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Original Research Article

Psychopharmacological Evaluation of the Anxiolytic and Antidepressant Effects of the Ethanolic Flower Extract of *Millingtonia hortensis* (EFEMH) in Experimental Animal Models

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Abstract: *Millingtonia hortensis* flowers have long been used in traditional medicine for their calming and anxiolytic properties, indicating potential therapeutic value in neurobehavioral disorders. However, scientific evidence supporting their anxiolytic and antidepressant activities is limited. The present study investigated the effects of the ethanolic flower extract of *Millingtonia hortensis* (EFEMH) in mice. Administration of EFEMH at 100 and 300 mg/kg, as well as the reference drug imipramine (10 mg/kg), produced significant behavioural effects across multiple psychopharmacological tests. EFEMH markedly reduced locomotor activity in the actophotometer test; decreased the number of entries and time spent in the open arms in the elevated plus maze; reduced head dips in the hole-board test; and decreased rearings and the number of steps climbed in the staircase test. EFEMH also significantly reduced immobility time in both the tail suspension test, indicating antidepressant-like activity. These findings provide scientific support for the anxiolytic and antidepressant potential of *Millingtonia hortensis* flower extract and help validate its traditional use in the management of emotional and neurobehavioral disorders.

Keywords: *Millingtonia hortensis*, Ethanolic flower extract, Anxiety, Depression, Psychopharmacology, Elevated Plus Maze, Tail Suspension Test, Mice.

1. Introduction

In 2021, nearly 1 in every 7 people (about 1.1 billion) worldwide were living with a mental disorder, with anxiety and depressive disorders being the most common. [1]. "Depression and anxiety are among the most common psychiatric disorders worldwide. Although several conventional anxiolytic and antidepressant medications—such as benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors, and noradrenergic and specific serotonergic antidepressants—are routinely used in clinical practice, they are often associated with adverse effects. These may include cardiovascular toxicity, sexual dysfunction, weight gain, and undesirable drug interactions [2], [3]. Therefore, there is an urgent need for the development of effective anxiolytic and antidepressant therapies without any or at least fewer adverse effects.

Millingtonia hortensis flower extract has long been valued in traditional medicine for its calming and anxiolytic effects, suggesting its therapeutic relevance in neurobehavioral disorders[4], [5].

Phytochemical investigations report that the flowers contain high levels of bioactive flavonoids, including hispidulin, scutellarein, scutellarein-5-galactoside, and hortensin, as well as other secondary metabolites. The essential oil obtained through hydrodistillation (yield 0.5–2%) possesses major constituents, including β-caryophyllene, α-bergamotene, linalool, nerolidol, trans-farnesol, and vanillin, many of which are known for their neuroactive and anxiolytic properties [6], [7].

Notably, hispidulin has demonstrated anticonvulsant action via modulation of GABA and benzodiazepine receptors, inhibition of glutamate release, and neuroprotective effects, while scutellarin has shown improvement in sensorimotor function and depressive behaviour in vivo[8], [9]. Additionally, Beta-Caryophyllene has been shown to be a

Cannabinoid Receptor Type 2 Selective Agonist. Considering these pharmacologically validated constituents and the plant's traditional use in anxiety-related conditions, the ethanolic extract of *Millingtonia hortensis* flowers has been selected for screening of anxiolytic and antidepressant activity. [10].

The present study evaluated the anxiolytic activity of EFEMH in mice at doses of 100 and 300 mg/kg/day using Irwin behavioural screening, the actophotometer test, the rota-rod test, the hole-board test, the staircase test, and the elevated plus maze test (EPMT). Its antidepressant effects were assessed using the tail suspension test (TST). Each experimental group consisted of six mice, and all behavioural assessments were conducted 60 minutes after oral administration of EFEMH. Diazepam and imipramine, administered intraperitoneally 15 minutes before testing, served as standard reference drugs for anxiolytic and antidepressant activities, respectively. A 10-day washout period was provided before initiating each subsequent experimental method to prevent carryover effects.

2. Materials and Methods

2.1 Plant Material and Preparation of the Aqueous Extract

Flowers of *Millingtonia hortensis* collected from the West Godavari district were cleaned, shade-dried, powdered, and stored in an air-tight container. About 150 g of the powdered flowers were macerated in 500 mL of ethanol for two weeks with intermittent stirring. The extract was filtered through muslin cloth and Whatman No.1 paper, then concentrated using a rotary evaporator at 50–60°C under reduced pressure. The resulting residue was further dried in a water bath and vacuum desiccator, yielding the ethanolic extract, which was stored at 4°C for subsequent pharmacological studies.

