

**IN VITRO ANTHELMINTIC SCREENING COMPARISON OF
VARIOUS CRUDE EXTRACTS OF THE FRUITS OF CUCUMIS
TRIGONUS ROXB. AND CUCUMIS SATIVUS LINN**

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ABSTRACT

Anthelmintic activity of the various crude extracts (Petroleum ether (40°-60°C), benzene, chloroform, ethanol and water) of the fruits of *Cucumis trigonus* Roxb. and *Cucumis sativus* Linn. was evaluated on Indian adult earthworms, *Pherentima posthuma* and compared. Time of paralysis and time of death of the worms were considered as the parameters to assess the anthelmintic action. The results revealed that the ethanolic extracts of both the fruits of *Cucumis trigonus* and *Cucumis sativus* showed significant anthelmintic activity at a higher concentration level of 100 mg/ml on time taken for both paralysis and death of the worms when compared to other extracts. Hence the ethanolic extracts of the fruits of *Cucumis trigonus* and *Cucumis sativus* were also tested in various doses (80mg, 60mg, 40mg, 20mg).

The results revealed that the ethanolic extract of the fruits of *Cucumis trigonus* showed excellent anthelmintic activity compared to that of *Cucumis sativus*. Albendazole was used as standard drug.

Keywords: *Cucumis trigonus* Roxb; *Cucumis sativus* Linn; Anthelmintic activity; *Pherentima posthuma*; Albendazole.

INTRODUCTION

Anthelmintic resistance creates a major hitch over the decades throughout the world. As per WHO only few drugs are frequently used in the treatment of helminth infestations in human beings. Anthelmintics are drugs which may kill (Vermicides) or expel (Vermifuge) infesting helminthes^[1]. Helminthiasis is prevalent globally, but is more common in the developing countries with poorer personal and environmental hygiene^[2]. Number of synthetic drugs like albendazole, piperazine citrate, pyrantel, and mebendazole are used to control and prevent from the infection, used as broad spectrum anthelmintic drugs^[3]. Long term use of these synthetic drugs will cause various adverse effects like tolerance, vomiting drowsiness, dizziness, and abdominal pain^[4]. Therefore, search for new drugs is essential from natural source.

Cucumis trigonus (Fam. Cucurbitaceae) is commonly known as “Thummittikai” in Tamil, “Bitter gourd” in English, and “Vishala” in Sanskrit. The fruit pulp of *Cucumis trigonus* is bitter, acrid, thermogenic, anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting^[5]. *Cucumis sativus* (Fam.Cucurbitaceae) is commonly known as “Vellari” in Tamil, “Cucumber” in English, and “Sakusa” in Sanskrit. *Cucumis sativus* is amongst the constituents of cosmetics marketed as treatments for skin inflammations and as skin protectants^[6]. Pulp of the fruit is useful in dysenteric-diarrhoea, dropsy, piles and leprosy. In the present work, the anthelmintic activity has been studied on the various extracts of the fruits of *Cucumis trigonus* and *Cucumis sativus* and compared.

EXPERIMENTAL

Collection of plant materials

The fruits of *Cucumis trigonus* and *Cucumis sativus* were collected in the month of March and July respectively from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai- 600 045, Tamil Nadu, India (Reg.No of the certificate: PARC/2013/2048 & PARC/2013/2047).

The voucher specimens (MSU/PHAR/HER-140 and MSU/PHAR/HER-141) have been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012, Tamil Nadu, India.

Extraction of plant material

The collected fruits were cut into pieces, shade-dried at room temperature and powdered.

The dried fruit powders (500 g) were successively extracted using petroleum ether (40°-60°C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure distillation and then vacuum dried. The dried crude extracts were used for the study.

Anthelmintic activity

Earth Worm collection

Pheretima pothuma (Earth worm) of nearly equal size (6-8 cm length and 0.2- 0.3 cm width) were collected from Ella –Farm, Vermi-culture Department (old-Goa) and washed with normal tap water to remove all fecal matter. The earthworm resembles both anatomically and physiologically to the intestinal roundworms parasites of the human being, hence can be used to study the anthelmintic activity^[7].

Drugs and chemicals

Albendazole, and distilled water were used during the experimental protocol. Albendazole Gift sample obtained from vera's pharmaceuticals, A.P and all other chemicals were of analytical grade.

