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Research

Evaluation of anti-oxidant and anxiolytic effects of ethanolic root extract of Cymbopogon flexousus in swiss albino mice

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Abstract

The study aimed to evaluate the antioxidant and anxiolytic potential of the ethanolic root extract of Cymbopogon flexuosus (lemongrass) in Swiss albino mice. Antioxidant properties were assessed using in vitro assays, including DPPH radical scavenging and reducing power activities, which demonstrated significant free radical neutralization by the extract. For the anxiolytic evaluation, behavioral studies such as the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark Box Test were conducted on Swiss albino mice after oral administration of varying doses of the extract (100, 200, and 400 mg/kg). The results indicated a dose-dependent reduction in anxiety-like behaviors, comparable to standard anxiolytic drug diazepam. Furthermore, the extract exhibited no observable toxic effects in acute toxicity studies, confirming its safety profile.

The presence of bioactive compounds such as flavonoids, phenols, and terpenoids in the extract likely contributed to its antioxidant and anxiolytic activities. These findings suggest that Cymbopogon flexuosus root extract has the potential to mitigate oxidative stress and anxiety, providing a natural therapeutic alternative for managing anxiety-related disorders. Further investigations, including mechanistic studies and clinical trials, are recommended to validate its efficacy and pharmacological properties.

Keywords: Cymbopogon flexuosus, antioxidant activity, anxiolytic effects, ethanolic root extract,

INTRODUCTION

In the face of demanding situations such as a pandemic, people are forced to face certain challenges that can be stressful and cause strong emotional changes in adults as well as children. The necessary precautions like social distancing can make an individual feel isolated and lonely which increases stress and anxiety[1]. Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive and behavioral components, associated with significant disability (including educational and occupational) which has a negative impact on the quality of life[2].

Anxiety disorders include Generalized anxiety disorder - GAD; panic disorder, social anxiety disorder and post-traumatic stress disorder. In these conditions, individuals present disturbances in their physiology, such as tachycardia,

dyspnea, muscle pain and behavioral changes, such as recurring negative thoughts and worries[4]. Anxiety disorder is increasingly recognized as a highly

prevalent and chronic disorder with onset during the teenage years, with an incidence of 18.1% and a lifetime prevalence of 28.8%. Individuals are spending lot of money to rid themselves of anxiety.

The neurotransmitters synthesized by the brain such as gamma amino butyric acid, serotonin, norepinephrine, acetylcholine, adrenaline, and dopamine play special roles in the neurophysiology of anxiety. Anxiety disorders have centre on the Gamma amino butyric acid mechanisms. Anxiety symptoms may be due to disrupted modulation within the central nervous system. Many believe that low serotonin system activity and elevated noradrenergic system activity are responsible for its development[3]. Benzodiazepines are now the most commonlyused drugs. However, the regular uses of the above synthetic drugs results in unpleasant side effects such as drug dependence, tolerance, rebound insomnia, amnesia, psychomotor impairment and potentiating other central depressant drugs[5].

Some plants have shown anxiolytic effects like *Abies pindrow Royle (Pinaceae), Acorus calamus Linn.(Araceae),Cannabis sativa Linn.(Cannabaceae),Clitoria ternatea Linn (Papilionaceae), Glycyrrhiza glabra Linn. (Leguminosae)*[6] etc, due to the presence of flavonoids one such search for plant with anti-anxiety and antioxidant effect, *Cymbopogon flexuosus* was chosen for the study on the basis of its traditional use. *Cymbopogon* is a genus of aromatic perennial grasses in the family Poaceae, native to warm regions in Asia, Africa, and Australia. These grasses are commonly known as lemon grasses due to their lemony scent and flavor. Lemongrass is widely cultivated for culinary, medicinal, and aromatic purposes[7].Overall, *Cymbopogon* species, particularly lemongrass, are valued for their culinary, medicinal, and aromatic qualities, making them versatile and popular plants around the world. *Cymbopogon flexuosu*s is commonly known as Cochin grass, East-Indian lemon grass or Malabar grass. In recent years medicinal and pharmacological significance of lemongrass essential oil and its major constituent citral has been rapidly increased.A number of studies have revealed many useful bioactivities such as antimicrobial, allelopathic, anthelmintic, anti-inflammatory, anticancer, antioxidant, insect and mosquito repellent of lemon grass extract, oil, citral and citral derived compounds[8]. The current study was carried out in an attempt to investigate antioxidant and anxiolytic effect of ethanolic root extract of *Cymbopogon flexuosus* in swiss albino mice using elevated plus maze (EPM) and spontaneous motor activity tests.