2.2 Animals

Swiss albino mice of either sex, weighing 20 to 25 g, were purchased from the *Vyas Labs*, Hyderabad. The animals were housed under a 12 h light/dark cycle, with controlled humidity (50 ± 10%) and temperature (22 ± 2°C), and were allowed free access to food and water. The animals were acclimatized for at least 3 days before they were tested [11]. All animal experiments were performed in compliance with the recommendations of the IAEC, A.K.R.G College of Pharmacy.

2.3. Drugs and Administration

For each test, mice were divided into four groups: vehicle control, standard treatment, and two experimental groups. Animals in the vehicle control group received normal saline (0.9% NaCl). The standard group was treated with 2 mg/kg I.P diazepam (DZP, Sigma) as an anxiolytic drug, while in the antidepressant tests, the standard group was treated with 10 mg/kg I.P Imipramine as an antidepressant drug 30 minutes before the test. Mice in the two experimental groups were administered ELEMH at doses of 100 and 300 mg/kg p.o., 60 minutes before the test. All administrations were performed at a volume of 0.4 mL per 25 g body weight. All drugs were administered once daily via gastric intubation between 10:00 AM and 3:00 PM [12].

2.4 Irwin Behavioural Screening

The Irwin test was used to assess the general behavioural profile of mice after EFEMH administration, focusing on autonomic, neurological, and motor responses. Motor activity was monitored to detect any stimulant or depressant effects. Behavioural screening tests were performed to observe changes in grooming, posture, reflexes, and exploratory activity [13].

2.5 Acute Toxicity Study

Toxicity studies were conducted separately for the EFEMH to establish a safe dose, following OECD 420 fixed-dose guidelines. Swiss albino mice (8–12 weeks, 170–190 g) were randomly selected, acclimatized for five days, and maintained under standard laboratory conditions (22 ± 3°C; 12-h light/dark cycle). As per the guideline, single animals received the test extract sequentially at fixed dose levels of 50, 200, 300, and 2000 mg/kg, selected based on expected toxicity thresholds [14].

2.6 Behavioural Test

General behavioural profiling was performed using the Irwin test. Adult albino mice were divided into 5 groups and treated according to the study protocol. Animals were observed for behavioural changes at 30-minute intervals during the first hour, then hourly for the next 6 hours. Parameters assessed included awareness, alertness, spontaneous activity, stereotypic movements, and reflex responses. Behavioural scores were assigned based on the degree of activity or response. Touch, pain, pinna, corneal, and flexor reflexes were evaluated using standard stimuli to detect any CNS depressant or stimulant effects of the extract [15].

2.7 Anxiolytic Activity Tests in Mice

Actophotometer Tests in Mice

Locomotor activity was assessed using a digital actophotometer equipped with photoelectric sensors that record movement interruptions as activity counts. Animals were acclimatized to the apparatus for 3 minutes, and baseline activity was recorded individually. Treatments followed the standard protocol. Activity was measured 1 hour after extract administration and 30 minutes after diazepam. Each mouse was placed in the actophotometer, and locomotor activity was recorded for 5 minutes [16].

Rota-rod Tests

The rota-rod apparatus consisted of a non-slippery rotating rod (3 cm × 30 cm) mounted above a base platform. The time each mouse remained on the rod without falling was recorded as performance time. Mice were trained through repeated trials, and only those able to stay on the rod for at least 10 seconds were included; poorly performing animals were excluded. Treatments followed the standard protocol. A cut-off time of 300 seconds was applied. Each dose was tested in a separate group of trained mice, and no animal was reused for drug testing [17].

Elevated Plus-Maze Test (EPMT)

The elevated plus maze consisted of two open arms and two closed arms (35 × 5 cm), connected by a 5 × 5 cm central platform, elevated 25 cm above the floor, in a dimly lit room. Treatments followed the standard protocol. Each mouse was placed at the centre facing a closed arm, and the number of entries and the time spent in the open and closed arms were recorded for 5 minutes. An arm entry was counted when all four paws entered the arm [18].

