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## Evaluation of anti-anxiety activity of ethanolic extract of *Jasminum sambac* leaves in experimental animals

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### Abstract

**Aims and Objectives:** The present study aimed to evaluate the anti-anxiety activity of the ethanolic extract of *Jasminum sambac* leaves in Swiss albino mice using the Elevated Plus Maze model.

**Methods:** Healthy Swiss albino mice (35-40 g, either sex) were randomly divided into four groups (n = 6). Group I (control) received normal saline (10 ml/kg, orally) once daily for 21 days. Group II (standard) received diazepam (2 mg/kg, orally) on the twenty-first day. Groups III and IV received ethanolic extract of *Jasminum sambac* leaves at doses of 250 mg/kg and 500 mg/kg, respectively, orally once daily for 21 days. On the final day, animals were tested in the Elevated Plus Maze one hour after treatment. The parameters recorded included number of entries and time spent in open and closed arms, total arm entries, percentage of open arm entries, and percentage of open arm time during a 300-second trial. Phytochemical screening of the extract was also carried out. Data were analyzed using one-way analysis of variance followed by Dunnett's post hoc test.

**Results:** The ethanolic extract of *Jasminum sambac* leaves at 500 mg/kg significantly increased the number of entries and time spent in open arms ( $p < 0.01$ ), with corresponding increases in the percentage of open arm entries and percentage of open arm time, without affecting locomotor activity. The 250 mg/kg dose produced no significant effect. Diazepam significantly enhanced all open arm parameters ( $p < 0.001$ ). Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenolic compounds, carbohydrates, saponins, and steroids.

**Conclusion:** The ethanolic extract of *Jasminum sambac* leaves exhibited significant dose-dependent anti-anxiety activity without impairing locomotion, supporting its traditional use. Flavonoids and alkaloids may contribute through modulation of the gamma-aminobutyric acid system.

**Keywords:** *Jasminum sambac*, ethanolic extract, anxiolytic activity, elevated plus maze, phytochemical screening, gabaergic system

### Introduction

Anxiety disorders, including generalized anxiety disorder, panic disorder, agoraphobia, social anxiety disorder, specific phobias, obsessive-compulsive disorder, and post-traumatic stress disorder, are among the most common neuropsychiatric conditions worldwide [1]. They affect a significant portion of the population annually and over a lifetime, contributing heavily to disability-adjusted life years [2]. Characterized by excessive fear, apprehension, and physical symptoms such as tachycardia and sweating, these disorders often begin during adolescence or early adulthood, with a higher prevalence in females than males [3]. They have a profound impact on quality of life, educational achievement, and work productivity, making them a serious public health concern [4].

The underlying mechanisms of anxiety involve dysregulation of neurotransmitter systems, particularly those mediated by gamma-aminobutyric acid (GABA), serotonin, and noradrenaline [5]. The amygdala plays a central role in fear processing, while GABA acts as the brain's primary inhibitory neurotransmitter, reducing neuronal excitability through GABA<sub>A</sub> receptors targeted by benzodiazepines [6]. Serotonin pathways influence mood regulation, and noradrenaline contributes to the physiological stress response [7]. Current pharmacological treatments, such as selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and benzodiazepines, are effective but often limited by adverse effects.

These can include insomnia, sexual dysfunction, gastrointestinal problems, sedation, tolerance, dependence, and withdrawal, leading to poor patient adherence and creating a need for safer therapeutic alternatives [8,9].

Complementary and alternative medicine has gained interest as a potential option for managing anxiety, with herbal remedies widely used due to their perceived safety, availability, and cultural acceptance [10]. Plants such as *Ginkgo biloba*, *Valeriana officinalis*, and *Passiflora incarnata* have shown potential anxiolytic effects by modulating neurotransmitter pathways, though evidence is often limited by methodological variations [11]. Among traditional medicinal plants, *Jasminum sambac*, commonly known as Arabian jasmine or Mogra, is widely used in Ayurveda and Unani systems of medicine for central nervous system conditions including anxiety and insomnia, as well as for various endocrine, gastrointestinal, and infectious disorders [12,13]. Native to the Indian subcontinent and thriving in tropical climates, this plant holds significant ethnomedicinal value [14].

