

Phytochemicals Screening and Anthelmintic Activity of Some Therapeutic Plants Against *Pheretima posthuma*

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DOI: 10.18811/ijpen.v11i02.04

ABSTRACT

Anthelmintic drugs refer to medicines used to indulge parasitic helminth worm infections in humans or animals. Anthelmintic drugs kill parasites by attaching themselves to muscle and nerve cells, which paralyzes and ultimately kills the parasite; preventing the cells from transporting glucose and preventing the development of new worms from adult worms. The objective of the present work is to examine the *in vitro* anthelmintic action of ethanolic extracts of three therapeutic plants such as *Tagetes erecta*, *Curcuma longa*, and *Laurus nobilis*, wherein *Pheretima posthuma* was used as a test worm. Phytochemical constituents of plants' ethanolic extracts were determined following standard procedure. All three plant extracts have shown antioxidant properties. All the plant extracts were applied *in vitro* against *Pheretima posthuma*, and the times of paralysis (ToP) and death (ToD) of the earthworm were determined. Albendazole was used as a reference compound, and distilled water was used as a control. The ethanolic extract of the rhizome of *Curcuma longa* showed a notable anthelmintic effect, as demonstrated by the worms' least paralyzing time and death time compared to other studied plant extracts. Every crude extract displayed potential anthelmintic activity in a concentration and time-dependent manner at all dosage levels. Of all the extracts, the maximum concentration at 15 mg/mL exhibited considerably ($p < 0.05$) higher nematocidal activity when compared to the control. All of the plants under investigation were shown to be effective against *Pheretima posthuma*, and further pharmacological and toxicological studies of the plants would be beneficial in the development of novel herbal anthelmintic medications.

Highlight:

- The therapeutic plant extracts that were chosen contained secondary metabolites like alkaloids, flavonoids, tannins, saponins, glycosides, etc.
- Extracts from *Curcuma longa* have shown promising *in vitro* anthelmintic action against *Pheretima posthuma*.
- The activity of the *curcuma* extract was almost as strong as that of the reference drug, such as albendazole.
- The anthelmintic properties and the presence of certain phytochemicals in the studied plants indicate the possibility of natural deworming agents.

Keywords: Helminth, Anthelmintic, Herbal Medications, Phytochemical.

International Journal of Plant and Environment (2025);

ISSN: 2454-1117 (Print), 2455-202X (Online)

INTRODUCTION

Helminths are parasitic worms that can afflict humans and other animals. Infections caused by helminths have been observed to be among the most prevalent illnesses in humans, affecting an important portion of the worldwide population (Idris *et al.*, 2019). Helminth infection is quite common in tropical and subtropical areas of the world, and it is expected to rise due to climate change (Weaver *et al.*, 2010). The infection is more frequent in communities with inadequate social infrastructure and poor sanitation. Additionally, it has been shown that helminths acclimatize and change in response to alterations in the climate, and resistance to medication has been found (Tinsley 2011). Their infections posture a serious risk to human health and cause growth retardation in both humans and animals. Most of the helminths inhabit the animals' gastrointestinal systems; however, some also live in tissues, or their larvae move into the tissues of the host. Therefore, they cause harm to the host by withholding food at the alimentary canal, shedding blood, injuring organs, obstructing lymphatic flow, and secreting toxins (Kumar *et al.*, 2014). Helminthic parasites are a serious risk to public health in underdeveloped nations, where they also increase the incidence of pneumonia, eosinophilia, anemia, and malnutrition (Ulrich and Schmid-Hempel 2015). Drugs used to treat parasitic illnesses, such as helminth infections and control, are known as anthelmintics. These drugs work by either

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How to cite this article: Sahu, P.M., Gayatri, T., Padhy, R., Das, M. (2025). Phytochemicals Screening and Anthelmintic Activity of Some Therapeutic Plants Against *Pheretima posthuma*. *International Journal of Plant and Environment*. 11(2), 259-264.

