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EVALUATION OF ANTI-ANXIETY ACTIVITY OF *CITRUS PARADISI* FRUIT EXTRACT ON MICE

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ABSTRACT

Aim and Objective: To evaluate the anti-anxiety activity of *Citrus paradisi* fruit extract on mice. **Methods:** The anti-anxiety activity of aqueous extract of *Citrus paradisi* fruit (AECPF) was investigated in mice by using elevated plus maze model (EPM) model. the Swiss albino mice (30-45g) of either sex was randomly divided into four groups of six animals each. Group Ⅰ animals treated with vehicle control (1ml/kg, p.o), Group Ⅱ animals treated with standard drug (diazepam, 2mg/kg, p.o), Group Ⅲ animals treated with aqueous extract of *Citrus paradisi* fruit (100mg/kg, p.o) and Group Ⅳ animals treated with aqueous extract of *Citrus paradisi* fruit (200mg/kg, p.o). The treatment was given through post oral route. The following parameters were measured; number of entries into open arms and closed arms, time spent in open arms and closed arms. The aqueous extract of *Citrus paradisi* fruit was estimated to assess the anxiolytic effect. **Results:** Aqueous extract of *Citrus paradisi* fruit (200mg/kg) treated animals showed increase in the time spent and the number of entries in the open arms of the elevated plus maze in comparison with the control animals. **Conclusion:** The finding of the present study provides evidence that the aqueous extract of *Citrus paradisi* exerts a dose dependent beneficial effect against anxiety.

KEYWORDS: *Aqueous extract of Citrus paradisi fruit (AECPF), anti-anxiety, elevated plus maze (EPM), Swiss albino mice.*

INTRODUCTION

Anxiety disorders are among the most prevalent neuropsychiatric conditions affect individuals worldwide, leading to significant impairment in quality of life. Although various synthetic anxiolytic agents, such as benzodiazepines, are available, their long-term use is often limited by side effects, dependence, and tolerance. Hence, there is growing interest in exploring natural alternatives with anxiolytic potential and fewer adverse effects. Anxiety disorder is increasingly recognized as a highly prevalent and chronic disorder with onset during the teenage years, with an incidence of 18.1% and a lifetime prevalence of 28.8%.[1]

Interest in alternative medicine and plant-derived medications that affect the "mind" has grown rapidly over the last two decades.[2] Several studies have been done on the anti-anxiety activity of medicinal plants, but a major constraint towards the development of a marketable formulation is the unsuitability of the tested plant material for human use and the non-availability of plant materials in bulk at economical rates. This study aims to develop of safe anti-anxiety economic drug of easy availability. At the same time, aromatic oils can be used topically or for inhalation purposes, whereas leaf extracts can be a better medium for formulation development, which can be easily administered.[3]

This study was conducted to evaluate the phytochemicals, proximate, and antioxidant contents of *Citrus paradisi* fruit. *C.paradisi* (grapefruit) is a rich source of diverse phytoconstituents that contribute to its nutritional and medicinal significance. Among the major groups are flavonoids (naringin, naringenin, hesperidin),

which are very well known for their antioxidant, anti-inflammatory, anti-anxiety and cardioprotective roles. The fruit also contains coumarins (bergamottin, bergapten), which are notable for their influence on drug metabolism through cytochrome P450 inhibition. Limonoids (limonin and nomilin) further enhance their bioactivity with anticancer and cholesterol-lowering potential. The peel and essential oils are abundant in monoterpenes (limonene), which impart the characteristic aroma and possess antimicrobial and chemoprotective properties. Additionally, grapefruit is enhanced with carotenoids (lycopene, β-carotene), which contribute to its antioxidant defence and health-promoting benefits. The presence of phenolic acids, organic acids, and vitamins enhances its free radical scavenging and anti-anxiety activity, while trace elements and fibres further improve its therapeutic profile.[4]

So, the present study was designed to develop an economic herbal-based drug substitute for existing anti-anxiety drug modules using aqueous extract of Citrus paradisi using the EPM, an exteroceptive behaviour animal model.