*Jasminum sambac* contains bioactive constituents such as flavonoids, alkaloids, terpenoids, essential oils, saponins, glycosides, tannins, and phenolics, which are associated with antioxidant, anti-inflammatory, antimicrobial, and neuropharmacological properties [15]. Flavonoids and alkaloids, in particular, are known to modulate GABAergic neurotransmission, providing a potential scientific basis for its traditional calming effects [16].

Despite its long-standing use for alleviating anxiety, no systematic studies have evaluated the anxiolytic effects of the ethanolic leaf extract of *Jasminum sambac*. The present study was undertaken to investigate the anti-anxiety potential of this extract in Swiss albino mice using the Elevated Plus Maze model, with diazepam as the reference standard. The study involved collection and authentication of plant material, preparation of the ethanolic extract by

maceration, phytochemical screening, and evaluation of anxiolytic activity in the Elevated Plus Maze (EPM) model.

## Materials and Methods

### Plant Material and Extraction

Leaves of *Jasminum sambac* were collected from Farangipete, Mangalore, in June 2025 and authenticated by Dr. Siddaraju M. N., Department of Botany, University College Mangalore.

The leaves were washed, shade-dried, and pulverized into a coarse powder (300 g). The powder was macerated in 1500 ml of 95% ethanol for 72 hours with occasional shaking. The extract was filtered, concentrated using a rotary evaporator at 40°C, and yielded 3.26% w/w semi-solid extract (dark green), which was stored at 4°C [17].

### Phytochemical Screening

Preliminary phytochemical tests were conducted per Khandelwal (2008) [18] to detect the presence of Alkaloid, Flavonoids, Tannins, Phenolics, Carbohydrates, Saponins, Steroids.

### Experimental Animals

Healthy Swiss albino mice (35-40 g, either sex) were procured from the animal house at Srinivas College of Pharmacy, Mangalore, and housed under standard conditions (22±2°C, 50±5% humidity, 12 h light/dark cycle) with ad libitum access to food and water [19]. The study was approved by the Institutional Animal Ethics Committee (IAEC, SCP/IAEC/27/JUN/2025-269 dated 14.06.2025) per CCSEA guidelines. Animals were acclimatized for one week.

### Dose Preparation

EJSL was suspended in distilled water using a sonicator. Doses were 250 mg/kg and 500 mg/kg (p.o.). Diazepam (2 mg/kg, p.o.) and saline (10 ml/kg, p.o.) were prepared similarly.

## Experimental Design

**Table 1:** Experimental Grouping, Treatments, Dosage, and Testing Schedule for the Anxiolytic Activity Study of *Jasminum sambac* Ethanolic Leaf Extract (EJSL)

Group	Treatment	Dose	Route	Duration	Testing Schedule
I (Control)	Saline (0.9%)	10 ml/kg	p.o.	20 days	Day 21, 2 h post-dose
II (Standard)	Diazepam	2 mg/kg	p.o.	Single dose on Day 21	Day 21, 1 h post-dose
III (Test 1)	EJSL	250 mg/kg	p.o.	20 days	Day 21, 2 h post-dose
IV (Test 2)	EJSL	500 mg/kg	p.o.	20 days	Day 21, 2 h post-dose

### Elevated Plus Maze (EPM)

The Elevated Plus Maze (EPM) apparatus consisted of two open arms (16 × 5 cm), two closed arms (16 × 5 × 12 cm), and a central platform (5 × 5 cm), elevated 25 cm above the floor. Each mouse was placed at the center of the maze facing an open arm and observed for 5 minutes. The parameters recorded included the number of open and closed arm entries (defined as all four paws entering an arm), time spent in open and closed arms (in seconds), total arm entries (sum of open and closed entries), percentage open arm entries (%OAE) calculated as (open entries / total entries) × 100, and percentage open arm time (%OAT) calculated as (open time / 300) × 100 [20].