Hole-board test

The hole-board apparatus consisted of a wooden chamber (40 × 40 × 25 cm) with 16 evenly spaced holes (3 cm diameter) and was elevated 25 cm above the floor to allow head-dipping behaviour. Treatments followed the standard protocol. Each mouse was then placed in the apparatus, and the number and duration of head dips were recorded over a 5-minute observation period [19].

Staircase test

The staircase apparatus consisted of a wooden structure with five identical steps (3 cm high, 15 cm wide, 3 cm deep) enclosed by vertical walls of uniform height. Treatments followed the standard protocol. Each mouse was placed at the bottom of the staircase and observed for 5 minutes. The number of steps climbed and rearings were recorded as indices of anxiety [20].

2.8 Antidepressant Test in Mice

Tail Suspension Test (TST)

The tail suspension test was performed according to the method of Steru et al. Treatments followed the standard protocol described under *Drugs and Administration*. Mice were suspended 5 cm above the floor using adhesive tape placed about 1 cm from the tip of the tail. The test was conducted in a darkened, quiet room. Immobility was defined as the absence of limb or body movements, except those required for respiration. The total duration of immobility during the 6-minute session was recorded by an observer blinded to the treatment groups.

Statistical Analysis

The data were statistically analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in GraphPad Prism 8. The results were expressed as the mean ± Standard Error of Mean (S.E.M.). Differences among groups were considered to be statistically significant at $P < 0.05$.

3. Results

Percentage of yield and characteristics of ELENC

Ethanolic Flower Extract of *Millingtonia hortensis (*EFEMH) showed the following characteristics, with a yield of 15.6%.

Table 1: Percent of yield and characters of ELENC

Form of Extract	Color	Consistency	Yield
Ethanolic	Dark yellow	Sticky	15.6%

Preliminary Qualitative Phytochemical Study of ELEMH

Table 1: Preliminary Qualitative Phytochemical Study of ELEMH

NO	Constituent	Test	Observation	Inference & Intensity
1	**Alkaloids**	Mayer's reagent	Creamy-white precipitate	+ve
		Wagner's reagent	Reddish-brown precipitate	+ve
		Dragendorff's test	Red-precipitate	+ve
2	Carbohydrates	Molisch test	No change	-ve
3	Steroids& Terpenoids	Biurette test	Purple coloration	-ve
4	Protein	Ninhydrin test	Purple coloration	-ve
	Phenols	Ferric chloride test	Deep blue colour	+ve
5		Liebermann's test	Deep green colour	+ve
6	Tannins	Ferric chloride test	Greenish black	+ve
		Ammonium test	Yellow colour	+ve
7	Flavanoids	Aluminum Chloride	Yellow ppt	+ve
8	Glycosides	Fehling's test	No precipitate	-ve
9	Saponins	Emulsion test	Emulsion formation	+ve

(+) denotes the presence of the respective class compound,

(-) denotes the absence of the respective class compound.

Acute oral toxicity studies

No observable signs of toxicity were observed in the animals at the limit dose of 2000 mg/kg. Therefore, two dose levels were selected for the evaluation of nootropic activity: a lower dose of EFEMH 100 mg/kg (p.o.) and a higher dose of ELENC 300 mg/kg (p.o.).

Effect of ELEMH on the general behavioural profile

Table 2: Effect of ELEMH on general behavioral profile in mice. (n=3)

Evaluation parameters	Control	Standard	ELEMH 300 mg/kg
Alertness	4	2	3
Stereotype movements	0	0	0
Spontaneous activity	3	1	2
Grooming	0	0	0
Passivity	0	0	0
Reactivity	4	3	4
Pain response	4	3	2
Touch response	4	3	4
Limb position	4	3	4
Body position	4	3	4
Corneal reflex	4	3	4
Righting reflex	0	1	0

ELEMH significantly affect spontaneous activity, reactivity, and passivity. They also depress animals' awareness and alertness. However, the righting reflex, corneal reflex, and vocalisation remain normal throughout the study. The above studies indicate that the extract has a sedative action on the animals. The loss of reactivity indicates an anxiolytic effect of the extract on the animal.

Reduction of Locomotor Activity by EFEMH in the Actophotometer Assessment

Mice treated with EFEMH at doses of 100 mg/kg and 300 mg/kg exhibited a significant ($p < 0.05$) dose-dependent reduction in locomotor activity compared to the control group. Control animals recorded 255 ± 5.62 counts, whereas treatment with 100 mg/kg and 300 mg/kg EFEMH reduced the counts to 163 ± 5.61 and 137 ± 2.84, respectively. This dose-related decrease suggests the extract's potential anxiolytic effect. The standard drug, diazepam (2 mg/kg), also significantly reduced locomotor counts from 255.32 ± 5.62 to 150.35 ± 3.01.

