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EVALUATION OF ANTI-ANXIETY ACTIVITY OF *LAURUS NOBILIS* ESSENTIAL OIL IN MICE

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1. ABSTRACT

Aim and Objective: The aim of this study is to evaluate anxiolytic activity of *Laurus nobilis* essential oil in experimental animals. **Methods:** The anxiolytic activity in mice was investigated by using Elevated plus maze (EPM) and Light and Dark Chamber (LDC), of *Laurus nobilis* essential oil (LNEO) were subjected for this study. The Swiss albino mice (22-27g) of either sex was divided into three groupsof six animals each. Group I animals received Vehicle control (1% Gum acacia 10ml/kg) orally, Group II received Standard drug (Diazepam 1mg/kg) (ip) and Group III received LNEO – 200mg/kg (p.o) for 21 days. All animals will be pretreated for 20 days except diazepam treated animals. On 21st day, animals will be treated, 30 min before the evaluation. In EPM model, the parameters measured were; number of open arm entries, number of closed arm entries and time spent in open and closed arms and in LDC, the parameters measured were; number of crossings between light and dark side and time spent in light and dark side. **Results:** The administration of the dose (200mg/kg) of LNEO produced significant reduction of number of entries, time spent in closed arm and dark side respectively. And increase in number of entries and time spent in open arm and light side when compared with control and standard groups, thus Laurus nobilis essential oilshows the significant anti-anxiety activity.

KEYWORDS: Anxiety, *Laurus nobilis* essential oil, EPM, Light and Dark Chamber.

2. INTRODUCTION

Anxiety usually refers to the experience of fear, apprehensiveness, nervousness, panic, restlessness, tension, and agitation. Manifest symptoms include trembling, fainting, headache,

and sweating, possibly elevated blood pressure, and changes in other psychophysiological induces such as heart rate, muscle tone, and skin conductance.[1] If the symptoms of anxiety interfere the daily life activities, then these are called anxiety disorder.[2]

Anxiety affects one-eighth of the total population of the world and has become a very important area of research interest in psychopharmacology.[2] Anxiety, a state of excessive fear and characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes.[3] Anxiety disorders are the most prevalent psychiatric disorders and are associated with a high burden of illness. With a 12-month prevalence of 10.3%, specific (isolated) phobiasare the most common anxiety disorders.[4] Although persons suffering from isolated phobias rarely seek treatment. Panic disorder with or without agarophobia (PDA) is the next most common type with a prevalence of 6.0%, followed by social anxiety disorder (SAD, also called social phobia; 2.7%) and generalized anxiety disorder (GAD; 2.2%). Women are 1.5 to two times likely than men to receive a diagnosis of anxiety disorder.[5]

The benzodiazepines are most widely used anxiolytic drugs. Their pharmacokinetic properties differ widely. Side effects are usually mild but dependence can supervene after long-term administration, even if the normal therapeutic doses are not exceeded. Careful monitoring of use is essential. The rate of onset of action depends on mode of administration, the dissolution of formulation, rapidly of absorption and rate of entry into brain. Diazepam is rapidly absorbed after oral use and enters the brain quickly, thus promptly relieving anxiety.[6]

Neurotransmitters involved in anxiety generation include serotonin, dopamine, nor-adrenaline, GABA, Corticotrophin releasing factor (CRF), Melanocyte stimulating hormone (MSH), neuropeptides and neurosteroids.[7] Benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects.[8] The recognition of anxiolytic effects of non-benzodiazepine azapirones agents, which act as 5HT1A partial agonists and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT1A receptor.[4] Although the azapirones display nanomolar affinity for 5-HT1A receptor sites.[9] Therefore, for the treatment of anxiety disorders, a novel agent with good therapeutic effect and better compliance is needed.[10]

Some of the herbal medicines with anti-anxiety effects are *Citrus papadisi* (Grapefruit), *Cirsium rivulare* (Ornamental thistle), *Drymaria cordata* (Tropical chickweed), *Colocasia esculenta* (Arvi), *Panax ginseng*, *Coriandrum sativum L* (Dhania), *Cinnamomum cassia* (Dalchini) and so on.