AIM AND OBJECTIVES

The preliminary aim of this study is to evaluate the antioxidant and anxiolytic effect of ethanolic root extract of *Cymbopogon flexousus* root in Swiss albino mice. Procurement and authentication of *Cymbopogon flexousus* root. Successive soxhlet extraction of the powdered roots of *Cymbopogon flexousus* using ethanol as a solvent. Phytochemical screening of the extraction obtained from the source of *Cymbopogon flexousus* root. Evaluation of invitro antioxidant activity in *Cymbopogon flexousus* root extract by hydroxyl radical scavenging activity. Evaluation of acute oral toxicity test in mice using *Cymbopogon flexousus* root extract.Evaluation of invivo behavioural assessment in *Cymbopogon flexousus* root extract using Elevated Plus maze method, Open field apparatus and Staircase model.

MATERIALS AND METHODS

Procurement of *Cymbopogon flexousus* roots

The Roots of *Cymbopogon flexousus* were collected from Mahadeva malai,Vellore and were authenticated by Dr. S. Sankaranarayanan, Head Of the Department, Medical Botany and Pharmacognosy, Govt. Siddha Medical College, Chennai – 600106.

Fig 1: *Cymbopogon flexousus*

Preparation of extracts

- Extraction is the preliminary step involved in the phytochemical studies.

- It is the separation of medicinally active portions of plant using selective solvents through standard procedures.
- The root was cleaned, washed and air-dried at 25°C for two weeks.
- Roots was pulverized to a coarse powder by grinding in mixer and stored in an airtight container.

Fig 2: Dried root

Soxhlet Extraction Method

- A weighed quantity of the powder (250g) was passed through sieve number 40 and subjected to hot solvent extraction in a soxhlet apparatus using ethanol at a temperature range of 60–80°C, respectively
- Before and after every extraction the powder bed was completely dried and weighed
- The filtrate were evaporated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator
- The percentage yield of ethanolic extract was calculated using the following formula

$$\textbf{Percentage Yield (\%)} = \frac{\text{Weight of the Plant Extract}}{\text{Weight of the Plant material}} \times 100$$

Phytochemical analysis[44]

Methodology for chemical analysis

Test for Alkaloids

a) Mayer's Test:
0.5ml of sample was treated with 1ml of Mayer's reagent (Potassium mercuric iodide solution] presence of alkaloids were produced cream colour precipitate.

b) Dragendroff's Test:
1ml of the sample was treated with 1ml of Dragendroff's reagent (Potassium bismuth iodide solution), it produced reddish brown precipitate.

c) Wagner's Test:
0.5ml of the sample was treated with 0.5ml of Wagner's reagent (Solution of iodine in potassium iodide), red dish brown were precipitated.

d) Hager's Test:
1ml of the sample was treated with 0.5ml of Hager's reagent (Saturated solution of Picric acid), were produced yellow colour precipitate.

Test for Carbohydrate

a) Molisch's Test:
To the 0.5ml of sample, few drops of alcoholic alpha-napthol was added and 0.2ml of concentrated sulfuric acid added slowly through the sides of the test tube, a purple to violet colour ring was appeared at the junction.

b) Benedict' Test:
1ml of sample was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath, it produced reddish brown precipitate.

c) Fehling's Test:
1ml of sample was treated with few drops of Fehling's solution A and B heated for few minutes, brick red precipitate were formed.

d) Barfoed's Test:
0.5ml of sample was treated few drops of Barfoed's reagent and heated for few minutes, red precipitate were formed.

Test for Glycosides

a) Legal's Test:
0.5ml of sample was treated with 0.3ml of pyridine and alkaline sodium nitroprusside solution, presence of glycosides were produced blood red colour

b) Baljet Test:
0.5ml of sample was treated with 0.3ml of sodium picrate, presence of glycosides were produced yellow to orange colour.

c) Borntrager Test:
1ml of sample was treated with 0.5ml of dilute sulphuric acid and boiled for few minutes, then it was filtered. The filtrate was treated with ether or chloroform, to the organic layer few drops ammonia solution were added, presence of glycosides were produced pink or violet colour

Test for Cardiac Glycosides

Keller killani Test [Test for Deoxy sugars]: 0.5ml sample was treated with 0.4ml of glacial acetic acid containing at race amount of ferric chloride. It were transferred to a small test tube and then 0.5ml of concentrated sulphuric acid was carefully added by the side of the test tube, blue colour were appeared in the acetic acid layer.

Test for Saponin Foam frothTest

1ml of sample was treated with 10ml of water and boiled for few mins the nit were filtered. The filtrate was shaken well and noted for the stable froth.

