Evaluation of anti-anxiety activity of the leaves of *Cyanthillium cinereum*

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Abstract
Anxiety is a mental disorder that alters a person's way of life and places a heavy burden on the patient's ability to afford therapy. You can start to perspire, become agitated and anxious, and have fast heartbeat.The object of present investigation was in order to ascertain whether *Cyanthillium cinereum* (L.) H. Rob's methanolic extract had any anti-anxiety effects on mice. The physiochemical, phytochemical, and anti-oxidant activity of C. cinereum was examined. Different significant parameters, including ash value, moisture content, fiber content, and extractive values for extract with methanol, extract with ethyl acetate, extract with chloroform, and extract with water, were reported by physiochemical assessment. The phytochemical investigation revealedthat *C. cinereum* contain alkaloid, saponin, flavonoid, terpenoids and phenol. The outcomedemonstrated that methanolic extract the better solvent for Enhancement of methanolic extraction by FTIR and LCMS analysis. The anxiety activity of methanolic extract was determined and result of antianxiety activity recorded that significantly($p< 0.0001$) increase the effect of diazepam 2mg/kg and plant extract 400mg/kg as comparedwith plant extract 100mg/kg and 200mg/kg compared data with control group. Thus, thestudy proved that methanolic extract of *C. cinereum*had good potential as anti-oxidant,antianxiety activity and thus this plant can be utilized as natural source for CNS activity.

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Introduction
History is a detailed narrative of humankind's past. It offers a way to assess and evaluate the development of human affairs and historical events, putting them into context. Through historical awareness and the present is more fully disclosed, judged, and understood, and it can be more successfully interpreted and described given how it has influenced and molded the past[1].Man is not only able to live in the present more successfully as a result of this improved understanding of the past via assessment and interpretation of the present, but he is also more equipped to forecast the

better future and to predict. As a result, history is a product of the past and the present, and the future will be a product of both as impacted by both past and present circumstances and situations[2].The emotion of anxiety predates the development of man. While anxiety manifests itself in different ways for children, adolescents, and adults, it can also be deduced from other people's physiological and psychological reactions in some cases[3]. You could feel more energized or more focused as a result of the worry.Anxiety that doesn't go away or grow better over time might be a symptom of an anxiety disorder.The risk factors for various anxiety disorders might differ.The symptoms can obstruct daily tasks including job performances, homework, and interpersonal connections. Even in reaction to the same stimuli, anxiety might vary in frequency and intensity amongst individuals. It is a widespread feeling of unease or foreboding. There are numerous reasons to worry. Our health, social interactions, tests, occupations, and environmental conditions are just a few examples of potential worry-causing factors.Being a little concerned about these areas of life is natural and even appropriate. When anxiety encourages us to study for exams or prompts us to seek out routine medical examinations, it serves a useful purpose[4].Changes in your lifestyle might successfully reduce some of the stress and worry you might experience daily.Among psychiatric conditions, anxiety disorders are among the most prevalent. A generalized, uncomfortable, hazy sense of unease is what defines anxiety. Autonomic symptoms including sweating, chest stiffness and palpitations, slight stomach discomfort are frequently present along with it. The main family of substances used to treat anxiety is called benzodiazepines, and these medications continue to be the most often prescribed ones Anxiety is connected yet distinct. When you are anxious about a specific event, such as an approaching test, presentation, wedding, or other significant shift in your life, stress is a common and appropriate response[5].

C. cinereum (L.) H. Ro. (Also known as purple fleabane, Kala Jira, or Sahadevi) was a type of annual erected herbal that grows up to 2meter long and belongs to at Asteraceae family. It is one of the most widespread Indian weeds, with populations ranging from the Himalayan foothills to the Kashia and Peninsular hills at elevations of up to 8000 feet. *C. cinereum* is an herb that grown annual& it is approximately 75 cm tall and is rarely decumbent Stems are rigid, slender, tubular, serrated and corrugated, and pubescent Ly striate and somewhat branched, up to 17 cm tall, with divaricated branches[6].The entire plant possesses a number of pharmacological qualities that may be used to treat a wide variety of ailments in traditional medicine, but its potential for usage as a commercial medication is underappreciated. The dried entire plant C. cinereum's substantial capacity to scavenge free radicals has been extensively examined and confirmed, and it may have therapeutic benefits due to its whole phenolic and flavonoid contents. Fruits are elliptical kernel with appressed white hairs and a terete (ribbed) shape. From August through April, flowers and fruits bloom. Pinkish purple flower with a violet center and a tiny head. Rounded or flat-topped corymbs, terminal corymbs There are numerous options. Divaricated blooms, around 20 at a time, 6 mm diameter Each flower head is accompanied by a little linear bract and peduncular forks with tiny bracts; involucre Corolla with linear-lanceolate bracts, awned and silky on the back, petal-right -sharp, awned, and smooth on the back thin lobes are lobes that have become pubescent over time[7]. The whole herb's decoction or infusion is used to treat bladder strangury and spasms, as well as fever and wounds and sores. Malaria is treated with a whole herb containing quinine. The liquid mixture of the whole herb is used to treat urinary infection, colic, and piles. Sunstroke and dropsy are treated using its root. Scorpion

