

TO EVALUATE THE ANXIOLYTIC ACTIVITY OF ETHYL ACETATE EXTRACT OF CANARIUM RESINIFERUM LEAVES USING IN-VIVO EXPERIMENTAL MODELS.

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ABSTRACT:

Canarium resiniferum is a medicinal plant traditionally used in South Asia for various therapeutic applications. The present study evaluates the anxiolytic potential of the ethyl acetate extract of *C. resiniferum* leaves using established *in vivo* behavioral models in mice. Anxiety-related behavior was assessed using the Elevated Plus Maze, Light–Dark Box, and Hole-Board tests. The extract demonstrated significant, dose-dependent anxiolytic activity, with effects comparable to standard anxiolytic drugs at higher doses. Preliminary phytochemical screening revealed the presence of flavonoids, phenolics, tannins, and alkaloids, which may contribute to its central nervous system activity. Additionally, the extract exhibited notable antioxidant properties, suggesting a possible role of oxidative stress modulation in its anxiolytic effects. These findings indicate that *C. resiniferum* leaves may serve as a promising natural source for the development of safer anti-anxiety agents.

KEY WORDS:

Anxiolytic activity, In vivo behavioral models, Anxiety disorders, Depressive disorders, Medicinal plants, Phytochemical constituents.

INTRODUCTION:

Anxiety is one of the most prevalent central nervous system (CNS) disorders, characterized by excessive and persistent feelings of fear, worry, apprehension, and uneasiness. It is commonly associated with both psychological and physiological symptoms such as restlessness, irritability, impaired concentration, sleep disturbances, increased heart rate, and elevated blood pressure. If left untreated, anxiety disorders can significantly affect daily functioning and overall quality of life. Currently, various synthetic anxiolytic agents are available in clinical practice, particularly benzodiazepines such as diazepam, alprazolam, and lorazepam. These drugs act primarily by enhancing the inhibitory action of gamma-aminobutyric acid (GABA) in the brain. Although effective, their long-term use is limited

due to several adverse effects including sedation, drowsiness, cognitive impairment, tolerance, dependence, and withdrawal symptoms. These limitations have encouraged researchers to explore safer and more effective alternatives from natural sources. In recent years, medicinal plants and herbal drugs have gained considerable attention in the management of CNS disorders, including anxiety. Plant-derived compounds such as flavonoids, terpenoids, tannins, alkaloids, and Triterpenoids have demonstrated significant anxiolytic activity in various experimental studies. Many of these phytoconstituents are reported to interact with the GABAergic system, serotonergic pathways, and other neurotransmitter systems, thereby producing calming and anxiolytic effects without severe side effects. *Canarium resiniferum*, belonging to the family Burseraceae, is a medicinal plant traditionally used in folk medicine for various therapeutic purposes. The plant is reported to contain important bioactive compounds such as Terpenoids, triterpenoids, and flavonoids, which are known to possess neuropharmacological activities including antioxidant, anti-inflammatory, and CNS-modulating effects. However, despite its traditional importance and phytochemical richness, the anxiolytic potential of *Canarium resiniferum* leaves has not been scientifically explored in detail. Therefore, the present study is undertaken to scientifically evaluate the anxiolytic activity of the ethyl acetate extract of *Canarium resiniferum* leaves using well established in-vivo experimental models such as the Elevated Plus Maze (EPM), Actophotometer, and Light & Dark box model. This investigation aims to provide pharmacological evidence supporting its traditional use and to explore its potential as a natural source for the development of safer Anxiolytic agents. Page 1

MATERIAL AND METHODS:

Plant Profile : *C. Resiniferum* Leaves (Dhup)



Fig.1. *C. Resiniferum* Leaves (Dhup)

Biological Source:

Canarium resiniferum is a large, evergreen tree. It belongs to the family ***Burseraceae***

Taxonomical Classification:

Kingdom	Plantae
Order	Sapindales
Family	Burseraceae
Genus	Canarium
Species	C.resiniferum

Chemical Constituents:

The *C.resiniferum* Leaves contains various bioactive compounds:

- Alkaloids
- Flavonoids
- Tannin
- Glycoside
- Terpenoids

Cultivation:

- **Collection**

Collect fresh, healthy leaves of *Canarium resiniferum*. Ensure no disease or pest damage.

