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Research Article

ANTIOXIDANT AND CNS ACTIVITY OF LEAVES EXTRACT OF *CYMBOPOGON FLEXUOSUS*

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ABSTRACT

Cymbopogon flexuosus commonly known as *"lemongrass"* is a perennial grass native to India, Sri Lanka, Burma and Thailand. The species is rich source of essential oil which can be extracted using alcohol. In the present study, essential oil extract of *Cymbopogon flexuosus* were evaluated for antioxidant and Central nervous system behavior using *In vitro* antioxidants models and screened for potential central nervous system activity by using open field models. The bioassay revealed significant inhibition of sample extract for claimed effect in exercised antioxidant models. On the other hand, central nervous system potential was evaluated using open field models such as Induced sleeping time, hole board method, open field and locomotor activity. The results obtained were significant in response to behavioral effect of administered extract doses of *Cymbopogon flexuosus* and explored extensive phytochemical nature of plant constituents for the claimed pharmacological activity.

Keywords: *Lemongrass*, essential oil, antioxidant, Central nervous system, bioassay.

INTRODUCTION

Cymbopogon flexuosus commonly known as *"*East Indian *lemongrass"* is a perennial grass which is widely used in indigenous system of medicine and is reported to contain essential oil in the form of monoterpenes fractions. The essential oil is renowned for its immense commercial significance in flavors, fragrance, cosmetics, perfumery, soaps, detergents and pharmaceuticals. The grass is aromatic and yields an essential oil upon hydro distillation or extraction with alcohol of their aerial parts.[1] The leaves are major source of essential oil which mainly consists of monoterpenes fractions with variety of chemical constituents in the form of citral, neral, geranial, citronellol, citronellal, linalool, elemol, 1, 8-cineole, limonene, geraniol, caryophyllene, methyl heptenone, geranyl acetate and geranyl formate. The essential oil components are greatly influenced by genetic, environmental and geographical conditions. The essential oils in *Cymbopogon flexuosus* are biosynthesized in leaves and stored in specific oil cells in the parenchymal tissues.[2] The leaves of *Cymbopogon flexuosus* were widely utilized in preparation of food stuffs, in curries and as flavouring agent in Asian sub-continent. Fresh leaves boiled with water are used to wash hair and as toilet water in India. In past years, studies also revealed biological significance of essential oil and its constituents of *Cymbopogon flexuosus* such as anti-cancer, anti-inflammatory, allelopathic and repellent activities. Literature review indicates that no studies combining the antioxidant and CNS effects of leaves of *Cymbopogon flexuosus* have so far been undertaken. Taking this in view and as a part of our ongoing research on Indian medicinal plants, the present study aimed to evaluate the antioxidant and CNS potential of hydro distilled leaves extract of *Cymbopogon flexuosus*.[3]

MATERIAL AND METHODS

Plant Material

The leaves of *Cymbopogon flexuosus* was collected in the month of July from Herbal Garden of IPS College of Pharmacy, Gwalior M. P. The leaves were dried, washed and stored in Polythene bags. The plant material was identified and authenticated by Dr. P. Jayaraman, National Institute of Herbal Science, Chennai (T. N.). The reference voucher of the plant material (Letter Ref. PARC/2012/1646) specimen has been maintained in the respective institute.

Preparation of the Plant extract

The leaves of *Cymbopogon flexuosus* were dried, chopped into small pieces weighed (1 Kg) and transferred into a 5 litre round bottomed flask to which 200 ml of deionised water was added and distilled for 3 hours using Clevenger Apparatus. The total distillation time was approximately 4 hours (including about 1 hour for the oil to start distilling) since the essential oil of *lemongrass* is less dense than water, the hydro distilled oil extract floats on top of water. Hydro distillation using Clevenger apparatus was applied for extracting essential oil from the leaves of *Cymbopogon flexuosus.* The yield of essential oil depends on various parameters such as weight of raw material, volume of water, size of raw material and nature of raw material. The yield of hydro distilled extracted oil obtained was 0.90% with yellowish brown colour.