stings, amoebiasis, and cough have all been treated using its leaves Herpes, eczema, ringworm, and guinea worm are all treated with a poultice of leaves[8]. C. *cinereum* can be treated with a paste made from the whole plant and a little ghee. When applied locally, oil made by boiling the leaves' juice has shown to be effective against elephantiasis. Its blooms can help with rheumatism and conjunctivitis. Flatulence, dysuria, leukoderma, psoriasis, and leprosy are all treated using its seeds. Asthma, bronchitis, and constipation are all treated by *C. Cinereum*[9]

C.cinereum has a broad range of medicinal uses in many forms of herbal around the world. The medicinal value of different portions of the plant varies. Traditional medicine use *C. cinereum* for a wide variety of ailments due to its potent pharmacological qualities, but it is underutilised as a source for commercial drugsCommonly found in highland crop regions, gardens, waste places, and by the sides of roads, C. cinereum has several medicinal advantages that are sometimes underappreciated. The plant is used in malarial infection, as a painkiller, inflammatory, diuretic, anti-cancerous, miscarriage, and peptic ulcer[10]

Material and methods

Fresh leaves of the plant *C. cinereum* was collected from local area of Varanasi, Utter Pradesh and authentication at CSIR India (NIScPR/RHMD/consult /2021/3990-91, 19/01/2022). After identification I was collect *C. cinereum* leaves and dried at room temperature up to 15-20 days them dried leave make powder it weighted 750 gm. 50gm of plant powder was used of the powder was taken in a thimble and extraction process up to 5 days according to solvent temperature. After extraction, by utilizing a water bath to concentrate it, the extract. The whole dried extract was measured and stored for future analysis purpose.

Qualitative Phytochemical screening

Preliminaryphytochemicalscreeningisveryimportantparameterwhichisanimportantscreening method for a new drug or to identify the appearance of active of bioactive compounds.The water, chloroform, methanol, and ethyl acetate,extractof *C. cinereum* leaves wasscreenedbyvariousphytochemicalscreening methodsliketheFehling'stestandtheMolisch'stestforcarbohydrate,Shinodatestandconcentrated H_2SO_4 test for flavonoid, biuret test, million's test, aminoacidsand proteins are identified by Salkowski's test and thefoamtesting forSaponinsaccordingto previousresearch[11].

Test for carbohydrates:

- **Molisch's Test:** It entails tend the substances made up of concentrated sulfuric acid and-naphthol along the test tube's sides. The intersection of two liquids generated a purple or reddish violet colour.
- **Fehling's Test:** Fehling's A and B added. It gives brick red precipitate on gentle heating.
- **Benedict's test:** Add 8 drops of the solution being studied to 5ml of Benedict's reagent. After thoroughly combining, boiling the mixture for two minutes, it should be cooled. Precipitate in red is produced.

Test for alkaloids:

- **Dragendroff's Test:** Take 1ml of Dragendroff's reagent and add to the extract, and an orange-red precipitate will result.
- **Wagner's test:** Add Wagner reagent to the extract, a reddish-brown precipitate form as a result.
- **Mayer's Test:** Mayer's reagent, in either 1 or 2 milliliter amounts, is added to the extract to produce a drab, white precipitate.
- **Hager's Test:** Take 3ml of Hager's reagent and add to the extract, it forms yellow precipitate.

Test for Steroids and Sterols:

- **Liebermann Burchard test:** Take a dry Test tube, dissolve the test sample in 2 ml of chloroform. Add 2 drops of strong sulfuric

acid and 10 drops of Acetic Anhydride now. Then colour of solution changes from red to blue and then into bluish green.

- **Salkowski test:** Add equal volumes of concentrated sulfuric acid and chloroform to the test solution sample to dissolve it. Chloroform layer has bluish red cherry red and purple hues, whilst acid exhibits pronounced as green fluorescence.

Test for Glycosides:

- **Legal's test:** The sample is dissolved into pyridine, and then an alkaline sodium nitroprusside solution is added. The colour pink red is created.
- **Baljit test:** Sodium picrate solution is added to the drug sample. The colour changes from yellow to orange.
- **Bontrager test:** Several ml of diluted sulfuric acid should be added to the test solution. Boil, filter, and then use ether or chloroform to extract the filtrate. When ammonia is given to the organic layer after it has been separated, the organic layer turns pink, crimson, or violet.
- **Killer Killani test:** Sample is moved to the surface of concentrated sulfuric acid after being dissolved in acetic acid with traces of ferric chloride. Reddish-brown colour is formed at the liquid junction, which progressively turns blue.