Submitted: 04/11/2024 **Accepted:** 15/05/2025 **Published:** 30/06/2025

eliminating the parasites or forcing them to leave the host's body. The usual side effects of anthelmintic medications include nausea, diarrhea, and stomach pain (Coakley *et al.*, 2016). Thus, there is an immediate need to find novel compounds because it has been seen that the parasites are becoming less sensitive to the anthelmintic medications now available on the market. Around the world, folk ethnic groups use a variety of medicinal herbs that have been demonstrated to have anthelmintic action in modern medicine. According to past research, certain plants exhibit good anthelmintic action, including *Combretum molle* (Ademola and Eloff 2010), *Zingiber officinale* (Ghafari *et al.*, 2021),

Ziziphus nummularia (Bachaya *et al.*, 2009), *Azadirachta indica* (Batool *et al.*, 2009), *Chenopodium album* (Choudhary *et al.*, 2021), and others.

Tagetes erecta, commonly called marigold, is a decorative plant found in India. This herb originated in Mexico and spread worldwide from the continent (Politi *et al.*, 2017). The plant has gorgeous, fragrant flower blooms in the winter. Due to its excellent healing abilities, marigold is widely used in medicine to treat wounds, inflammation, and skin disorders. Marigold tincture is an effective treatment for various skin conditions, such as burns, cuts, bruises, and irritated skin. Because of its antifungal qualities, it can effectively cure ringworm, athlete's foot, and *Candida albicans* (Kashif *et al.*, 2015). Additionally, they are used to treat dermatitis, allergic responses, and insect bites. The plant is effective in curing conjunctivitis and lowering inflammation and redness in the eyes due to its high content of antioxidants such as lutein, zeaxanthin, and lycopene (Politi *et al.*, 2016). The petals of marigolds are used in sitz baths to treat hemorrhoids, mend stitches from delivery, and treat vaginal and bladder infections since it is thought to have some antispasmodic properties.

Curcuma longa L., also known as turmeric, is a rhizomatous herbaceous perennial plant used in traditional medicine to manage, prevent, and treat a wide range of illnesses. *Curcuma longa* has several medicinal applications since its anti-inflammatory, anti-cancer, antidiabetic, anti-diarrheal, antibacterial, antiviral, and antioxidant potentials (Verma *et al.*, 2018). Since ancient times, curcumin, a yellow polyphenolic pigment derived from the rhizome of turmeric, has been utilized in food coloring and cooking and in various medical applications. Today, curcumin has been extensively used in the culinary and biotechnology sectors due to its possessing of anti-inflammatory, neuroprotective, hepatoprotective, cardioprotective, antioxidant, and anticancer properties that are relevant to health promotion and illness prevention (Rajkumari and Sanatombi 2017).

The evergreen tree known as bay laurel (*Laurus nobilis*) belongs to the Lauraceae family. It may typically grow to a height of 2 to 5 meters, but under highly favorable circumstances, it has been reported to reach as high as 20 meters. The leaves are leathery and lanceolate and have lustrous top surfaces and matted undersides. The bark is smooth and reddish brown. Because of their fragrant properties, the leaves and fruits are used. The plant is mostly used to relieve discomfort for specific disorders, including bronchitis, flu, and upper respiratory tract infections, and to increase hunger by secreting more digestive juices (Dobroslavic *et al.*, 2022). The fruits are believed to induce abortions, and the extract of leaves has been used to start menstruation, to treat dandruff, and as a compress for sprains and bruises. It possesses insect-repelling abilities, and dried leaves can be used to make fresheners to protect against insects (Yilmaz Deniz 2017). Moreover, since the leaves release their flavor gradually, they should be used sparingly when cooking.

Synthetic anthelmintic medications are typically administered to treat helminthic issues. They take the medications orally. The medications frequently cause headaches, nausea, and stomachaches as side effects. Suppression of bone marrow is one of the potentially dangerous side effects.

Finding some possible plants that could be employed as herbal anthelmintic medications was the goal of the current study. Thus, with the three plants above having enormous therapeutic potential in mind, an *in vitro* study is carried out to determine the plants' effectiveness as anthelmintic potential.

MATERIALS AND METHODS

Collection of plant samples and authentication

The fresh and disease-free plant materials of three species, namely *T. erecta*, *C. longa*, and *L. nobilis*, were collected in and around campus areas of GIET University, Gunupur, Odisha. Then, a taxonomist of the Botany Department at Berhampur University identified the collected plants. The voucher herbarium specimens of each of the three species were kept at the university for forthcoming use.