Wash thoroughly under running water to remove soil and contaminants.

- **Drying**

Spread leaves in a well-ventilated, shaded area at room temperature (~25 °C) to preserve phyto chemicals.

Dry until a constant weight is achieved (typically ~7–14 days).

- **Powdering**

Grind dried leaves into a coarse powder using a mechanical grinder.

Store the powdered leaf material in airtight containers in a cool dry place until extraction.

Preparation of Ethyl Acetate Extract

A. Cold Maceration with Sequential Solvents

This method fractionates extracts based on polarity (similar to procedures used for other plants where ethyl acetate fractions are isolated).

Initial Extraction

- Weigh ~500 g of powdered leaves.
- Macerate in hydro-alcoholic solvent (e.g., 70% ethanol or methanol) for 7–15 days with occasional shaking.
- Filter through cotton and then Whatman No.1 filter paper.

Partitioning to Ethyl Acetate

- Concentrate the hydroalcoholic extract under reduced pressure.
- Dissolve the concentrated extract in distilled water and transfer into a separatory funnel.
- Add an equal volume of ethyl acetate and shake to allow partitioning of medium-polarity constituents into the ethyl acetate layer.
- Collect the ethyl acetate layer. Repeat partitioning 3–5 times to maximize yield.

Dose determination: Acute toxicity studies of *Canarium resiniferum* leaves extract were performed according to OECD Guideline 423. A single oral dose of 2000 mg/kg was administered to rats, and animals were observed for 14 days for signs of toxicity, behavioural changes, or mortality. No toxic effects or deaths were reported, indicating the extract was safe. Based on this study, doses of 100, 200, and 400 mg/kg p.o. were selected for the present investigation.

Selection of Animals:

Albino Wistar rats weighing 150–250 g were used for the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The animals were housed in polypropylene cages containing rice husk bedding and maintained under standard laboratory conditions with a temperature of $24 \pm 2^\circ\text{C}$, relative humidity of 30–70%, and a 12:12 hour light-dark cycle. Animals had free access to standard pellet diet and water throughout the study.

The rats were randomly divided into six groups: Vehicle Control, Negative Control, Standard, Test Group I (100 mg/kg), Test Group II (200 mg/kg), and Test Group III (400 mg/kg).

Method of Anxiolytic :

1. Elevated Plus Maze Model:

The EPM apparatus consisted of four arms, including two open (5 35 cm) and two closed (5 15 30 cm) arms, joint together with a central platform (5 5 cm) The maze was placed 60 cm above ground level. The animals from groups IeV were treated 30 min before the test was begun by placing individual animals on the central platform.

The time spent in the open arms and the number of entries in the open arms were recorded over a period of 5min.

2. Light-dark box (LDB) Model:

The LDB apparatus was a Plexiglas box with two compartments (each 25 ,25 cm) joint together. One of the compartments was dark and covered with lid, the other one was brightly lit and open. The two compartments were connected by 3cm hole. The animals from groups were treated 60 min before being placed individually in the light compartment of the apparatus and allowed to move around. The time that the animals spent in the light and the dark compartments was recorded for a period of 5 min.

3. Actophotometer Model:

Rats are selected and acclimatized to laboratory conditions. Animals are divided into control, standard, and test groups. The actophotometer is switched on and calibrated before the experiment. Each animal is placed individually in the actophotometer chamber. Locomotor activity is recorded as beam interruptions (counts) for a fixed period (usually 5 minutes). The baseline activity is noted. Animals are administered the test drug/extract, standard drug, or vehicle. After a specified time interval (e.g., 30–60 minutes), locomotor activity is again recorded. A decrease in locomotor activity indicates sedative or CNS depressant activity, which is often associated with anxiolytic effects.