Test for Sterols

a) Salkowski Test:
To 0.5ml of sample about 0.3ml of chloroform with few drops of concentrated sulphuric acid was added, shaken well and allowed to stand for some time, red colour were appeared at the lower layer indicating the presence of steroids, formation of yellow coloured lower layer was indicate the presence of Triterpenoids.

b) Libermann Burchard Test:
0.5ml of sample was treated with 0.3ml of chloroform, small amount of acetic anhydride and concentrated sulphuric acid. The colour were changed from red to bluish green.

Test for phenolic compounds

Ferric chloride Test:
To 1ml of sample, 1ml of water was added and boiled for few minutes the nit were filtered. The filtrate were with ferric chloride solution, bluish black colour was produced.

Test for Tannins

a) Gelatin Test:
To 1ml of sample, 0.5ml of 1% gelatin and 10% sodium chloride was added, a white precipitate were formed.

b) Lead acetate Test:
1ml of sample was treated with 0.5ml of lead acetate solution, formation of white precipitate were indicate the presence of tannins.

c) Potassium dichromate Test:
0.5ml of sample was treated with 0.5ml of potassium dichromate solution, presence of tannins were produced yellow precipitate.

d) Potassium ferric cyanide Test:
1ml of sample was treated with 0.5ml potassium ferric cyanide solution and few drops of ammonia solution was added, red colour were formed.

Test for Flavonoids

a) Shinoda Test (Magnesium hydrochloride reduction test):
To 1ml of sample, few fragments of magnesium ribbon was added, then few drops of concentrated hydrochloric acid was added, presence of flavonoids were produced magenta colour.

b) Alkaline reagent Test:
To 2ml of sample, 1ml of sodium hydroxide was added, presence of flavonoids were produced yellow colour.

c) Mineral acid Test:
To 1ml of sample,few drops of concentrated sulphuric acid was added, presence of flavonoids were produced orange colour.

d) Boric acid Test:
To 0.5ml of sample, few drops of boric acid was added, presence of flavonoids were produced yellow colour.

Test for Proteins and Amino acids

a) Millon's Test:

To 1ml of sample, 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added, white precipitate was appeared, which were turned into red upon gentle heating.

b) Ninhydrin Test:

To 1ml of sample, 0.5ml of 0.2% solution of Ninhydrin (Indane1,2, 3 trione hydrate) was added and boiled in a water bath, appearance of violet colour were indicate the presence of amino acids and proteins.

c) Biuret Test:

To 1ml of sample, 1ml of 10% sodium hydroxide, 1% copper sulphate was added, violet colour were appeard.

a) Xantho protein Test:

To 0.5ml of sample, few drops of concentrate nitric acid was added, orange colour were appeared.

b) Tannic acid Test:

To 1ml of sample, 0.5ml of tannic acid solution was added, white colour were appeared

Test for Fats and Fixed oils

a) Stain Test:

The small quantity of sample was pressed between two filter papers ; the stain on a filter paper were indicate the presence of fixed oils

b) Saponification Test:

To 1ml *sample*, 0.5ml of 0.5N alcoholic potassium hydroxide and phenolphthalein was added and then it was heated for 1-2 hours, it produces disappearance of pink colour indicated the presence of fixed oils

ANTIOXIDANT ACTIVITY

Antioxidant is any substance that, when present at low concentration compared with those of an oxidizing substrate, significantly prevents or delays the oxidation of that substrate. Neutralizes free radicals and prevents cell damage which may lead to cancer. Any nutrient or chemicals that react with and neutralize free radicals to prevent oxidative damage to cells (e.g., oxidation of lipid membranes, DNA damage). A good biological antioxidant is able to accept an unpaired electron to form a free radical intermediate with a relatively long half-life in the normal biological environment. There is a complex intracellular enzymatic antioxidant system, including superoxide dismutase, catalase and enzyme of the glutathione peroxidase family. Non enzymatic antioxidant includes arginine, vitamins A, C, E, B carotene, glutathione, polyphenols and minerals (selenium and zinc) [49].