MATERIALS AND METHODS

Plant Material and Extraction

The fresh fruit of *Citrus paradisi* was collected from local region of Mangalore, Dakshina Kannada and authenticated by Dr. Siddaraju M.N. Assistant professor and Research guide, Department of Botany, university college Mangalore, Hampanakatta, Mangalore-575001.

The extract was prepared by separating the pulp from the peel which was then subjected homogenization to maximize the yield of bioactive compounds. It was then macerated for 48-72 hours by using distilled water (q.s). The extract was filtered with Whatman filter paper. A dark brown viscous liquid was obtained and preserved in air tight container and kept at 4-5°C until further use.[5]

Phytochemical Screening

Preliminary phytochemical tests were conducted 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. 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

AECPF was suspended in Tween 80 using a sonicator. Doses were 100 mg/kg and 200 mg/kg (p.o.). Diazepam (2 mg/kg, p.o.) and saline (10 ml/kg, p.o.) were prepared similarly.[5]

Experimental Design

Table No. 01: Experimental grouping, treatments, dosage, and testing schedule for evaluation of the anti-anxiety of *Citrus paradisi* fruit extract on mice.

Group	Treatment	Dose	Route	Duration	Testing Schedule
I (Control)	Tween 80	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)	AECPF	100 mg/kg	p.o.	20 days	Day 21, 2 h post-dose
IV (Test 2)	AECPF	200 mg/kg	p.o.	20 days	Day 21, 2 h post-dose

Elevated plus maze model (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 centre 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.[6]

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 31.19g (31.19 w/w) of dark brown, viscous extract.



Fig. 1: Aqueous extract of *Citrus paradisi* fruit.

Phytochemical Screening[7]

Aqueous extract of *Citrus paradisi* tested positive for alkaloids (Mayer's, Dragendorff's), flavonoids (Shinoda, alkali), tannins/phenolics (ferric chloride, lead acetate), carbohydrates (Molisch's, Benedict's), and steroids (Salkowski, Liebermann-Burchard).

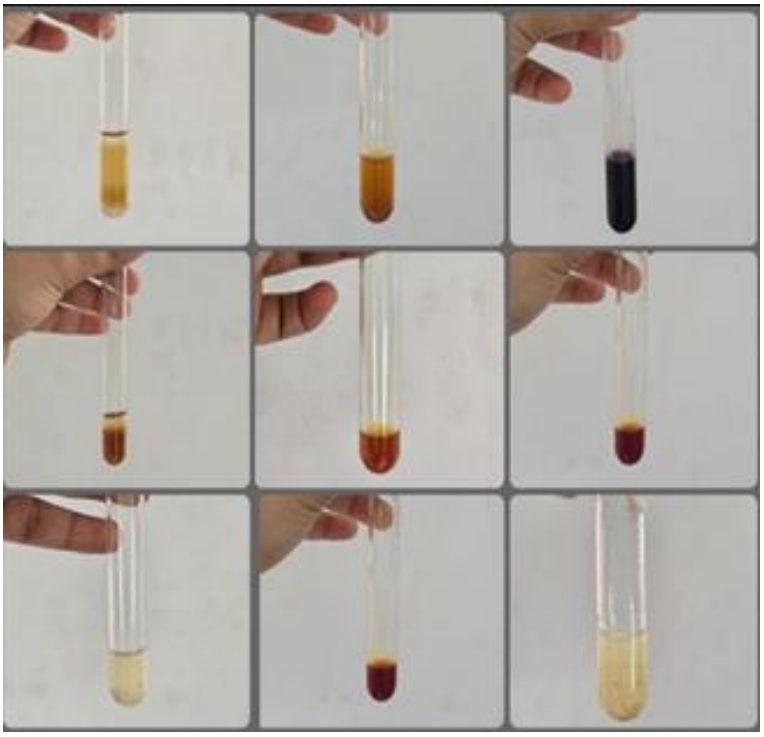


Fig. 2: Phytochemical analysis of Aqueous extract of *Citrus paradisi* fruit.