Reduction of fall-off time Activity by EFEMH in the Rotarod test

In this study, EFEMH administered at 100 mg/kg and 300 mg/kg did not significantly alter fall-off time on the rotarod test compared with the control group (21.00 ± 1.67 seconds). In contrast, treatment with diazepam resulted in a marked reduction in fall-off time, to 17.80 ± 0.60 and 12.20 ± 0.87 seconds, respectively, indicating impaired motor coordination.

Reduction in the number of entries into the open arm and the open arm time spent by EFEMH in the Elevated Plus Maze

In the present study, administration of EFEMH increased the time spent in the open arms and produced a significant ($p < 0.05$) increase in the number of open-arm entries in the Elevated Plus Maze. These findings suggest that the extract reduces anxiety and fear associated with open, illuminated spaces. In the control group, animals recorded 4.50 ± 0.43 open-arm entries. Treatment with EFEMH at 100 mg/kg and 300 mg/kg increased these entries to 4.67 ± 0.33 and 5.67 ±

0.33, respectively. Similarly, open-arm duration increased from 14.33 ± 0.72 seconds in the control group to 32.83 ± 1.17 seconds and 44.33 ± 1.38 seconds at 100 mg/kg and 300 mg/kg, respectively. This dose-dependent increase highlights the extract's anxiolytic potential. The standard drug, diazepam (2 mg/kg), also significantly increased the number of open-arm entries (6.17 ± 0.48) and open-arm duration (47.83 ± 2.82 seconds)..

Reduction in the number of head dips by EFEMH in the Hole board test

In the present study, EFEMH administered at 100 mg/kg and 300 mg/kg produced a significant ($p < 0.05$) reduction in head-dip counts in the hole-board test compared to the control group. Control animals showed 16.33 ± 0.76 head dips, whereas treatment with EFEMH reduced the counts to 11.50 ± 0.56 and 9.17 ± 0.87 at 100 mg/kg and 300 mg/kg, respectively. This reduction reflects the extract's anxiolytic potential. The standard drug, diazepam (2 mg/kg), also significantly decreased head-dip counts from 16.33 ± 0.76 to 7.83 ± 0. 60..

Reduction in the number of rearings and the number of steps climbed by EFEMH in the Staircase test

In the staircase test, animals treated with the ethanolic extract (EFEMH) showed a significant ($p < 0.05$) reduction in rearings compared to the control group, indicating the extract's anxiolytic potential. Control animals exhibited 20.33 ± 0.99 rearings. Treatment with EFEMH at 100 mg/kg and 300 mg/kg reduced rearings to 15.67 ± 0.56 and 11.33 ± 0.67, respectively. Similarly, the number of steps climbed decreased from 18.17 ± 0.60 in the control group to 11.67 ± 0.67 at both 100 mg/kg and 300 mg/kg. The standard drug, diazepam (2 mg/kg), produced a marked reduction in rearings (6.50 ± 0.62) and decreased the number of steps climbed from 18.17 ± 0.60 to 6.00 ± 0. 68..

Table 3: Summary of Behavioural Test Outcomes for Anxiolytic Evaluation

S.No	Behavioral Test	Control	Diazepam	EFEMH 100mg/kg	EFEMH 300mg/kg
1.	Actophotometer test	255.32 ± 5.62	150.35 ± 3.01**	163.24 ± 5.61*	137 84± 2.84**
2.	Rotarod test (Fall of time in sec)	21.00 ± 1.67	12.20 ± .87**	17.8 ± .60ns	14.30 ± 0.72ns
3.	Elevated plus-maze test No. of entries into Open arm	4.50 ± 0.43	6.17± 0.48**	4.67± 0.33ns	5.67± 0.33**
	Open arm time in sec	14.33 ± 0.72	47.83 ± 2.82**	32.83 ± 1.17*	44.33 ± 1.38**
4.	Hole Board test	16.33 ± 0.76	7.833 ± 0.60**	11.50 ± 0.56*	9.17 ± 0.87*
5.	Staircase test No of rearings	20.33 ± 0.99	6.50 ± 0.62**	15.67 ± 0.56ns	11.33 ± 0.67*
	No of steps climbed	18.17 ± 0.60	6.00 ± 0.68**	11.67 ± 0.67*	11.67 ± 0.67**

Values are expressed as Mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's post hoc test was used for statistical analysis. *$P < 0.05$ and **$P < 0.01$ compared with the control group.