IN-VITRO ANTIOXIDANT ACTIVITY

Oxidative stress is an imbalance between cellular production of reactive oxygen species and the counteracting antioxidant mechanisms. The brain with its high oxygen consumption and a lipid-rich environment is considered highly susceptible to oxidative stress or redox imbalances. Therefore, the fact that oxidative stress is implicated in several mental disorders including depression, anxiety disorders, schizophrenia and bipolar disorder, is not surprising[50]. Some of the studies suggest that oxidative stress causes anxiety-related behaviors but do not explain the neurobiological pathways underlying the effect of oxidative stress on anxiety symptoms. Some articles showed the use of antioxidant in the prevention or reduction of high anxiety[51]. This may be due to the reason that GABA receptors activities are enhanced by antioxidants[52-55]. The benzodiazepines act by binding to GABA receptor which is used as anti anxiety drug predominantly by patients. Anti-oxidants like poly phenols and flavonoids are therefore very helpful in reduction of stress factors and free radical formation which inhibits GABA binding activity. It has become evident that flavonoids are able to exert enhancement of GABA binding activity even at low concentration[56]. Based on this assumption between the anti-oxidant and the anxiety disorder, the antioxidant activity was conducted for ethanolic extracts of *Cymbopogon flexousus* root using the Hydroxyl radical scavenging activity. In vitro antioxidant activity was done for the ethanolic extracts of *Cymbopogon flexousus* using Hydroxyl radicals scavenging activity.

INVIVO STUDY

Experimental animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirement of national guidelines of CPCSEA/IAEC approval no. 1917/GO/ReBi/S/16/CPCSEA, 20/09/2021 and for the protocol approval no. 04/AEL/IAEC/MMC, Date: 14-08-2024, 27 Swiss albino mice (Either sex) used for this study were procured from Animal house, Madras Medical College, Chennai, India.

Maintenance Of Animals

Quarantine and acclimatization

Quarantine is the separation of newly received animals from those already in the facility until the health status of the newly received animals have been determined. The newly procured albino rats was quarantined for a period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop the physiological and nutritional stabilization before their use.

Housing

The animals was housed in the well-ventilated animal house maintained at a constant temperature (22± 2c) and relative humidity of 55-65%. The animals was housed in spacious polypropylene cages and paddy husk were utilized as bedding material. The bedding material were changed frequently.

Diet and water

The animals was maintained on standard pellet diet and purified water. The animals were provided with food and water ad libitum except during fasting.

Drug administration

Drug was administered by oral gavage using feeding tube fixed to a syringe needle to administer the required quantity

Animal identification

- All animal cages used in the study were given a proper identification.
- Each animal in the cage was marked on head or body or tail with the permanent marker for their appropriate identification.

Acute Oral Toxicity

The acute oral toxicity study of *Cymbopogon flexousus* root extract was performed by giving a single dose (2000mg/Kg)because already the other parts of the plant were used for various experiments. The animals was observed for 14 days. The procedure was performed according to OECD guidelines 423.

Procedure

- According to OECD guidelines 423 the ethanolic extract was administered in a single dose by using a oral gavage for three animals.
- Animals was fasted prior to dosing . Following the period of fasting, the animals were weighed and the extract was administered.
-

After the substance has been administered, food was withheld for 1-2 hours in mice.

- Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days
- It was determined by the toxic reactions, time of onset and length of recovery period.
- All observations were systematically recorded with individual records being maintained for each animal.

INVIVO ANTI-ANXIETY ACTIVITY

Experimental study design

Animals was acclimatized for 1week and then randomly assigned into 4 groups, each bearing six animals

Table 1: Experimental study Design

GROUP NO	GROUP NAME	TREATMENT &ROUTE OF ADMINISTRATION	DURATION	NO.OFANIMALS
Group 1	Control	Normal Saline 10ml/kg/day,1-10th day, orally	10 days	6
Group 2	Standard drug	Diazepam 2.5mg/kg/day,1-10th, orally	10 days	6
Group 3	C.F extract	200mg/kg/day,1-10th, orally	10 days	6
Group 4	C.Fextract	400mg/kg/day,1-10th, orally	10 days	6

ANTI-ANXIETY METHODS

Elevated Plus Maze[46, 56-61]

- The plus maze apparatus consisted of two open arms, measuring 16×5cm,and two closed arms, measuring 16 × 5 × 12 cm, connected to a central platform
  (5 ×5cm).
- The maze elevated to a height of 25 cm above the floor.
- Each mice was placed individually at the center of elevated plus maze with its head facing toward an open arm and observed for 5min to record the number of entries into open arm, closed arm and time spent in each arm.

- In EPM test ,the percent time spent on the open arms was determined as follows:

Staircase test[74]

The staircase test was carried out according to the method of Simiand et al,. It consisted of placing an experimentally naive mouse in an enclosed staircase with five steps (2.5 × 10 × 7.5 cm). The apparatus having 45 cm in length, with one end 12 cm and the other 25 cm in height. Each mouse was placed individually on the floor of the box with its back to the staircase, and then the locomotory behavior was noted. The numbers of steps climbed and rearing were counted for 3 min. A step was considered to be climbed only if the mouse had placed all four paws on the step. Rearing was recorded when the mouse rose on its hind legs either on the step or against the wall to sniff the air.

