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ORIGINAL ARTICLE **OPEN ACCESS**

Comparative anti- anxiety Activity Evaluation of Aerial Parts of *Nepeta Cataria* Linn, *Costus Speciosus* Koen and *Jasminum Mesnyi* Hance

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ABSTRACT
The present study has been made with an aim of making a comparative analysis of the anti-anxiety activity of three conventionally used medicinal plants- Nepeta cataria Linn, Costus speciosus Koen and Jasminum mesnyi Hance. The elevated plus maze model was employed to evaluate the efficacy of different extracts (Petroleum ether, chloroform, methanol, and water) derived from all three plants. The investigations were performed on Swiss albino mice, and oral route was used for the administration of test materials. The highest and considerable effect on the elevated plus maze was observed at 200 mg/kg for the methanol extract of N. cataria aerial parts. These findings were comparable to those of the standard antianxiety drug diazepam (2 mg/kg). So Nepeta cataria was considered most active plant for detailed investigation and also found that there was no apparent adverse effect in experimental animals at the dose level tested. Current investigation validates conventional use of Nepeta cataria in curing the anxiety and tension.
Keywords: *Nepeta cataria, Costus speciosus, Jasminum mesnyi, antianxiety, elevated plus maze*

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INTRODUCTION

Catnip, also known as Catmint, is a perennial flowering plant that belongs to the Mint family (Lamiaceae). It is a native plant found in southeast Europe, the Orient, Southwest Asia, and the Western temperate Himalayas. Specifically, it grows naturally from Dalhousie to Kashmir at elevations of up to 1500 meters. (1). Traditionally, Catnip has been used for therapeutic purposes such as treating kidney and liver diseases, flatulence, and dysmenorrhea. The flowers and leaves of Catnip are prepared as a calming herbal tea, promoting a restful sleep. Additionally, it has been employed in the treatment of colic, diarrhea, cancer, the common cold, anxiety, and tension (2). *Costus speciosus* Koen (Family-Costaceae) is a significant medicinal and ornamental plant. The indigenous hill tribes of South India, known as "Kannikars," have traditionally used this plant as both medicine and food. This plant has been administered for pneumonia, rheumatism, dropsy, urinary disease, jaundice using its rhizomes, while its leaves are used for mental disorders (3). Furthermore, *Costus speciosus* is utilized for treating eye and ear infections. It is even mentioned in the Kama Sutra that the plant's extracts were used as an ingredient in cosmetics for enhancing sexual attractiveness, particularly for eyelashes (4). *Jasminum mesnyi* Hance (Oleaceae), commonly known as primrose Jasmine*, peeli malati,* and *peeli chameli*, is an evergreen rambling shrub native to the Himalayas region. It features long and slender arching stems that climb like a sprawling vine. Various parts of this plant are employed in traditional folk remedies to address a wide array of ailments and illnesses. The leaves have been widely used since ancient times to alleviate conditions such as diabetes, muscular pain, and CNS disorders (5). However, despite its long history of traditional medicinal use for ailments like anxiety and mental tension, scientific studies supporting the therapeutic claims of *N. cataria, C. speciosus*, and *J. mesnyi* are lacking, as highlighted in a literature review (6). Medicinal plant-based preparations are commonly used for basic healthcare in underdeveloped countries due to their minimal adverse effects and compatibility with the human body (7). Considering the traditional uses of *N. cataria*, *C. speciosus*, and *J. mesnyi* for their potential anti-anxiety activity, a recent study aimed to evaluate and compare the anti-anxiety properties of different extracts, including, chloroform, methanol, petroleum ether, and water

extracts, obtained from these plants. Natural remedies derived from plants have demonstrated anxiolytic effects in both humans and animals (8).

MATERIAL AND METHODS

Procurement and verification of plant material

The aerial parts of *N. cataria* were procured from JK Medicinal Plant Introduction Center (a Govt. unit of Jammu and Kashmir). Identity was confirmed through Head, (R&D) Dr. Sheikh Gulzar, JK medicinal plant introduction center, the vide letter dated 22.9.2016.

The aerial parts of *Costus speciosus* and *Jasminum mesnyi* were procured from the nurseries of Himachal Pradesh. Dr. Avneet Pal Singh, Assistant Professor at the Department of Botany, Punjab University of Patiala, confirmed the identity through letters dated 14th December 2016 and 27th July 2016.