### Statistical Analysis

Data were expressed as Mean ± SEM. Statistical analysis was performed using GraphPad Prism software, version 10.5.0 (GraphPad Software, San Diego, CA, USA). One-way ANOVA followed by Dunnett's post-hoc test was used to compare each treatment group with the control group, with  $p < 0.05$  considered statistically significant. Derived parameters, including %OAE and %OAT, were calculated from the mean values prior to analysis.

## Results

**Extraction Yield:** Maceration yielded 3.26 g (3.26% w/w) of dark green, semi-solid extract.



**Fig 1:** Ethanolic extract of *Jasminum sambac* leaf.

### Phytochemical Screening

EJSL tested positive for alkaloids (Mayer's, Dragendorff's), flavonoids (Shinoda, alkali), tannins/phenolics (ferric

chloride, lead acetate), carbohydrates (Molisch's, Benedict's), saponins (foam), and steroids (Salkowski, Liebermann-Burchard).



**Fig 2:** Phytochemical analysis of Ethanolic extract of *Jasminum sambac* leaf.

**Elevated Plus Maze:** EJSL (500 mg/kg) significantly increased the number of entries into open arms ( $8.33 \pm 0.80$  vs. control  $4.67 \pm 0.21$ ,  $p < 0.01$ ) and the time spent in open arms ( $152.50 \pm 4.81$  s vs.  $134.00 \pm 1.34$  s,  $p < 0.01$ ), while significantly reducing closed arm entries ( $5.50 \pm 0.56$  vs.  $7.83 \pm 0.31$ ,  $p < 0.01$ ) and time spent in closed arms ( $150.17 \pm 4.24$  s vs.  $166.00 \pm 1.34$  s,  $p < 0.01$ ). EJSL at 250 mg/kg produced no significant changes compared to the control. Diazepam (2 mg/kg) markedly increased open arm

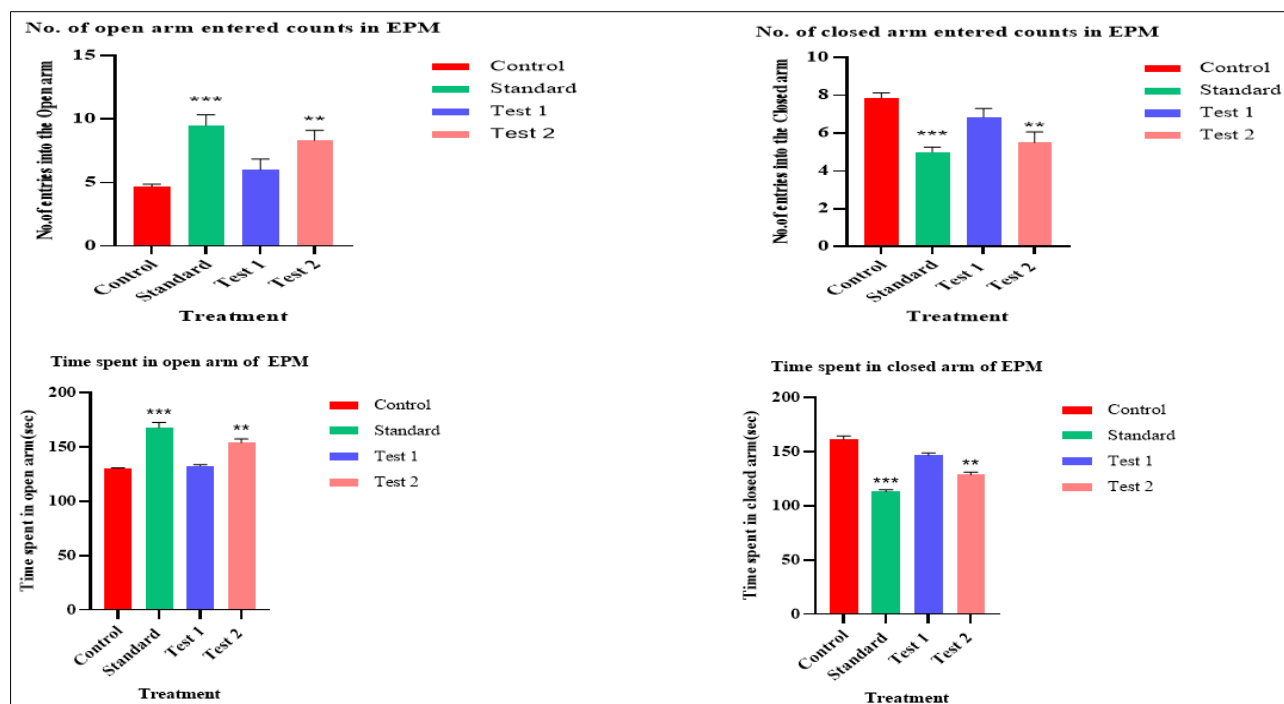
entries ( $9.50 \pm 0.85$ ,  $p < 0.001$ ) and time ( $157.00 \pm 4.28$  s,  $p < 0.001$ ), while reducing closed arm parameters.