3. METHODOLOGY

3.1 EXPERIMENTAL ANIMALS

Healthy Swizz albino mice (18 to 25gms) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol (Approval no SCP/IAEC/F150/P128/2022). All the animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use. All the procedures were performed in accordance with Institutional Animal ethics committee constituted as per the direction of the CPCSEA, under ministry of animal welfare division, Government of India, New Delhi, India.

3.2 ETHICAL CONSIDERATIONS

All efforts were made to minimize animal suffering and to reduce the number of animals used in experiment. The animals received humane care and all the experiments were conducted strictly in accordance with the approved guidelines by the IAEC regulated by the CPCSEA according to Government of India accepted principles for lab animal's use and care. The study protocol was approved by IAEC, Srinivas College of Pharmacy, Valachil, Mangalore (Ref no.SCP/IAEC/F150/P128/2022)

3.3 CHEMCIALS AND INSTRUMENTS

- Gum acacia
- HCL (Hydrochloric acid)
- NH4OH (Ammonium hydroxide)
- FeCl3 (Ferric chloride)
- Fehling's solution

- Oral Feeding Needle.
- Electronic Weighing Balance
- Stop Watch
- Distilled water

3.4 COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The leaves of *Laurus nobilis* used for the present studies were collected from online stores (Ayurvedic store online) on September 2022. It was authenticated by Dr. U. Srinivasa, Professor and HOD, Dept. of pharmacognosy Srinivas college of Pharmacy Mangalore.

Preparation of Aqueous Extract of *Laurus nobilis* leaves: The shade dried leaves were powdered and used for extraction of essential oil. The powdered leaves were taken in one-liter flat-bottomed flask and 400 ml of distilled water was added. The contents were thoroughly mixed and flask was kept overnight. The contents were refluxed at 100ºC for about five hours. The oil extracted using hydro distillation method. The essential oil layer containing little water was collected in a conical flask. The essential oil layers were partitioned using diethyl ether. The ether layer was dried over sodium sulphate and excess solvent was evaporated using vacuum and finally oil was stored in a stoppered tube. The essential oil was insoluble in waterand soluble in alcohol, diethyl ether and glacial acetic acid.[11]

3.5 Preliminary Qualitative Phytochemical Analysis[12]

Table 1: Preliminary Phytochemical analysis.

S. No	Phytochemicals	Test	Observation	Inference
1	Alkaloids	Wagner's Test	Reddish brown precipitate	Presence of Alkaloids
2	Alkaloids	Mayer's test	White or creamy precipitate	Presence of Alkaloids
3	Saponins	Froth's test	Foams formation	Presence of saponin.
4	Tannins	Ferric chloride test	Dark green coloror blue-green	Presence of tanins.
5	Carbohydrates	Fehling's Test	Formation ofBrick red ppt	Presence of carbohydrates.

3.6 PREPARATION OF STOCK SOLUTION OF THE EXTRACT FOR DOSING

The aqueous extract of *Laurus nobilis* was weighed and suspended in 1% gum acacia. Each time fresh preparation of the extract was prepared before administration. The extract was administered post orally at a constant volume of 200mg/kg for each animal.

Dose Selection: Dose of 200mg /kg body weight was chosen as per previous works.[13]

3.7 ANXIOLYTIC ACTIVITY

Preparation of animals

The animals were selected in such a way that they were free from illness, injury, disease and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Only those animals which are healthy having weights 18-25 g were selected and maintained at standard laboratory conditions.

Preparation and administration of doses: All the doses were prepared in distilled water using 1% gum acacia solution as suspending agent and administer orally. In all cases, the concentrations were prepared in 1ml/100g of body weight. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 hours.

OBSERVATIONS

Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavioral pattern were also done. Attention was also given to observations of tremors and convulsions.

