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Research Article

EVALUATION OF THE ANTHELMINTIC ACTIVITY OF CITRUS LIMON JUICE SACS



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Abstract

Citrus limon fruit empty juice sacs was investigated for its anthelmintic activity. Petroleum Ether, chloroform, and alcoholic extract of *Citrus limon* juice sacs were evaluated separately for anthelmintic activity on 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 a control group.

Keywords: - Citrus limon, Anthelmintic activity, *Pheretima posthuma*(Annelida), Piperazine citrate.

Introduction

Helminths are recognized as a major constraint to livestock production throughout the tropics and elsewhere [1]. The economic impact of parasitic gastroenteritis (PGE), which is caused by mixed infection with several species of stomach and intestinal round worms, as a production disease in ruminants lies not only in direct losses such as mortality associated with the clinical form of the disease but also indirect insidious losses as a result of weaknesses, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity [2]. Winrock International (1992) indicated that over \$4 billion is lost in animal productivity as a result of animal diseases, with over half of this loss due to internal parasites such as helminthes. In an effort to reduce losses due to effects of helminth parasites on livestock industry, approximately \$1.7 billion are spent annually worldwide in control measures [3]. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Most

diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Helminthiasis or infection with parasitic worms, affects over two billion people worldwide causing malnutrition, blindness, debility, disfigurement and death [4]. There has not been enough emphasis on the research efforts and consequently not enough new agents discovered in the last 30 years to cope with the spreading of parasitic infections. This study was undertaken to evaluate the anthelmintic activity of Petroleum Ether, chloroform, and alcoholic extract of in adult Indian earthworms, *Pheretima posthuma* at different doses.

Citrus limon (Family-Rutaceae) is cultivated throughout India in plains and hills in areas upto 1200m elevation. It is a much branched thorny shrub with spreading branches; leaves unifoliately compound, rachis winged, leaflet elliptic-oblong, alternate, coriaceous, entire or crenulate; flowers white in short racemes; fruits large, globerries with thick or thin rind, pulp pale, very acid; seeds many, horizontal, testa coriaceous. Only fruits are useful. The fruits are sour, thermogenic, digestive, carminative, stomachic laxative, anthelmintic, antiscorbutic, stimulant, antiseptic and mosquito repellent and are useful in vitiated conditions of kapha and vata, dyspepsia, flatulence, colic, constithornypation, anorexia, helminthiasis, scabies, fatigue, halitosis, vomiting trembling of the limbs, hemicrania, psychopathy phyryngopathy, cough, bronchitis and heartburn [5]. This study was undertaken to evaluate the

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anthelmintic activity of Petroleum Ether, chloroform, and alcoholic extract of *Citrus limon* empty juice sacs in adult Indian earthworms, *Pheretima posthuma* at different doses.

Material and Method:-

Plant materials:

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

Preparations of extract:

The peels, seeds and juice of the fruit were removed. The solid bio-mass was washed with distilled water filtered at suction pump and dried at room temperature. The dried solid bio-mass was then powdered using grinder to have uniform size. 100 gm 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 obtained a greenish-black colour sticky residue. The prepared extracts were tasted for anthelmintic activity.

Worms Collection and authentication:

Indian earthworm *pheretima posthuma* (Annelida) were collected from the water logged areas of soil and identified at the Department of Zoology, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra.

Phytochemical screening:

In order to determine the presence of alkaloids, glycosides, flavonoids, tannins, terpenes, sterols, saponines, fats, and sugars, a preliminary phytochemical study (colour reaction) with various plant extract and fraction was performed [6].

Study of anthelmintic activity:

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 E. O. et al with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the

intestinal roundworm parasites of human beings[7,8,9]. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug was advocated earlier [10,11,12]. 50 ml formulations containing three different concentrations, each of crude alcoholic 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 50⁰ C[13,14,15]. Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control.

Result and discussion:

Preliminary phytochemical screening has shown the presence triterpenes, carbohydrates, flavonoids in methanolic extracts of plants. The data revealed that methanolic extracts of seeds of the plant *Citrus limon* fruit empty juice sacs showed significant anthelmintic activity at 50 mg/ml concentrations whereas Chloroform, showed moderate activity and Petroleum ether extract is having least anthelmintic activity. Results are comparable with standard drugs Piperazine citrate. (Table 1) reveals that total methanolic extract of *Citrus limon* juice sacs showed best anthelmintic activity.

Table 1. Anthelmintic activity of *Citrus limon* empty juice sacs.

Sr.No	Extract	Conc (mg/ml)	Time (min) taken for paralysis (P) and for death of <i>Pheretima posthuma</i> worms (D) in min.	
			P	D
1)	Petroleum ether extract	10	64.39±0.24	73.05±0.81
		25	58.03±0.13	62.50±0.71
		50	26.44±0.29	32.20±0.21
2)	Chloroform extract	10	53.13±0.95	60.48±0.55
		25	49.75±0.82	53.15±0.16
		50	22.78±0.37	38.12±0.37
3)	Methanol extract	10	42.12±0.16	48.30 ±0.32
		25	15.02±0.12	36.25±0.22
		50	13.30±0.62	25.30±0.24
4)	Piperazine citrate	10	20.16±0.16	65.25 ±0.32

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