Phytochemical studies

As per the literature survey and potential screening of plant extract for proposed pharmacological activity the important

bioactive phytoconstituents present are essential oil and flavonoids. The essential oils are present in the form of neral; geranial and geraniol whereas flavone and flavanol are the major contents of flavonoids.[4] The leaves also contain terpenoids and phenolic compounds. The varieties of phytochemical compounds present in the investigated plant material contribute to the therapeutic and pharmacological activity of the plant.[5]

Drugs and Chemicals

All the chemicals and reagents were purchased from Nike Chemicals India (Pvt) Ltd, Muzaffarnagar (U.P.) such as DPPH (α,α- diphenyl β-picryl hydrazyl), ethanol, Nitro blue tetrazolium (NBT), Riboflavin, EDTA. The standard drug sample of Pentobarbiton was obtained from JPEE Drugs, Haridwar and diazepam gift sample from Paksons Pharmaceuticals (P) Ltd. Bahadurgarh. All chemicals used were of analytical reagent grade.

Animals

Eighteen young male albino rats weighing between 100-200 gm were used for the experiment. They were kept in standard environment conditions (at 24 ± ºC and 55-65% relative humidity and 12-hour light / dark cycle) for one week for accumulation after their purchase and fed CCRAS animal research lab formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment was approved by the institutional animal ethical committee with approval number (CPCSEA Reg. No.-1498/PO/a/11/CPCSEA).[6]

Acute Toxicity

The 50% lethal dose (LD_{50}) of *Cymbopogon flexuosus* in rats was estimated by the up and down method.[5] Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

***In vitro* antioxidant activity**

Antioxidant activity of plant drug was evaluated by two experimental models and the results obtained were highly significant in reducing oxidative stress as compared with the control group.

DPPH (α,α- diphenyl β-picryl hydrazyl) Free Radical Scavenging activity

The activity was measured by spectrophotometric method.[7] A stock solution of DPPH (1.5 mg / ml in ethanol) was prepared such that 75 µl of it in 3 ml of ethanol gave an initial absorbance of approx. 0.9. Decrease in the absorbance in presence of sample extract at different concentration (100-500 µg/ml) was noted after 15 min.

Superoxide Scavenging Activity (N.B.T)

This method was given by McCord and Fridovich. The method was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system.[7] The reaction mixture contains EDTA, riboflavin, nitro blue tetrazolium (NBT), various concentrations of drug extract and phosphate buffer (pH 7.8) in a final volume of 3 ml.

Percentage reduction was calculated as

$$\% \text{ Reduction} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

***In vivo* CNS Activity**

Pentobarbital-induced sleeping time

Eighteen young male albino rats weighing between 100-200 gm were used for the experiment. Group A was kept as control received vehicle (1% Tween 80 in water), group B and group C were given oral dose of extracted oil of *Cymbopogon flexuosus* (100, 200 mg/kg body weight) respectively 30 min before administration of pentobarbitone (30 mg/kg body weight i. p.) and latency to sleep and duration of sleep was measured. The sleeping time was measured as the duration for which the righting reflex was lost.[8]

Hole board Test

The Hole Board apparatus consist of wooden box (40 x 40 x 25 cm) with 16 holes (diameter, 3 cm) evenly distributed in the floor.[9] The Hole board was elevated to the height of 25 cm. Twenty-four young male albino rats weighing between 100-200 gm were used.

Group A was kept as normal received vehicle, group B and group C were given oral dose of extracted oil of CF (100, 200 mg/kg body weight) respectively. Group D was given standard dose of diazepam (0.5 mg/kg) or vehicle (5 ml/kg). 30 min before placing on the apparatus and the number of head poking during 5 min period was recorded.

Open field Test

The apparatus consists of a wooden box (96 x 96 x 5 cm). The floor of the box was painted black and divided into sixteen squares of the same size.[10] Twenty-four young male albino rats weighing between 150-200 gm was used. Group A was kept as normal received vehicle while group B and C was given extracted oil of CF (100, 200 mg/kg body weight). Group D was administered standard dose of diazepam (1 mg/kg) or vehicle (5 ml/kg) and were placed individually in one of the corner squares of the box. The time required for leaving the square was recorded as transfer latency. The number of rearing and the number of squares traversed were counted for 5 minutes.