ELEVATED PLUS MAZE

Aqueous extract of *Citrus paradisi* fruit(AECPF) (200 mg/kg) showed significant increase in the number of entries into open arms (9.00±0.67 vs. control 4.66±0.33, $p<0.01$) and the time spent in open arms (151.33±1.33s vs 146.83±1.62s, $p<0.01$), while significantly reducing closed arm entries (8.83±0.36 vs. 6.50±0.24, $p<0.01$) and time spent in closed arms (139.33±2.21 s vs. 125.33±1.28 s, $p<0.01$). AECPF at (100mg/kg) produced less significant to negligible changes compared to the control. Diazepam (2 mg/kg) markedly increased open arm entries (9.50±0.56, $p<0.001$) and time spent in open arm (161.00±2.18 s, $p<0.001$), while reducing closed arm parameters. Total arm entries were comparable across groups (Control: 11.16; Diazepam: 18.17; AECPF 100: 14.50; AECPF 200: 17.83), confirming that locomotor activity was unaffected. Derived parameters showed that AECPF (200 mg/kg) increased % open arm entries (%OAE: 50.05%) and % open arm time (%OAT: 42.01%) compared to control (51.8% and 53.9%, respectively), though slightly lower than diazepam (52.3% and 53.3%).

Table No. 02: Effect of AECPF on the Elevated Plus Maze model.

Group No.	Drug Treatment	Dose (mg/kg)	Number of entries (Mean±SEM)		Time spent in seconds (Mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	10ml	4.66±0.33	6.50±0.24	146.83±1.62	125.33±1.28
II	Diazepam	2	9.50±0.56***	8.67±0.49***	161.00±2.18***	141.00±1.31***
III	Test	100	6.50±0.42*	8.00±0.25*	159.33±1.91*	130.83±0.61*
IV	Test	200	9.00±0.67**	8.83±0.36**	151.33±1.33**	139.33±2.21**

Note: All the results are expressed in term of Mean ± SEM, n=6 animals in each group; Statistical Significance was determined by ANOVA followed by Dunnett's test. *p<0.05, **p<0.01 and ***P< 0.001 statistically significant compared to control group.