Test for Saponins:

Foam test: Take1ml sample of alcohol and diluted separately with 20ml of water and stir it for 15 minutes in cylinder. The formation of foam by 1 cm shows the presence of saponins.

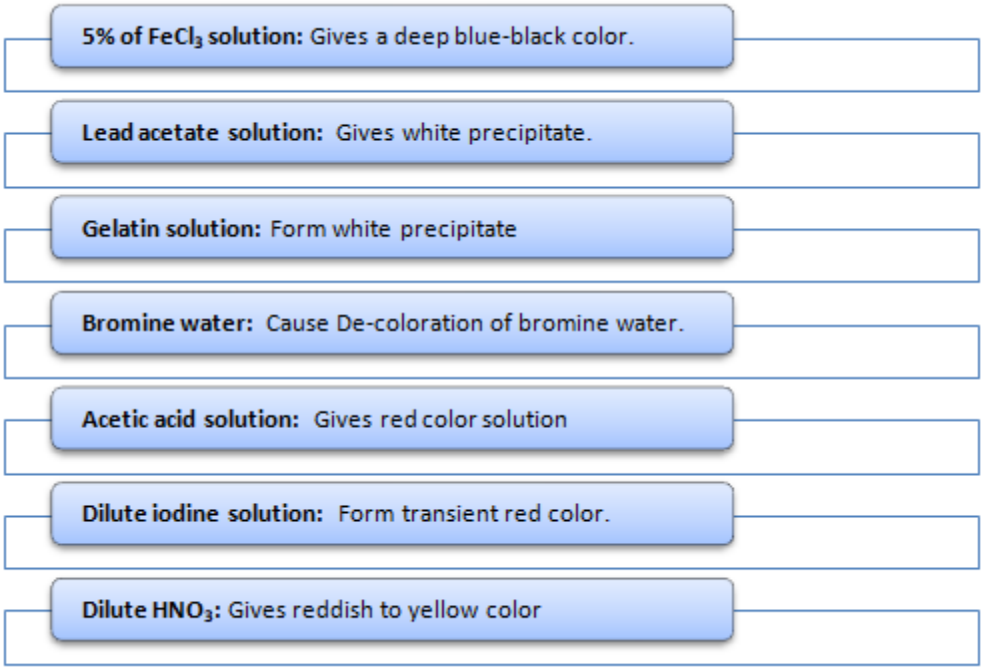
Test for Flavonoids:

- **Shinoda test:** When concentrated hydrochloric acid is introduced, followed by the sample, magnesium turnings, a red colour results.
- **Ferric chloride test**: A blackish red colour would appear in test solution, the presence of flavonoids confirms.
- **Lead acetate solution Test**: Test solution would produce a yellow precipitate when exposed to a few drops of lead acetate (10%) solution.

Test for Tri-Terpenoids: Two or three tin grains were placed in the test tube, together with a 2 ml solution of thionyl chloride and the test solution. Triterpenoids are present as evidenced by the production of a pink colour.

Tests for Tannins and Phenolic Compounds:

By using a spectroscopic approach, the amount of phenol in the raw material of the causative in aspen extract was calculated. Take 2-3 ml of extract add few drops of respective reagent to it.



Counting the amount of phenol in total

The amounts of phenolic components in methanol, chloroform and ethyl acetate extracts of C. cinereum were determined using standard procedures. Varying amounts of plant extracts or standard solutions were added to 2.5 ml of Chocalteus Folin reagent (10-fold diluted with water) and 2.5 ml of Na2CO3 solution (7.5%). The optical density of the reaction mixture at 760 nm was measured after incubation at 25 °C for 20 minutes. The results of triplicate experiments were presented as averages with standard error of the mean (SEM), and the results were expressed as mg gallic acid equivalent (GAE)/g dry extract[12].

Counting the amount of all flavonoids

A previously published aluminum chloride colorimetric method was used to investigate the total flavonoid content of *C. cinereum* methanol extract, chloroform and ethyl acetate. Today, flavonoids are used in a variety of pharmacological, therapeutic, cosmetic and nutraceutical applications. 5.6 ml of distilled water, 3.0 ml of methanol, 0.2 ml of 10% AlCl3, 0.2 ml of 1M potassium acetate and 1 ml of each concentration of plant extract were added. The reaction was then terminated by incubating the reaction mixture at room temperature for 30 minutes. The absorbance of the mixture at 420 nm was quantified. Values are expressed as quercetin equivalents per gram of sample (QE/g) and the study was performed in triplicate. Results were presented as mean SEM[12].

