ORIGINAL ARTICLE

Investigation of phytochemicals and anti-convulsant activity of the plant *Coleus amboinicus* (lour.)

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Objectives: The present study has been designed to evaluate the comparative anticonvulsant activity of different parts of *Coleus amboinicus* as it has been mentioned in the various literatures regarding the use of this plant in the treatment of epilepsy, but no specific scientific reports are available in this regard. **Materials and Methods:** The *in vitro* anticonvulsant activity of leaf, stem and roots of *C. amboinicus* has been evaluated by maximal electric shock-induced seizures (MES) and Pentylenetetrazole (PTZ)-induced seizures models in Swiss albino mice. The drug/extracts were administered through intra-peritoneal route (100 mg/ml), in both experimental models and the effect was compared with Phenytoin in MES and PTZ-induced convulsion. **Results:** All the three studied extracts have shown significant anticonvulsant activity in both the models. However, the alcoholic leaf extract has shown highest activity by abolishing the MES-induced convulsions after 60 minutes of drug administration. The duration of convulsions in PTZ model was also significantly reduced ($P < 0.001$) compared to the control group. **Conclusion:** The alcoholic leaf extract of the *C. amboinicus* has shown the significant anticonvulsant activity in both the studied models, followed by stem and root extracts. The presence of alkaloids, flavonoids, and saponins in these extracts may be responsible for this activity.

Key words: Anti-convulsant, *Coleus amboinicus,* maximal electric shock, Pentylenetetrazole, Phenytoin

INTRODUCTION

Convulsion is one of the most common diseases of brain, affecting at least 50 million people in worldwide. Convulsion is a medical condition where body muscles contract and relax rapidly and repeatedly, resulting in an uncontrolled shaking of the body.[1] The plant *Coleus amboinicus* (synonyms: *Plectranthus amboinicus, Coleus aromaticus*) commonly known as Country borage or Indian borage. It is a dicotyledonous plant belonging to the family *Lamiaceae*.[2,3] The plant is a folkloric medicinal plant, used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsions, and epilepsy.[4-6] It is used to treat colds and cough as well as arthritic inflammations.[7] Its insect-repellent properties also have been tested.[8] Studies performed in India demonstrated the "fungi static" properties of the essential oil of this plant.[9] The phytochemical study reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin, and volatile oils in the leaves[10] of the plant.

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MATERIALS AND METHODS

Collection and Preparation of Plant Material

The plant *Coleus amboinicus* was collected from local areas of Shimoga, Karnataka. The collected material was authenticated by Prof. D. Ruddrappa, Head of the Dept. of Botany, Shayadri Science College, Shimoga. The leaves, stems, and roots are cleaned, separated, air-dried, coarsely powdered, and preserved in air-tight containers till further use.

Extraction of Plant Material

The dried plant materials such as root, stem, and leaf were subjected for cold maceration using alcohol as solvent for 2 days. The extracts were collected by filtration. Marc was subjected for Soxhlet-extraction by using ethanol. Both the extracts were combined concentrated in a rotary flash evaporator under vacuum at 45°C.

Preliminary Phytochemical Investigation

All the three extracts were subjected to preliminary phytochemical screening for the determination of major chemical groups by standard procedures.[11]

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Experimental Animals

Swiss albino mice weighing between 18 and 22 g were used. The animals were obtained from animal house, National College of Pharmacy, Shimoga, Karnataka. They were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 24 ± 2°C and relative humidity of 30-70%. A 12:12 light/day cycle was followed. All animals were allowed to free access for water and feed with standard commercial pelleted rat/mice chaw (M/s. Hindustan Lever Ltd, Mumbai). All the animals were kept for fasting 12 hours before starting the experiment, but allowed to free access to water. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC (NCP/IAEC/CLEAR/07/2007-08).