Locomotor activity

Twenty-four young male albino rats weighing between 150-200 gm were used. Group A was kept as normal received vehicle, animals of group B and C were given oral dose of extracted oil of CF (100, 200 mg/kg body weight). Group D was given standard dose of diazepam (1 mg/kg) or vehicle (5 ml/kg) the spontaneous locomotor activity of each rat was recorded individually for 10 min using actophotometer.[11]

Statistical Analysis

All values for *in vitro* and *in vivo* data were analyzed as the mean with one-way ANOVA followed by Dunnett's multiple comparison tests. AP < 0.05 was considered statistically significant in all the cases.

Table 1: Evaluation of DPPH scavenging activity of extracted oil of *Cymbopogon flexuosus*

Group	Concentration (µg/ml)	Absorbance	% Inhibition
Control	75	0.840 ± 0.02	59.51
Extracted oil of *Cymbopogon flexuosus*	100	0.652 ± 0.01*	22.38*
	200	0.582 ± 0.03*	30.72*
	300	0.563 ± 0.02*	32.94*
	400	0.498 ± 0.01*	40.71*
	500	0.486 ± 0.03**	42.13**

All values are expressed as mean ± SEM; n = 3; **P < 0.01; *P < 0.05 considered significant as compared to control

Table 2: Evaluation of Superoxide Scavenging Activity of extracted oil of *Cymbopogon flexuosus*

Group	Concentration (µg/ml)	Absorbance	% Inhibition
Control	75	0.923 ± 0.01	63.54
Extracted oil of *Cymbopogon flexuosus*	100	0.662 ± 0.01*	28.25*
	200	0.591 ± 0.03*	35.98*
	300	0.568 ± 0.02*	38.43*
	400	0.502 ± 0.02*	45.61*
	500	0.406 ± 0.01**	56.01**

All values are expressed as mean ± SEM; n = 3; **P < 0.01; *P < 0.05 considered significant as compared to control

Table 3: Effect of extracted oil of *CF* on Pentobarbitone-induced sleep in albino rats

S. No.	Treatment (mg/kg)	Latency to sleep (in second) mean ± SEM	Duration of sleep (In Minutes) mean ± SEM
Gr. A	Vehicle (1% Tween 80 in water)	15.4 ± 0.24	86.25 ± 6.5
Gr. B	Extracted oil of CF (100 mg/kg b. w)	14.4 ± 0.24	84.2 ± 5.4
Gr. C	Extracted oil of CF (200 mg/kg b. w)	13.8 ± 0.24	85.2 ± 6.7

All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Dunnett's test. *P < 0.05 compared to vehicle treated group.

Table 4: Effect of extracted oil of *CF* on number of head poking in hole board apparatus in albino rats

S. No	Treatment (mg/kg)	Number of Head poking Mean ± SEM
Gr. A	Vehicle	56.20 ± 2.16
Gr. B	Extracted oil of CF (100 mg/kg b. w.)	55.10 ± 2.12
Gr. C	Extracted oil of CF (200 mg/kg b. w.)	54.40 ± 2.16
Gr. D	Diazepam (0.5 mg/kg)	7.12 ± 0.23*

All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Dunnett's test. *P < 0.05 compared to vehicle treated group.

Table 5: Effects of extracted oil of *CF* on rearing and number of squares traversed in the open field apparatus in albino rats

S. No.	Treatment (mg/kg)	No. of Rearing Mean ± SEM	No. of Squares traversed Mean ± SEM
Gr. A	Vehicle	3.0 ± 0.10	68.0 ± 4.30
Gr. B	Extracted oil of CF (100 mg/kg b. w.)	2.0 ± 0.10	61.0 ± 3.21
Gr. C	Extracted oil of CF (200 mg/kg b. w.)	3.0 ± 0.10	65.0 ± 3.30
Gr. D	Diazepam (1 mg/kg b. w.)	28.2 ± 2.60****	130.8 ± 6.82****

All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Dennett's test. *P < 0.05 compared to vehicle treated group