FourierTransformInfraredSpectroscopy(FTIR)

Perhaps the most effective tool for determining the types of chemical bonds (functional groups) present in a substance is Fourier Transform Infrared Spectrophotometry (FT-IR). Dry powdered methanol extracts of plant materials were used for FT-IR studies. 100 mg of KBr granules contains 100 mg of dry extract powder. For the manufacture of transparent discs with samples. The powder sample scanning range is 400 to 4000 cm-1 with a resolution of 4 cm-1 [13].

LiquidChromatographyElectroscopyIonization MassElectroscopy(LC-ESI-MS)

20 mg of *C. cinereum* extract was dissolved using 1 ml of 10% methanol and the resulting solution was filtered through a 0.45 µM

membrane filter and added to the HPLC column. For LC-ESI-MS studies, a Shimadzu LC-MS-2020 quadrupole mass spectrometer (Kyoto, Japan) equipped with an electrospray ionization source (ESI) and operating in negative ion mode was used. The mass spectrometer was connected online to an ultrafast liquid chromatography system containing an LC-20AD XR binary pump system, a SIL-20AC XR autosampler, a CTO-20AC column oven, and a DGU-20A 3R degasser (Shimadzu, Kyoto, USA). .). Japan). Analysis was performed using an Aquasil guard column (10 mm 3 mm, 3 m, Thermo Electron) and an Aquasil C18 column (150 mm 3 mm, 3 m, Thermo Electron, Dreieich, Germany).The mobile phase, eluted using a linear gradient over times of 0-45 min and 45-55 min, contained components A and B (0.1% formic acid in methanol and HO, respectively). There was a prescribed 5 min equilibration time between each run. The column temperature was maintained at 40°C, the amount of liquid injected was 5 L, and the flow rate of the mobile phase was 0.4 ml/min. Spectra were processed using Shimadzu Lab Solutions' LC-MS software when viewed in Selected Ion Monitoring (SIM) mode.The mass spectrometer has a full spectrum scan from 50 to 2000 Da, a capillary voltage of approximately 3.5 V, a spray gas flow rate of 1.5 L/min, a dry gas flow rate of 12 L/min, and a block source temperature of 250. °C and voltage detector 1.2V[14].

Pharmacological Activity

Animal

For the study, the animal house had albino mice that weighed 15-20 g and were between 6- and 8-weeks old animal house at UIPS Chandigarh University, Mohali.All the test animals had unrestricted food and water availability as well as 12-hour light-and-dark cycle.Criteria of the treatment or maintenance on the animals were built by the Committee for the Control and Supervision of Animal Experiments in India. The experimental protocol (CU/2021/IAEC/4/11) was approved by UIPS Chandigarh University's institutional Animal Ethics Committee, registration number 2068/PO/ ReRc/s/19/CPCSEA.

Drug

Diazepam was obtained from Consern Pharmaceutical Pvt Ltd. The drugs were diluted in normal saline and freshly prepared before drug administration.

Experimental Design

Mice were placed into five groups with five animals in each group to explore the anti-anxiety activity utilizing the EPM and the light and dark box models, as follows[15].

Group 1: normal saline by oral route

Group 2: Diazepam (2 mg/kg) by oral route

Group 3: *C. cinereum* leaves extract (100mg/kg in CMC by oral route)

Group 4: *C. cinereum* leaves extract (200mg/kg in CMC by oral route)

Group 5: *C. cinereum* leaves extract (400mg/kg in CMC by oral route).

Acute oral toxicity test

The doses of *C. cinereum* methanolic extract given to the animals were 600 mg per kg, 1000 mg per kg, and 1500 mg per kg.

Experimental modal to test anxiolytic activity

The test substance's ability to reduce anxiety in albino mice was evaluated using the elevated plus maze (EPM) and light dark box tests.

Elevated Plus Maze

The EPM consisting of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) was used. One of the tests that is most frequently used to gauge anxiety-like behaviour is the elevated plus maze test. The test is based on mice's innate aversion to high, open spaces as well as on their spontaneous natural inquisitive tendency in unfamiliar surroundings.The maze was elevated to height of 25 cm above the floor. Saline, Diazepam and Test solution was administered once daily at 10 AM for a period of 8 days. On the 8 days , animals were placed individually at the center of the EPM with their head facing towards the open arm. Indicators of open space-induced anxiety in mice include the frequency of entrances and the amount of time spent in the

open arms.The walls are translucent and the platform is white. The raised plus maze's equipment comes in a variety of colours and materials.Total time of stay and number of entries in different arms were recorded during 5 min period. Number of rearing and head dipping were also counted. An arm entry is defined as the presence of all four paws in the arm. EPM was cleaned with 70% ethanol after each reading to eliminate any possible bias due to odor[16](18).