Chemicals

All the chemicals of HPLC grade like Petroleum ether, chloroform, methanol (Merck Specialties Ltd), were used for the extraction of plant material. Jackson Pharmaceutical Pvt. Ltd., Amritsar provided the sample of diazepam.

Preparation of extracts

The Soxhlet apparatus was used to extract 1 kg of powdered aerial plant parts in a sequence of solvents with increasing polarity, starting from petroleum ether, followed by chloroform, methanol, and finally water. Extensive extraction was done using these solvents. The solvents from extracts were separated on a Buchi 461 rotary vacuum evaporator. In order to prevent moisture, the dried extracts were stored in vacuum desiccators.

Experimental animals

The experiment utilized male and female Swiss albino mice weighing between 20-30 grams. These mice were procured from the Animal House at Chitkara College of Pharmacy, Chitkara University in Rajpura, Punjab. The mice were housed in a controlled environment with a 12-hour light/dark cycle and a constant temperature of 25°C. They were provided with standard pellet diet and unrestricted access to water.

Prior to the experiments, the mice underwent a fasting period of 18 hours, during which they had access to water. The mice were then divided into several experimental groups, with each group consisting of six mice. The study was conducted in accordance with the guidelines provided by the Institutional Animal Ethical Committee under approval number IAEC/CCP/18/PR-001. The welfare of the animals was ensured by following the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Government of India.

Dose Preparation:

To achieve dosages of 100, 200, and 400 mg/kg for the extracts, and 2 mg/kg for diazepam, the experimental samples were mixed with the appropriate vehicle at predetermined concentrations. These doses were administered orally to mice using a tuberculin syringe equipped with an oral cannula, with a volume ranging from 0.20 to 0.30 ml.

Pharmacological Evaluations:

Acute Oral Toxicity Studies: Following OECD guidelines 425, acute toxicity studies were conducted for all the extracts.

Elevated Plus-Maze Model of Anxiety:

The modified Elevated Plus-Maze Model was utilized to assess the anxiolytic effects of *Nepeta cataria*, *Costus speciosus*, and *Jasminum mesnyi* extracts, as well as the standard antianxiety drug 'diazepam' (Montgomery, 1958; Pellow et al., 1985; Lister, 1987). The plus-maze apparatus consisted of two open arms (16x5cm), two closed arms (16x5x12cm) with an open roof, and was elevated 25cm from the floor. The plus-maze apparatus utilized in the experiment comprised of two open arms measuring 16x5 centimeters each, along with two closed arms of dimensions (16x5x12cm) but with an open roof. The entire apparatus was elevated at a height of 25 centimeters from the floor. To administer the test substances, including the extracts and diazepam, a cannula equipped on a tuberculin syringe was used. All substances were suspended in a 1% carboxymethyl cellulose vehicle. The extracts were administered orally at doses of 100, 200, and 400 mg/kg, while diazepam was given at a dose of 2 mg/kg. The administration occurred 45 minutes prior to the mice being placed on the Elevated Plus-Maze apparatus. Each mouse was positioned in the center of the model, facing the open arms. During a five-minute observation period, the following measurements were documented: (a) the count of entries into both the open and closed arms, and (b) mean duration of time spent by the mouse in each arm. Throughout the experiment, the mice were allowed to interact with each other, and precautions were taken to ensure that no external stimuli induced anxiety in the mice.

Statistical Analysis

The statistical analysis involved the utilization of one-way analysis of variance (ANOVA) to ascertain significant distinctions among the various groups. Dunnett's multiple comparison tests were employed for conducting the analysis with GraphPad Prism software version 5. The outcomes are expressed as the mean ± standard error of the mean (SEM). Significance was defined as $p < 0.05$.

Phytochemical screening

To determine the primary constituents, present in the extract, a phytochemical screening was conducted on the extract that displayed the highest anti-anxiety activity.

RESULTS AND DISCUSSION

Oral acute toxicity studies

During the fourteen-day study period, the extracts of *N. cataria, C. speciosus*, and *J. mesnyi* did not demonstrate any signs of toxicity or cause mortality at doses of 2000 and 5000 mg/kg. The results can be found in **Table 1.**

Table 1: Effect of petroleum ether, chloroform, methanol and water extract of *Nepeta cataria, Costus speciosus* and *Jasminum mesnyi* on acute oral toxicity test in Swiss Albino mice.

