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In-vitro anthelmintic activity of *Coleus aromaticus* root in Indian Adult Earthworm

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ABSTRACT

Objective: Anthelmintic resistance creates a major hitch over the decades throughout the world. As per WHO only synthetic drugs are frequently used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effects. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug. **Methods:** All the prototypes and the standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (2% v/v Tween 80 in normal saline), and Piperazine Citrate (10 mg/ml) and prototypes (10, 20 and 50mg/ml). **Results:** All the investigational extract acquired the anthelmintic activity at minimal dose of 10 mg/ml. its significant activity ($P < 0.05$) at 10 mg/ml for time taken to paralysis and death when compared to the standard drugs Piperazine citrate used at 10 mg/ml respectively. **Conclusions:** Herbal drugs and synthetic drugs have equally effective in helminth infestations but methanolic extract has the maximum anthelmintic activity potential than other root extract of *Coleus aromaticus*.

1. Introduction

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates [1]. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs, infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation [2]. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity, including lymphatic

filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis [3]. As per WHO only synthetic drugs are frequently used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

The *Coleus aromaticus* (Lamiaceae) commonly known as Patta Ajwain is a native species from Asia. It is a large succulent aromatic perennial herb. Much branched fleshy highly aromatic herb [4]. *Coleus aromaticus* are used to treat various diseases like stomach pain, vomiting diarrhoea, etc [5].

2. Materials and Methods

2.1 Drugs and Chemicals

Saline water (Clariflife sciences Ltd, Ahmedabad) and Piperazine citrate (pure drugs) use as standard and drug extracts were prepared as per standard procedure vehicle (2% v/v Tweens 80 in distilled water) were used [6–7]. All the prototypes were dissolved in minimum quantity of 2% v/v Tween80 and then the volume was adjusted to 10 ml with

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normal saline for making the concentration of 10, 20 and 50 mg/ml).

2.2 Experimental Model Indian earthworm

Pheretima posthuma), were used to study the anthelmintic activity. Earthworms were obtained from water logged area of pond of Gudamba, Lucknow. Worms were authenticated by Preeti Bajpai, Parasitologist, Department of Biotechnology, Integral university, Lucknow, India and washed with normal saline to remove all the fecal matter and waste surrounding their body. The earth worms (*Pheretima posthuma*) 3–5 cm in length and 0.1–0.2 cm in width weighing 0.8–3.04 g were used for all experiment protocols. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the anthelmintic activity [8–9].

2.3 Anthelmintic Investigation

Indian adult earth worm 4–5 cm in length and 0.1–0.2 cm in width were used for the invitro anthelmintic bio assay of methanolic, ethanolic and aqueous extracts. The worms were divided into the respective groups containing six–earth worms in each group. All the prototypes were dissolved in minimum quantity of 2% v/v Tween 80 and the volume was adjusted to 10 ml with normal saline for making the concentration of 10, 20 and 50 mg/ml. All the prototypes and the standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (2% v/v Tween 80 in normal saline), and Piperazine Citrate (10 mg/ml) and prototypes (10, 20 and 50 mg/ml) the anthelmintic

activity was determined.

Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color. Six worms of about the same size per petridish were used. They were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms.

2.4. Statistical Analysis

All results are expressed as mean \pm SEM. Groups of data was compared with analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Values would be considered statistically significant, when $P < 0.05$.

3. Results

Each crude extract containing 10, 20, and 50 mg/mL, produced dose–dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. As evident from the available literature, Anthelmintic activities of all prototypes were tested in this bioassay at various concentrations of 10, 20 and 50 mg/ml (Table 1). All the investigational extract acquired the anthelmintic activity at minimal dose of 10 mg/ml. its significant activity ($P < 0.05$) at 10 mg/ml for time taken to paralysis and death when compared to the standard drugs Piperzine citrate used at 10 mg/ml. [6]

4. Discussion

The assay of biological activity, alcoholic and aqueous

Table 1

Anthelmintic activity of roots extracts of *Coleus aromaticus*

Anthelmintic activity of root extracts of <i>Coleus aromaticus</i> .				
Groups.	Drugs treatment	Concentration(mg/ml)	Pheretima posthuma (Earthworm)	
			Time taken For paralysis (P) in min. (Mean and SEM)	Mean Time taken for death (D) in min.(Mean and SEM)
I	Control	-----	-----	-----
II	Piperazine citrate	10	21.67 \pm 1.07	56 \pm 2.81
III	Methanol Extract	10	34.70 \pm 1.01***	96.33 \pm 1.08***
IV	Methanol Extract	20	28.33 \pm 1.07	85.33 \pm 1.78***
V	Methanol Extract	50	23.67 \pm 1.41***	67.33 \pm .82*
VI	Ethanol Extract	10	40.33 \pm 1.07***	106 \pm 2.37***
VII	Ethanol Extract	20	35 \pm 1.87	90.67 \pm 1.46***
VIII	Ethanol Extract	50	30 \pm 1.41***	69.67 \pm 1.77**
IX	Aqueous Extract	10	55.67 \pm 1.46**	262.67 \pm 2.75***
X	Aqueous Extract	20	48 \pm 1.41***	187.33 \pm 1.08***
XI.	Aqueous Extract	50	47 \pm 1.86***	140.33 \pm 1.77***

Results are expressed as mean \pm SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test.

* Vs. group II: * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$

extracts were used to evaluate anthelmintic activity has shown dose dependant activity. The Mean \pm S.E.M. values were calculated for each extracts. The result of anthelmintic activity on earthworm *pheretima posthuma* was given in table–1 reveals that, the different concentration used for both aqueous and alcoholic extracts has shown paralysis and death of earthworms and it was compared in the same concentration with Piperazine citrate as reference drug. Piperazine cause hyperpolarization of worms muscle by GABA agonistic action opening Cl^- channels that cause relaxation and depresses responsiveness to contractile action of A.Ch. (Acetylcholine). By increasing chloride ion conductance of worm muscle membrane produced hyperpolarization and reduced excitability that led to muscle relaxation and flaccid paralysis [10].

The root extracts of *Coleus aromaticus* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time. Methanol extract of roots of *Coleus aromaticus* shows highest efficacy and potency towards anthelmintic activity than other extract of root, Preliminary phytochemical tests of the crude methanol, root extract of *Coleus aromaticus* showed the presence of flavonoids, terpenoids, alkaloid and tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities. Chemically tannins are polyphenolic compounds [11]. It is possible that tannins and terpenoids contained in the root extracts of *Coleus aromaticus* produced similar effects. Possible mechanism anthelmintic effect of root extract of *Coleus aromaticus* due to presence of tannins and terpinoids is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. In addition, tannins or their metabolites have a direct effect on the viability of the pre– parasitic stages of helminthes and other phytochemicals may responsible for have an anthelmintic effect include essential oils, flavonoids and terpenoids [12]. This speculation is supported by the varying rates of effectiveness of *Coleus aromaticus*,

6. Conclusion

Biological parameter can be concluded that the plant *Coleus aromaticus* has significant anthelmintic activity. Further studies using in vivo model are required to find out and to establish effectiveness and pharmacological rationale for the use of root as anthelmintic drug. In the light of above mentioned pharmacological effects, it may be concluded that methanolic extract has the maximum anthelmintic activity

potential than other root extract of *Coleus aromaticus*.

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8. Conflict of interest statement

We declare that we have no conflict of interest.

9. References

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