Light/dark box

The rat natural aversion to bright lights and their spontaneous exploratory behaviour in response to modest stressors, such as a new habitat and light, are the foundations of the light/dark test.The light dark box apparatus consisted of two compartments (20 × 20 × 20 cm), one painted white and illuminated and other painted black which is kept dark. These two compartments connected to each other by a door (5 × 5 cm). The light source kept at 50 cm above the compartment's roof. Saline, Diazepam and Test solution was administered once daily at 10 AM for a period of 8 days. On the 8 days, animals were placed individually at the center of the illuminated white chamber (light compartment) with their head facing the side wall. Total time of stay and number of entries in light compartment were recorded during 5 min period. An entry is defined as the presence of all four paws in the respective compartment. The light and dark box were carefully wiped with 70% ethanol after each trial, to eliminate the possible bias due to the odor of the previous animal[16] (18).

Statistical analysis

Statistical analysis was reported - "Using GraphPad Prism Version 9.0, one-way analysis of variance (ANOVA) and Dunnett's test were used to evaluate the data (GraphPad Software Inc, San Diego, CA). Standard error of the mean (SEM) and p values were used to express all values."

Result

Phytochemical Parameter

Preliminary phytochemical revealed the presence of alkaloids, saponin, terpenoid, Phenol, Flavonoid, and sterol in leaves of *C. cinereum* show in table 1.

Table. 1 Findings from phytochemical testing of various C. cinereum extracts

S. No	Phytochemical Component	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract	Water Extract
1.	Alkaloid	+	+	+	+
2.	Steroid/ sterol	-	-	-	+
3.	Saponin	+	+	+	+
4.	Glycoside	-	-	-	-
5.	Flavonoid	+	-	+	-
6.	Terpenoid	+	+	+	+
7.	Phenol/tannins	-	-	+	-

8.	Carbohydrate	-	-	-	-

Present (+) absent (-)

Glucose hyperglycemia, antioxidant, anticancer, and anti-inflammatory uses for saponins are discussed[17]. Alkaloids, which have properties including being analgesic, anti-inflammatory, diuretic, antiviral, antihypertensive, antiulcer, and anticancer[18]. Terpenoids offer a wide range of potential uses, including neuroprotection, immune modulation, antioxidation, antiaging, and insect resistance[19]. For example, flavonoids exhibit anti-inflammatory, anti-atherosclerotic, anti-ulcer, anti-hepatotoxic, anti-thrombogenic, anti-osteoporotic, anti-tumor, antiviral, antibacterial, and antifungal activity[20]. Additionally, polyphenols have a broad range of advantageous pharmacological action. Currently, they are used mostly to treat disorders like cancer, diabetes, heart disease, arthritis, neurodegenerative illnesses, and many more. The sources, makeup, and useful pharmacological properties of polyphenols are the main topics of this review [21].

Quantitative phytochemical parameter

Total Phenolic Content

The Folin-Ciocalteu reagent was used to determine the phenolic content of each extract. The outcomes were expressed as gallic acid equivalents (GAE) per gramme of dry extract weight (table. 2). According to the findings, methanolic extract had a higher TPC than chloroform and ethyl acetate extracts, with GAE concentrations of 85.940.24 mg for methanolic extract, 55.830.19 mg for chloroform extract, and 32.380.14 mg for ethyl acetate extract, respectively. Since the bioactivity of the methanolic extract is caused by its higher phenolic content, this extract is predicted to have good antioxidant activity.

Total Flavonoid Content

Aluminum chloride was used in a colorimetric process to assess the number of flavonoids in the extracts. Quercetin equivalents (QE) per gramme of dry extract weight were used to express the results (Table. 14). According to the findings, the methanolic extract shown higher TFC than the chloroform and ethyl acetate extracts. Methanolic extracts have a GAE content of 64.44 0.14 mg/g, 36.83 0.19 mg/g, and 25.77 0.14 mg/g, respectively, when compared to chloroform and ethyl acetate extracts.

