



EVALUATION OF ANTI – CLAUSTROPHOBIC EFFECT OF COSTUS IGNEUS (LEAVES) IN SWISS ALBINO MICE

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Abstract: Background: A phobia is an anxiety disorder characterized by a fear that seriously impairs a person's capacity to function in daily life. Many indigenous medicinal plants with natural neuroprotective and antioxidant properties have shown to be beneficial in a variety of behavioral disorders such as anxiety disorders. Many allopathic drugs are available to treat anxiety disorders, among which benzodiazepines are most commonly used which possess various systemic side effects. A specific sort of phobia known as "claustrophobia" is the fear of enclosed spaces. The present study was designed to study anxiolytic property of methanolic extracts of *Costus Igneus* an important and commonly used for its medicinal properties belongs to Rosaceae family. **Materials and Methods:** Methanolic extracts of *Costus Igneus* leaf at doses 50 mg/kg, 100mg/kg and 200 mg/kg were compared with standard (Diazepam 1mg/kg) and control (distilled water). Six Swiss albino mice (5 each of male mice) weighing 25-30g were used for each group. Anxiety induced by chronic restraint stress model. Level of anxiety was studied using the behavioural models Elevated Plus Maze and Light Dark Box test. Phytochemical analysis of the extracts was done and compared. **Statistical Analysis:** Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test at $P = 0.05$. The results were represented as Mean \pm SEM. **Results:** MECI 200mg/kg has shown significant increase in time spent in open arm and number of entries to open arm when compared with control and other group in EPM model. The same dose was found to be significant for time spent in light compartment and number of light compartment entries in LDB model. Preliminary phytochemical screening showed the presence of bioactive component flavonoids the extracts, but saponins were absent in extract. **Conclusion:** The methanolic extract of *Costus Igneus* leaves at a dose of 200mg/kg has showed significant anti-anxiety activity. Means anti-claustrophobic effect.

KEYWORDS: Anxiety, Claustrophobia, Diazepam, Chronic Restraint Stress, Elevated Plus Maze, Light Dark Box, Phytochemical Analysis, *Costus Igneus*.

1. INTRODUCTION

A phobia is an anxiety disorder characterized by a fear that seriously impairs a person's capacity to function in daily life. Avoiding the situation or thing that causes fear is an example of life impairment. The diagnostic and statistical of mental disorders (DSMD) lists numerous categories for anxiety disorders. These consist of panic disorder, social anxiety disorder, separation anxiety disorder, selective mutism, and specific phobia. There are more subcategories of the specific phobia; including animal, natural, environmental, situational, and blood injection harm types. Specific phobias are intense fears of particular situations, people, things, or activities. Avoidance caused by specific triggers such as people, locations, or animals is linked to phobic

disorders. Children often have fears. In kids between the ages of 7 and 11, the frequency of among children aged 7 and 11 years, the prevalence of specific phobias is approximately 2.4% and 0.9%, respectively.

Claustrophobia

A specific sort of phobia known as "claustrophobia" is the fear of enclosed spaces (the term "claustr" suggests closed). Enclosed environments include things like engine rooms, small, lockable rooms, cellars, tunnels, elevators; MRI machines, subway trains, and crowded locations. Individuals who suffer from specific phobias typically exhibit avoidance behaviors towards the specific object or scenario that causes them to feel afraid. In a phobic situation, the dread may manifest as a fear of injury, disgust, or bodily symptoms. While most patients find strategies to cope by avoiding tiny or enclosed spaces, it can be uncomfortable and distressing. Individuals who respond to one of the trigger events may react to all of them. Additionally, recognized as a symptom of claustrophobia is the fear of being confined, such as when waiting in a long line or sitting in a dentist chair. Those who suffer from Claustrophobia are afraid of potential dangers rather than confined areas in and of them. While agoraphobia is more widely understood to be a fear of potential events that could occur in public, including having a panic attack, claustrophobia can also be understood in this way the majority of claustrophobic individual's report feeling subjectively imprisoned. The majority of closed spaces involve some degree of entrapment in addition to movement restrictions. People may be vulnerable, as well as animals, "in confinement scenarios"; placing an animal in a restricted place has the potential to quickly cause experimental neuroses. Suffocation fear affects those who are claustrophobic. Those who have claustrophobia see this extremely strong and expected aspect of the condition as a serious threat.



