IN-VITRO ANTHELMINTIC ACTIVITY OF *CITRUS SINENSIS* SEED COATS

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Abstracts
The crude extracts of *Citrus sinensis* seed coats were evaluated for anthelmintic activity using adult earthworms. Petroleum ether, chloroform and methnolic extracts of *Citrus sinensis* seed coats were evaluated separately for anthelmintic activity by using adult Indian earthworms, *Pheretima posthuma (Annelida).* Various concentrations of all extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/mL) was used as a reference standard and distilled water as control group.
Keywords: anthelmintic, *Citrus sinensis, Pheretima posthuma*

1. Introduction

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Infections with helminthes or parasitic worms affect more than two billion people worldwide. In region of rural poverty in the tropics, where prevalence is greatest simultaneous infection with more than one types of helminthes is common. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastrointestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases[1,2,3]. The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs for their primary health care needs. The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs. Hence there is an increasing demand towards natural anthelmintics[4].

Citrus sinensis cultivated throughout west and south India. Fruits are round with an abundant, sweet, solid pulp and spindle-shaped juice sacs. Fruits get reddish-yellow colour when ripe. Fruit is having a distinctively farrowed fruit with (areole) around the apical end. Flowers and fruits are useful. Fruits are a source of anti-oxidants and chemical exfoliants in specialized cosmetics. The fruit is appetizer and blood purifier. It is used to allay thirst in people with fevers and also treats catarrh. The fruit juice is useful in the treatment of bilious affections and bilious diarrhoea. The fruit rind is carminative and tonic. The fresh rind is rubbed on the face as a cure for acne. The dried peel is used in the treatment of anorexia, colds, coughs etc[5]. This study was undertaken to evaluate the anthelmintic activity of petroleum ether, chloroform and alcoholic extract of seed coats of *Citrus sinensis* in adult Indian earthworms, *Pheretima posthuma* at different doses.

2. Materials and Methods

2.1 Plant material: Fruits of *Citrus sinensis* were collected from National Research Center for Citrus (NRCC), Nagpur and authenticated by Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

2.2 Extraction: The seed are taken out from the *Citrus sinensis* fruits. Then seed coats were removed from the seeds. The seed coats which are colorless and plastic in nature have been washed thoroughly with water, filtered at suction pump and dried at room temperature. The dried seed coats were then powdered using grinder to have uniform size. A 100 g of powder subjected to extraction by Soxhlet apparatus using various solvent such as petroleum ether, chloroform and alcoholic extract. The solvent was then removed under reduced pressure which gives a brownish-black color sticky residue. The prepared extracts were tested for anthelmintic activity.

2.3 Animals: Indian earthworm *pheretima posthuma* (Annelida, Megascolecidae) was used due to its anatomical and physiological resembalence with the intestinal roundworm parasites of human beings[6,7,8] for evaluating the

anthelmintic activity of crude extract using the reference substance for comparison. Earthworms were collected from the water logged areas of soil and identified at the Department of Zoology, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra.

2.4 Phytochemical screening: In order to determine the presence of alkaloids, glycosides, flavonoids, tannins, terpenes, sterols, saponins, fats and sugars a preliminary phytochemical study (color reaction) with various plant extract and fraction was performed.

2.5 Anthelmintic activity: For the anthelmintic activity of seed coats of *Citrus sinensis* fruits, Indian adult earth worms (*Pheretima posthuma)* of 3-5 cm in length and 0.2-0.4 cm in width were used. *Pheretima posthuma* are easily available and used as a suitable model for screening of anthelmintic drug was advocated earlier[9,10,11,12,13,14]. Samples for *in vitro* study were prepared by dissolving and suspending 2.5 g of crude methanolic extract in 25 mL of distilled water to obtain a stock solution of 100 mg/mL. From this stock solution different working dilutions were prepared to get concentration range of 10, 25 and 50 mg/mL. The anthelmintic assay was carried as per the method of Ajayieoba et al.[15] with minor modifications. 50 mL formulations containing three different concentrations, each of crude methanolic extract and its various fractions (10, 25 and 50 mg/mL in distilled water) were prepared and five worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^{o}C[16,17]. Piperazine citrate (10 mg/mL) was used as reference standard while distilled water was used as the control.