Table. 2 The total phenolic and flavonoid content of methanolic extract of *C. cinereum*

Extracts	Phenolic content (mg/g GAE)	Flavonoid (mg/g QE)
ME	85.94±0.24	64.44±0.14
CE	55.83±0.19	36.83±0.19
EAE	32.38±0.14	25.77±0.14

"ME= Methanolic extract, CE= Chloroform extract EAE= Ethyl acetate extract"

Fouriertransforminfraredspectroscopy(FTIR)

Obtain an infrared spectrum of methanolic extract of *C. cinereum*. Spectrum recorded between 4000 to 400 cm^{-1} show in table and figure

Table. 3 FTIR Peak Values of Methanolic Extract of *C. cinereum*

S. No	Peak Value cm^{-1}	Frequency Range	Functional group
1.	3261.14	3500-3200	alcohols and phenols present in the O-H stretching vibration

2.	2917.08	3000-2850	Alkenes cause a C-H stretching vibration.
3.	2850.91	3000-2850	presence of alkene and C-H stretching vibration
4.	1562.69	1640-1550	Primary and secondary amines and amides present in N-H bending vibration
5.	1377.08	1400-1000	Fluoride presence and C-X stretching vibration
6.	1039.74	1250-1020	Existence of aliphatic amines causes the C-N stretching vibration.
7.	934.40	1000-690	Alkanes' C-H out-of-plane bend
8.	895.11	910-665	the presence of primary and secondary amines in the N-H stretching vibration
9.	718.38	910-665	Primary and secondary amines present with N-H stretching vibration

"Fourier transform infrared spectroscopy studies were used to analyze compounds' functional groups based on their peak values (cm-1). Alcohol, Phenol, Alkanes, Amide, Amine, Fluoride, and intensity peaks for primary and secondary amines have all been identified. Table. 3 shows the peak values for 3261.14 cm-1, 2917.08 cm-1, 2850.91 cm-1, 1562.69 cm-1, 1377.08 cm-1, 1039.74 cm-1, 934.40 cm-1, 895.11 cm-1, and 718.38 cm-1."

LiquidChromatographyElectroscopyIonizationMass spectroscopy (LC-ESI-MS)

Table. 4 Result of LCMS Analysis of *C. cinerarium* Extract

Compound Name	Molecular Formula	Molecular Weight
Beta-Caryophyllene	C15H24	204
Caryophyllene oxide	$C_{15}H_{24}O$	220
Guaiol	$C_{15}H_{26}O$	222
1-Heptadecene	$C_{17}H_{34}$	238
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
1-Nonadecene	$C_{19}H_{38}$	266
n-Tetracosanol-1	$C_{24}H_{50}O$	354
Tricosyl trifluoroacetate	$C_{25}H_{47}F_3O_2$	436
Tetra tetracontane	$C_{44}H_{90}$	618

Pharmacological activity

Anti-anxiety activity

Elevated Plus Maze Model

Table. 5 Result of anxiety related behavior use Elevated Plus Maze Model

Sr. No.	Group	Time spends in open arm	Number of entries in open arm
1	Control	25.20±1.393	1.600±0.2449
2	Low dose (100mg/kg)	52.20±0.8602*	$3.000±0.3162^{ns}$
3	Mid dose(200mg/kg)	84.20±3.338**	5.000±0.4472*