Fig 1: Claustrophobia from enclosed areas. ^[1]

Breathlessness and the fear of suffocation are closely related symptoms that many persons with claustrophobic tendencies encounter when in confined spaces. Claustrophobia is an anxiety disease that affects between 15% and 37% of the global population. The Latin word *claustrum* means "confined in place," while the Greek Word *Phobic* means fear. Claustrophobia is anxiety caused by a restriction in one's freedom of movement. Claustrophobia is classified by the Diagnostic and Statistical Manual of Mental Disorders as a situational anxiety disorder having two relatively connected components. The two most prevalent phobias associated with claustrophobia are suffocation and imprisonment. Despite this, claustrophobia affects just about 3% of

people worldwide. Between 0.7% and 14.0% of those who have an appointment for magnetic resonance imaging may be affected.

Rather than being fearful of enclosed spaces in general, those with claustrophobia are afraid of what could occur in one. Confined spaces include small, closed rooms, cellars, tunnels, and other similar settings that limit or obstruct movement. Individuals who respond fearfully to any of this Claustrophobia: An Issue with Suggested Remedies It is quite probable that 16 scenarios will all show the same kind of response in comparable circumstances. People's fear of small spaces has been the subject of conjecture; claustrophobic emotions have been compared to those displayed by animals whose escape is restricted to a small area. This is a plausible rationale for the fear of crowds. One way to conceptualize claustrophobia as a residual fear that prevents. The tendency of claustrophobic patients to overestimate their chances of developing claustrophobic symptoms may be explained by "rules-of-thumb.

" The first rule of availability states that those who are claustrophobic tend to recall uncomfortable situations more quickly. Second, those with claustrophobia are more inclined to imagine situations in which they feel claustrophobic, according to the simulation rule. The third factor is representativeness. Individuals who suffer from claustrophobia sometimes overstate the negative effects of being claustrophobic in comparison to the relatively benign situations that statistics show.

MRI (magnetic resonance imaging) scans are a good example of a claustrophobic problem since they are a perfect example of a constrained environment that can cause claustrophobic reactions in people who have never had similar symptoms before. Due to frequent interruptions from claustrophobic reactions, which can result in claustrophobia, this puts medical sciences, which employ MRI equipment frequently, in a time-consuming and financially challenging situation. MRI equipment is used for examinations and research. ^[1]

Costus igneus commonly known as fiery costus, ^[2] Step ladder or Spiral flag or Insulin plant, is native to South and Central America. *Costus igneus* is member of the Rosaceae family, is often referred to as the "insulin plant" in India. [3] This is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant. It is widely grown in gardens as ornamental plant in South India and also run wild in many places. It is used in India to control diabetes, and it is known that diabetic people eat one leaf daily to keep their blood glucose low. Leaves of *C. igneus* were one among the plants known to be effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district, Tamilnadu. In Mexican folk medicine, the aerial part of *C. pictus* is used as an infusion in the treatment of renal disorders.

^[4] The use of medicinal plants as a rich source of therapeutic substances for the treatment and prevention of diseases and ailments has been recognized for millennia and is highly regarded worldwide. ^[5] Both in developing and developed nations, there is a growing need for plant-based medications, health goods, pharmaceuticals, food supplements, cosmetics, etc. Medicinal use astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant and useful in burning sensation, constipation,

leprosy, worm infection, skin diseases, fever, asthma, bronchitis, inflammations and anemia, effective in bringing blood sugar levels under completely under control. [6]

Costus igneus leaf has various medicinal properties like antioxidant, anti-bacterial, anti-fungal, anti-inflammatory, anti-diabetic, gastroprotective, Microbiological Activity, Antiurolithiatic Property, radioprotective activities, Effect on Cancer, Effect of *Costus Igneus* on Learning and Memory, Hepatoprotective Activity and contraceptive effects as revealed from past studies. [7]

2. MATERIALS AND METHODS

2.1. Material

The leaves of *costus igneus* are collected from Shree Kshetra Bhimashankar Ayurved, Mumbai, and Identified by the botanist of Vidyabharti Mahavidyalaya, Amravati.