Reduction in Duration of Immobility by EFENH in TST

Administration of EFEMH at doses of 100 mg/kg and 300 mg/kg produced a significant ($p < 0.05$) reduction in total immobility duration in the Tail Suspension Test, in which immobility decreased from 185.2 ± 2.85 seconds (control) to 157.7 ± 3.45 seconds and 138.2 ± 3.16 seconds at 100 mg/kg and 300 mg/kg EFEMH, respectively. Imipramine (10 mg/kg) further reduced immobility to 130.0 ± 2.37 seconds.

Table 4: Summary of Behavioural Test Outcomes for Antidepressant Evaluation

S.No	Immobility	Control	Imipramine 10 mg/kg i.p	EFEMH 100mg/kg	EFEMH 300mg/kg
1.	Tail suspension test	185.2 ± 2.85	130.0 ± 2.37**	157.7 ± 3.45 *	138.2 ± 3.16**

Values are expressed as Mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's post hoc test was used for statistical analysis. *$P < 0.05$ and **$P < 0.01$ compared with the control group.

4. Summary and Conclusion

Nearly one-fifth of the global adult population experiences psychiatric disorders such as anxiety, depression, or psychosis. Growing access to information has increased awareness of the adverse effects of conventional psychopharmacological drugs, including sedation, drowsiness, and withdrawal symptoms. As a result, many individuals, particularly in Western societies, are turning to alternative therapies that are perceived as safer and more acceptable. [21], [22]. Herbal therapy has become increasingly popular worldwide, with the use of medicinal plants rising even in developed countries. Despite this growing demand, pharmaceutical research in phytotherapy has slowed in recent years due to its complex, time-consuming nature and limited marketability. Strengthening scientific investigation in this field and providing evidence-based justification may enhance its clinical relevance and industrial potential[23]. Therefore, we undertook this study to evaluate the potential of *Millingtonia hortensis* flowers using different animal models and to examine the plant's effect on their exploratory behaviour.

Plant-derived natural products have long been used in traditional medicine. Millingtonia hortensis has been investigated for multiple pharmacological activities based on its traditional use and chemical composition. Different parts of the plant—flowers, leaves, roots, stem, and bark—are traditionally used for conditions such as fever, sinusitis, cough, and as general tonics. Literature also reports antifertility activity of the flowers and the use of aqueous ethanolic extracts of aerial parts for managing constipation and diarrhoea [24].

In traditional medicine, the leaves and roots of *Millingtonia hortensis* are used for their anti-asthmatic and antimicrobial properties, and leaf extracts have shown notable antimicrobial activity. The tree is valued ornamentally for its fragrant flowers, while its wood and bark serve as timber and a substitute for cork. Leaves are sometimes used as a low-cost tobacco substitute. The plant contains alkaloids, tannins, flavonoids, and phenolic compounds, with key flavones such as hispidulin and hortensin isolated and characterised

Several flavonoids have been isolated from the flowers of *Millingtonia hortensis*. Fresh flowers yielded a new glycoside, scutellarein-5-galactoside, along with scutellarein. Other reported constituents include scutellarein, hispidulin, and scutellarein-5-glucuronide. Hispidulin has also been isolated from dried flowers using TLC. Powdered flowers yielded hortensin (3,4-dihydroxy-6,7-dimethoxyflavone), which was characterised spectroscopically and found to differ from the synthetic standard. The Neuropharmacological Profile study suggested by Irwin provides clues for classifying the active compounds for further testing. In this procedure, mice are observed after drug administration for an initial undisturbed phase, followed by phases in which they are subjected to mild to increasingly intense stimuli. Broadly, the neuropharmacological profile is divided into behavioural, neurological, and autonomic changes after administration of increasing doses of drugs [6], [8].