Experimental design

The anthelmintic activity was performed as per the method^[8] of Ghosh et al., (2005). The assay was performed on adult Indian earth worm (*Phertima posthuma*) due to its anatomical and physiological resemblance to the human intestinal roundworm parasites^[9-12]. Due to their ready availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*^[13-15]. The earth worms were divided into ten groups containing six earth worms in each group. Albendazole was used as standard reference and distilled water as control. All the extracts and standard drug solution were freshly prepared in double distilled water. Various extracts and standard drug solutions were poured in different petri plates. All the earth worms were released into 10 ml of formulation as follows: Control (double distilled water), Petroleum ether extract (100 mg), Benzene extract (100 mg), Chloroform extract (100 mg), Ethanol extract (20, 40, 60, 80, 100 mg), Aqueous extract (100 mg) of the fruits of *Cucumis trigonus* and Albendazole (15 mg). Observations were made for the time taken to paralysis and death of worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility when dipped in warm water (50°C)

followed with fading away of their body colors. Three sets of experiments were carried out for statistical significance.

RESULTS AND DISCUSSION

Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues. Helminthiasis or infections with parasitic worms are pathogenic for human beings^[9,10]. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Tannins produce anthelmintic activity by binding to free protein in the gastrointestinal tract of the host animal^[16] or glycoprotein on the cuticle of the parasite^[17] and phenolic compounds by uncoupling oxidative phosphorylation hinder the energy production in helminth parasites^[18] and may cause death. Alkaloids may act on central nervous system and cause paralysis of the earthworm^[19]. The effect would be due to presence of the steroidal alkaloid oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which could interfere in lical homeostasis which is essential for the development of helminthes^[20]. Phytochemical analysis of the fruits of *Cucumis trigonus* and *Cucumis sativus* revealed the presence of tannins, alkaloids and phenolic as compounds.

The results of anthelmintic activity of the ethanolic extract of the fruits of *Cucumis trigonus* and *Cucumis sativus* are presented in [Table 1].

Table 1: *In vitro* anthelmintic activity of various extracts of the fruits of *Cucumis trigonus* Roxb and *Cucumis sativus* Linn.

Group	Treatment	Concentrations (mg)	Time in minutes for paralysis / Death of worms			
			<i>Cucumis trigonus</i> Roxb.		<i>Cucumis sativus</i> Linn.	
			Paralysis	Death	Paralysis	Death
1	Control	-	-	-	-	-
2	Pet ether (40°-60°C) extract	100	29.85±0.22	74.57±0.54	35.74±0.53	78.59±0.82
3	Benzene extract	100	24.72±0.14	71.36±0.72	28.36±0.14	70.43±0.52
4	Chloroform extract	100	22.63±0.47	68.49±0.71	25.15±0.17	68.36±0.17
5	Ethanol extract	20	21.42±0.55	65.25±0.44	23.75±0.18	65.29±0.19
		40	19.41±0.11	60.44±0.75	22.18±0.19	61.75±0.84
		60	15.45±0.91	49.52±0.73	19.58±0.76	58.36±0.14
		80	12.96±0.49	44.25±0.41	16.75±0.21	47.11±0.82
		100	10.14±0.50	32.45±0.72	15.25±0.98	45.72±0.73
6	Water extract	100	13.47±0.29	41.48±0.53	18.47±0.52	58.82±0.74
7	Albendazole	15	8.17 ±0.22	18.16 ±0.46	8.17 ±0.22	18.16 ±0.46

Ethanollic extract of the fruits of *Cucumis trigonus* was most effective in causing death of earthworms at all concentrations than that of the *Cucumis sativus*. The effect was found to be more than the standard drug, albendazole. The rate of paralysis as well as death is found to be concentration dependent. 100 mg/ml dose exhibited higher anthelmintic activity. The decreasing order of anthelmintic activity is noticed when we move from high polar to less polar solvents. In the case of petroleum ether, benzene, and chloroform extracts, paralysis was caused earlier but the death time was found to be longer. Pet ether extract is the least active among four extracts. In the water extract death time was longer but shorter than that of the petroleum ether, benzene and chloroform extracts. But in the case of ethanol extract of *Cucumis trigonus*, the death time was longer at lower dose (20 mg) but shorter at higher doses (100 mg) than the *Cucumis sativus*. Anthelmintic effect of the various extracts of the fruits of *Cucumis trigonus* Roxb and *Cucumis sativus* Linn. against *Pheretima prosthuma* are presented in **Fig. 1.** and **Fig. 2.** respectively.