2.2 Experimental Animals

Experiments are performed in accordance with the committee for the purpose of control and supervision of experimental animal (CPCSEA) guideline after the approval of the experimental protocol by the institutional animal ethical committee (IAEA). Swiss albino mice weighing 24-27gm are obtained from the animal house of Department of pharmacology, Vidyabharati College of pharmacy, Amravati. The entire animal is acclimatized to the animal house prior to use. they are kept in case in animal house with a 12 hrs. light and 12hr dark cycle at temperature (25°C 1°C) with 50-55% of relative humidity. Animals are fed on pellets and tap water ad libitum. The care and handling of animals in accordance with the internationally accepted standard guidelines of use of animals (CPCSEA).

2.3. Drug and chemicals

Standard drug Diazepam 10mg/2ml ampule purchased from Matoshree Medical Stores, Valgaon, Dist. Amravati, Maharashtra.444801.all the treatment (test drug, standard drug and the vehicle) were given orally with the help of oral gavage. Drug and vehicle were given in the form of liquid suspension which was freshly prepared at the time of administration to the animals.

2.4 Preparation of Dose

Diazepam was diluted to 2mg/10 ml with distilled water. two different concentrations (50 mg/kg, 100mg/kg and 200mg/kg) of the MECI were prepared by dissolving the extracts in distilled water. All solutions were freshly prepared at the time of administration to the animals. Extract solution and vehicle (0.9% NaCl) were given orally and standard drug (diazepam) intraperitoneal.

2.5 Treatment Protocol

Sr. No.	No. of Animals	Group	Treatment/Dose	Route of Administration
1.	6	Control group	Normal Saline With (CRS)1ml/Kg	Oral
2.	6	Standard group	Diazepam 1mg/Kg	Oral
3.	6	Test group 1	Extract of <i>Costus Igneus</i> 50mg/kg	Oral
4.	6	Test group 2	Extract of <i>Costus Igneus</i> 100mg/kg	Oral
5.	6	Test group 3	Extract of <i>Costus Igneus</i> 200mg/kg	Oral

2.6 Evaluation of Anxiolytic Activities

2.6.1 Stress Procedure (INDUCING MODEL)



Fig 2: Induction of CRS in mice

Stress procedure for chronic restraint stress (CRS) was employed for 30 days. CRS consisted of 1 hr. session of restraint stress for selected days and the evaluation of behavioral testing for anxiolytic activity was done on 10th day, 20th days and 30th days. From day 1 to day 10 animals were stressed for 7 days except day 8 and 9 and again stressed on day 10 for behavioral testing to determine anxiolytic activity and 10 days after testing same CRS procedure were applied for next 10 days except day 17 and 18 and again behavioral testing with CRS procedure on day 20 were carried out. After 20 days' animals were not stressed from 23 to 29 days and then again stressed on 30 days for behavioral testing. Restraint stress was carried out by placing the animal in a plastic restraint device adjustable in size depending on the animal's weight) for 1hr from the first day (day 1) 30 minutes before the stress procedure drugs were given once per day. Group I received normal saline with stress, Group-II received standard drug (DZP 1 mg/kg), Group-III, Group-IV, Group- V received Test drug (50, 100 and 200 mg/kg respectively) [8]

2.6.2 Behavioral Tests

On day 10, 20 and 30th of experimental procedure the following anti-anxiety models assessed the effect of *Costus Igneus* for evaluation of anxiolytic activity. All assessment was done 30 minutes after CRS.

