobility in mice was observed in test groups when compared to control group. The high dose of test compound (200mg/kg) exhibited a significant reduction in immobility compared to other test group of animals. The results were shown in the table no.2. The results coincided with the previous reported article^[17].

Table No.2 - EFFECT OF ETHANOLIC EXTRACT OF *AMMANIA BACCIFERA* ON IMMOBILITY TIME IN THE FORCED SWIM TEST (FST) USING MICE

S.No	Treatment	Duration of immobility (seconds)	
		Day 1	Day 14
1.	Group I (Propylene glycol-5ml/kg)	111.2±0.21	117.5±0.32
2.	Group II (Fluoxetine – 20mg/kg)	46.7±0.19	43.5±0.56
3.	Group III (EEAB – 100mg/kg)	63.6±0.26	60.9±0.60
4.	Group IV (EEAB – 200mg/kg)	56.3±0.31	52.4±0.48



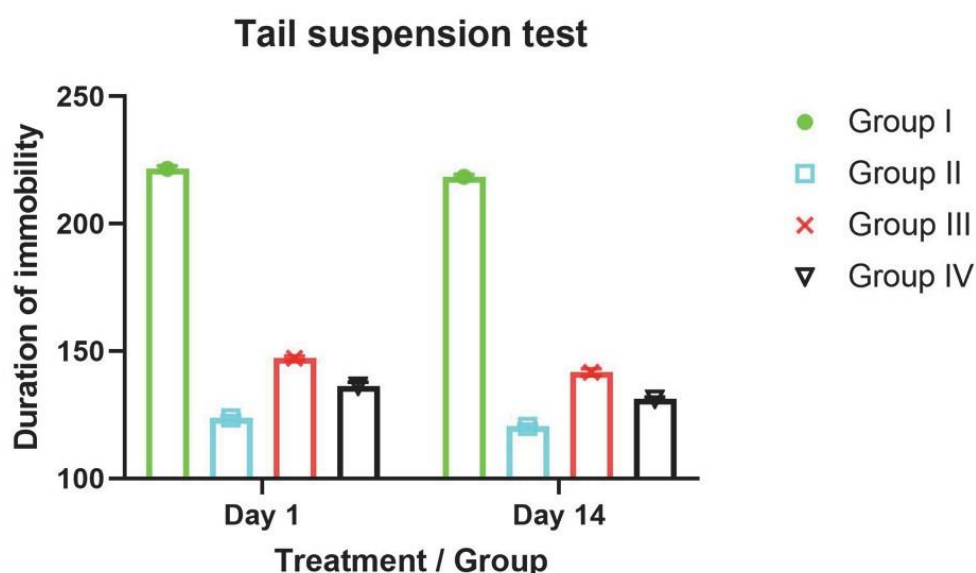
TAIL SUSPENSION TEST

Group I animals showed duration of immobility for 221.6±1.06 seconds on day 1 and on day 14, duration of immobility was found to be 218.4±0.96 seconds. On day 1, Group II animals treated with fluoxetine showed reduction in duration of immobility for about 123.8±0.89 seconds and on day 14, they showed further reduction in the duration of immobility for about 120.4±0.94. Group III and Group IV (100 mg/kg and 200mg/kg of extract) also showed reduction in the duration of immobility.

A significant decrease in duration of immobility in mice was observed in test groups when compared to control group. The high dose of test compound (200mg/kg) exhibited a significant reduction in immobility compared to other test group of animals. The results were shown in the table no.3. The results coincided with the previous reported article ^[18]

Table No.3 - EFFECT OF ETHANOLIC EXTRACT OF *AMMANIA BACCIFERA* ON IMMOBILITY TIME IN THE TAIL SUSPENSION TEST (TST) USING MICE

S.No	Treatment	Duration of immobility (seconds)	
		Day 1	Day 14
1.	Group I (Propylene glycol- 5ml/kg)	221.6±1.06	218.4±0.96
2.	Group II (Fluoxetine – 20mg/kg)	123.8±0.89	120.4±0.94
3.	Group III (EEAB – 100mg/kg)	147.2±0.83	141.7±1.41
4.	Group IV (EEAB – 200mg/kg)	136.3±1.45	131.2±0.71



DISCUSSION

The results of phytochemical analysis showed the presence of phytoconstituent other than Flavonoids like Alkaloids, Triterpenes, Tannins, saponins, glycosides etc., in EEAB.

Previous research reports reveal that the presence of saponins and flavonoids [19] attributed to the antidepressant activity of several plants.

Depression is characterized by emotional symptoms such as hopelessness, apathy, and loss of self-confidence as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances and loss of appetite. The study was conducted to assess the antidepressant activity of EEAB using FST and TST. Exposure to stress plays an important role in depression. FST and TST models of depression which provides a rapid and reliable behavior screening test for antidepressants.

In this study, treatment of EEAB decreased the duration of immobility in FST and in TST which reveals the antidepressant activity of EEAB.

The presence of Phytoconstituents like saponins and flavonoids could attribute to the antidepressant activity of EEAB. However further studies like receptor binding assay interaction studies and neurotransmitter assay

are required for elucidating the mechanism of action.

CONCLUSION

The present study was designed to evaluate the anti-depressant activity of selected EEAB using Forced Swimming Test and Tail Suspension Test using the standard drug Fluoxetine (20 mg/kg).

In conclusion, EEAB showed significant anti depressant activity without producing motor in coordination in mice. Hence EEAB may become potential resource for natural psychotherapeutic agent against various anxiety related disorders and behavioral depression with fewer side effects compared to current available therapies.

Further, extensive studies are required to determine the exact mechanism and its active ingredient responsible for the anti-depressant activity

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