Maximum Electric Shock-induced Convulsions

In this model,[12] animals were divided into mainly two sets. The animals of each set were divided into five groups of animals with six animals in each group. The animals of each set had one vehicle control which received 0.9% w/v of saline (1 ml/kg). Second group has received Phenytoin (25 mg/kg body weight i.p.) and treated as positive control. Third, fourth, and fifth Groups of received *C. amboinicus* leaf, stem, and root extracts (CALE, CASE, and CARE) of 100 mg/kg BW, respectively. In the first set, drug was administered to the animals intraperitoneally 30 min prior to the electroshock, and the second set, 60 min prior to the electroshock. The electroshock induced in the animals by passing a current of 6 mA for 0.2-sec-duration through electro-convulsiometer (Techno India) using ear electrodes. The incidence and duration of flexion, extensor, clonus, and stupor were noted.

Pentylenetetrazole-induced Seizure Model

In this type of seizure model,[13,14] the mice were divided into five groups with six animals in each group. Group I served as solvent control and received 0.9% w/v of saline (1 mL/kg), Group II received Phenobarbitone (20 mg/kg) and treated as positive control and Group III, IV, and V received *C. amboinicus* leaf, stem, and root extracts (CALE, CASE, and CARE) of 100 mg/kg BW, respectively. All the drugs were administered by intraperitoneally 60-min prior to the administration of Pentylenetetrazole (PTZ) (80 mg/kg) by i.p. route. The animals were observed for 1 hour by placing in a separate cage. The duration of seizures (tonic-clonic convulsions) were recorded.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). $P < 0.05$ were considered statistically significant.

Thin Layer Chromatography

All the three extracts were subjected for thin layer chromatography. Various mobile phases were tried and the one with maximum number of spots was selected. The most suitable mobile phase was found to be toluene: dioxan: Glacial acetic acid (90:25:4). All the three plant extracts were dissolved in methanol and applied to pre-coated TLC silica gel plates (silica gel 60 F_{254}, AluGram, Germany). Chromatograms were developed in solvent systems and were examined under UV and daylight as well as after spraying with Anisaldehyde-sulphuric acid reagent to detect the presence of different phytoconstituents.

RESULTS

Preliminary Phytochemical Investigation

The preliminary phytochemical screening revealed the presence of alkaloids, flavanoids, tannins, triterpenoids, saponins in all the three extracts. Carotenoids were detected in leaf and stem extracts.

Maximal Electroshock-induced Convulsions

C. amboinicus leaf, stem and root extracts exhibited a significant reduction in various phases of epileptic seizures on comparison with the reference standard Phenytoin (25 mg/kg BW). There was also a significant reduction in the time required for righting reflex (recovery) in the extract treated group [Tables 1 and 2]. In the second set of animals, which were treated with the extracts 60-min prior to the induction of convulsion by MES method, exhibited a highly significant anticonvulsant effect.

In the first set, the leaf extract significantly reduced the extensor phase compared to the stem and root extracts;

Table 1: Effect of *Coleus amboinicus* extracts on maximal electric shock-induced convulsion in mice after 30 min

Drug used	Duration of various phases of convulsions (sec.)				Recovery/death
	Flexion	Extensor	Clonus	Stupor	
Control	3.68±0.37	13.80±0.63	12.94±0.47	30.48±3.13	Recovered
Phenytoin (25 mg/kg)	Nil	Nil	Nil	Nil	Recovered
CALE (100 mg/kg)	2.49±0.28**	7.78±1.07**	9.19±0.85**	21.84±0.86**	Recovered
CASE (100 mg/kg)	3.07±0.19**	8.61±0.93**	10.83±0.69**	23.46±0.93**	Recovered
CARE (100 mg/kg)	3.29±0.20*	11.51±0.76**	11.55±0.82**	28.50±1.16	Recovered

*P<0.05, **P≤0.001. Values are in mean±SEM (n=6). CALE – *C. amboinicus* leaf extract; CASE – *C. amboinicus* stem extract; and CARE – *C. amboinicus* root extract

however, in the second set i.e., 60 min after the extract administration it completely abolished the extensor phase. The other two extracts reduced the extensor phase in both the sets but the results were not as significant as that of leaf extract. Compared to the first set of animals all the three extracts decreased the recovery time, where the control and standard groups showed almost similar results as first set animals.

