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ANTHELMINTIC AND ANTIOXIDANT ACTIVITY OF ALCOHOLIC EXTRACTS OF DIFFERENT PARTS OF *COLEUS AMBOINICUS* LOUR

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ABSTRACT

The present study reports anthelmintic activity of alcoholic extracts of leaf, stem and root of *Coleus amboinicus* against Indian earthworms *Pheritima posthuma*. The results revealed that all the tested extracts of *Coleus amboinicus* possessed significant anthelmintic activity in a dose-dependent manner. The activities were comparable with the reference drug Piperazine citrate and Albendazole. Among the tested extracts, the leaf extract was found to be more promising in comparison to stem and root extracts. Hence the present study justifies its use in the folklore remedies as an anthelmintic drug.

Whereas, antioxidant activity of the extracts were performed by four methods, DPPH free radical scavenging activity, Hydrogen-peroxide scavenging activity, nitric oxide scavenging activity and Reducing power assay using Ascorbic acid as standard. The antioxidant potency was found to be maximum with leaf extracts in all the models among all the three extracts.

KEYWORDS: Coleus amboinicus, anthelmintic, *Pheritima posthuma*, Piperazine citrate, albendazole, antioxidant.

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INTRODUCTION

The plant *Coleus amboinicus* (synonym: *Plectranthus amboinicus, Coleus aromaticus*) commonly known as Country borage or Indian borage, is a dicotyledonous plant belonging to the family Lamiaceae[1,2]. The plant is distributed through out India and cultivated in the gardens. It 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[3,4,5]. The phytochemical study reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil in the leaves[6]. The insect repellent properties of *Coleus amboinicus* have been tested[7] and it has been found to cause reduction in egg laying capacity, retard in adult emergence and weight loss in the pulse beetle *Callosobruchus maculatus* F[8]. A moderate allelopathic effect of the powdered leaves of *C. amboinicus* against the water hyacinth is also on record[9]. Studies performed in India demonstrated the "fungistatic" properties of the essential oil of this plant[10]. Warrier *et al* mentioned the traditional use of *Coleus amboinicus* leaf as anthelmintic[11]. Pushpa et al reported has the Anti-epileptic and antioxidant activity of *Coleus amboinicus* leaf juice[12].

In this contest it has been decided to evaluate the different parts of the *Coleus amboinicus* for their anthelmintic and antioxidant activity in order to give the scientific proof for its traditional use.

MATERIALS AND METHODS

Plant Material

The plant *Coleus amboinicus* was collected from local area of Shimoga, Karnataka. The collected material was authenticated by Prof. D. Ruddrappa, Dept. of Botany, Sahyadri Science College, Shimoga. The leaves, stems, and roots were separated, cleaned, air-dried, coarsely powdered, and subjected for Soxhlet-extraction by using ethanol. The extracts were evaporated to dryness by using Rotary-evaporator under reduced pressure at 45° C and stored in refrigerator at 4° C till the further use.

Anthelmintic activity

Adult Indian earthworms, *Pheretima postuma,* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[13,14,15] were used in the present study. All earthworms were of approximately of equal size. They were collected from local place, washed and kept in water.

The alcoholic leaf, stem and root extract of *Coleus ambionicus* were tested in various doses in each group. Normal saline water (0.9% Sodium chloride) was used as control. Piperazine citrate (Antepar-GSK) and Albendazole (Zentel-GSK) were used as the standard drugs in various doses for comparative study with the extract.

The method of *Nargund*[16] was followed for the screening of anthelmintic activity. Seventeen groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were made. Each group was treated with one of the followings: - Vehicle (0.9% Sodium chloride), Piperazine Citrate (10mg/ml, 20mg/ml, and 25mg/ml), Albendazole (10mg/ml, 20mg/ml, 30mg/ml, and 50mg/ml), *Coleus amboinicus* alcoholic leaf, stem and root extracts (25mg/ml, 50mg/ml, and 100mg/ml). Observations were made for the time taken to paralyze and/or death of individual worms. Paralysis was said to be occurred when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colors (**Table1**).

Antioxidant Activity

Comparative antioxidant effect of leaf, stem and root alcoholic extracts was evaluated by Free Radical scavenging activity (DPPH Model), Hydrogen peroxide Scavenging activity, Nitric acid Scavenging activity and Reducing power assay. Ascorbic acid is used as a standard in above models.

a. DPPH free radical scavenging activity[17]

The free-radical scavenging activity of all the three extracts was measured as decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493, and 5 ml of this stock solution was added to 1 ml of extract solution at different concentrations (50, 100, 150, 200, and 250 µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 µg/ml). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Anti-radical activity} = \frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Scavenging of Hydrogen Peroxide

Solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (250, 500, 1000, 1500, 2000 and 2500 µg/ml) of all the extracts were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of extracts and standard compounds was calculated using the following formula:

$$\% \text{ scavenged } [H_2O_2] = [(A_o - A_1)/A_o] \times 100$$

Where A_o was the absorbance of the control, and A_1 was the absorbance in the presence of the sample and standard[18].