1. Elevated plus maze model

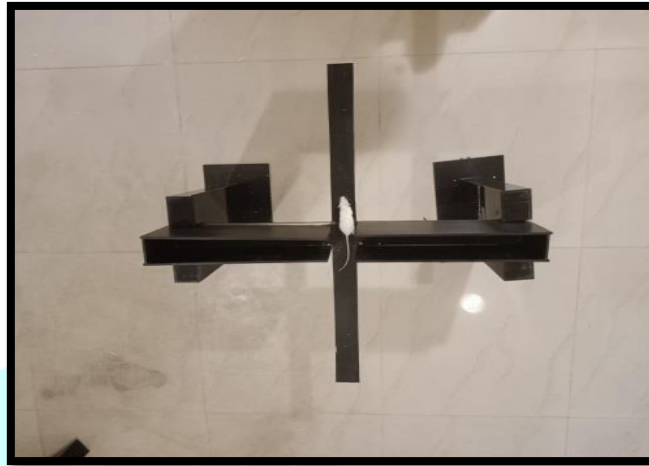


Fig 3: Elevated plus maze apparatus

One of the most often utilized animal models for evaluating the anxiolytic effects of novel medications is the elevated plus-maze model. The elevated plus-maze apparatus consists of two open arms (35 x 5 cm), two closed arms (30 x 5 x 15 cm) that extend from a common central platform (5x5 cm) and the entire maze elevated 50 cm from the floor. The animals were kept to fast overnight before the starting of experiment and were selected randomly on the day of the experiment and grouped into 5 of 6 animals each. Group-1 received normal saline water and served as control. Groups-II received standard drug (Diazepam 1mg/kg). Group-III, Group-IV and Group-V received test extract (50, 100 and 200 mg/kg body weight of mice) respectively.

2. Light-dark box test

The apparatus consisted of two square boxes separated by wooden wall each measuring 50 cm × 50 cm × 50 cm. One box was dark and another box illuminated with 7W/12V bulb. In the center of the wooden wall, there was an opening (6 cm × 6 cm) which can be opened or closed using a transparent plexy glass sliding door from which the animals can move on either side. The mice were placed individually in the center of the light box and observed for the next 5 min. The time spent in both boxes was measured in seconds. The numbers of crossings between the boxes are also noted. The mice were treated with MECI extract, diazepam, and 30 min before being placed in the light box. [9,10]

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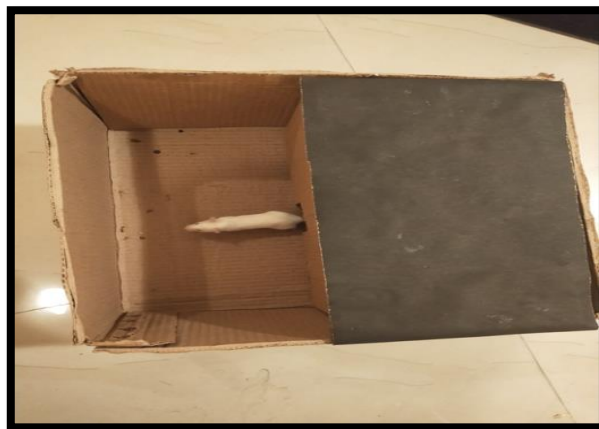


Fig 4: Light-dark box model

2.7. Methodology for Soxhlet Extraction

The leaves of *Costus Igneus* was washed with clean water, air-dried, pulverized using pestle and mortar and sieved with 0.3 mm sieve the powdered material is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent i.e. ethanol, methanol is heated on a water bath. As the solvent boils, its vapours rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off. Leaving the organic substance behind. Afterwards the methanolic extract of *costus igneus* transfer in a clean and dried beaker and are concentrated by placing on a water bath and then cool, keep it in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out. ^[11]



Fig.no.5. Soxhlet Extraction Method.

3. RESULT

3.1 Extraction yield

Weight of dried leaves powder - 900 gm.

Weight of extract obtained -116 gm.

Percent Practical yield = Practical yield + Theoretical yield \times 100

$$= 116 \div 900 \times 100$$

Percent Practical yield = 12.88

3.2 Phytochemical Investigation of Methanolic Extract of *Costus igneus*.

Sr. No.	Phytochemical Analysis	Test Performed	Result
1.	Flavonoids	Alkaline Reagent Test	(+)
2.	Alkaloids	Mayer's Test	(+)
3.	Tannins	Ferric Chloride Test	(-)
4.	Saponins	Foam Test	(+)
5.	Glycosides	Legal's Test	(+)
6.	Steroids	Lieberman's Test	(+)
7.	Carbohydrate	Molisch's Test	(+)
8.	Proteins	Million's Test	(+)
9.	Phenols	Ferric Sulphate Test	(-)
10.	Triterpenoid	Horizon Test	(+)
11.	Quinones	Sulfuric acid test	(+)

Where indicates (-) absent, (+) indicates Present

Phytochemical testing carried out find out the secondary metabolite because secondary metabolic possess biological activity. The data of above table reveals that alkaloids, flavonoids, carbohydrates, glycoside, tannins, saponins, steroids, proteins, phenols, Triterpenoid, Quinones, were present in methanolic extract of *Costus Igneus* leaves.