Total arm entries were comparable across groups (Control: 12.50; Diazepam: 14.50; EJSL 250: 12.83; EJSL 500: 13.83), confirming that locomotor activity was unaffected. Derived parameters showed that EJSL (500 mg/kg) increased % open arm entries (%OAE: 60.24%) and % open arm time (%OAT: 50.83%) compared to control (37.34% and 44.67%, respectively), though slightly lower than diazepam (65.52% and 52.33%).

**Table 2:** Effect of Ethanolic Leaf Extract of *Jasminum sambac* (EJSL) on Elevated Plus Maze Parameters in Swiss Albino Mice

Group	Drug Treatment	Dose (mg/kg)	Number of Entries (Mean $\pm$ SEM)		Time Spent in Seconds (Mean $\pm$ SEM)		Total Entries (Mean)	%OAE (Mean)	%OAT (Mean)
			Open Arm	Closed Arm	Open Arm	Closed Arm			
I	Control	10 ml	$4.67 \pm 0.21$	$7.83 \pm 0.31$	$134.00 \pm 1.34$	$166.00 \pm 1.34$	12.50	37.34%	44.67%
II	Diazepam	2	$9.50 \pm 0.85^{***}$	$5.00 \pm 0.26^{***}$	$157.00 \pm 4.28^{***}$	$144.67 \pm 3.71^{***}$	14.50	65.52%	52.33%
III	EJSL 250	250	$6.00 \pm 0.86^{ns}$	$6.83 \pm 0.48^{ns}$	$141.67 \pm 2.59^{ns}$	$158.33 \pm 2.59^{ns}$	12.83	46.75%	47.22%
IV	EJSL 500	500	$8.33 \pm 0.80^{**}$	$5.50 \pm 0.56^{**}$	$152.50 \pm 4.81^{**}$	$150.17 \pm 4.24^{**}$	13.83	60.24%	50.83%

All the results are expressed in term of Mean  $\pm$  SEM, n=6 animals in each group; Statistical Significance was determined by ANOVA followed by Dunnett's test.  $^{**}p < 0.01$ ,  $^{***}p < 0.001$  statistically significant compared to control group. "ns" indicated non-significant differences.



**Fig 3:** Effect of Ethanolic Leaf Extract of *Jasminum sambac* (EJSL) on Elevated Plus Maze Parameters in Swiss Albino Mice

## Discussion

The present study was undertaken to investigate the anxiolytic-like potential of the ethanolic leaf extract of *Jasminum sambac* (EJSL) in Swiss albino mice using the Elevated Plus Maze (EPM) paradigm, with diazepam employed as a reference standard.

Anxiety disorders are multifactorial in origin, involving dysregulation of the GABAergic, serotonergic, and noradrenergic neurotransmitter systems<sup>[21]</sup>. Benzodiazepines, such as diazepam, are highly effective in attenuating anxiety by enhancing GABA<sub>A</sub> receptor-mediated chloride influx, thereby promoting neuronal hyperpolarization<sup>[22]</sup>. However, their use is often constrained by undesirable effects including sedation, cognitive impairment, tolerance, and dependence. Selective serotonin reuptake inhibitors (SSRIs) offer an alternative, but delayed onset of action and adverse effects such as sexual dysfunction remain problematic<sup>[23]</sup>. These limitations highlight the need for novel, plant-based therapeutic agents with improved safety profiles.