ANXIOLYTIC MODELS

Elevated Plus Maze Test[14]

Purpose and rationale

This rodent model of anxiety has been extensively used for evaluation of novel anxiolytic agents and to investigate psychological and neurochemical basis of anxiety.

This test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect. The primary measures are the proportion of entries into the open arms and the time spent on the open arm expressed as a percentage of the total time spent on both open and closed arms.

Procedure

Prior to starting the experiment, the mice were handled daily to reduce stress. Two hours after the oral administration of the test drugs and 30 min after the i.p. administration of diazepam,

the animal was placed in the center of the maze, facing one of the enclosed arms.

Following parameters measured

1. Number of open and closed arm entries.
2. Percentage time spent in open and closed arm.

An arm entry being defined when all four paws are in the arm.

EXPERIMENTAL DESIGN

The Swiss albino mice (22- 27gms) of either sex were selected. The mice were divided into following groups (n=6) as follows:

Group I: Vehicle control (1% gum acacia 10 ml/kg)

Group II: Standard group (Diazepam-1mg/kg)(i.p)[15]

Group III: *Laurus nobilis* essential oil (200mg/Kg)(p.o)[13]

TREATMENT

The treatment was given through oral route. All animals were pretreated for 20 days except diazepam treated animals. On 21st day, animals was treated, 30 min before the evaluation.

Evaluation

The above-mentioned parameters of standard and test compounds were carefully evaluated and compared to find the anxiolytic activity of the test compound.

Light dark Test[14]

Purpose and rationale

Crawely and Goodwin (1980) Crawley (1981) described a simple behavior model in mice to detect compounds with anxiolytic effects. Mice tends to explore a novel environment but to retreat from the aversive properties of a brightly – lit open field and a dark corner, they show more crossings between the two chambers and more locomotor activity after treatment with anxiolytic. The number of crossings between the light and dark sites are recorded.

Procedure

In a two chambered system, where the animals can freely move between a brightly- lit open field and a dark corner, they show more crossings between the two chambers and more locomotor activity after treatment with anxiolytics.

The animals were treated 30 min before the experiment with test drugs or vehicle i.p. and then observed for 10 min; groups of 3 animals are used for each dose.

The following behavior was measured

1) The number of entries in dark and light chamber.
2) Time spent in minutes in dark and light chambers.

EXPERIMENTAL DESIGN

The Swiss albino mice (22- 27gms) of either sex were selected. the mice were divided into following groups (n=6) as follows:

Group I: Vehicle control (1% gum acacia 10 ml/kg)

Group II: Standard group (Diazepam-1mg/kg)(i.p)[15]

Group III: *Laurus nobilis* essential oil (200mg/Kg) (p.o)[13]

TREATMENT

The treatment was given through oral route. All animals were pretreated for 20 days except diazepam treated animals. On 21st day, animals were treated, 30 min before the evaluation.

Evaluation

The above-mentioned parameters of standard and test compounds were carefully evaluated and compared to find the anxiolytic activity of the test compound.

Statistical Analysis

Results are prepared as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by multiple comparison tests. For all tests a "P" value of 0.05 or less was considered for statistical significance.

ANOVA (Analysis of variance)

In statistics, analysis of variance is a collection of statistical models and their associated procedures, in which the observed variance is partitioned into components due to different explanatory variables. In its simplest form ANOVA gives a statistical test of whether the means of several groups are all equal and therefore generalize Dunnett's multiple comparison test s to more than two groups.

4. RESULTS

Preliminary phytochemical screening

Results of preliminary phytochemical investigation of aqueous extract of LNEO extract are shown in Table 4

Table 2: **Preliminary phytochemical screening of LNEO.**[12]

Sl. No.	Test	Result
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Saponins	+ve
4.	Tannins	+ve
5.	Starch	-ve

Screening of LNEO for its anxiolytic activity

In present study, anxiolytic model namely Elevated plus maze model and Light and Dark Chamber were employed.

Elevated plus maze model: EPM was proposed as model to test for anxiolytic activity. The test has been proposed for selective identification of anxiolytic drugs. Anxiolytic compounds decrease anxiety and increase open arm exploration time.