Plant Extract Preparation

Parts of the plants, such as flowers of *T. erecta*, rhizomes of *C. longa*, and leaves of *L. nobilis*, were considered for the anthelmintic study. After thoroughly cleaning the fresh plant materials of each plant under running water to get rid of any dirt, fungi, and other foreign objects, the materials were left to dry for five days in the shade. With the aid of a grinder, about 1000 g of each shade-dried plant material was ground into a powder and stored in an airtight container. After that, a Soxhlet apparatus was used to carry out the solvent extraction of the phytocompounds. Using ethanol as a solvent, about 50 g of powdered plant material was placed into a filter paper cover, and extraction was carried out till the extractor siphon tube's solvent became colorless. Each plant material undergoes the same process. Using Whatman No. 1 filter paper, the extracts were filtered. Lastly, a dried extract was obtained by freeze-drying the filtrate in a lyophilizer while it was prior concerted on rotavapor at 40°C with lower pressure. Once the dried extracts were ready, they were put into vials and refrigerated until needed later. Following that, the phytoconstituents of the extracts were first analyzed, and then their anthelmintic abilities were assessed *in-vitro*.

Phytoconstituent analysis

The conventional procedures were used to evaluate all extracts for the presence and absence of several phytochemicals. Standard techniques were utilized to conduct a first examination of the extracts to detect the existence of different phytoconstituents. The presence of alkaloids was determined by Mayer's test (Patil *et al.*, 2023), flavonoids by Shinod's test (Pandey and Tripathi 2014), glycosides by Keller Killiani test, tannins, and phenolic chemicals by ferric chloride test, presence of saponins by sodium carbonate test, steroids by Salkowski test, terpenoids by Horizontal test and phlorotannins by HCl test (Ingle *et al.*, 2017). The antioxidant activity of the extract was measured following the standard method with little modifications (Christel 2000). Then absorbance was measured at 230 nm contrary to a blank and a measure of the hydrogen peroxide scavenging percentage was taken. by using the following formula:

$$\% \text{ Scavenged } [H_2O_2] = [(Ac - As) / Ac] \times 100$$

Ac = Control's absorption and As = Sample absorbance.

Collection of *Pheretima posthuma*

Pheretima posthuma (Annelida), an adult Indian earthworm, is anatomically and physiologically analogous to the roundworm parasite that lives in the human gastrointestinal tract. It was utilized to assess the anthelmintic properties of several plant extracts. Earthworms with an average size of 5 to 6 cm and free of disease were collected from the vermicompost division, department of agriculture at GIET University. All excrement was eliminated by carefully washing the worms in steady saline water. To provide them with a stress-free habitat, all of the worms were kept in a moist, shaded area before the experiment.

Evaluation of anthelmintic property

Plant parts were utilized for the *in vitro* assessment of their anthelmintic properties, including the leaves of *Laurus nobilis*, the rhizomes of *Curcuma longa*, and the flowers of *Tagetes erecta*. All of the previously obtained dried extracts are diluted to 5 mg/mL, 10 mg/mL, and 15 mg/mL with 5% dimethyl sulfoxide (DMSO). 20 ml of various test solutions were added to a petri dish containing ten earthworms of roughly equal size in each group. The same number of worms was presented to an individual Petri dish for each concentration. A concentration of 10 mg/mL of albendazole was applied as a reference anthelmintic drug, and 5% dimethyl sulfoxide in distilled water was used as a control. Throughout the entire experimental process, freshly made plant test solutions and standard drug solutions were applied. It was observed how long it took for each earthworm in the Petri dish to become paralyzed and die. The total time needed for each treatment during which the earthworms in the Petri dish did not move aside from shaking is referred to as the paralyzing time. In a similar vein, the absence of movement and the fading of the body color of the earthworm in the Petri dish was considered death to the worms, and the time required to reach that stage is considered death time (Sisay *et al.*, 2012).

Data Analysis

The experimental data were collected carefully, properly prepared, and analyzed using SPSS Version 20. Tukey's HSD multiple comparison was performed after a one-way ANOVA

was employed to assess the outcomes of the anthelmintic assays. The study of earthworm paralysis and death assay results was expressed using descriptive statistics (mean \pm SEM). A statistically significant p-value was defined as p less than 0.05.