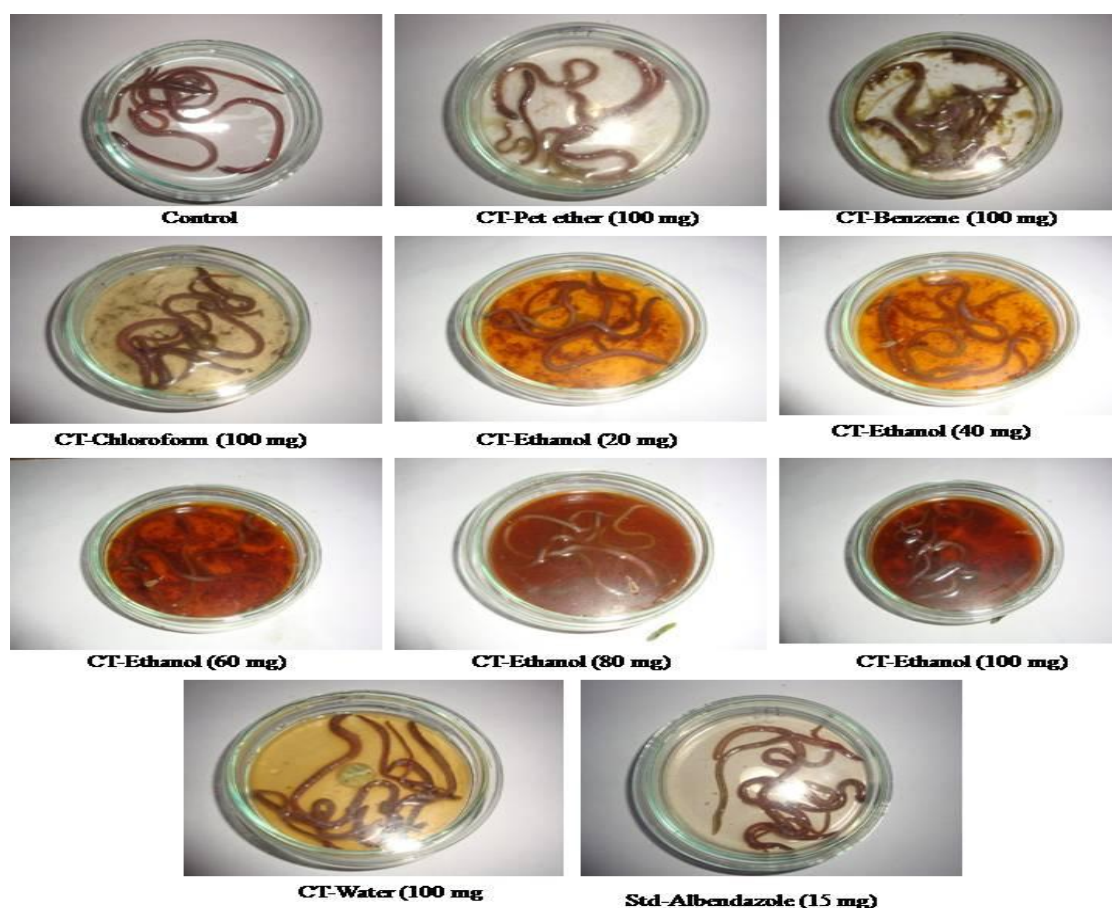


Figure1:Anthelmintic activity of the various extracts of the fruits of *Cucumis trigonus* Roxb.