Table 3: Effect of LNEO on EPM paradigm in mice.

Group No.	Drug Treatment	Dose	Number of entries (mean ±SEM)		Time spent in sec. (mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
1.	Control	10ml/kg	2.00 ± 0.36	13.33±0.86	37.00±6.5	267.0±19.07
2.	Standard (Diazepam)	1mg/kg	17.33±1.5***	8.50±0.42***	316.7±12.82***	170.0±11.25***
3.	Test (LNEO)	200mg/kg	15.83±1.24***	10.00±0.57**	313±16.40***	196.7±9.5**

Values are expressed as mean ±SEM. n=6 for each group. Expressed as the time (in sec). Dataanalysis was performed using Tukyes test. *P<0.05, **P<0.01, ***P<0.001 vs. control.

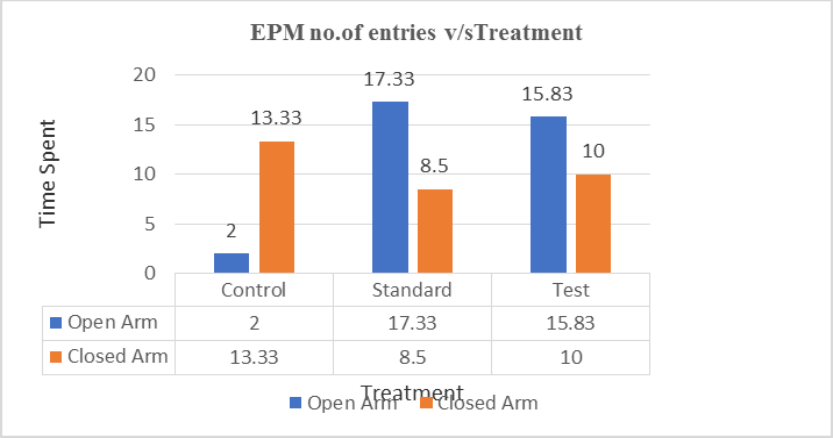

Fig 1: Comparative profile of No. of entries to open and closed arm in EPM after oraladministration of 200mg/kg of LNEO.

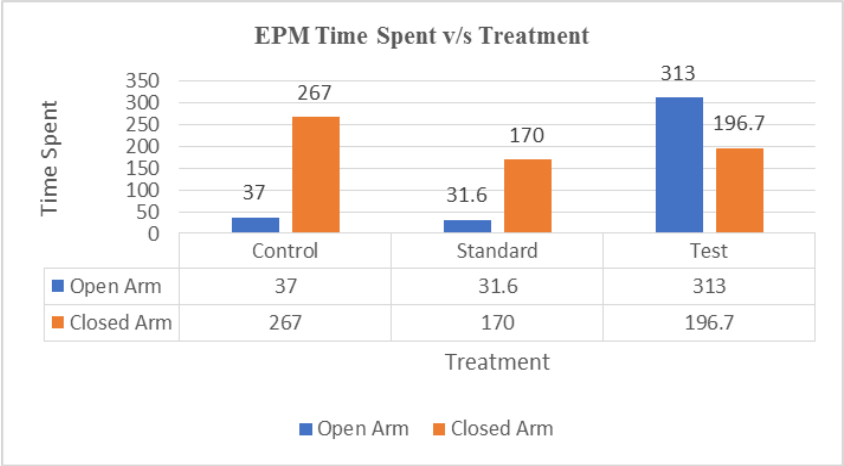

Fig. 2: Comparative profile of time spent in open and closed arm in EPM after oral administration of 200mg/kg of LNEO.

Light and Dark Chamber: Light and Dark Chamber has been described as simple behavior model in mice to detect compounds with anxiolytic effects.

Table 4: Effect of LNEO on Light and Dark chamber.