RESULTS AND DISCUSSION

Plant materials such as *Tagetes erecta* flowers, *Curcuma longa* rhizomes, and *Laurus nobilis* leaves were utilized. Table 1 provides information about the various plant species, their families, parts that are working for research, and the medicinal potentials of the plant. All three of these plants used in the anthelmintic study are very therapeutic and have significant ethnobotanical value.

Using ethanol as a solvent, the phytochemicals found in plant parts such as *Tagetes erecta* flower, *Curcuma longa* rhizome, and *Laurus nobilis* leaf were extracted. Table 2 shows the investigated plants' yield percentages of solid extracts of phytochemicals utilizing ethanol as a solvent. *Tagetes erecta* flowers had the lowest total phytoconstituent (35%) and the highest total phytoconstituent (68%) in the leaves of *Laurus nobilis* leaf. Several phytoconstituents were found in the plant extracts when qualitative preliminary secondary metabolite screening was performed.

The phytochemicals found in the plant extracts, including triterpenoids, alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins, steroids, and phlorotannins, were analysed using established techniques, and these phytochemicals are found in varying amounts in all plants. However, steroids and phenolic compounds are not detected in the petals of *Tagetes*, triterpenoids and phlorotannins in the rhizome of *Curcuma*, and saponin and phlorotannins in the leaves of *Laurus nobilis*. Different plants have varying concentrations of secondary metabolites in their parts (Table 3).

Plants yield compounds known as secondary metabolites that help them thrive in their particular environments. There are medicinally useful secondary metabolites found in some plants (Sahoo *et al.*, 2024). Both the plant and other living things are impacted by these secondary metabolites in a variety of ways. They support perennial growth, promote flowering,

Table 1: The details of the plant species, their families, parts used for study, and medicinal properties used in the nematocidal study

Species	family	English Name	Local name	Parts used for study	Medicinal properties
<i>Tagetes erecta</i>	Asteraceae	marigold	Genduphula	flower	stomach problems, coughs, colds, and many other conditions
<i>Curcuma longa</i>	Zingiberaceae	Turmeric	Haladi	rhizome	cancer, diabetes, Arthritis, diarrhoea, inflammation, psoriasis, hepatobiliary diseases, gastric and peptic ulcers
<i>Laurus nobilis</i>	Lauraceae	bay laurel	Teejpatta	leaves	anti-fungal, diuretic, bronchitis and to stimulate appetite

Table 2: Percentage yield of the test plants using ethanolic as extraction process

Plant name	Sample taken in gram	Yield in gram	% yield in (W/W)
<i>Tagetes erecta</i>	100	35	35
<i>Curcuma longa</i>	100	54	54
<i>Laurus nobilis</i>	100	68	68

Table 3: Phytochemical constituents of ethanolic extracts of plants such as *Tagetes erecta*, *Curcuma longa* and *Laurus nobilis*

Secondary metabolites	<i>Tagetes erecta</i>	<i>Curcuma longa</i>	<i>Laurus nobilis</i>
Alkaloids	++	+++	+++
Flavonoids	+	++	+++
Glycosides	++	+++	++
Phenolic compounds	-	+	+
Tannins	+	+	++
Saponins	++	++	-
Steroids	-	+	++
Triterpenoids	++	-	+
Phlobatannins	+	-	-
antioxidant property	+	+++	++

Note: +++: highest constituent; ++: moderate constituent; +: least constituent; —: not found.

Table 4: *In vitro* nematocidal examination of crude extracts of *Tagetes erecta*, *Curcuma longa* and *Laurus nobilis*. (Values expressed as average (n=3) ± SEM)

Plant	Treatment Conc. (mg/ml)	<i>Pheretima posthuman</i> (Test Organisms)	
		Paralyzing time (min)	Death time (min)
Distilled water	-	90	120
<i>Tagetes erecta</i>	5	58.66±0.67*	80.66±0.22
	10	32±0.34	50±0.81*
	15	25.33±0.53*	38.33±0.17 ^{ns}
<i>Curcuma longa</i>	5	55.33±0.63*	72.33±0.32
	10	26±0.80*	35±0.24
	15	20.33±0.33	30.30±0.56
<i>Laurus nobilis</i>	5	62.33±0.52*	88.33±0.29
	10	47.56±0.46	80.23±0.67*
	15	37.33±0.36	54.33±0.66*
albendazole	10	12±1.16	20±1.76

