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IN VITRO ANTHELMINTIC ACTIVITY OF *SYZYGIUM AROMATICUM* FLORAL BUDS AND *CINNAMOMUM ZEYLANICUM* BARK AQUEOUS EXTRACTS

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ABSTRACT

Cloves (*Syzygium aromaticum*) are the aromatic dried flower buds of a tree in the Myrtaceae family and Cinnamon (*Cinnamomum zeylanicum*) bark are common Indian spices with enormous medicinal value. This study investigates *in vitro* anthelmintic activity of aqueous extracts of *S. aromaticum* (floral buds) and *C. zeylanicum* (bark). Phytochemical analysis of the extracts of *S. aromaticum* and *C. zeylanicum* revealed the presence of alkaloids, tannins, phenolics, phlobatannins, cardiac glycosides, anthraquinone glycosides, hydroxyl anthraquinones, flavonoids and terpenoids. Aqueous extracts of *S. aromaticum* and *C.zeylanicum* were evaluated for anthelmintic activity against Indian earthworm *Pheritima posthuma*. Albendazole (10 and 20 mg/ml) was used as a reference standard. Various concentrations (5, 10 and 20 mg/ml) of both plant extracts were tested and results were expressed in terms of time taken for paralysis (PT) and death (D) of worms. The potency of the above extracts was found to be inversely proportional to PT and D. Dose dependent activity was observed in the plant extracts. At higher dose of 20 mg/ml *C. zeylanicum* showed significant activity as compared to *S. aromaticum*.

KEYWORDS: **Anthelmintic activity, *Syzygium aromaticum, Cinnamomum zeylanicum, Pheretima posthuma,* Phytochemical screening, Albendazole.**

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INTRODUCTION

Helminthiasis is a macro parasitic worm disease of humans and animals in which a part of the body is infected with parasitic worms such as pinworm, roundworm, or tapeworm. It is one of the most common infections in mankind affecting a large proportion of the world population. Parasitic diseases may cause severe morbidities including lymphatic filariasis and onchocerciasis[1]. Anthelmintics are drugs that may act locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminths or development forms that invade organs and tissues[2]. Chemotherapy is the commonly used effective tool to cure and control helminth infection, as effective vaccines have not been developed so far[3] Indiscriminate use of the existing drugs causes adverse effects and made the parasites to develop drug resistance[4]. Development of resistance to most of the commercially available anthelmintics became a problem world-wide. These factors paved the way for herbal remedies as an effective alternate anthelmintics[5,6,7]. These days modern research focuses on phytoremedies to overcome adverse effects and drug resistance problems[3]. A number of medicinal plants have been used to treat parasitic infections in man and domestic animals[8,9]. Therefore an attempt has been made to evaluate anthelmintic activity of *S. aromaticum* floral buds and *C. zeylanicum* bark on adult earthworm *Pheretima posthuma*. From time immemorial, medicinal plants have been an indispensable source for treating a variety of ailments and acquired global importance. Indigenous medicines like Ayurveda, Siddha and Unani deploy a variety of herbal ingredients. Cloves (*Syzygium aromaticum*) are the aromatic dried flower buds of a tree in the family Myrtaceae. Cloves are used in Indian ayurvedic medicine, Chinese medicine, and Western herbalism. The clove tree is an evergreen that grows to a height ranging from 8–12 m, having large leaves and sanguine flowers in numerous groups of terminal clusters. The floral buds are at first of a pale color, gradually become green and develop into bright red at maturity. Cloves are harvested when 1.5–2 cm long, and consist of a long calyx, terminating in four spreading sepals. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. Cloves are also said to be a natural anthelmintic[10]. *C. zeylanicum* is a evergreen aromatic tree belonging to the family, Lauraceae. The species of *Cinnamomum* have aromatic oils in their leaves and bark. Both powdered and in whole, or "stick" form are used as a flavouring agent in food and therapeutic agent in ayurvedic medicine[11].

MATERIALS AND METHODS

Collection and identification of plant material

S. aromaticum floral buds and bark of *C. zeylanicum* used in this study were purchased from local market, Tirupati, Andhra Pradesh. The materials were identified and authenticated by Dr. P. S. T. Jaya Devi, Dept. of Biological Sciences, Sree Vidyanikethan Degree College, Tirupati, Andhra Pradesh. A voucher specimen was deposited in the department for future reference.

Drugs and chemicals

Albendazole procured from Ranbaxy pharmaceuticals Ltd. was used as reference standard for anthemintic activity. Sodium Chloride (NaCl) and Tween-80 were procured from Merck, India and normal saline was prepared by dissolving 0.9 g of NaCl in sterile distilled water.

Preparation of extract

About 50g of each plant material i.e., floral buds of *S. aromaticum* or bark of *C. zeylanicum* were weighed and blended into coarse powder. The contents were suspended in about 250 ml of distilled water in a conical flask with stopper and kept in shaker for one day by thoroughly monitoring for every one hour. The extracts were filtered by using a muslin cloth and kept in a water bath at 50°C to concentrate it. The concentrated extracts were stored in a brown glass bottle at 4°C.