4	High dose(400mg/kg)	127.6±2.064***	8.600±0.5099**
5	Diazepam (2mg/kg)	154.4±1.077****	12.20±0.3742***

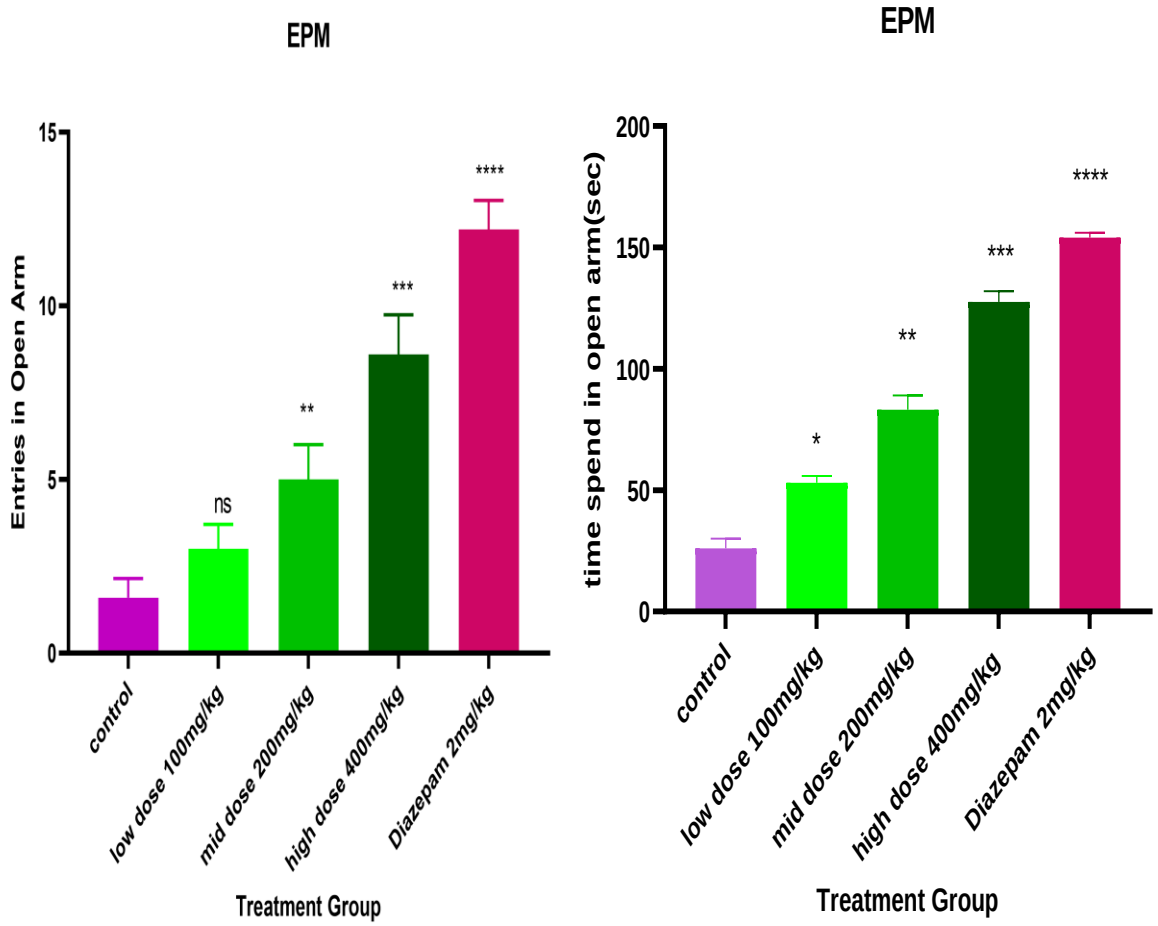


Figure. 1"Effect of *C. cinereum* extract time spent in open arm of the EPM during 5-minute test session (n=5) at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. Data is presented as mean Sem. Utilizing ANOVA and a post hoc Turkey multiple comparison test, comparisons were done. (A) Compared to the control group, C. cinereum significantly increased the number of entries into the open arm (100 mg/kg, p<0.0001 n=5, 200 mg/kg, p<0.0001 n=5, and 400 mg/kg, p<0.0001). Diazepam's (2 mg/kg) effects were comparable to those of (p<0.0001). (B). When compared to the control group, the time spent in the open arm was much longer when C. cinereum (400mg/kg) was taken. Similar results were also shown with diazepam (p0.0001 n=5). 100 mg and 200 mg of *C. cinereum* had minimal effects".

Behavioral assessment in the EPM is mainly used to assess exploration and anxiety States.Figure demonstrates that as compared to the control group, diazepam at 2 mg/kg considerably lengthened the time spent and increased the number of entries with open arms, Additionally, extract at a low dose of 100 mg/kg significantly increased both the amount of time spent and the number of entries into open arms when

compared to the control group, but at the same level, there was no significant difference between these two outcomes compared to the standard dose. In addition, a medium dose of 200mg/kg considerably increased the time spent and the number of entries into open arms as compared to the control group and low dose group, but at the same dose, the time spent and the number of entries into open arms was lower than with the standard dose. In comparison to the control group, low dose group, and medium dose, as well as high dose 400mg/kg, the time spent and number of entries into open arms are much higher. However, at the same dose, there is no difference between the two parameters. The findings imply that MECC (methanolic extract of *C. cinereum*) markedly reduced anxiety-like behaviors in experimental mice in a dose-dependent way.

Light/Dark Model

Table. 6 Result of anxiety related behavior use light/dark Model

S. No.	Group	Time spends in light area	Number of entries in light area
1.	Control	26.20±0.5831	2.200±0.3742
2.	Low dose (100mg/kg)	52.20±0.8602 ^{ns}	5.400±0.4000*
3.	Mid dose (200mg/kg)	84.20±3.338*	8.200±0.412**
4.	High dose (400mg/kg)	127±2.064**	11.40±0.6000***
5.	Diazepam (2mg/kg)	164.6±1.435***	16.60±0.5099****