3.3. Acute Toxicity study

Table: Represent Acute Toxicity in Mice According to Dose Concentration

Sr. No	Dose (mg/kg)	Observation
1.	50	No death
2.	100	No death
3.	200	No death
4.	300	No death
5.	2000	No death
6.	5000	No death

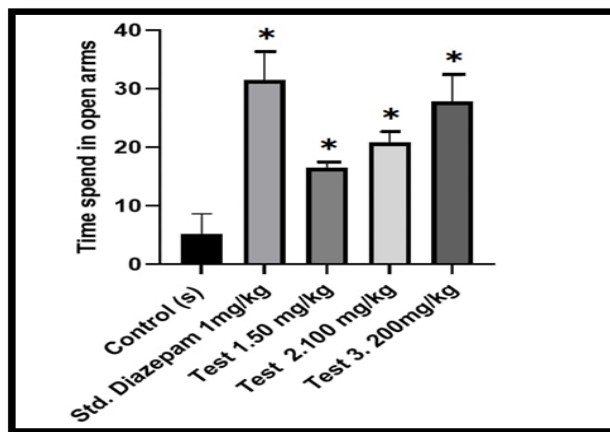
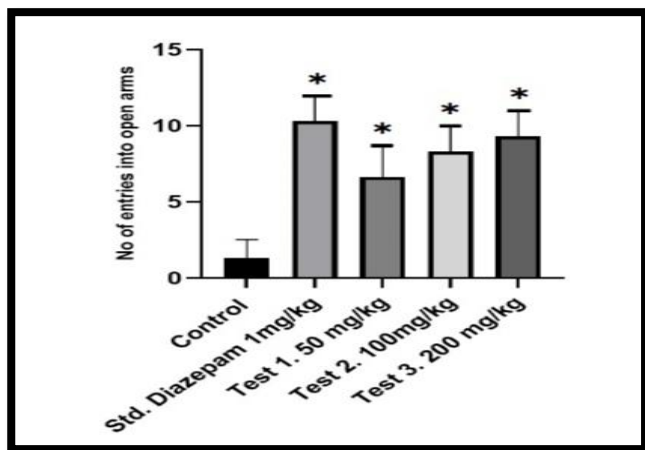
According to oral toxicity studies (OECD-425 guideline) fruit extract of *Costus igneus* showed no toxicity up to doses of 1000mg/kg and the dose above 1000 produce mild degree of sedation & imbalance in locomotor activity but, no death observed up to dose of 5000 mg/kg. Therefore, the LD50 of methanolic extract of fruit of *Costus igneus* was found to beyond 5000 mg/kg. So, for present experimental studies the 1/10th as a low dose & 1/5th as a high dose of was selected i.e. 50mg/kg, 100mg/kg and 200mg/kg.

3.4 Pharmacological Study

A. The effect of MECI on Elevated plus maze test in mice (open arms)

Sr. No.	Treatment	Dose	%Time spends in open arms (S)	% No Entry into open arms (S)
1.	Control	1 ml/kg	5.167±1.424	1.333±0.4944
2.	Standard	1mg/kg	31.50±1.996*	10.33±0.6667*
3.	<i>Costus Igneus</i> extract	50 mg/kg	16.50±0.4282*	6.667±0.8433*
4.	<i>Costus Igneus</i> extract	100 mg/kg	20.83±0.7491*	8.333±0.6667*
5.	<i>Costus Igneus</i> extract	200 mg/kg	27.83±1.815*	9.333±0.6667*

All data are expressed as mean ± SEM (n = 6). One-way Anova followed by Dunnett's multiple comparisons. Values are statistically Significant *P <0.0001 for parameter as compared with Positive control in open arm.