Pentylenetetrazole-induced Convulsions

The leaf, stem, and root extracts of *C. amboinicus* increased the threshold of PTZ-induced convulsions in mice and offered protection against convulsion. In this model, the durations of convulsion in root extract was highest (7.07 min) than stem extract (5.21 min). Whereas leaf extract has shown the lowest duration of convulsion (1.98 min). In addition, the recovery was 100% in all the animals tested with the extracts. Similarly the recovery time more in root extract (53.96 min) than stem extract (34.73 min) and it was found to be minimum with the leaf extract (16.23 min). Whereas, the animals of control group, duration of convulsion was high compared to test drug treated animals, and all the animals showed mortality.

Thin Layer Chromatographic Profile

The solvent system toluene: dioxan: glacial acetic acid (90:25:4) showed maximum separation of constituents for all the three extracts. In visible light and in UV both short and long wavelengths, the R_f value of quercetin matches with the R_f values of the first spots of all the three extracts. This indicates the presence of quercetin in all the three extracts. In visible light and in UV both short and long wavelengths leaf extract showed presence of about 10 components, whereas stem and root extract showed less components. Post-chromatographic derivatization with anisaldehyde-sulphuric acid reagent, about 14 spots have been detected with both leaf and stem extracts, whereas about 16 spots detected with root extract [Figure 1].

DISCUSSION

The results in MES model indicated that the extracts increased the threshold of seizure. It has been found that the drugs which raise the threshold for production of electrically induced seizures are generally effective against absence seizures, where those that reduce the duration and spread of electrically induced convulsions, are effective in generalized tonic-clonic seizures.[15] In addition to identifying against generalized tonic-clonic seizures, it has been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures. In clonic seizures induced by PTZ is reported to be blocked by drugs that reduce T-type calcium current (Ethosuximide) and drugs that enhance inhibitory neurotransmission by $GABA_A$ receptors (Benzodiazepine, Phenobarbitone and Valproate).[16] Convulsants those actions previously unexplained (including penicillin and PTZ) may act as

Table 2: Effect of *Coleus amboinicus* extracts on maximal electric shock-induced convulsion in mice after 60 min

Drug used	Duration of various phases of convulsions (sec.)				Recovery/death
	Flexion	Extension	Clonus	Stupor	
Control	3.77±0.34	19.89±0.70	9.24±0.67	30.33±1.49	Recovered
Phenytoin (25 mg/kg)	Nil	Nil	Nil	Nil	Recovered
CALE (100 mg/kg)	Nil	Nil	Nil	18.51±2.35**	Recovered
CASE (100 mg/kg)	2.81±0.28**	7.89±0.77**	7.04±0.53**	20.22±1.13**	Recovered
CARE (100 mg/kg)	2.93±0.24*	10.39±0.89**	8.09±1.15*	25.50±1.42**	Recovered

*P<0.05, **P≤0.001. Values are in mean±SEM (n=6). CALE – *C. amboinicus* leaf extract; CASE – *C. amboinicus* stem extract; and CARE – *C. amboinicus* root extract

