



**IN VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF
ETHANOLIC LEAF EXTRACT OF *THESPESIA POPULNAE* ON
INDIAN EARTHWORMS *EISENIELLA FETIDA***

**T. VINCIYA^{1*}, M. SUDHA², I. NOORUL ALAM³, N. HARIKRISHNAN⁴ AND S.
ABDUL SAMEER⁵**

1: Assistant Professor, Faculty of Pharmacy, Dr.M.G.R. Educational and Research Institute,
Chennai, India

2: Associate Professor, Faculty of Pharmacy, Dr.M.G.R. Educational and Research Institute,
Chennai, India

3: Associate Professor, College of Pharmacy, Mother Theresa Post Graduate and Research Institute
of Health Sciences, Puducherry, India

4: Professor, Faculty of Pharmacy, Dr.M.G.R. Educational and Research Institute, Chennai, India

5: Faculty of Pharmacy, Dr.M.G.R. Educational and Research Institute, Chennai, India

***Corresponding Author: Dr. T. Vinciya: E Mail: vinciya.pharm@drmgrdu.ac.in**

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ABSTRACT

The purpose of the current study was to assess the effectiveness of the anthelmintic properties of the leaves of *Thespesia populnae* against Indian earthworms (*Eiseniella fetida*). With varying doses, *Thespesia populnae* ethanolic leaf extract was utilised to test on *Eiseniella fetida* for anthelmintic activity. The earthworms were categorized into 7 groups, with Saline serving as the control (Group 1), Albendazole serving as the standard at 2.5 mg/ml, 5 mg/ml, and 10 mg/ml (Group 2,3,4), and TPEE serving as the test drug at 2.5 mg/ml, 5 mg/ml, and 10 mg/ml (Groups 5, 6, and 7). The plant showed significant anthelmintic activity but less potent than the standard drug. The phytoconstituents may have an anthelmintic action, according to these findings. Tannins was detected in the alcoholic *Thespesia populnae* leaf extracts. Hence, presence of tannins might display anthelmintic activity of TPEE. TPEE displayed anthelmintic activity depending on the dose.

Keywords: *Thespesia populnae*, in-vitro, Anthelmintic activity, Albendazole

INTRODUCTION:

Plant profile:

Thespesia populnea, commonly known as the **Portia tree**. **Pacific rosewood (Indian tulip tree, or milo)**, is a species of flowering plant belonging to the mallow family, *Malvaceae* [1]. It is a tree found commonly on coasts around the world. Although it is confirmed to be native only to the Old World tropics, other authorities consider it to have a wider, possibly pantropical native distribution. It is thought to be an invasive species in Florida and Brazil [2].

Leaf description:

Simple leaf, heart shaped with long stalk, stipules 4-10 mm long, free, lateral, linear to lanceolate, cauducous; petiole 5-10 cm, slender, swollen tipped, scaly; lamina 5-12.7 x 5.5-15 cm, orbicular or ovate, base cordate or truncate, apex acute or acuminate, margin entire or dentate, coriaceous, with peltate scales above, glabrescent or stellate-tomentose beneath; 5-7 nerved from the base, palmate, prominent, lateral nerves 4-5 pairs, pinnate, prominent, intercostae subscalariform, prominent, often a glandular pore in one or more of the intercostal spaces beneath [3].



Thespesia populnea

Treatment:

Albendazole was highly effective against *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Enterobius vermicularis*. Significant improvement was also observed in patients having infections due to *Trichuris trichiuria* [4].

The primary drugs used for cestode infections are albendazole and praziquantel. It is a broad-spectrum anthelmintic affecting both flukes and tapeworms. Diethylcarbamazine and ivermectin, used for treating filarial worm infections, are absorbed from the intestinal tract [5].

Benzimidazoles, macrocyclic lactones, levamisole, piperazine and amino-acetonitrile derivatives may be used to treat almost all these parasites and lower infection levels below clinical relevance. However, helminth resistance to these medications have been documented in certain publications, which is typical in the field of veterinary medicine [6].

Plants produce a broad spectrum of secondary metabolites or phytochemicals which aid in several biological activities including the defence of the plant against pests and diseases. The major classes of phytochemicals include phenolic, alkaloids, flavonoids and terpenoids compounds. These phytochemicals make some plants a good source of remedy for ailments [7].

Plant's secondary metabolites have been successfully used in ethnomedicine and are generally used for insecticide, pesticidal, molluscicidal, antimicrobial, antiparasitic and other ailments [8].

Drug resistance in helminths threatens human and animal health across the world. Understanding the genetics and mechanisms of helminth medication resistance is critical for preventing resistance to newly discovered anthelmintic treatments, reducing the dissemination of resistant parasites, and effectively managing parasite management at all phases of their lifecycle [9].