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the extract (50, 100, 200, 300 and 400 µg/ml)) prepared in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was used. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standard (ascorbic acid) [19].

Reducing power assay

The reducing power of extracts was determined as per the reported method. Different concentrations of the three extracts (500, 1000, 2000 and 3000 µg/ml) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, p^H 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power[20].

RESULTS AND DISCUSSIONS

Anthelmintic activity

Preliminary phytochemical screening of all the three extracts of *Coleus amboinicus* revealed the presence of polyphenols, alkaloids, flavonoids, saponins, triterpenoids and tannins. From the results shown in table 1, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper-polarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.

All the three extracts of *Coleus amboinicus* produced a significant anthelmintic activity in dose-dependant manner as shown in Table 1. The anthelmintic activity of all the three extracts was comparable with that of standard drugs. From the results it is observed that the leaf extract shows higher anthelmintic activity, then stem extract and at last the root extract. The doses of 50mg/ml and 100mg/ml of leaf extract showed potent anthelmintic activity compared to piperazine citrate at 20mg/ml & 25mg/ml as well as albendazole at 30 and 50mg/ml doses respectively. Where as doses of 50 and 100mg/ml of stem extract showed good anthelmintic activity compared to piperazine citrate 10mg/ml as well as albendazole 30mg/ml and 50mg/ml doses respectively. The root extract at 50 and 100mg/ml doses are comparable with albendazole 10mg/ml and 30mg/ml doses respectively.

Phytochemical analysis of the crude extracts revealed presence of flavonoids and tannins in all the three extracts. Polyphenolic compounds show anthelmintic activity (28). Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (29). It is possible that phenolic content in the extracts of *Coleus amboinicus* produced similar effects.

Future scope involves need of isolation of phytoconstituent(s) responsible for the activity of all the three extracts of *Coleus amboinicus*.

Antioxidant Activity

DPPH free radical assay

DPPH free radical scavenging activity is the most widely reported method for screening of antioxidant activity of many plants. It involves reaction of specific antioxidant with a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). As a result there is a reduction of DPPH concentration, and DPPH radical is scavenged, the colour of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength of 517nm.

Figure 1 shows the scavenging activity against DPPH radicals of alcoholic extracts of leaf, stem and root, while ascorbic acid was used as standard. All the four samples exhibit dose-dependent anti-DPPH radical activities. Absorbance of DPPH against methanol as blank was served as control, which absorbance was 1.507 at the concentration of 90µg/ml. reduction in the absorbance by different concentration of test samples and ascorbic acid was recorded.

Hydrogen-peroxide-free radical assay

Hydrogen-peroxide, a reactive non-radical, is very important as it can penetrate biological membranes. Although H_2O_2 itself is not very reactive, it may convert into more reactive species such as singlet oxygen and hydroxyl radicals. Hydrogen-peroxide scavenging activity of leaf, stem and root extracts of *Coleus amboinicus* is shown in the figure-2. Ascorbic acid was used as standard. All the three extracts were capable of scavenging activity in a concentration dependant manner, i.e. with increasing concentration, there is a decrease in the absorbance of all the three extracts and as well as the standard at 230nm.

Nitric-oxide scavenging assay

The extracts of *Coleus amboinicus* decreased the amount of nitrites generated from the decomposition of sodium-nitroprusside *in vitro*. Suppression of NO released might be partially attributed to direct NO scavenging by the extracts. The scavenging of NO by the plant extracts increased with increase in the concentration of the extracts. While IC_{50} value of leaf extract is 162.69µg/ml, stem extract is 262.04µg/ml, root extract is 341.68µg/ml, and ascorbic acid is 38.22µg/ml.

Reducing power assay

Increased absorbance of the reaction mixture indicated increased reducing power of the extracts. As seen in the graph, the reducing power of the three extracts was much lower than that of Ascorbic acid. The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of antioxidant activity of the extracts.