2.6 Statistical analysis: The data were analyzed using one way analysis of variance and p<0.05cosidered as statistical significance. All the results were expressed as a mean ± SEM of six animals in each group.

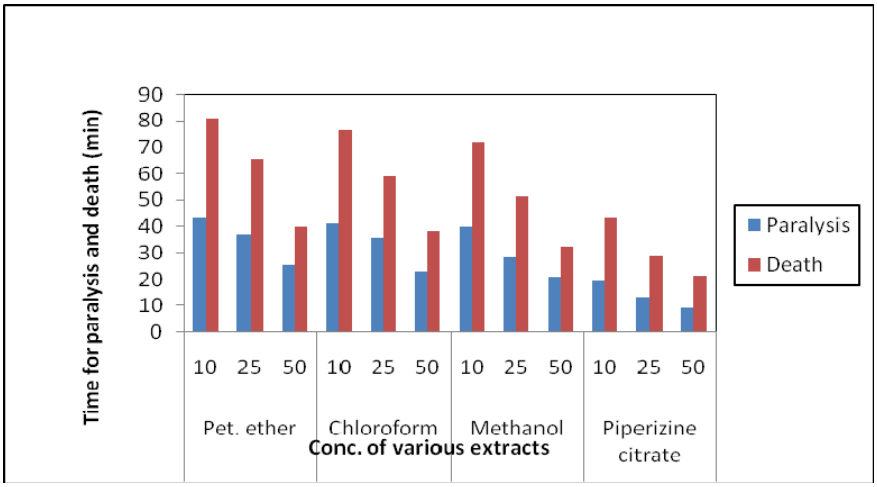
3. Result and Discussion

Preliminary phytochemical screening showed the presence of triterpenes, carbohydrates, tannins like phytoconstituents in the extracts of *Citrus sinensis* seed coats. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity when compared to the standard drug. The data revealed that methanolic extracts of seed coats of *Citrus sinensis* fruit showed significant anthelmintic activity at 50 mg/mL concentrations where as chloroform showed moderate activity and petroleum ether extract is having least anthelmintic activity. Results are comparable with standard drug piperazine citrate. Table 1 and figure 1 reveals that total methanolic extract of seed coats of *Citrus sinensis* showed best anthelmintic activity. All the values are expressed as mean ± SEM (n = 6). From the above results, it is concluded that all the extracts of *Citrus sinensis* seed coats have potent anthelmintic activity when compared with the conventionally used drug and is equipotent to standard anthelmintic drug. Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Citrus sinensis* seed coats as an anthelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituents responsible for anthelmintic activity.

Table 1. Anthelmintic activity of various extracts of *Citrus sinensis* seed coats

S.N.	Compounds	Concentration	Time taken for paralysis (P) and death (D) (Minutes)	
			P	D
1.	Pet. ether	10	43.16±0.61	80.52±0.41
		25	37.29±0.28	65.29±0.13
		50	25.53±0.41	40.15±0.59
2.	Chloroform	10	41.31±0.60	76.26±0.61
		25	35.42±0.81	58.93±0.57
		50	22.81±0.92	38.35±0.90
3.	Methanol	10	39.85±0.62	71.66±0.14
		25	28.26±0.23	51.19±0.83
		50	21.17±0.81	32.11±0.92
4.	Piperizine citrate	10	19.26±0.68	43.19±0.21
		25	13.19±0.21	29.13±0.23
		50	9.13±0.23	21.33±0.27

Figure 1. Anthelmintic activity of various extracts of *Citrus sinensis* seed coats



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