Resistance to gastrointestinal nematodes, as well as other parasitic worms such as liver fluke, has been shown to be high in ruminants. As a result, parasitological research into the causes of anthelmintic resistance is required in order to create new treatment techniques and medications for helminth control [10].

The major approach for discovering novel anthelmintic medications were centred mostly on screening new compounds using *in vitro* and *in vivo* test system.

Helminthiasis:

Helminthiasis, commonly known as **worm infection**, is a macro parasitic disease in which a part of the body

is infected with parasitic worms or helminths [11].

There are several species of parasites and they often reside in the digestive tract of the host body. Helminths either live as parasites, or free of a host, in aquatic and terrestrial environments. The most common worldwide are intestinal nematodes or soil-transmitted helminths (STH), schistosomes (parasites of schistosomiasis) and filarial worms, which cause lymphatic filariasis (LF) and onchocerciasis [12].

Soil-transmitted helminthiasis and schistosomiasis are the most important helminthiasis and are among the neglected tropical diseases. A parasitic worm's lifecycle may be quite complicated, with several hosts for different stages; also, a significant adaptation utmost parasitism of a worm is a complex lifecycle incorporating tropic transmission. Some helminths (soil-borne nematodes) such as Strongyloides and Hookworms have a free-living stage (Rhabditiform larvae) and a parasitic stage (Filariform larvae) that may need a different host or environment [13].

EXPERIMENTAL METHODS: [14]

The plant sample was washed with distilled water for several times and subjected to air dried under the shade. After drying they were ground by an electrical mixer until it become powder. About 160 g of dried sample powder was weighed and

extraction process is carried out in Soxhlet extractor by using 200 ml of ethanol in rotary shaker at 150 rpm at 25°C temperature for 72 h. The extracts were filtered using Whatman No.1 filter paper, the filtrate was concentrated by a rotary evaporator, and the residual ethanolic extract of *Thespesia populnea* (TPEE) were dried under vacuum desiccator, and stored for further use of phytochemical studies.

PRELIMINARY PHYTOCHEMICAL SCREENING: [15-19]

The preliminary phytochemical analysis of TPEE was carried out for the presence of terpenoids, flavonoids, steroids, anthraquinones, glycosides, carbohydrates, alkaloids, quinones, phenols, tannins, saponins, proteins and amino acids.

1. Test for alkaloids: Mayer's Test: To the extract, 2 ml of Mayer's reagent was added; formation of reddish-brown precipitate indicates the presence of alkaloids.

2. Test for saponins: To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

3. Test for tannins: To the extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

4. Test for cardiac glycosides: Keller-Killani test: To 1ml of the extracts, 2 ml of glacial acetic acid containing a drop of FeCl₃. Equal volume of conc. H₂SO₄ was

added from the sides of the tube. A brown color ring indicates the presence of cardiac glycosides.

5. Test for flavonoids: Alkaline reagent test: Extract was treated with 10% NaOH solution; formation of intense yellow colour indicates presence of flavonoid.

6. Test for phenols: Lead acetate test: The extract was taken; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

7. Test for steroids: 1 ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H₂SO₄ was added from the side of test tube. The upper layer turns red and H₂SO₄ layer showed yellow with green fluorescence This indicates the presence of steroid.

8. Test for terpenoids: Salkowski test: 5 ml of extract was mixed in 2 ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

9. Test for Quinones: The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of quinones.

10. Test for Proteins: Ninhydrin test: The extract was taken and few drops of freshly prepared Ninhydrin reagent was added and heated. The appearance of pink or purple

colour indicates that the presence of proteins, peptides or amino acids.

IN-VITRO ANTHELMINTIC ACTIVITY: [20]

The anthelmintic activity was tested on the Indian adult earthworm *Eiseniella fetida* using technique described by Ghosh et al. The standard medicine, Albendazole was diluted with normal saline to produce concentration of 2.5 mg/ml, 5 mg/ml and 10 mg/ml and placed onto Petri plates.

Ethanollic extracts (TPEE) were diluted with normal saline to reach concentrations of 2.5 mg/ml, 5 mg/ml and 10 mg/ml. The negative control was plain saline (0.9 percent NaCl). All of these dilutions were carefully put onto the petri dishes. The study used six earthworms (n = 6). At room temperature, earthworms roughly identical size (approximately 8cm) was inserted in each Petri dish.

The time for paralysis was recorded when no movement of any kind

could be detected, save when the worms were severely agitated. After determining that the worms did not move when shook forcefully or dunked in warm water, the time of death was recorded (50°C). The paralysis and fatal times were measured in minutes.