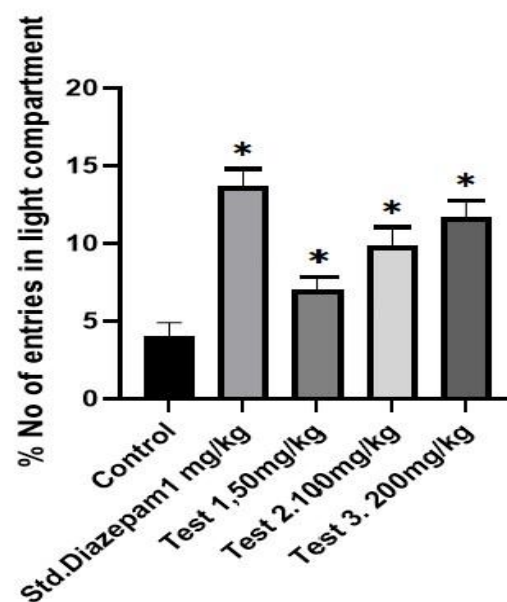
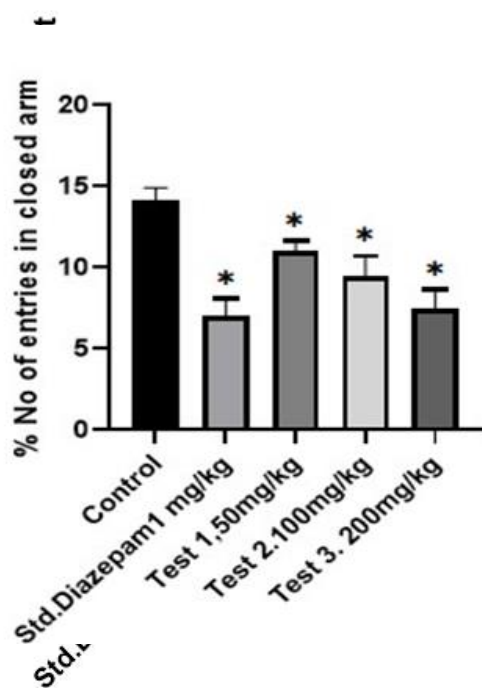
Graph 1. Number of entries in open arms.

Graph 2. % Time spent in open arms.

The effect of MECI on Elevated plus maze test in mice (closed arms)

Sr. No.	Treatment	Dose	%Time spends in closed arms (S)	% No Entry into closed arms (S)
1.	Control	1 ml/kg	21.50±1.088	14.17±0.3037
2.	Standard	1mg/kg	9.833±0.5426	7.000±0.4472
3.	<i>Costus Igneus</i> extract	50 mg/kg	15.17±0.8333	11.00±0.2582
4.	<i>Costus Igneus</i> extract	100 mg/kg	12.50±0.5627	9.500±0.5000
5.	<i>Costus Igneus</i> extract	200 mg/kg	8.833±0.5426	7.500±0.5000

All data are expressed as mean ± SEM (n = 6). One-way Anova followed by Dunnett's multiple comparisons. Values are statistically Significant *P <0.0001 for parameter as compared with Positive control in closed arm.



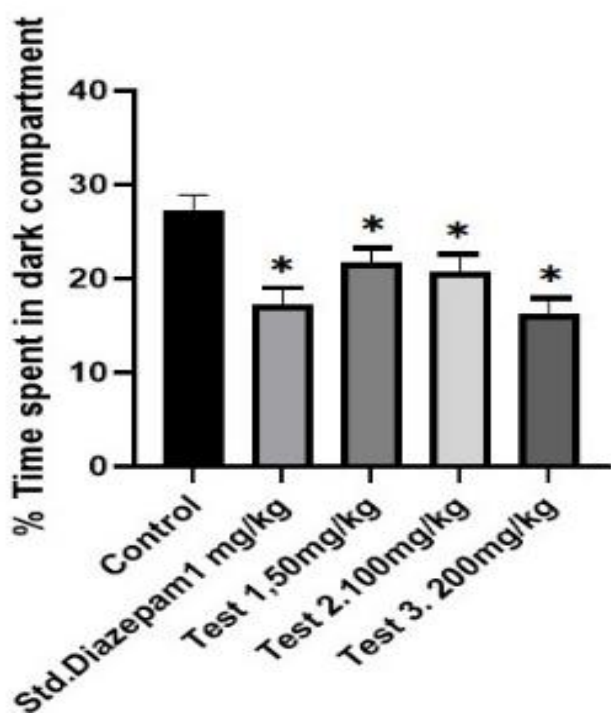
Graph 3. Number of entries in closed arms.