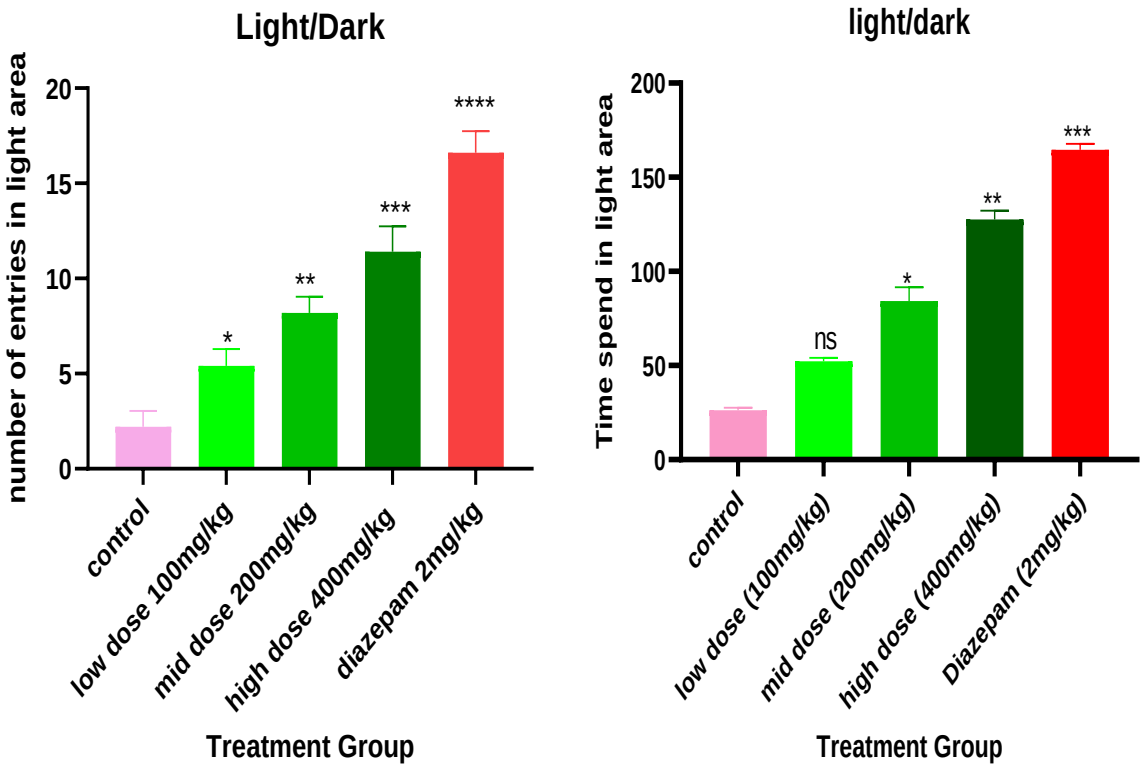


Figure. 2"*C. cinereum* extract (100, 200, and 400 mg/kg) time spent in the light section of the light / dark model during a 5-minute test session (n = 5). Data is presented as mean Sem. Utilizing ANOVA and a post hoc Turkey multiple comparison test, comparisons were done. (A) C. In comparison to the control group,*C. cinereum* significantly increases the frequency of entry into the light region (100mg/kg $p<0.0001$ n=5, 200mg/kg $p<0.0001$ n=5 and 400mg/kg $p<0.0001$). Diazepam's (2 mg/kg) effects were comparable to those of ($p<0.0001$). (B). C. When compared to the control group, the time spent in a light area rose significantly ($p<0.0001$ n=5) with cinereum (400mg/kg). Similar results were also shown with diazepam ($p<0.0001$ n=5). C. Cinereum (100 mg/kg, 200 mg/kg) has minimal effects."

The major purposes of behavioral evaluation in the light and dark are to measure exploratory and anxious moods. The figure demonstrates that 2 mg/kg diazepam significantly increased the time spent and the number of entries in the light area when compared to the control group, as did extract at a low dose of 100 mg/kg. However, at the same dose, the time spent and the number of entries in the light area were less than the standard dose. In comparison to the control group and low dose group, the time spent and the number of entries into the light area greatly rise with medium dose 200mg/kg as well, but at the same dose, there is no difference between the two when compared to the standard dose. In comparison to the control group, low dose group, and medium dose, as well as high dose 400mg/kg, the time spent and number of entries into the light region are significantly increased, although at the same dose, there is a non-significant decrease compared to the standard dose. The findings imply that MECC considerably reduced anxiety-like behavior in experimental mice in a dose-dependent way.

Conclusion

In study, my result the presence of phenol, flavonoid, terpenoid, saponin and alkaloid in the methanolic extract *C. cinereum*, the anxiolytic properties of this plant were observed in this study, and the anxiolytic activity was dependent on the phytochemical

parameter. demonstrates that oral administration of *C. cinereum* part of leaves methanolic extract may have an anxiolytic activity in mice. The future focus is on the correct mechanism of action incorporating therapeutic and toxic doses of each form of anxiety condition, as well as clinical and pre-clinical testing. To identify more chemicals, additional phytochemical analysis can be performed. To verify the anti-anxiety activity, more animal behavioral models can be used. to discover the molecular mechanism of action for the activity and to ascertain whether the separated compounds were accountable for the antianxiety effect. So that they can soon be employed as therapeutic agents following clinical trials, more research is hoped to identify the precise mechanism of action of the extract and isolated chemical for its Anti-Anxiety efficacy.

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