Group No.	Drug Treatment	Dose	Number of entries (mean ±SEM)		Time spent in sec. (mean±SEM)	
			Light	Dark	Light	Dark
1.	Control	10ml/kg	6.8±0.6	15.17±1.13	78.33±5.3	263.3±14.06
2.	Standard (Diazepam)	1mg/kg	18.67±0.3***	3.5±0.6***	183.3±17.14 ***	138.3±13.76***
3.	Test (LNEO)	200mg/kg	16.0+_1.29***	6.5+_0.71***	141.7±13.76 *	183.3±15.85**

Values are expressed as mean ±SEM. n=6 for eachgroup. Expressed as the time (inmin). Dataanalysis was performedusing Tukyes test. *P<0.05, **P<0.01, ***P<0.001 vs. control.

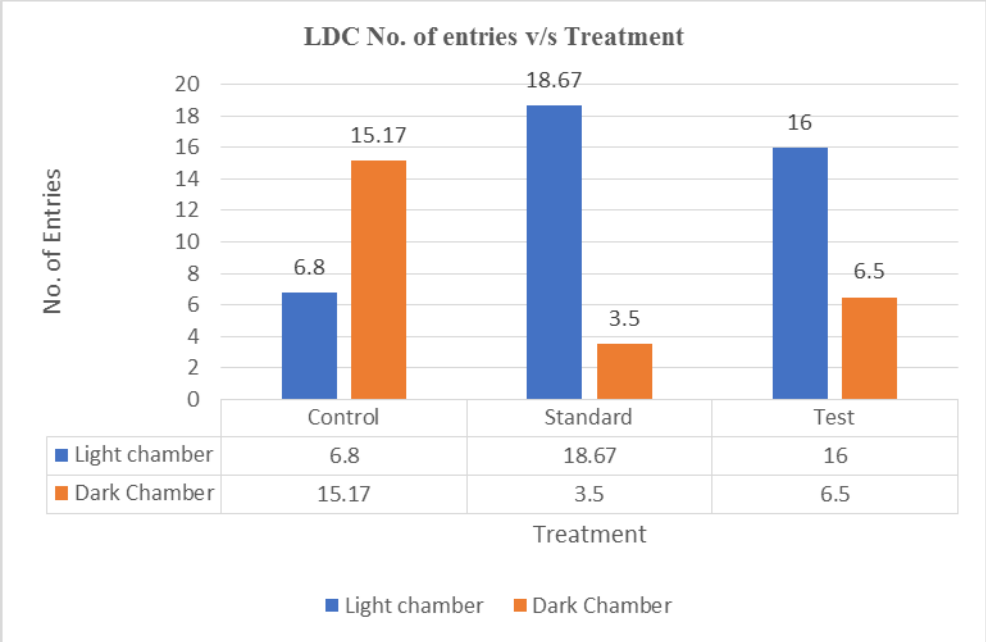

Fig 3: Comparative profile of No. of entries to Light and Dark chamber in LDC after oraladministration of 200mg/kg of LNEO.