The number of steps descended was not counted. In some experiments, locomotor patterns of mice were analyzed. The number of climbing to each step of the staircase was counted**.**

Open field model[47,62]

- Each mice was placed in a open-field apparatus (45×45×40cm), made of wooden floor and glass sides.
- The floor carved into 16 equal sized squares (15×15cm). An hour before dropping the individual mice in one of the corner of the box (i.e. 60 min prior), the different groups were administered with respective treatments (Normal saline, diazepam 2.5mg/kg, extract doses of 200mg/kg, 400mg/kg) and then locomotion (number of both central and peripheral crossings) were recorded

Statisticalanalysis[63]

The results were expressed as mean ± SEM. The data was statistically analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison test using Graphic padprism software version 8.0.2. One way ANOVA was used to correlate the statistical difference between the variables. P values such as $P<0.05$, $P< 0.01$ and $P<0.001$ were considered statistically significant.

RESULTS

EXTRACTION

The Percentage yield of the ethanolic Extract of the *Cymbopogon flexousus* obtained through Soxhlet Extraction was calculated by using the formula,

$$\textbf{Percentage Yield (\%)} = \frac{\text{Weight of the Plant Extract}}{\text{Weight of the Plant}} \times 100$$

Table 2: Percentage yield

Name of the Extract	% of Yield
Ethanolic extract of *Cymbopogon flexousus* root	4.5 w/w

PHYTOCHEMICAL ANALYSIS

- The *Cymbopogon flexousus* root extract was subjected to Phytochemical analysis for identification of Phytoconstituents.
- The obtained phytochemicals results were illustrated in the Table No. 3

Table 3: Phytochemical analysis

S. NO	PHYTOCHEMICAL	CHEMICAL TESTS	RESULT
1.	Test for Carbohydrates	Molisch's Test Benedict's Test Fehling's Test	+ + +
2.	Test for Proteins and Amino acids	Millon's Test Biuret Test Xanthoprotein Test	+ + +
3.	Test for Alkaloids	Mayer's Test Dragendorff's Test Hager's Test	+ + +

4.	Test for Glycosides	Legal's Test	+ + +
5.	Test for Cardiac Glycosides	Kellar-Killani Test	+ + +
6.	Test for Phenolic compounds	Ferric Chloride Test	+ + +
7.	Test for Flavonoids	Alkaline reagent Test	+ + +
8.	Test for Saponins	Foam froth Test	+ + +
9	Test for Tannins	Gelatin Test Lead acetate Test	+ + +
10	Test for Terpenoids	Noller's Test	- - -

(+) presence,(-) absence

IN VITRO ANTIOXIDANT ACTIVITY

The invitro antioxidant activity of ethanolic root extract of *Cymbopogon flexousus* werestudied using Hydroxyl radical-scavenging activity (HRSA)

Hydroxyl radical– scavenging activity (HRSA)[45]

Table 4: Hydroxyl radical scavenging activity of ascorbic acid, ethanolic extracts

S.NO	Concentration(µg/ml)	% of scavenging of Hydroxyl radicals	
		Ascorbic acid	Ethanolic Extract
1.	200	49.528	48.58
2.	400	69.974	57.33
3.	600	77.857	58.14
4.	800	93.361	62.58
5.	1000	96.266	72.27
	IC50 (µg/ml)	213	239

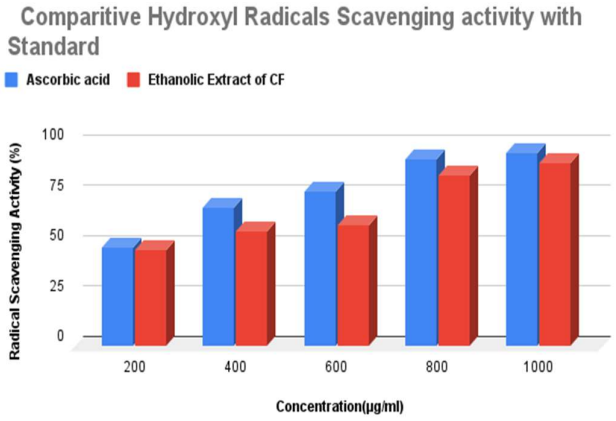

Fig.3. Comparative Hydroxyl radicals scavenging activity with Standard

ACUTE ORAL TOXICITY (OECD guidelines 423)

The acute oral toxicity were observed over a period of 14 days, following the procedure given in OECD guidelines 423. The results obtained were then presented and expressed in Table no.5