Response	Animal						
	After treatment						
	Before treatment	*Nepeta cataria*		*Costus specusus*		*Jasminum mesnyi*	
		2000 mg/kg	5000 mg/kg	2000 mg/kg	5000 mg/kg	2000 mg/kg	5000 mg/kg
Alertness	**N**	**N**	N	N	N	N	**N**
Restlessness	A	A	A	A	A	A	A
Irritability	N	N	N	N	N	N	N
Fearfulness	N	N	N	N	N	N	N
Defecation	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N
Touch	N	N	N	N	N	N	N
Pain response	N	N	N	N	N	N	N
Irritability	N	N	N	N	N	N	N
Gait	N	N	N	N	N	N	N
Mortality	A	A	A	A	A	A	A

N: Normal, A: Absent

Antianxiety activity

In current preclinical research on anxiety, the raised plus maze is a widely used animal model. Animals experience anxiety when placed on the EPM due to their fear of heights. The antianxiety activity of various test extracts, including those from *Nepeta cataria, Costus speciosus*, and *Jasminum mesnyi*, was evaluated using the EPM apparatus in mice at different dose levels (100 mg/kg, 200 mg/kg, and 400 mg/kg). The results were compared with the standard antianxiety drug diazepam (Figure 1-4). Anxiety and fear lead to a decrease in motor activity, causing the animals to prefer the closed arm, which is considered a safer place. The utilization of antianxiety agents may lead to enhanced motor activity, which can be quantified through observing the duration and frequency of entries made by animals in the open arm. The outcomes of this research indicate that the methanol extract derived from N. cataria demonstrated the most notable antianxiety effects when administered at a dosage of 200 mg/kg, as compared to the control group (Figure 3).

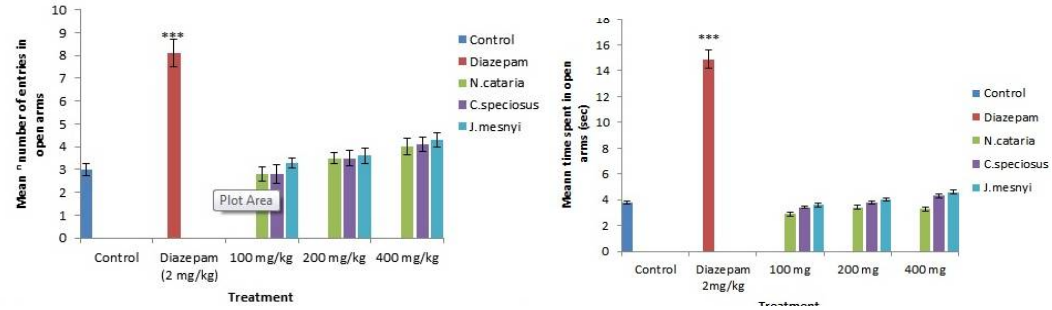

Figure 1: Antianxiety profile of petroleum ether extract of aerial parts of *N. cataria, C. speciosus* and *J. mesnyi* using EPM

The data was presented as the mean ± standard error (SE) with a sample size of n=6. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test to compare the experimental groups to the control group. Statistical significance was indicated as *P<0.05, **P<0.01, and ***P<0.001.

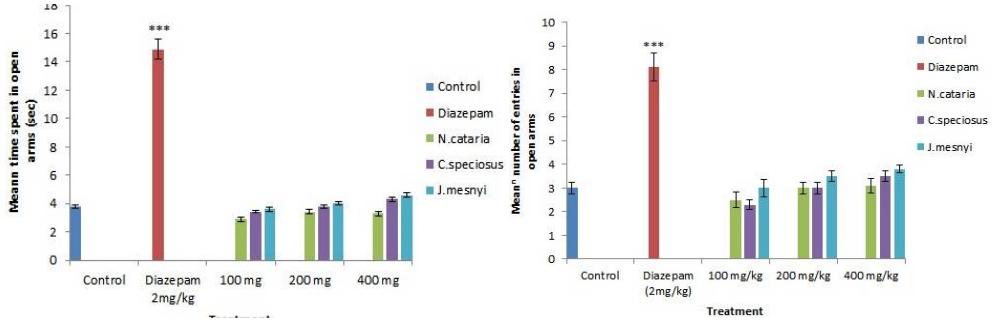

Figure 2: Antianxiety profile of chloroform extract of aerial parts of *N. cataria, C. speciosus* and *J. mesnyi* using EPM

The data was presented as the mean ± standard error (SE) with a sample size of n=6. A one-way ANOVA followed by Dunnett's multiple comparison test was conducted to compare the experimental groups to the control group. Statistical significance was denoted as *P<0.05, **P<0.01, and ***P<0.001.