RESULTS AND DISCUSSION:

The phytochemical screening of ethanolic leaf extract of *Thespesia populnae* revealed the presence of steroids, cardiac glycosides, tannins, terpenoids, and quinones (**Table 1**).

IN-VITRO ANTHELMINTIC ACTIVITY:

In-vitro anthelmintic activity was measured by both the paralysis and fatal times. The **Table 2** shows the paralysis and death times of both standard and ethanolic extracts, as well as the control. The study found that both standard and ethanolic extract of *Thespesia populnea* exhibited substantial efficacy at higher concentrations (10mg/ml).

Table: 1 Preliminary phytochemical screening of TPEE

TEST	TPEE
Alkaloids	-
Flavonoids	-
Saponins	-
Tannins	+
Phenols	-
Cardiac glycosides	+
Steroids	+
Terpenoids	+
Quinones	+
Proteins	-

+ Present; - Absent

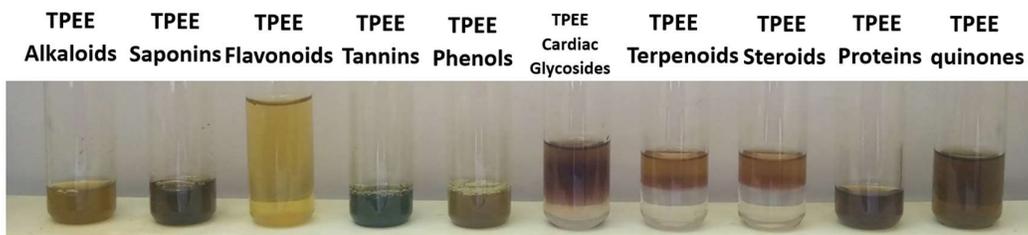


Figure 2” Phytochemical constituents of TPEE



Figure 3a: Control saline



Figure 3b: Albendazole 2.5mg/ml



Figure 3c: Albendazole 5mg/ml



Figure 3d: Albendazole 10mg/ml



Figure 3e: Ethanolic extract 2.5mg/ml



Figure 3f: Ethanolic extract 5mg/ml



Figure 3g: Ethanol extract 10mg/ml

Figure 3: Photographs of In vitro anthelmintic activity of TPEE

Table :2 Anthelmintic activity of Ethanol extract of *Thespesia populnae* on Indian earthworms (*Eiseniella fetida*)

S. No.	Treatment	Time taken for paralysis	Time taken for Death
1.	Control (Saline water)	0.00	0.00
2.	Albendazole (2.5mg/ml)	53.80 ± 3.45	110.38 ± 3.45
	(5 mg/ml)	46.62 ± 5.31	100.61 ± 1.34
	(5 mg/ml)	35.92 ± 2.34	84.32 ± 4.76
3.	Ethanol extract (2.5 mg/ml)	80.15 ± 2.64	145.62 ± 2.55
	(5 mg/ml)	70.61 ± 1.90	138.73 ± 4.73
	(5 mg/ml)	52.64 ± 3.46	118.42 ± 2.61

The three concentrations of extract TPEE viz 2.5 mg/ml, 5 mg/ml, and 10 mg/ml were subjected to anthelmintic activity with Albendazole as standard drug. Albendazole at 2.5mg/ml paralyzed the worm at 53.80 ± 3.45 minutes and caused death at 110.38 ± 3.45 minutes. The ethanol extract showed a close range of paralysis and death similar to albendazole at 10mg/ml. At this concentration the worms paralyzed at 52.64 ± 3.46 minutes and death at 118.42 ± 2.61 minutes. From the observations made, higher concentration of extract TPEE showed anthelmintic activity and based on the dose-dependent manner, it was giving shortest time of paralysis (P) and death (D).

The specific phytoconstituents may have an influence on the *in vitro* anthelmintic action. A number of studies conducted with

nematode parasites of ruminant livestock have demonstrated that direct anthelmintic effects of extracts from tannin-containing plants in *in vitro* assays, with *in vivo* verification of these results [21, 22]. The alcoholic *Thespesia populnae* leaf extracts consist of tannins, which might be the cause of *in vitro* anthelmintic action.

CONCLUSION:

The whole plant extract of *Thespesia populnae* was subjected to anthelmintic activity. The ethanol extract showed a good anthelmintic property. The phytoconstituents were also investigated and analysed. Albendazole was used as a standard drug for anthelmintic activity. The ethanol extract showed a moderate paralysis and death in the worms. These results indicate that the phytoconstituents may possess an anthelmintic

activity. The anthelmintic effect of the extract *in-vitro* may either be the effect of the individual phytoconstituents. The presence of tannins in the alcoholic *Thespesia populnae* leaf extracts may be responsible for the *in vitro* anthelmintic effect of the TPEE.