Table 5: Acute Oral Toxicity study

Days Observation	30 ms	1 hr	2 hr	4 hr	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	8 Day	9 Day	10 Day	11 Day	12 Day	13 Day	14 Day
Alertness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aggressiveness	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Touch response	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gripping	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Motor co ordination	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drowsiness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Food consumption	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(+) indicates presence of parameter, (-) indicates absence of active parameter, (N) indicates Normal

INVIVO STUDIES

I ANTIANXIETY ACTIVITY IN MICE USING ELEVATED PLUS MAZE METHOD

The result with Ethanolic extracts of *Cymbopogon flexousus*root in the dose of 200mg/kg and 400mg/kg was significant when compared to control as shown in table 6

Table 6: Effect of Ethanolic extracts of *Cymbopogon flexousus* root in elevated plus maze

S.NO	Treatment group	% open arm entries	%time spent in open arm
1	Control	12.43±1.14	7.6±0.67
2	Standard Diazepam 2.5mg/kg/orally	66.81±0.58**	69.93±0.41**
3	CF in the dose of 200mg/kg/orally	40.55±0.52**	46.73±0.63**
4	CF in the dose of 400mg/kg/orally	50.28±0.9***	56.50±0.39**

All values are expressed as Mean± SEM(n=6). One way ANOVA followed by Dunnet's test. *** P<0.001 when compared to control.

II SPONTANEOUS LOCOMOTOR ACTIVITY

Spontaneous locomotor activity is considered as an index of alertness and can be helpful to confirm the general depressive activity of any drug. The decrease in motor activity gives an indication of the level of depression of CNS[67]. Since, an anxiolytic also produces sedation and hypnosis; these activities were evaluated with spontaneous locomotor activity in Open field test and Staircase apparatus.

1. Open field method

The anxiolytic activity obtained for the extracts was statistically significant and the results were shown in table 7.

Table 7: Locomotor activity of mice in open field

S.No	Treatment Group	Before Drug Administration	After 30 Min	After 60 Min
1.	Control	165.83±1.5	170±1.28	164.7±1.26
2	Standard Diazepam 2.5mg/kg	159±1.16	99.33±2.24 ***	72.67±1.22***
3.	CF in the dose of 200mg/kg	153.17±2.5	136.17±2.03**	101.83±1.6***
4.	CF in the dose of 400mg/kg	156.5±2.9	117.67±2.8**	98.5±2.0***

All values are expressed as Mean ± SEM(n=6). One way ANOVA followed by Dunnet's test.

**P<0.01, *** P<0.001 when compared to control

2. Staircase Apparatus

In Staircase test, number of climbing decreases in test groups compared to control group. The obtained results were shown in table 8

Table 8: Locomotor activity of mice in Staircase method

S.No	Treatment Group	Before Drug Administration	After 30 Min	After 60 Min
1.	Control	196.17±2.6	190.67±2.38	189.83±2.5
2.	Standard Diazepam 2.5mg/kg	199.17±3.03	117.5±2.24***	80.5±2.6***
3.	CF in the dose of 200mg/kg	192.16±3.07	162.17±1.58*	135.83±2.4***
4.	CF in the dose of 400mg/kg	181.67±2.49	153±2.9**	119.3±1.8***

All values are expressed as Mean±SEM (n=6). One way ANOVA followed by Dunnet's test.
*P<0.05, **P<0.01 and *** P<0.001 when compared to control

DISCUSSIONS

Cymbopogon flexousus root was procured and authenticated. Then the root was dried and using ethanol as a solvent the root was extracted. Then by using Percentage yield formula we get the yield.After the extraction of CF is taken for Phytochemical screening. Antioxidant activity was evaluated by using Hydroxyl radical scavenging activity method

- In the reaction mixture, 2-deoxy-2-ribose, KH_2PO_4-KOH buffer, $FeCl_3$, ethylene diamine tetra-acetic acid (EDTA), H_2O_2, ascorbic acid and *Cymbopogon flexousus* root extract were added and controls shows their inhibition power against hydroxyl radicals
- The % inhibition of ethanolic extract and standard ascorbic acid values are mentioned in table 5, the extracts was able to neutralize hydroxyl radicals in the concentrations dependent manner at a concentration range of 200-1000 µg/ml and IC50 (µg/ml) value has been calculated and was found to be 213 µg/ml for ascorbic acid, whereas ethanolic extract showed IC50 (µg/ml) of 239 µg/ml respectively.
- From this studies, the ethanolic extract has significant antioxidant activity in comparison to the standard (ascorbic acid). Recent studies shows that antioxidant are responsible for antianxiety.
- Therefore, the ethanolic extract have been used in invivo studies. Then it was taken for acute oral toxicity studies

Table no. 5 represents daily observations for various parameters over a 14-day period. The observations include alertness, aggressiveness, touch response, gripping, motor coordination, salivation, diarrhea, tremors, convulsions, piloerection, drowsiness, food mortality. Throughout the 14-day period, the animals showed positive responses in terms of alertness, touch response, gripping, motor coordination, righting reflex, food consumption and water consumption.