Phytochemical investigations of *J. sambac* have demonstrated the presence of flavonoids, alkaloids, and essential oils, many of which possess central nervous system (CNS) activity, making this species a rational candidate for anxiolytic evaluation.

In the EPM, EJSL at a dose of 500 mg/kg produced a significant increase in both open arm entries and time spent in open arms, without altering total arm entries. This profile suggests genuine anxiolytic activity devoid of sedative or motor-impairing effects, an important advantage over many benzodiazepines. The magnitude of the effect was comparable to, though slightly less pronounced than, that of diazepam, indicating that while EJSL may not match the potency of the reference standard, it is capable of producing meaningful behavioral modulation. The lower dose of EJSL (250 mg/kg) failed to elicit significant anxiolytic-like effects, indicating a dose-dependent relationship and suggesting that a minimum threshold concentration of bioactive constituents is required to achieve

pharmacological efficacy. The derived parameters—percentage open arm entries (%OAE) and percentage open arm time (%OAT)—were consistent with the raw behavioral data, further validating the reliability of the findings.

The mechanism of action underlying the anxiolytic-like activity of EJSL is not yet fully elucidated; however, existing phytochemical and pharmacological evidence provides plausible hypotheses. Flavonoids such as luteolin and apigenin, reported in *J. sambac*, are known positive allosteric modulators of GABA<sub>A</sub> receptors, acting at benzodiazepine-binding sites to enhance inhibitory neurotransmission. Alkaloids and terpenoids present in the extract may exert complementary effects via serotonergic or dopaminergic modulation, contributing to the overall anxiolytic profile. Moreover, essential oils such as linalool, identified in the species, have been shown to influence CNS activity by interacting with glutamatergic and GABAergic systems. The similarity of EJSL's behavioral effects to those observed in other flavonoid-rich plants, such as *Passiflora incarnata* and *Valeriana officinalis*, further supports the hypothesis of GABA<sub>A</sub>-mediated action. Nonetheless, the possibility of multi-receptor involvement cannot be excluded, given the polypharmacological nature of plant extracts.

While the present findings support the traditional use of *J. sambac* in the management of anxiety, certain limitations must be acknowledged. The study employed a single behavioral assay, which, although well-validated, cannot comprehensively capture the complexity of anxiety disorders. Inclusion of additional models—such as the open field test, light-dark box, or novelty-suppressed feeding test—would strengthen the behavioral evidence. Furthermore, no biochemical or receptor-binding assays were performed to confirm the putative mechanisms of action. Future studies should incorporate GABA<sub>A</sub> antagonists (e.g., flumazenil) to verify receptor involvement, along with neurochemical assays to quantify changes in neurotransmitter levels. Chronic dosing studies will also be necessary to assess tolerance development and long-term safety. Overall, these



results provide promising preliminary evidence that *J. sambac* possesses significant anxiolytic-like activity at higher doses, warranting further exploration as a potential source of novel, plant-derived anxiolytic agents.

### Conclusion

The present study demonstrates that the ethanolic leaf extract of *Jasminum sambac* (EJSL) at 500 mg/kg produces significant, dose-dependent anxiolytic-like effects in the Elevated Plus Maze model, without evidence of locomotor suppression. These findings lend pharmacological support to the plant's traditional use in anxiety management. The observed activity is plausibly attributable to the presence of flavonoids and alkaloids, which may exert their effects through positive modulation of GABA<sub>A</sub> receptor function and possibly other neurotransmitter systems. To advance the therapeutic potential of *J. sambac*, future research should focus on bioassay-guided fractionation to isolate active constituents, detailed mechanistic studies including receptor-binding and neurochemical analyses, and comprehensive safety profiling under acute and chronic dosing regimens. Such investigations could facilitate the development of standardized, plant-derived anxiolytic formulations with improved efficacy and safety profiles compared to existing synthetic agents.

**Conflict of interest:** None.

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