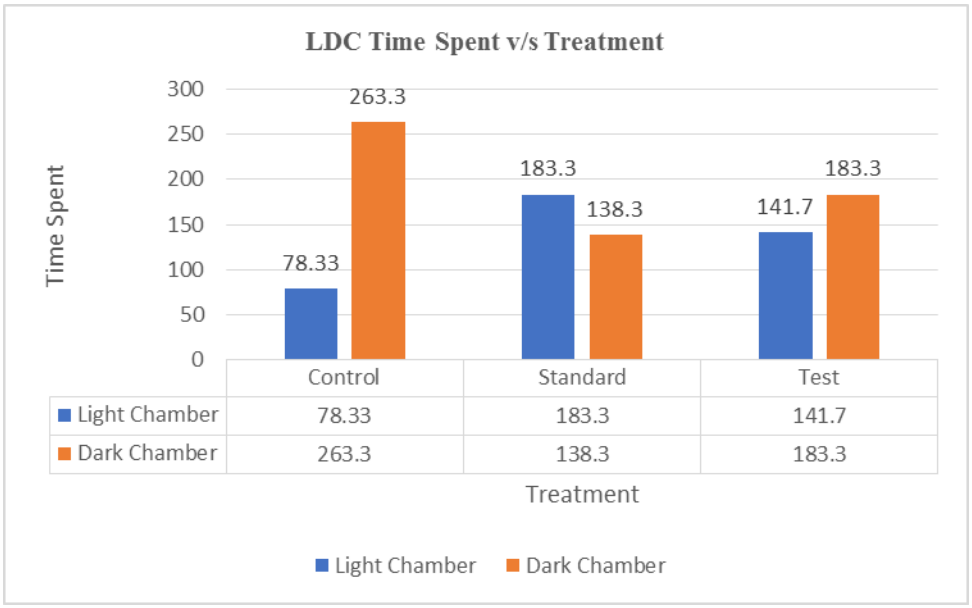

Fig. 4: Comparative profile of time spent in Light and Dark chamber in LDC after oral administration of 200mg/kg of LNEO.

DISCUSSION

The purpose of this study was to evaluate anxiolytic activity of LNEO by using anxiolytic models. Anxious reaction is an adaptive reaction of an individual when confronted with danger or threat. One of the most widely used animal models for screening putative anxiolytic is the elevated plus-maze.[16]

The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform.[17]

In EPM animals treated with single dose of LNEO (200mg/kg) showed increase in the number of entries to open arm which was extremely significant when compared with control. Also showed decrease in the number of entries to closed arm which was moderately significant when compared with control.

Similarly, shows increase in the time spent at open arm which was extremely significant when compared with control. Also showed decrease in time spent at closed arm which was moderately significant when compared with control.

The light and Dark chamber is also widely used for rodents as a model for screening anxiolytic or anxiogenic drugs. A two chambered system where the animals can freely move between a brightly-lit open field and a dark corner. After treatment with anxiolytics they show more crossings and locomotor activity between these two chambers.

In LDC animals treated with single dose of LNEO (200mg/kg) showed increase in the number of entries to light chamber which was extremely significant when compared with control. Also showed decrease in the number of entries to dark chamber which was extremely significant when compared with control.

Similarly, shows increase in the time spent in light chamber which was significant when compared with control. Also showed decrease in time spent in dark chamber which was moderately significant when compared with control.

The effects of LNEO on the EPM and light–dark test, were almost equivalent to that of 1mg/kg diazepam. In the present study, the anxiolytic activity of the *Laurus nobilis* essential oil was observed at dose of 200mg/kg in mice. These observations clearly indicate that *Laurus nobilis*exerts an anxiolytic activity.

Mechanism of action by which LNEO shows anxiolytic activity may be similar to that of diazepam [that acts via the gamma-aminobutyric acid (GABA) A receptor complex]. LNEO exerts anxiolytic activity due the presence of 1,8-cineole and linalool acetate. Essential oils havebeen used for centuries as traditional medicine in the management of anxiety, depression

and stress-related disorders.[18] We observed that following oral administration of LNEO demonstrated significant (compared to control treated group) results which indicate it has a significant anxiolytic effect in all this paradigms.

CONCLUSION

The present study can be concluded with the fact *Laurus nobilis* essential oil reveals significant anxiolytic effect in Swiss albino mice using animal models of anxiety namely, Elevated plus maze, light and dark chamber.

The data obtained was satisfactory and conclusive so as to achieve our objectives. In the conclusion, present data indicates that administration of LNEO to mice shown anxiolytic activity supporting the folk information regarding anxiolytic activity of the LNEO.

The extraction and preliminary phytochemical studies of LNEO revealed the presence of chemical and phytochemical constituents such as 1,8-cineole, linalool acetate, alkaloids, carbohydrates, saponins, tannins.

The exact mechanism underlying anxiolytic activity is not clear but it may be apparently related to active compounds present in the essential oil. Hence further studies would be necessary to evaluate the contribution of active chemical constituents for the observed anxiolytic activity as it still remains to be determined which components were responsible for these effects.

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