Table 6: Effect of extracted oil of *CF* on locomotor activity in albino rats assessed using Actophotometer

S. No.	Treatment (mg/kg)	Actophotometer score in 10 min
Gr. A	Vehicle	310.70 ± 11.54
Gr. B	Extracted oil of CF (100 mg/kg b. w.)	302.70 ± 9.56
Gr. C	Extracted oil of CF (200 mg/kg b. w.)	308.70 ± 6.54
Gr. D	Diazepam (1 mg/kg b. w.)	95.00 ± 7.52*

All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Dennett's test. *P < 0.05 compared to vehicle treated group

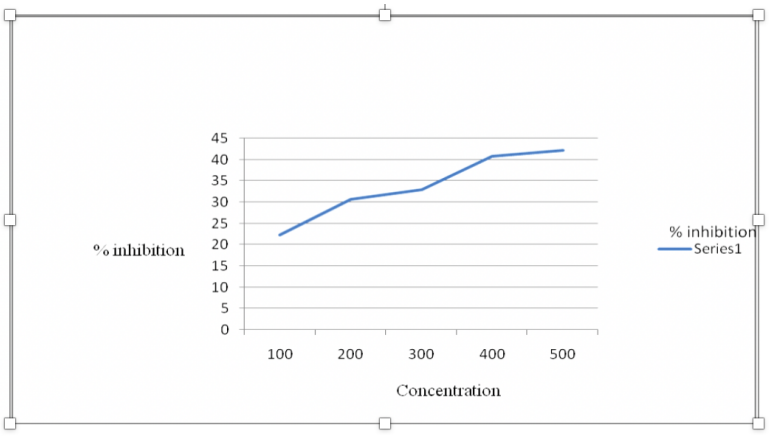

Figure 1: DPPH scavenging activity of extracted oil of *Cymbopogon flexuosus*

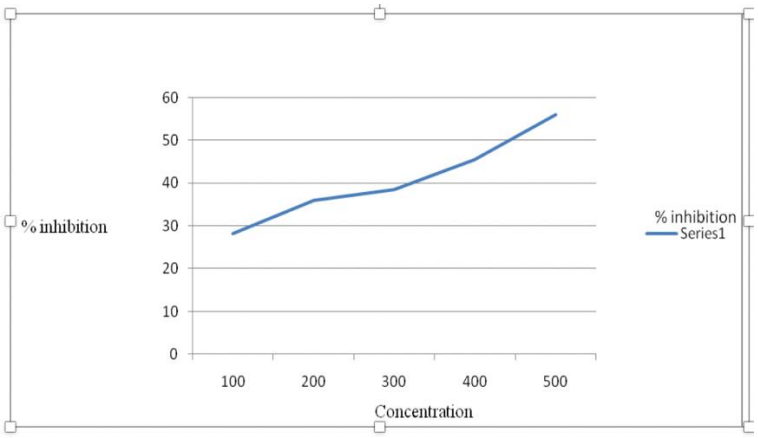

Figure 2: Superoxide scavenging activity of extracted oil of *Cymbopogon flexuosus*

RESULT

The qualitative chemical analysis of *Cymbopogon flexuosus* showed the positive result for the presence of hydrocarbon terpenes, monoterpene aldehyde mainly geranial (40 to 62%) and neral (25 to 38%). It also contains flavonoids and phenolic compounds. The screening of the phytoconstituents particularly contributes to their antioxidant and CNS behavioral activities.

Acute toxicity

Oral administration of graded doses of *Cymbopogon flexuosus* (100-200 mg/ Kg body weight) did not cause any death in different dose groups. The LD_{50} value for oral administration of the hydro distilled essential oil was found to be greater than 200 mg/kg.