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Table 1: Anthelmintic activity of leaf, stem and root extracts of *Coleus amboinicus*

Groups	Drug Used	Concentration (in mg/ml)	Time taken for paralysis (in min)	Time taken for death (in min)
1	Control (Normal saline)	---	---	---
2	Piperazine citrate	10	6.39±0.32	18.50±1.05
		20	4.56±0.51	13.42±0.20
		25	2.49±0.19	6.45±0.45
3	Albendazole	10	35.46±0.40	47.17±1.12
		20	21.02±0.31	39.17±0.27
		30	12.02±0.13	28.50±0.32
		50	10.05±1.01	20.30±0.22
4	CALE	25	25.30±0.59	39.32±1.03
		50	9.44±0.12	16.25±0.31
		100	1.28±0.19	3.09±0.26
5	CASE	25	54.30±1.26	111.10±1.58
		50	12.33±0.42	26.15±0.39
		100	8.40±0.21	13.55±0.28
6	CARE	25	57.53±1.09	121.42±1.21
		50	34.31±0.29	48.17±1.01
		100	14.63±0.30	27.16±0.55

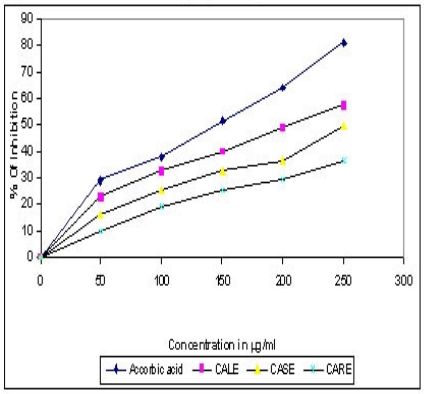
All values represent Mean± SEM from six observations.
CALE - *Coleus ambionicus* leaf extract, CASE - *Coleus ambionicus* stem extract, CARE - *Coleus ambionicus* root extract

Table 2: Antioxidant activity and IC_{50} Values of the extracts

Sample tested	IC_{50} Value			Reducing power (abs. at 700nm)
	DPPH Scavenging	H_2O_2 Scavenging	Nitric oxide Scavenging	
CALE	207.57	1059.88	162.69	2.723 (3g/ml)
CASE	>250	1824.95	262.04	1.640 (3g/ml)
CARE	>250	1902.11	341.68	0.699 (3g/ml)
Ascorbic acid	31.0	16.34	38.22	3.106 (0.6g/ml)

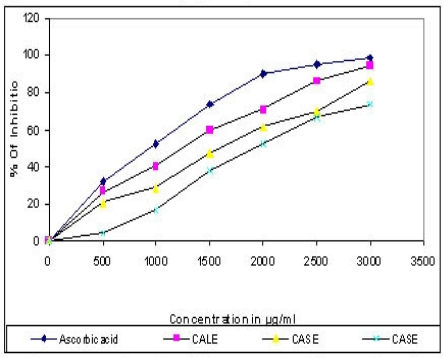
CALE - *Coleus ambionicus* leaf extract, CASE - *Coleus ambionicus* stem extract, CARE - *Coleus ambionicus* root extract

Figure-1: Free radical scavenging activity of alcoholic extracts of *Coleus amboinicus* of DPPH.



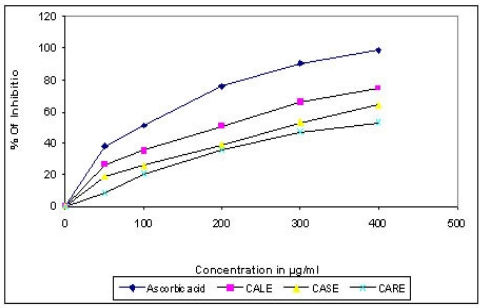
Results are mean ± SD of parallel measurements. Concentration of extracts expressed in µg/ml, whereas concentration of Ascorbic acid in 10X µg/ml.

Figure-2 Free radical scavenging activity of alcoholic extracts of *Coleus ambonicus* of H_2O_2



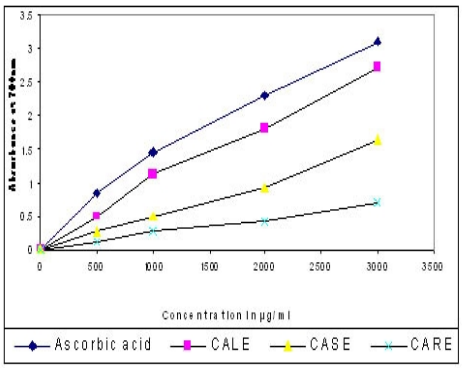
Results are mean ± SD of parallel measurements. Concentration of extracts expressed in µg/ml, whereas concentration of Ascorbic acid in 50X µg/ml.

Figure-3 Free radical scavenging activity of alcoholic extracts of *Coleus amboinicus* of Nitric Oxide



Results are mean ± SD of parallel measurements. Concentration of extracts expressed in µg/ml, whereas concentration of Ascorbic acid in 2X µg/ml.

Figure-4 Reducing power assay of alcoholic extracts of *Coleus ambonicus*



Results are mean ± SD of parallel measurements. Concentration of extracts expressed in µg/ml, whereas concentration of Ascorbic acid in 5X µg/ml.
CALE - *Coleus ambionicus* leaf extract, CASE - *Coleus ambionicus* stem extract, CARE - *Coleus ambionicus* root extract

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