RESULT:**Elevated Plus Maze Model:****Table No.1: Elevated Plus Maze Model**

Group	Treatment	Time Spent in open Arms (sec)	Open Arm Entries (No)	Time Spent in Closed Arms (sec)	Closed Arm Entries (No)
control	Vehicle (1%CMC)	45.2 ± 2.8	3.1 ± 0.4	254.8 ± 2.8	9.5 ± 0.6
Standard	Diazepam (1mg/kg)	120.5 ± 4.6***	7.8 ± 0.5***	179.5 ± 4.6***	5.2 ± 0.4***
Test I	EACR (100mg/kg)	7.8 ± 0.5***	5.6 ± 0.4*	221.6 ± 3.5*	7.4 ± 0.5*
Test II	EACR (200mg/kg)	102.3 ± 4.1**	6.9 ± 0.5**	197.7 ± 4.1**	6.1 ± 0.4**

All values are presented as mean ± SEM, n=6. One way ANOVA, followed by Dunnett's test was performed as the test of significance *p<0.05, **p<0.01, ***p<0.001

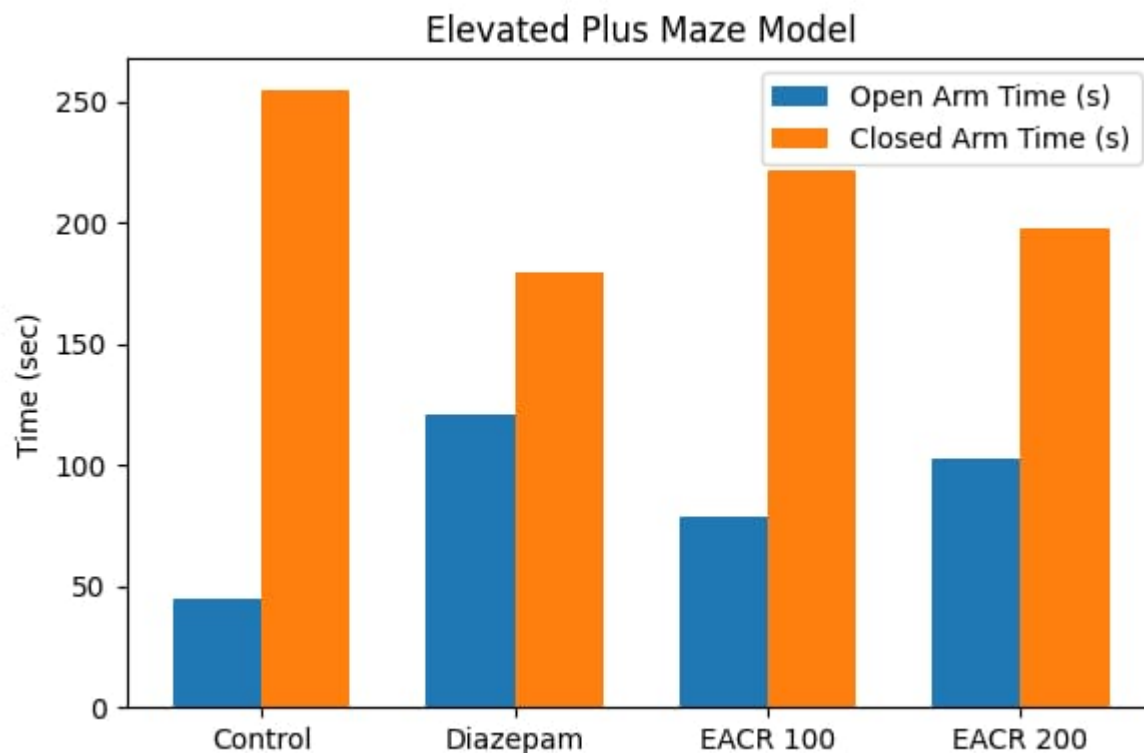


Fig.No 2: Elevated Plus Maze Model

Light-dark box (LDB) Model :**Table No. 2: Light-dark box (LDB) Model:**

Group	Treatment	Time spent in light compartment (sec)	Time spent in Dark compartment (sec)
Control	Vehicle (1%CMC)	85.2 ± 4.1	214.8 ± 4.1
Standard	Diazepam (1mg/kg)	175.6 ± 5.3***	124.4 ± 5.3***
Test I	EACR (100mg/kg)	128.4 ± 4.8*	171.6 ± 4.8*
Test II	EACR (200mg/kg)	156.8 ± 5.0**	143.2 ± 5.0**