In vitro antioxidant activity

DPPH Free Radical Scavenging Activity

It was measured by the spectrophotometric method. The decrease in the absorbance in presence of sample extracts at different concentration (100-500 µg/ml) was noted after 15 minutes. The percentage (%) inhibitory values of different concentration (100, 200, 300, 400, 500 µg/ml) of hydro distilled extracted oil of *Cymbopogon flexuosus* was 22.38, 30.72, 32.94, 40.71 and 42.13. (Table 1, Figure 1)

Superoxide Scavenging Activity (N.B.T)

The method was determined by Nitro blue tetrazolium (NBT). Different concentrations of test sample (100 µl) of hydro distilled extracted oil of *Cymbopogon flexuosus* with 100 µl riboflavin, 200 µl EDTA, 200 µl ethanol and 100 µl NBT solution was mixed in a test tube and the reaction mixture was diluted up to 3 ml with phosphate buffer. The absorbance of solution was measured after illumination for 15 minutes at 590 nm. The percentage (%) inhibition of different concentration (100, 200, 300, 400, 500 µg/ml) of hydro distilled extracted oil of *Cymbopogon flexuosus* was found to be 28.25, 35.98, 38.43, 45.61 and 56.01 (Table 2, Figure 2).

In vivo CNS activity

Pentobarbitone -induced sleeping time

Pentobarbitone induces sleep for 86.25 ± 6.5 minutes in vehicle treated group A (1% Tween 80 in water) after a latency of 15.4 ± 0.24 second. The hydro distilled extract dose of *Cymbopogon flexuosus* (100, 200 mg/kg bw) shows normal latency of sleep. (Table 3)

Hole board test

In case of sedation, reference standard diazepam (0.5 mg/kg b.w.) used as sedative which exhibits decrease in the number of head poking in comparison with hydro distilled extract dose of

Cymbopogon flexuosus (100,200 mg/kg bw) which shows normal sedative action (Table 4).

Open field Test

In the open field test group, A received vehicle (1% Tween 80 in water) and perform the minimum rearing and traversed while group B and C received extracted oil of *CF* (100, 200 mg/kg b. w.) shows normal rearing and traversed. The Group D administered reference standard diazepam (1 mg/kg) increased both rearing and traversed value significantly ($P < 0.05$). (Table 5)

Locomotor activity

In case of locomotion, Group A received vehicle showed the maximum response while group B and C was administered hydro distilled extracted dose of *Cymbopogon flexuosus* (100, 200 mg/kg b. w.) shows normal locomotive action as that of Group A, while group D was administered the standard drug diazepam (1 mg/kg b. w.) shows most significant results ($P < 0.05$) (Table 6).

DISCUSSION

There are varieties of *in –vitro* methods to determine the efficacy of natural anti-oxidants either as pure compound or as plant extract that act by several mechanisms.[12] The knowledge of anti-oxidant activity can be useful in the analysis of changes in the plasma anti-oxidant activities related to oxidative stress or the understanding of structure –activity relationships of pure anti-oxidant species. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is usually used as a substrate to evaluate the antioxidant activity of a compound. In super-oxide scavenging activity, it was revealed that hydro-distilled extract doses of *Cymbopogon flexuosus* did show the proton donating ability and could serve as a super-oxide inhibitor or scavenger. The presence of hydrocarbon terpenes in plant extract is the major cause of antioxidant activity in different models. The effect of hydro-distilled extract of *Cymbopogon flexuosus* on CNS was evaluated. The results indicated that the hydro-distilled extract of *Cymbopogon flexuosus* shows moderate to decreased CNS response in phenobarbitone-induced sleeping, open field, hole board test and locomotor activity. The locomotor activity is a measure of the level of excitability of the CNS and any decrease in this activity may be closely related to sedation resulting from depression of the central nervous system.[13] The extract doses of *Cymbopogon flexuosus* acts by potentiating the GABAenergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the plant extracts.[14] The phytoconstituents present in plant extracts are mainly responsible for the moderate CNS depressant activity and the marker compounds should be explored much more for the targeted therapeutic response in many CNS disorders.[15]

CONCLUSION

In conclusion, this work has demonstrated that the hydro-distilled plant extract of *Cymbopogon flexuosus* (Poaceae) possess mild to moderate antioxidant and CNS depressant potential, thereby supports the traditional use of the plant in preventing oxidative degradation pain and inflammatory disorders. However, further studies are needed to be conducted to understand the exact mechanisms of such actions and to formulate herbal medicinal product based on therapeutic efficiency for patients suffering from oxidative stress and central nervous system complications.

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