Graph 4. % Time spent in closed arms.

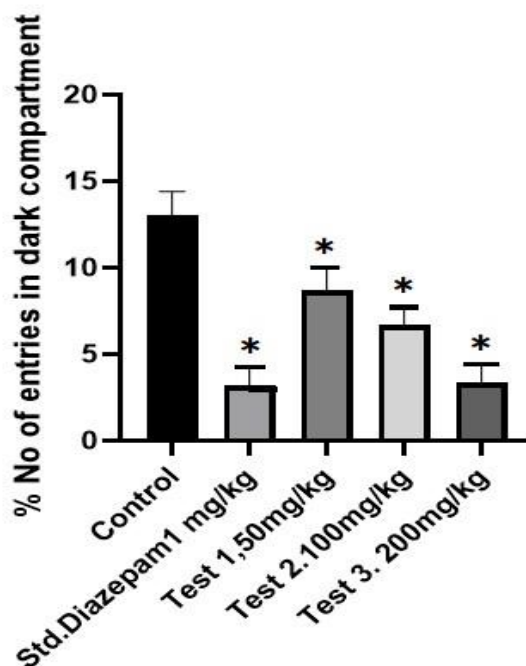
B. The effect of MECI on light-dark box test in mice. (Light compartment)

Sr. No	Treatment	Dose	% Time spent in light compartment	% No entries in light compartment
1	Control	1 ml/kg	5.000±1.155	4.000±0.3651
2	Standard	1mg/kg	32.67±1.145*	13.67±0.4216
3	<i>Costus Igneus</i> extract	50 mg/kg	18.50±1.607*	7.000±0.3651
4	<i>Costus Igneus</i> extract	100 mg/kg	25.50±2.029*	9.833±0.4773
5	<i>Costus Igneus</i> extract	200 mg/kg	30.17±1.108*	11.67±0.4216

All data are expressed as mean ± SEM (n = 6). One-way Anova followed by Dunnett's multiple comparisons. Values are statistically Significant *P <0.0001 for parameter as compared with Positive control in light compartment.



Graph 5. Time spent in light compartment.



Graph 6. No of entries in light compartment

The effect of MECI on light-dark box test in mice. (Dark compartment)

Sr. No	Treatment	Dose	% Time spent in dark compartment	%Number entries in dark compartment
1	Control	1 ml/kg	27.17±0.7032	13.00±0.5774
2	Standard	1mg/kg	17.17±7032	3.167±0.4773
3	<i>Costus Igneus</i> extract	50 mg/kg	21.67±0.7601	8.667±0.5578
4	<i>Costus Igneus</i> extract	100 mg/kg	20.17±0.7032	6.667±0.4216
5	<i>Costus Igneus</i> extract	200 mg/kg	16.17±0.7032	3.333±0.4216

All data are expressed as mean ± SEM (n = 6). One-way Anova followed by Dunnett's multiple comparisons. Values are statistically Significant *P <0.0001 for parameter as compared with Positive control in dark compartment.

4.DISCUSSION

Posttraumatic stress disorder (PTSD), a prevalent psychiatric disorder, is caused by exposure to a traumatic event. Individuals diagnosed with PTSD not only experiencesignificant impairments but also have higher rates of physical morbidity and mortality requiring chronic drug treatment. The problems associated with available current therapy with synthetic chemicals are poor response, remission and severe undesirable side effects. Hence, the search for novel drug continues and medicinal plant source became an importantsource for new drug development for this CNS ailment.

Since the last 40 to 50 years, benzodiazepines have been widely used to treat a variety of anxiety disorders. However, because of their unfavorable side effects, alternative treatment options, particularly in primary care settings, are being investigated. Finding novel treatments for certain diseases can often be found by looking to medicinal plants. Herbal psychopharmacology research has revealed a number of interesting drugs that may be useful in the treatment of stress and anxiety disorders. Natural anxiolytic drugs have been studied in the hunt for a more targeted, potentially cost-free therapy. *Costus Igneus* is a medicinal herb with several therapeutic use. *Costus Igneus* is an herb, indigenous ingredients of ayurvedic medicine. It is mainly used for its memory enhancing property, anticonvulsant, antianxiety, antidiuretic, and antiulcer properties.