The Irwin test evaluates the behavioural, neurological, and autonomic profiles of animals, including awareness, mood, motor activity, CNS excitation, posture, coordination, reflexes, and autonomic responses. In the present study, the ethanolic flower extract of *Millingtonia hortensis* (ELEMH) was examined based on earlier reports indicating its sedative properties, while its major constituent, hispidulin, has demonstrated anxiolytic activity. Therefore, ELEMH was expected to reduce spontaneous activity and exhibit anxiolytic potential. Based on the AOT study, no signs of toxicity were observed, and two doses—100 mg/kg and 300 mg/kg—were selected for further evaluation. Subsequent behavioral tests were carried out to establish the anxiolytic and antidepressant properties of the extract.

The Elevated Plus Maze (EPM) is a standard rodent model used to screen potential anxiolytic or anxiogenic compounds. It relies on the natural conflict between exploring open arms, which are perceived as risky, and preferring the safer closed arms. Although several neurotransmitter systems contribute to EPM behavior, many aspects remain unclear. However, pharmacological studies consistently show that GABA receptor modulation, particularly through agonists, plays a key role in reducing anxiety-like responses in this model [25]. Evidence suggests both GABAergic and serotonergic pathways influence EPM behavior. Diazepam, acting through the GABA receptor complex, increased open-arm entries and the time spent in open arms. Similarly, EFEMH (100 and 300 mg/kg) enhanced open-arm exploration and reduced closed-arm time, indicating a strong anxiolytic effect possibly mediated through GABA or serotonergic mechanisms.

The Rota-Rod test assesses motor coordination and is commonly used to detect neurotoxicity or muscle-relaxant effects of CNS-acting drugs. A decrease in fall time indicates impaired coordination or muscle relaxation[26]. In this study, the ethanolic extract at 100 and 300 mg/kg did not significantly reduce fall time compared with the vehicle group, suggesting that the extract does not produce notable muscle-relaxant effects.

Locomotor activity is considered an index of alertness, and a decrease in this indicates an anxiolytic and sedative effect. One hypothesis regarding the significance of motor activation in relation to anxiety-like states is that an increase in locomotor stimulation may indicate a stress-like state; a decrease in behavioural arousal, as reflected in the suppression of locomotor activity, may indicate an anxiolytic-like state. Following administration of 100 mg/kg and 300 mg/kg ethanolic extract, both doses showed a significant decrease in locomotor activity compared to the control. These results show that the ethanol extracts of *Millingtonia hortensis* affect the stress-induced exploration behaviour of the animals.

The hole-board test evaluates an animal's response to an unfamiliar environment and is commonly used to assess anxiety. Head-dipping behaviour is sensitive to emotional changes, with reductions generally indicating an anxiolytic effect [27]. In this study, EFEMH at 100 and 300 mg/kg produced a dose-dependent decrease in head-dip counts, demonstrating a significant anxiolytic effect of *Millingtonia hortensis* in this model..

The staircase test is a simple and reliable model for screening anxiolytic agents, where the number of steps climbed and rearings reflect exploratory and locomotor activity. Anxiolytic drugs typically reduce these behaviours [28]. In the present study, animals treated with the ethanolic extract showed a significant decrease in rearings and steps climbing compared with controls, indicating the anxiolytic potential of the extract.

Depression is a widely studied disorder, with extensive research over the past decades on its epidemiology, risk factors, neurobiology, symptoms, diagnosis, impact, and treatment, including the effectiveness and safety of various antidepressants. The Tail Suspension Test (TST) is a widely used model to assess antidepressant activity, as they measure the shift from active escape behaviours to immobility when rodents are exposed to an acute, inescapable stressor [29], [30]. This immobility is considered analogous to depressive-like behaviour. In the present study, *Millingtonia hortensis* extract (100 and 300 mg/kg) significantly reduced immobility time in both FST and TST, demonstrating a dose-dependent antidepressant effect.

5. Conclusion

From the above study, it was concluded that *Millingtonia hortensis* flower extract possesses significant anxiolytic and anti-depressant activities. These findings give a scientific rationale for the traditional use of *Millingtonia hortensis*.

6. Future scope for the research

Further research is needed to clarify the neuropharmacological mechanisms underlying the anxiolytic and antidepressant potential of *Millingtonia hortensis*. The plant shows neuroprotective and antidepressant-like effects, but detailed studies—such as receptor-binding assays targeting GABA, dopamine, and 5-HT systems—are required to identify its active pathways. Exploring such herbal sources may yield safer, more effective anxiolytic agents. Although many medicinal plants are promising and economical, challenges in standardization continue to limit their widespread use.

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