No signs of aggressiveness, salivation, diarrhea, tremors, convulsions, piloerection, drowsiness, or mortality were observed. No mortality or morbidity was observed in animals exposed to a concentration of 2000 mg/kg of *Cymbopogon flexousus* root extract . The animals displayed normal behavior and physiological responses, indicating their well-being. The absence of adverse effects suggests that *Cymbopogon flexousus* root extract did not exhibit significant toxicity at the tested concentrations. Overall, these findings indicate the safety of *Cymbopogon flexousus* root extract in the experimental animal model.

The elevated plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drug acting at the $GABA_A$ benzodiazepine complex. The EPM test is used to evaluate the psychomotor performance and emotional aspects of mice. EPM is considered as one of the well-established model for unconditioned anxiety to detect anxiolytic/anxiogenic like activity by investigating aspects of physiological and pharmacological behavior.

In EPM, mice was normally prefer to spend much of their allotted time in enclosed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. In the EPM test increased number of entries and time spent into the open arm are taken as the index/reliable indicators of decreased anxiety or indicating the anxiolytic-like activity of a compound[64-66].

The percentage open arms entry with control group was 12.43±1.14 and the percentage time spent in open arms was 7.6±0.67 seconds. With diazepam 2.5mgs/kg in group II, the percentage open arms entries was 66.81±0.58 and the percentage time spent in open arm was 69.93±0.41 seconds when compared to control groups.

With test group III i.e. Ethanolic extract of *Cymbopogon flexousus* 200mg/kg, the percentage of open arm entry was 40.55±0.52 and percentage time spent in open arm was 46.73±0.63 sec. With test group IV i.e. ethanolic root extract of *Cymbopogon flexousus* 400mg/kg, the percentage of open arm entry was 50.28±0.9 and percentage time spent in open arm was 56.50±0.39 seconds.

In the present study, mice treated with ethanolic extract of *Cymbopogon flexousus* at the doses of 200 mg/kg and 400 mg/kg produced significant (P< 0.001) anxiolytic effect. In the EPM test when compared to control as evidenced by increased percentages of both open arm entries and time spent in open arm when compared to control group of animals

In open field apparatus consists of a square open area which is divided by lines into16 equal squares. The locomotor activity was determined by manually counting the number of lines crossed by the animal during a fixed time of 10 minutes.

Locomotor activity was evaluated by using open field method. The spontaneous locomotor activity made by a mice was noted in control, standard and extract group before 30 min and 60 min after the administration of control, standard and extracts.

- The average number of counts at before 30 min and 60 min after the administration of control group of mice was 165.83±1.5, 170±1.28 and 164.7±1.26 respectively.
- The average number of counts at before 30 min and 60 min after the administration of standard group of mice was 159±1.16, 99.33±2.2 and 72.67±1.22 respectively.
- The average number of counts at before 30 min and 60 min after the administration of test group (ethanolic extract of CF 200mg/kg) of mice was 153.17±2.5, 136.17±2.03 and 101.83±1.6 respectively.
- The average number of counts at before 30 min and 60 min after the administration of test group (ethanolic extract of CF 400mg) of mice was 156.5±2.9, 117.67±2.8 and 98.5±2.0 respectively.

In the present study, mice treated with ethanolic extract of *Cymbopogon flexousus* root at the doses of 200mg/kg and 400 mg/kg showed a statistically significant (P< 0.001) reduction in spontaneous locomotor activity in Open field after 60 min of administration of standard and C.f extract were noted in comparison with control group of animals that signifies anxiolytic activity.

Each mice was placed in the staircase and its activity was measured for 10 minutes. Locomotor activity was evaluated by using Staircase method. The spontaneous locomotor activity made by a mice was noted in control, standard and extract group before 30 min and 60 min after the administration of control, standard and C.f extract .

- The average number of climbing at before 30 min and 60 min after the administration of control group of mice was 196.17±2.6, 190.67±2.38 and 189.83±2.5 respectively.
- The average number of climbing at before 30 min and 60 min after the administration of standard group of mice was 199.17±3.0, 117.5±2.2 and 80.5±2.6 respectively.
- The average number of counts at before 30 min and 60 min after the administration of test group (Ethanolic extract of C.f 200mg/kg) of mice was 192.16±3.0, 162.17±1.6 and 135.83±2.4 respectively.
- The average number of counts at before 30 min and 60 min after the administration of test group (Ethanolic extract of C.f 400mg/kg) of mice was 181.67±2.49, 153±2.9 and 119.3±1.8 respectively.