REFERENCE:

- [1] https://en.wikipedia.org/wiki/Thespesia_populnae
- [2] https://dbpedia.org/page/Thespesia_populnae
- [3] <https://indiabiodiversity.org/species/show/231322>
- [4] Misra PK, Pande NK, Jagota SC. Albendazole in the treatment of intestinal helminthiasis in children. *Current Medical Research and Opinion* 1985 9(8) 516–519. doi:10.1185/03007998509109627
- [5] <https://www.britannica.com/science/anthelmintic>
- [6] Holden-Dye L, Walker RJ. Anthelmintic drugs and nematicides: studies in *Caenorhabditis elegans*. In: *WormBook: The Online Review of C. elegans Biology* [Internet]. Pasadena (CA): WormBook; 2005-2018. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK116072/>
- [7] Divekar PA, Narayana S, Divekar BA, Kumar R, Gadratagi BG, Ray A, Singh AK, Rani V, Singh V, Singh AK, Kumar A, Singh RP, Meena RS, Behera TK. Plant Secondary Metabolites as Defense Tools against Herbivores for Sustainable Crop Protection. *Int J Mol Sci.* 2022 Feb 28;23(5):2690. doi: 10.3390/ijms23052690. PMID: 35269836; PMCID: PMC8910576.
- [8] Al-Snafi, Ali. Antiparasitic, antiprotozoal, molluscicidal and insecticidal activity of medicinal plants (part 2) – plant based review. *Scholars Academic Journal of Pharmacy.* 2016 5. Doi:10.21276/sajp.2016.5.6.1.
- [9] Fissiha W, Kinde MZ. Anthelmintic Resistance and Its Mechanism: A Review. *Infect Drug Resist.* 2021 Dec 15 14 5403-5410. doi: 10.2147/IDR.S332378. PMID: 34938088; PMCID: PMC8687516.
- [10] Shalaby HA. Anthelmintics Resistance; How to Overcome it? *Iran J Parasitol.* 2013 Jan 8(1) 18-32. PMID: 23682256; PMCID: PMC3655236.
- [11] <https://en.wikipedia.org/wiki/Helminthiasis#:~:text=Helminthiasis%2C%20also%20known%20as%20worm,tapeworms%2C%20flukes%2C%20and%20roundworms>
- [12] Mascarini-Serra L. Prevention of Soil-transmitted Helminth Infection. *J Glob Infect Dis* 2011 Apr 3(2) 175-82. doi:

- 10.4103/0974-777X.81696. PMID: 21731306; PMCID: PMC3125032.
- [13] Keiser PB, Nutman TB. *Strongyloides stercoralis* in the Immunocompromised Population. Clin Microbiol Rev 2004 Jan 17(1) 208-217. doi: 10.1128/CMR.17.1.208-217.2004. PMID: 14726461; PMCID: PMC321465.
- [14] Ali, Mortadha A, Tahseen A. Al-Hattab, Imad A Al-Hydary. Extraction of date palm seed oil (*Phoenix dactylifera*) by soxhlet apparatus. International Journal of Advances in Engineering & Technology 8.3 (2015): 261.
- [15] Bate-Smith EC. The phenolic constituents of plants and their taxonomic significance. Dicotyledond J Linn Soc Bot 1962 58 95-173.
- [16] Yadav M, Chatterji S, Gupta SK, Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci 2014 6(5) 539-42.
- [17] Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies 2020 Mar 8(2) 603-8.
- [18] Bhandary SK, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. Journal of Health and Allied Sciences NU 2012 Dec 2(04) 34-8.
- [19] Mungole AJ, Awati R, Chaturvedi A, Zanwar P. Preliminary phytochemical screening of *Ipomoea obscura* (L): A hepatoprotective medicinal plant. International Journal of PharmTech Research 2010 Oct 2(4) 2307-12.
- [20] Yadav AK, Tangpu V. Anthelmintic activity of ripe fruit extract of *Solanum myriacanthum* Dunal (Solanaceae) against experimentally induced *Hymenolepis diminuta* (Cestoda) infections in rats. Parasitology research 2012 Feb 110(2) 1047-53.
- [21] Hoste H, Martinez-Ortiz-De-Montellano C, Manolaraki F, Brunet S, Ojeda-Robertos N, Fourquaux I, Torres-Acosta JF, Sandoval-Castro CA. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. Vet Parasitol 2012 May 4 186(1-2) 18-27. doi: 10.1016/j.vetpar.2011.11.042.

Epub 2011 Nov 20. PMID:
22188981.

- [22] Nasasira M, Dominguez-Montero G, Betancourt-Valladares M. Antihelminthic activity of crude saponins and tannins isolated from *Tetradenia riparia* leaf extracts. *Revista Cubana de Plantas Medicinales* 2018 24 (1).