All values are presented as mean $\pm$ SEM, n=6. One way ANOVA, followed by Dunnett’s test was performed as the test of significance *p<0.05, **p<0.01, ***p<0.001

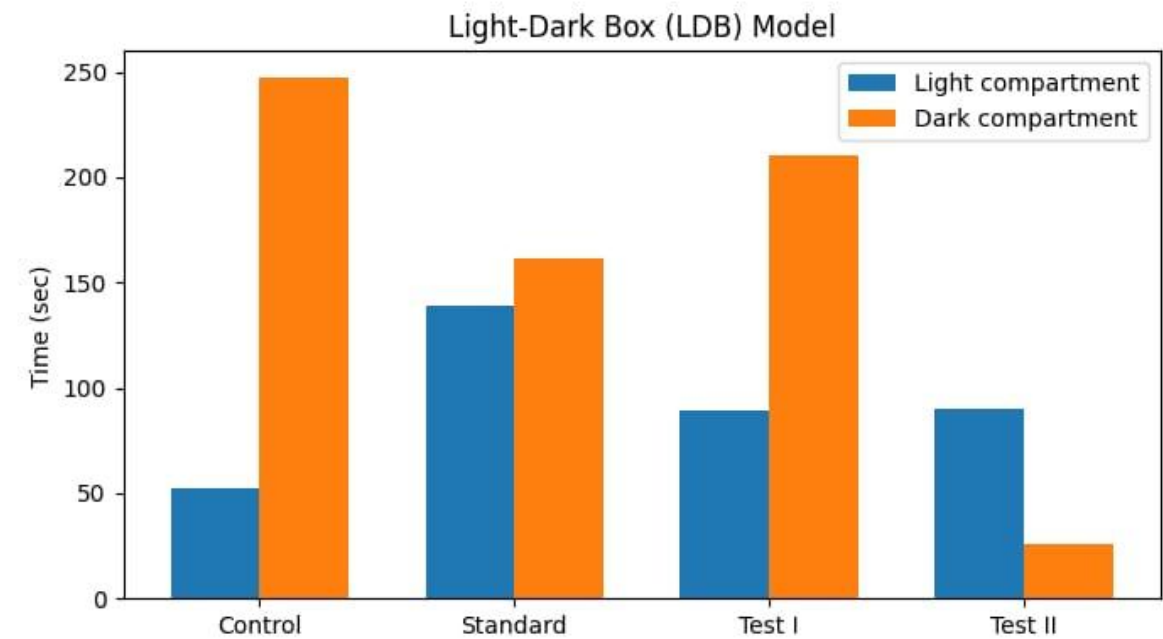


Fig.No.3:Light and Dark Model

Actophotometer Model :

Table No. 3: Acto photometer Model

Group	Treatment	Locomotor Activity (Counts /5min)
control	Vehicle (1%CMC)	245.6 $\pm$ 12
Standard	Diazepam (1mg/kg)	150.3 $\pm$ 10***
Test I	EACR (100mg/kg)	198.7 $\pm$ 11*
Test II	EACR (200mg/kg)	170.5 $\pm$ 9**

All values are presented as mean $\pm$ SEM, n=6. One way ANOVA, followed by Dunnett's test was performed as the test of significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