In the present study, mice treated with ethanol extracts of *Cymbopogon flexousus* at the dose of 200mg/kg and 400mg/kg showed a statistically significant (P<0.001) reduction spontaneous locomotor activity in Staircase apparatus after 60 minutes of administration of standard and C.f extracts were noted in comparison with control group of animals that signifies anxiolytic activity . Despite intensive efforts to develop novel psychiatric drugs for anxiety disorders over the past two decades, all drugs have so far failed to minimize side effects. In this respect, herbal medicines could be an attractive candidate as the therapeutic strategies for this conditions. A major role for plant derived compounds based on the reported immunomodulatory effects has emerged in recent times and has led to the rigorous scientific examination to determine efficacy and safety[68].

However, the anxiolytic activity of the Ethanolic extracts of *Cymbopogon flexousus* plant was measured by using EPM suggested when the C.f extract increases open arms entries without altering the total number of arm entries. Diazepam has been used as a standard anxiolytic and also frequently employed in behavioral pharmacology as a reference compound of potentially anxiolytic-acting substances. But the fractions of plant extract at 200 mg/kg and 400 mg/kg body weight in mice showed significant increase in the percentage of entries into open arms and time spent in the open arms of the maze.

Analyzing the results of present study, it can be inferred that the ethanolic extracts of *Cymbopogon flexousus* plant roots at the dose of 200mg/kg and 400mg/kg possess a antioxidant and anxiolytic activity. Therefore, this extract could be considered for the treatment of anxiety and related neuropsychiatric disorders by conducting further pharmacological studies and mechanism of anxiolytic action, as well as to identify the active compound(s) responsible for this bioactivity in the animal model.

SUMMARY& CONCLUSION

Anxiety related disorders are the most common mental illness and a major cause of disability in the world. Mental disorders have been found to be common, with over a one third of people in most countries reporting them with sufficient criteria to be diagnosed at one point in their life. Despite a phenomenal development of modern drug industry, medicinal plants still constitute an important part of pharmacopoeias in both the developed and developing countries. These plants are important elements of traditional medicine and can be developed as potential drug after scientific validation. However, many of these traditionally used plants have not yet been studied scientifically. *Cymbopogon flexousus* is an important medicinal plant of family Poacea commonly known as Lemon grass. A number of pharmacological activities have been reported such as antiulcer, antibacterial, antifungal, hepatoprotective, antidiabetic, hypolipidaemic activity, anti-inflammatory and analgesic activity. The results of Preliminary phytochemical investigation shown the presence of various phytochemical constituents like flavonoids, phytosterol, carbohydrate, terpenoids and Protein& amino acids, etc

In vitro studies in Hydroxyl radical scavenging assay was carried out to select the significant extract. based on the results of ethanolic extract of *Cymbopogon flexousus* root were selected for further in vivo studies. In-vivo study was done using elevated plus maze test for the evaluation of anti-anxiety activity of the extracts. Anxious mice mostly preferred the closed arm, after administration of the extracts the animal spent more time in open arm due to the anti anxiety activity of

the extracts. Further two well established animal models, Staircase and open field methods were used to evaluate locomotor activity of mice. The animal treated with Ethanolic extracts showed decrease in locomotor activity. The result of the study showed that extract *Cymbopogon flexousus* at the dose of 200mg/kg and 400mg/kg has antianxiety that was statistically significant**.** Preliminary phytochemical investigation was done for the ethanolic extracts of *Cymbopogon flexousus* roots. It was found to contain flavonoids, phytosterol, carbohydrate, terpenoids and Protein & amino acids, etc… The present study shows that the ethanolic extracts of *Cymbopogon flexousus* root have higher antioxidant activity which was determined using the method (Hydroxyl radical scavenging activity).

The study shows that extracts of *Cymbopogon flexousus* root at the dose of 200mg/kg and 400mg/kg has significant antianxiety and antioxidant activity. The study also shows that the antianxiety of ethanolic extracts of *Cymbopogon flexousus* at the dose of 200mg/kg and 400mg/kg is less efficacious when compared to the standard drug diazepam at the dose of 2.5mg/kg. The results from the experiments confirmed that the ethanolic extract from *Cymbopogon flexousus*root posses a significant antioxidant and anxiolytic potential. However, the further studies in other models and extensive phytochemical analysis are necessary to identify the exact chemical compound and its possible mechanism of action underlying the anxiolytic effect of *Cymbopogon flexousus* root.

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