The present study was aimed at evaluating the antianxiety property of *Costus Igneus* in comparison with control and standard drugs using animal models. The preliminary phytochemical studies revealed the presence of alkaloids, saponins, flavonoids, tannins, glycosides, sugars, steroids, terpenoids and phenolic compounds in *Costus Igneus*. Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids and phenols possess activity against many CNS disorders. The present study revealed that CRS exposure produced representative anxiety-like behavior, as evidenced by the fact that CRS exposed mice/animals significantly decreased their percentage of time spent and number of entries into the open arms, increased the time spent in the dark section and reduced the duration of stay in the light section and comparison with control group and other group with CRS. It is found that the same condition (i.e. anxiety) was produced in mice even after 10 days of rest period without CRS procedure in the mice. *Costus Igneus* treatment for 30 days daily prior to CRS reduced anxiety-like behavior.

The EPM is currently one of the most widely used models of animal anxiety and has been validated for use with both the sexes of mice. Therefore, this test was chosen to investigate the anxiolytic potential of the aqueous extract of coriander seed. The indices of anxiety in this test, no of entries in open arm and closed arm. The sensitivity to agents acts via the gamma-aminobutyric acid receptor complex, justifying the use of diazepam as a positive control in this study. Diazepam increased the entries of open arm and the time spent in the open arms confirming its anxiolytic effects. The results of the present study showed that the administration of *Costus Igneus* extract by oral route in mice produced a significant anxiolytic effect in two well-consolidated anxiety animal models. Elevated plus maze, in this animal model, the anxiety-related behaviors in mice were significantly decreased indicates that anxiety in mice was relieved

after treatment with extract. In the present study, administration of *Costus Igneus* extract has produced an increase in time spent open arm by mice in the illuminated side indicating an anxiolytic effect of plant which was confirmed by the increase in time spent in the EPM.

The light/dark test is based on mice' intrinsic aversion to brightly lighted places as well as their spontaneous exploratory behavior in response to moderate stressors such as unfamiliar environment and light. As a result, this test may be beneficial for predicting anxiolytic or anxiogenic activity in mice. Transitions have been claimed to be an indication of activity- exploration due to habituation over time, and time spent in each compartment to be a reflection of aversion. In this study, *C. Igneus* (50-200 mg/kg) significantly increased the latency of entry into the dark compartment and time spent in the light box. Diazepam also significantly increased the time spent in the light box and reduced the duration of stay in the dark section. The effects seen with *C. Igneus* during the light/dark exploration test point to the presence of anxiolytic activity, with peak response being evoked at dose of 200 mg/kg. The effect of 200 mg/kg *Costus Igneus* on the EPM test was almost equivalent to that of 1mg/kg diazepam. These observations clearly indicate that coriander seed exerts an anxiolytic activity. In this study, the anxiolytic activity of the *Costus Igneus* extract occurred at a dose of 50, 100, and 200 mg/kg in mice.

5. CONCLUSION

In conclusion, the methanolic extract of *Costus Igneus* Leaves considerably reduced anxiety disorder caused by the Elevated plus maze method and the Light and dark box. According to the findings of this investigation, the leaves of *Costus Igneus* have anti-anxiety properties. The results obtained in this study suggest that the methanolic extract of *Costus Igneus* leaves possesses anxiolytic activity due to one or a combination of phytoconstituents identified in the extract, probably due to the presence of phytoconstituents like flavonoids and phenolic compounds. However, further studies are needed to isolate the bioactive compound(s) and demonstrate the precise molecular mechanisms responsible for the pharmacological actions of methanolic extract of *Costus Igneus*.

The present results indicated that CRS followed by repeated reminders should be a reliable long-lasting animal model for PTSD. The plants *Costus Igneus* have potential anti PTSD activity and serotonin and GABA may play a role in the anti PTSD effects of *Costus Igneus*. Flavonoids and saponins present in both the plants must be having important role in results obtained in present investigation. *Costus Igneus* may provide right pharmacotherapy and alternative treatment approach to work as adjuvant phytochemical substances. In future, studies are required to ascertain mechanism of action for the said activity.

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