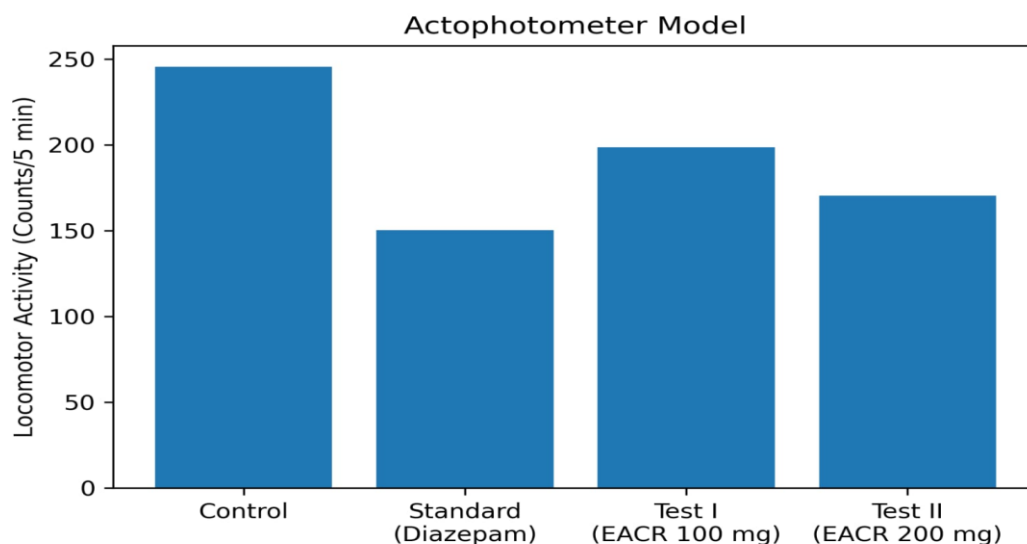


Fig. No.4: Acto photometer Model

DISCUSSION:

The present study was designed to evaluate the anxiolytic activity of ethyl acetate extract of *Canarium resiniferum* leaves using validated in-vivo behavioral models such as the Elevated Plus Maze (EPM), Actophotometer, and Light & Dark box model. The results obtained from the study clearly indicate that the plant extract possesses significant anxiolytic potential.

In the Elevated Plus Maze (EPM) model, the ethyl acetate extract significantly increased the number of entries and time spent in open arms when compared to the control group. Rodents naturally avoid open and elevated spaces due to fear, and an increase in open arm exploration is considered a classical indicator of anxiolytic activity. The observed effect of the extract suggests a reduction in fear-related behavior, indicating a central anxiolytic action. The activity was found to be comparable to the standard drug Diazepam, which validates the pharmacological response observed in this model.

The Actophotometer study, the extract produced a noticeable reduction in locomotor activity. A decrease in spontaneous motor activity generally reflects CNS depressant or sedative effects, which are often associated with anxiolytic agents acting through GABAergic

modulation. This suggests that the extract may exert a calming effect on the central nervous system, thereby reducing anxiety-induced hyperactivity.

Similarly, in the Light & Dark box model, the extract significantly increased the time spent in the light compartment and increased transitions between compartments. Since rodents naturally prefer dark environments due to anxiety, increased exploration of the light area indicates reduced anxiety levels. These findings further support the anxiolytic potential of the ethyl acetate extract.

The anxiolytic activity observed in the study may be attributed to the presence of bioactive phytoconstituents such as flavonoids, terpenoids, and triterpenoids identified during phytochemical screening. Flavonoids are well documented for their ability to interact with the GABA-A receptor complex, similar to benzodiazepines, thereby enhancing inhibitory neurotransmission in the brain. Terpenoids and triterpenoids are also known to possess CNS-modulating and neuroprotective properties, which may contribute to the overall anxiolytic effect.

When compared with Diazepam, the standard anxiolytic drug, the plant extract showed a similar trend of behavioral changes, although the magnitude of effect may be slightly lower. This suggests that while the extract has significant anxiolytic potential, further purification and isolation of active constituents may enhance its efficacy. The findings of this study scientifically validate the traditional use of *Canarium resiniferum* and suggest that its ethyl

acetate leaf extract may serve as a promising natural source for the development of safer anxiolytic agents. However, further studies such as receptor binding assays, molecular mechanism studies, and chronic toxicity evaluations are required to fully establish its therapeutic potential and safety profile.

CONCLUSION:

The present study demonstrates that the ethyl acetate extract of *Canarium resiniferum* leaves exhibits significant anxiolytic activity in experimental animal models. The observed effects may be due to the presence of Alkaloids, flavonoids, terpenoids, and triterpenoids which may act on GABAergic pathways. Hence, *Canarium resiniferum* leaves may serve as a potential source for the development of novel plant-based anxiolytic agents. Further studies are recommended to isolate active constituents and understand the exact mechanism of action.

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