Significant: *P<0.05, as compared to standard; ns= not significant

**Fig 1:** *In vitro* effect of plant extracts on paralysis and death of *Pheretima posthuma* a. control ; b: *Tagetes erecta* flower extract; c: *Curcuma longa* rhizomes extract; d: *Laurus nobilis* leaves extract; e: Albendazole, the reference drug. (a-e : paralyzed worms; a₁,e₁ : died worms).

fruit formation, and abscission, or specify deciduous behavior. They serve as repellents or, on the other hand, attractants and antimicrobials (Twaij and Hasan 2022). Plant secondary metabolites are the source of many contemporary medications as well as medicinal herbs. A phytochemical examination of a chosen medicinal plant that is both qualitative and quantitative is important to establish the medicinal properties of the plant (Sahoo *et al.*, 2024). All three aqueous extracts of plants confirmed antioxidant properties; however, compared to the other two extracts, the *Laurus nobilis* extract exhibits lower activity. Higher antioxidant properties are shown in the extracts of *Tagetes erecta* and *Curcuma longa*. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) radicals like superoxide, hydroxyl, and nitric oxide can oxidize cell lipids and proteins and damage DNA in biological systems. (Bassil *et al.*, 2005). Medicinal plants and food contain a wide variety of naturally occurring antioxidants. The advantages these naturally occurring antioxidants provide for biology, particularly those of

polyphenols and carotenoids, are numerous and include anti-inflammatory, anti-aging, anti-atherosclerosis, and anti-cancer properties (Changa *et al.*, 2002). The mean paralysis and death time caused by the different plant compounds against adult earthworms is displayed in Table 4.

Each plant extract was treated to the earthworms in the petri plates at three different concentrations, such as 5 mg/l, 10 mg/l, and 15 mg/l. The drug applied as a reference was albendazole at a concentration of 10 mg/ml, while the control was 5% DMSO in distilled water. The results of the experiment indicate that each of the three plants exhibited anthelmintic activity. The paralyzed and death times of treated earthworms were reliable on the extracts of all plants at 10 mg/l and 15 mg/l, but the *Curcuma long* extract (26±0.80) revealed a time that was extremely close to the reference drug (12± 1.16). The concentration of 15 mg/l of all extracts had the shortest time of both paralysis and death of the earthworm (Fig. 1).

Earthworms treated with *Laurus nobilis* leaf extract established the least effect, with a relatively high duration of paralysis time (47.56 ± 0.46) and death time (80.23 ± 0.67). Due to its pure form and commercial recommendation as an anthelmintic medicine, albendazole was found to be more potent at a concentration of 10 mg/ml.

CONCLUSION

Medicines that cure illnesses caused by helminth parasites in humans or animals are collectively referred to as anthelmintic drugs. They can also be referred to as vermifuges, those that kill helminths, or vermifuges, those that stun the worm. The condition known as helminthiasis is treated with anthelmintic drugs in patients infected with helminths. The results of this investigation demonstrated that the three medicinal plants have various secondary metabolites that may serve as anthelmintic sources. In addition, *Curcuma longa* extracts have demonstrated encouraging *in vitro* anthelmintic activity against *Pheretima*

posthuma, hence supporting the traditional usage of these herbs as anthelmintics. The *in vitro* anthelmintic activity of the investigated plants is considered by the paralyzing and death time of *Pheretima posthuma*, and the effectiveness of all plant extracts increases with increasing concentration and span of exposure. It is, therefore, suggested that the plant may help manage ruminant and human gastrointestinal parasites. This approach is essential in the battle against helminth infections, especially those that have developed treatment resistance, since it closes the gap between conventional empirical information and scientific confirmation of the products. To further assess the anthelmintic activity of these plants, fractionation of the crude extracts and compound isolation will be necessary, which will result in the creation of herbal anthelmintics.

ETHICAL APPROVAL

This investigation has been approved by the Research and Ethical Committee of GIET University, Gunupur.

CONFLICTS OF INTEREST

The authors of this work state that they have no competing interests.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Dean, School of Agriculture, GIET University, Gunupur for providing earthworms for the study.

AUTHOR CONTRIBUTIONS

PMS, TG: conceptualization, writing-original draft, formal analysis and editing.

MD, RP: conceptualization, data curation, review and editing, fund acquisition.

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