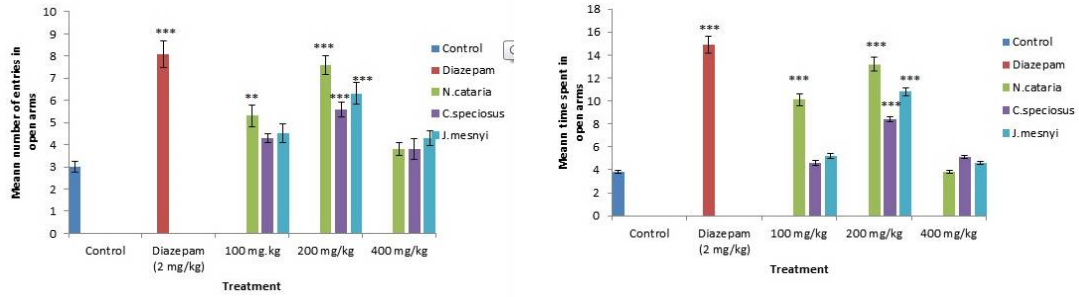

Figure 3: Antianxiety profile of methanol extract of aerial parts of *N.cataria, C. speciosus* and *J. mesnyi* using EPM

The data was presented as the mean ± standard error (SE) with a sample size of n=6. To compare the experimental groups with the control group, a one-way ANOVA followed by Dunnett's multiple comparison test was performed. Significance levels were indicated as *P<0.05, **P<0.01, and ***P<0.001.

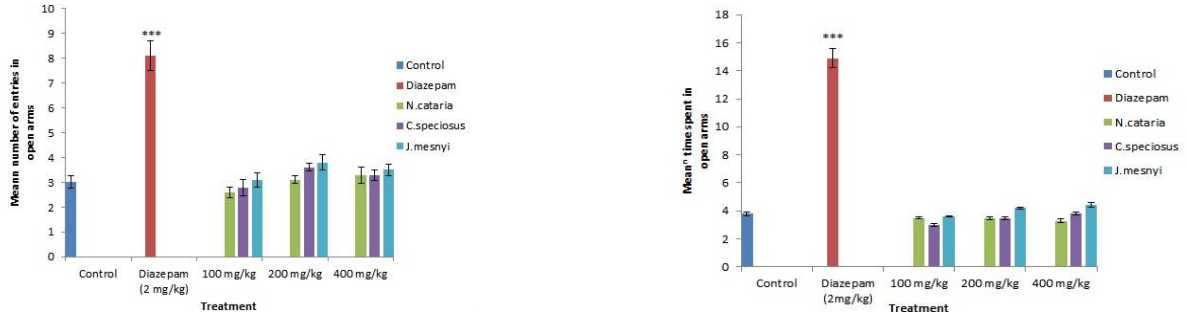

Figure 4: Antianxiety profile of aqueous extract of aerial parts of *N. cataria, C. Speciosus* and *J. Mesnyi* using EPM

The data was represented as the mean value with its standard error (SE) for a sample size of n=6. Statistical analysis using a one-way ANOVA followed by Dunnett's multiple comparison test was employed to compare the experimental groups against the control group. The significance levels were indicated as *P<0.05, **P<0.01, and ***P<0.001.

Phytochemical screening

The preliminary phytochemical analysis of methanol extract (aerial portions) indicated that it includes a number of phytoconstituent groups, including flavonoids, tannins, and phenol.

CONCLUSION

The current analysis finds that out of 12 extracts from the three plants that were being studied, *Nepeta cataria, Costus speciosus and Jasminum mesnyi* using EPM model, only methanol extract of *N. cataria* (aerial parts) displays remarkable levels of anti-anxiety effectiveness, reaching a statistical equivalence to diazepam, a widely recognized compound in the same category exhibits maximum and significant activity. These plants extracts showed no apparent adverse effects in experimental animals at the dose level tested. To identify the active ingredient(s) responsible for *N. cataria's* antianxiety action, more research is being done.

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ANIMAL ETHICS

Approval no. IAEC/CCP/18/PR-001

CONFLICTS OF INTEREST

Declare conflicts of interest or state “The authors declare no conflict of interest.”

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