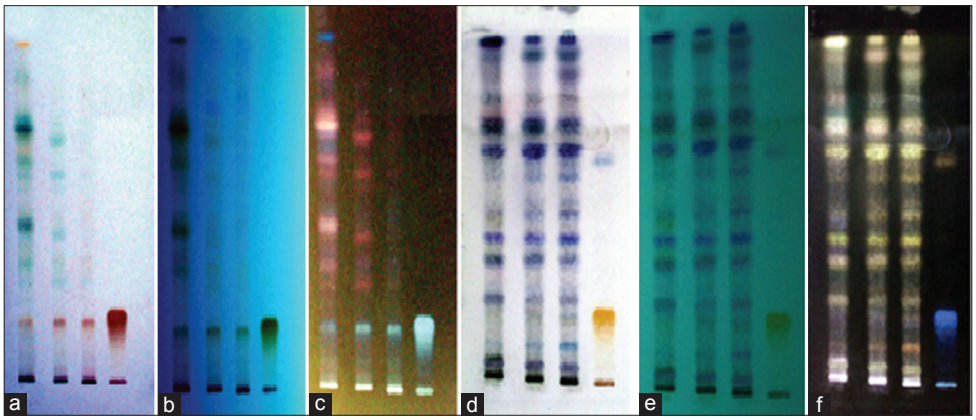

Figure 1: TLC profile of the alcoholic extract of various parts of *Coleus amboinicus* (a) Visible light; (b) UV-(366 nm); (c) UV-(254 nm), and after spraying with Anisaldehyde- sulphuric acid reagent; (d) Visible light; (e) UV-(366 nm); (f) UV-(254 nm). In each plate spots are as of follows from left-CALE, CASE, CARE, and Quercetin. CALE – *C. amboinicus* leaf extract; CASE – *Coleus amboinicus* stem extract; CARE – *C. amboinicus* root extract

relatively selective antagonists of the action of GABA at $GABA_A$ receptors, the major inhibitory neurotransmitter which is implicated in epilepsy.[17,18] The fact is, the extracts protected the animals against PTZ-induced seizures as well as delayed the occurrence of PTZ-induced convulsions. This may suggest that the three plant extracts contain compound (s) that facilitate GABA-aminergic transmission mechanism (s) to exert anticonvulsant effect by increasing the level of GABA. It has been found empirically that the drugs which inhibit PTZ-induced convulsions are generally effective against absence seizures.[19] Whereas those that reduce the duration of convulsions are effective in tonic-clonic seizures. The results indicate that the leaf, stem, and root extracts of *C. amboinicus* are effective in absence seizures as well as tonic-clonic seizures [Table 3]. The results from both the models indicate that, the leaf, stem and root extracts of *C. amboinicus* have broad-spectrum anticonvulsant activity. An increase in the doses of all the three extracts may show higher anticonvulsant activity. Different phyto constituent's presences may individually or co-elaborately show the anticonvulsant property in this drug.

Table 3: Effect of *Coleus amboinicus* extracts on Pentylenetetrazole-induced convulsions in mice after 60 min

Drug used	Duration of convulsion (min)	Recovery/ death (min)
Control	10.26±0.79	Death
Phenobarbitone (20 mg/kg)	Nil	NA
CALE (100 mg/kg)	1.98±0.37**	Recovered (16.23±1.21)
CASE (100 mg/kg)	5.21±0.49**	Recovered (34.73±1.88)
CARE (100 mg/kg)	7.07±0.86**	Recovered (53.96±2.11)

*P<0.05, **P≤0.001. Values are in mean±SEM (n=6). CALE – *C. amboinicus* leaf extract; CASE – *C. amboinicus* stem extract; and CARE – *C. amboinicus* root extract

CONCLUSION

The plant, *C. amboinicus* is being used traditionally in the treatment of many ailments like cold, sore throats, and nasal congestion; but also for a range of other problems such as infections, rheumatism, flatulence, and in epilepsy. In the present study, preliminary phytochemical investigation and anticonvulsant activity of the alcoholic extracts of leaf stem and root parts of the plant has been evaluated individually.

Apigenin is a naturally occurring flavonoid which was reported to be present in the plant *Coleus amboinicus*.[20] The apigenin and its derivatives are ligand for the benzodiazepine-binding site in the Gamma-Aminobutyric acid (GABA) receptor type A ($GABA_A$) and have anxiolytic properties.[21] So, anxiolytic effect of apigenin and its presence in the plant may contribute to the anticonvulsant property of the extracts. The actual phytoconstituents (s) responsible for anticonvulsant activity were needed to be determined. Hence, there is a further scope in detail phytochemical investigation and activity guided isolation of active constituents from the plant *C. amboinicus*. The parameters such as phytochemical evaluation and the TLC system will help in further standardization of *C. amboinicus*.

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