Phytochemical screening

Preliminary phytochemical screening was employed to detect various secondary

metabolites in the extracts of *S. aromaticum* and *C. zeylanicum.* The extracts were tested for the presence of alkaloids, phenolics and tannins, glycosides, flavanoids, phlobatannins, carbohydrates, steroids and terpenoids[12].

Preparation of standard drug and test formulations

Albendazole is the first drug of choice for the treatment of worm infections. It is the first reported anthelmintic which promises to have useful activity against all the types of helminth parasites infecting human beings and domestic animals[13,14]. It was prepared by dissolving in normal saline at the concentrations of 10 and 20 mg/ml. Test formulations to evaluate *in vitro* anthelmintic activity were prepared by adding 0.1% Tween-80 and suspending respective amounts of each extract in 50 ml of normal saline to obtain 5, 10 and 20 mg/ml concentration.

Animals used

Indian adult earthworms *Pheretima posthuma* were used to study anthelmintic activity. The earthworms were collected from a local place and washed with normal saline to remove the faecal matter. They were identified and authenticated by Dr. S. V. Ravikanth, Department of Biological Sciences, Sree Vidyanikethan Degree College, Tirupati, Andhra Pradesh. The earthworms were used for the evaluation of anthelmintic activity due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings, uninfectious nature to humans and easy availability[15,16,17]. The earthworms of 3-5 cm in length and 0.1- 0.2 cm in width were used for all experimental protocols.

Anthelmintic activity

The Anthelmintic activity of aqueous extracts of *S. aromaticum* floral buds and *C. zeylanicum* bark was performed according to the methodology followed by Ajayieoba *et.al*.[18] on adult Indian earthworm, *Pheretima posthuma*. The earthworms were randomly divided into 9 groups. Each group of six earthworms was released into the desired formulation. Each formulation of 50 ml contains different concentrations of standard drug or aqueous extract of plant (5, 10 and 20 mg/ml in normal saline and 0.1% Tween-80). Observations were made for the time taken to set paralysis and death of the individual worms. Mean time for the paralysis (PT) in minutes was noted when no movement of any sort could be observed, except when the worm was shaken vigorously. Death was said to be occurred when the worms lost their motility followed by fading of their body color. Time of death (D) in minutes was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50°C).

Statistical analysis

The results were expressed as mean ± SEM (standard error mean of six worms). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test, using graph pad software. *P* values <0.01 were consider to be significant.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of aqueous extracts of *S. aromaticum* floral buds and C. *zeylanicum* bark revealed the presence of alkaloids, phenolics and tannins, glycosides, flavanoids, phlobatannins, carbohydrates and terpenoids as indicated in Table 1. In the present study, the control earthworms in vehicle (normal saline and 0.1% Tween-80) were exhibiting usual motility even after completion of anthelmintic activity evaluation as shown in Figure 1a.

Table 1
Phytochemical screening of S. aromaticum and C. zeylanicum extracts

Screening tests	Phytochemical constituents	*Syzigium* aqueous	*Cinnamomum* aqueous
Dragendroff's test Mayer's test Wagner's test	alkaloids	+	+
Ferric chloride test Gelatin test Lead acetate test	phenolics and tannins	+	+
Phlobatanins test	phlobatannins	+	+
Legal's test Keller-Killiani test	cardiac glycosides	+	+
Borntrager's test	anthraquinone glycosides	+	+
Hydroxy anthraquinones test	hydroxy anthraquinones	+	+
Shinoda's test Alkaline reagent test	flavonoids	+	+
Molisch's test Fehling's test Benedict's test	carbohydrates	+	+
Liberman-Burchard's test Sulphur powder test	terpenoids	+	+
Salkowski's test	steroids	-	-

The standard drug used for comparison of anthelmintic activity was Albendazole at two different concentrations of 10 and 20 mg/ml (Figures 1b and 1c). The anthelmintic effect of aqueous extracts of *S. aromaticum* floral buds and bark of *C. zeylanicum* was studied at various doses of 5, 10 and 20 mg/ml as represented in Figures 2 and 3 respectively.