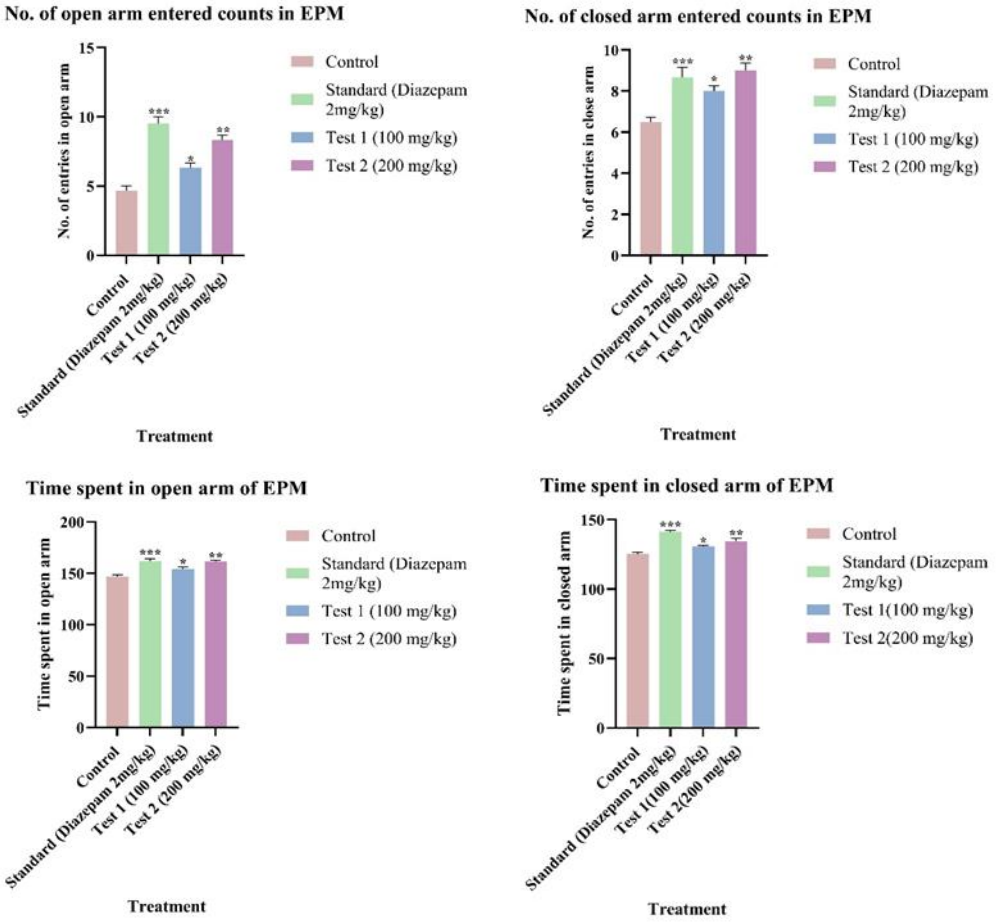


Fig 3: Aqueous extract of *Citrus paradisi* fruit (AECPF) on elevated plus maze parameters in Swiss albino mice.

DISCUSSION

In the persistent pursuit of effective psychiatric drugs for anxiety and depression, the last two decades have witnessed notable challenges in minimizing side effects. In response to this, herbal medicines are emerging as a promising therapeutic strategy. Despite the frequent use of the synthetic drug diazepam as a standard anxiolytic, its associated side effects prompt the exploration of alternative solutions. This study investigates the anxiolytic effects of the aqueous extract of fruit of *Citrus paradisi* using the Elevated Plus Maze (EPM).

The Elevated Plus Maze stands as a well-established animal model for anxiety, renowned for its sensitivity to both anxiolytic and anxiogenic drugs. Typically, mice exhibit a preference for enclosed arms in the EPM, reflecting a natural aversion to open arms and a fear of open spaces. Indicators such as increased entries and time spent in the open arm are deemed reliable markers of decreased anxiety, signifying an anxiolytic-like activity.

In the present study, the administration of a high dose of the aqueous extract of *Citrus paradisi* fruit (200 mg/kg) (P<0.001) significantly increased the time spent in the open arm and the number of transitions towards the open arm, compared to lower dose the aqueous extract of *Citrus paradisi* fruit (100 mg/kg) that has minimal significance thus underscoring its potent anxiolytic potential in the EPM model.

These promising results warrant an in-depth investigation into the specific bioactive compounds and mechanisms underpinning the anxiolytic properties of the aqueous extract of *Citrus paradisi* fruit, paving the way for potential therapeutic applications in anxiety management.

CONCLUSIONS

The present study can be concluded with the fact the aqueous extract of *Citrus paradisi* fruit unveils significant anxiolytic effect in Swiss albino mice using anxiolytic models namely Elevated Plus Maze. The data obtained was sufficient and conclusive so as to accomplish our objectives. In the conclusion, present data illustrates that administration of the aqueous extract of *Citrus paradisi* fruit on mice shows anxiolytic activity.

The extraction and preliminary phytochemical studies of the aqueous extract of fruit *Citrus paradisi* manifested the presence of chemical and phytochemical constituents such as Alkaloids, Carbohydrates, Flavonoids, Tannins, Phenolic Compounds and Steroids.

The exact mechanism emphasizing anxiolytic effect is not clear but it may be apparently related to the compounds present in *Citrus paradisi*. Hence further studies would be requisite to evaluate the contribution of active chemical constituents for the perceived anxiolytic activity as it still remains to be determined which components were responsible for these effects.

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