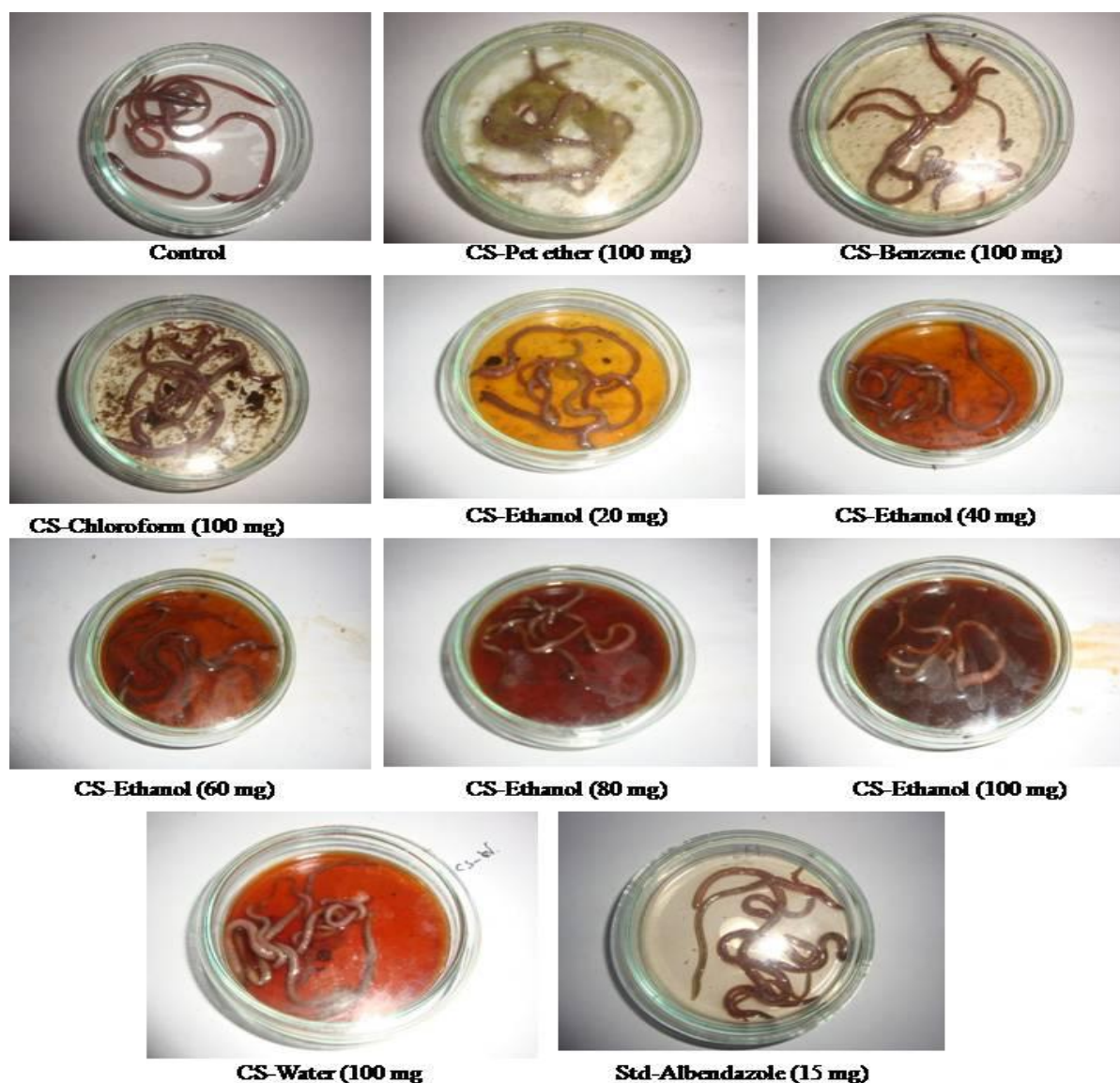


Figure 2:Anthelmintic activity of the various extracts of the fruits of *Cucumis sativus* Linn.

CONCLUSION

From the results it may be concluded that, all the extracts of the fruits of *Cucumis trigonus* and *Cucumis sativus* exhibited dose dependent anthelmintic activity against the earthworms, (*Pheretima prosthuma*) when compared to that of the standard drug, albendazole. The anthelmintic activity of the ethanolic extract of the fruits of *Cucumis trigonus* was found to be higher than that of the ethanolic extract of the fruits of *Cucumis sativus*. The results also indicate that the active constituents responsible for anthelmintic activity are present in ethanolic extract of the fruits of *Cucumis trigonus* and *Cucumis sativus*.

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