Figure 1
Vehicle and anthelmintic activity of standard drug

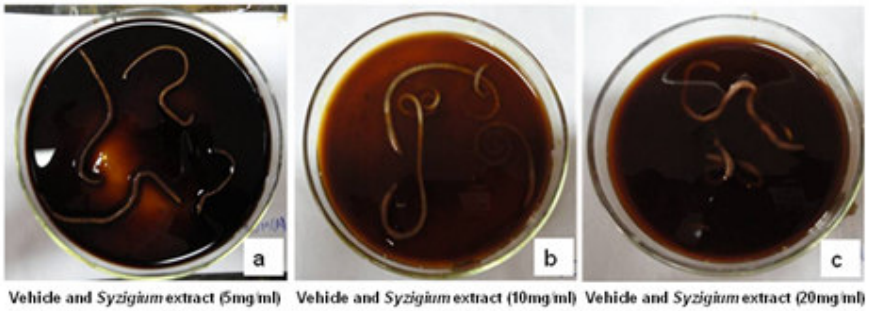

Figure 2
Anthelmintic activity of S. aromaticum

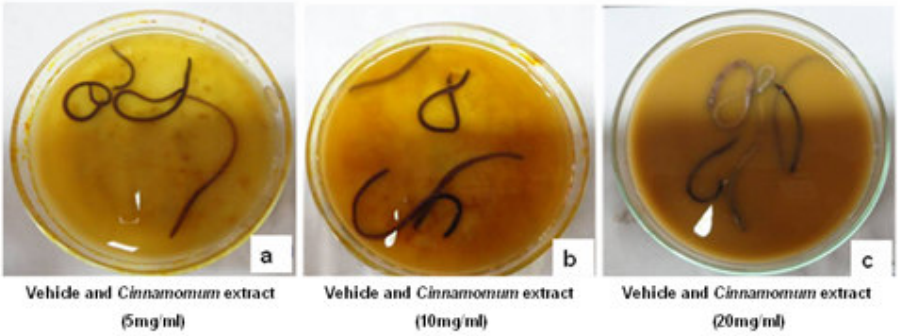

Figure 3
Anthelmintic activity of C. zeylanicum

The time taken for paralysis and death of individual earthworm was recorded, expressed as mean ± SEM of six worms and were significant at 99% level of confidence (P<0.01) as represented in Figure 4. In case of standard drug (10 mg/ml), the time taken for paralysis was 95 ± 1.33 minutes followed by death at 111 ± 1.52 minutes. Similarly, at higher dose of 20 mg/ml, PT was reduced to 56 ± 1.31 minutes and death occurred at 76 ± 1.54 minutes as shown in Figure 4. As the concentration of drug increased, a decrease in PT and D was observed.

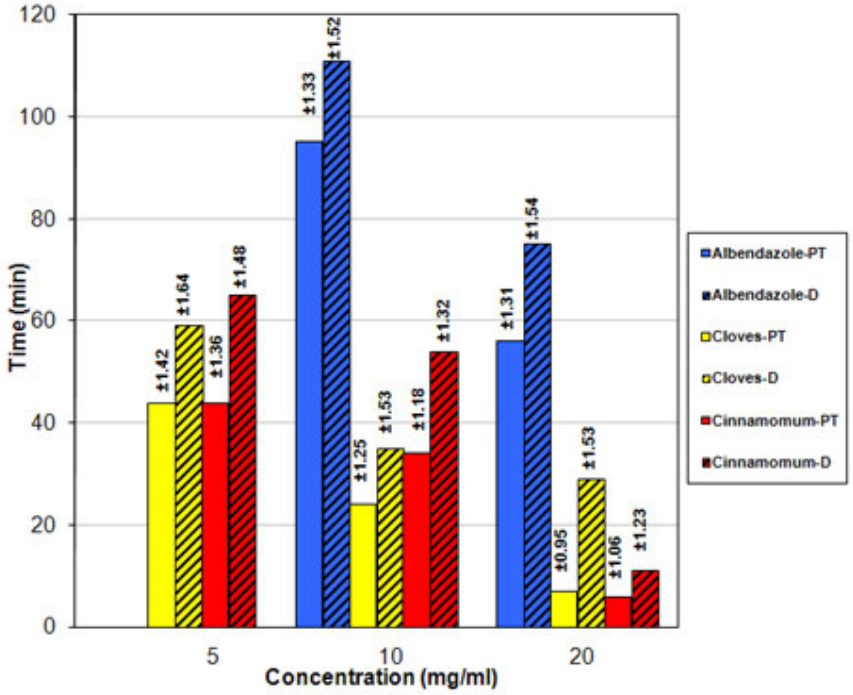

Figure 4
Comparison of PT and D of earthworms treated with Albendazole and plant extracts

The plant extracts viz. *S. aromaticum* and *C. zeylanicum* were able to produce dose dependent paralysis and death in earthworms. Higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all worms. Phytochemical screening of crude extracts revealed presence of tannins which are shown to produce anthelmintic activity[19,20,21]. Some synthetic phenolic anthelmintics like niclosamide, oxiclonazide and bithionol were shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. Another possible

anthelmintic effect of tannins is that, they can bind to free proteins in the gastrointestinal tract of host animal or glycoproteins on the cuticle of parasite and cause death[22]. At lower dose of 5 mg/ml, both the extracts were showing almost comparable anthelmintic activity. Whereas, at higher dose of 20 mg/ml, *C. zeylanicum* bark extract was more effective than *S. aromaticum* as indicated by significant decrease in time required for paralysis i.e., 5 ± 1.06 minutes at 20 mg/ml followed by death at 11 ± 1.23 minutes as shown in Figure 4. Both the extracts evaluated for the anthelmintic activity exhibited dose related effect and it was greater than standard drug Albendazole at similar concentrations. The results revealed that the tested phytoremedial anthelmintics are potential in devastating worms as compared to standard drugs available.

CONCLUSION

From the present study it is evident that both the plant extracts viz. *S. aromaticum* floral buds and *C. zeylanicum* bark were showing enhanced anthelmintic activity when compared to Albendazole at similar concentration. The anthelmintic activity exhibited was dose dependent. Tannins present in the plant extract may be responsible for this activity.

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