

## Research Article



## Comparative Anthelmintic Activity and Phytochemical Evaluation of *Tridax procumbens* Linn Whole Plant Extract and *Piper nigrum* Linn Seed Extract on Indian Adult Earthworm

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### ABSTRACT

The aqueous and ethanolic extract of *Tridax procumbens*, linn (Asteraceae) and *Piper nigrum*, linn (Piperaceae) (commonly known as Coat Buttons and black Pepper respectively) were evaluated for Anthelmintic activity. The dried powder of *Tridax procumbens* (leaves, stems, roots and flowers) containing chemical constituent procumbent in and Black pepper (dried fruit) containing chemical constituent piperine were extracted and the activity was studied. Both aqueous and ethanolic extract collected were screened for preliminary phytochemical studies and also tested for Anthelmintic activity against Indian adult earthworm *Pheritima posthuma* (Annelida) and recorded the time taken for induction of paralysis and death. Various concentration (25 mg/ml, 50 mg/ml and 75 mg/ml) of aqueous and ethanol were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Albendazole suspension (25mg/ml) was used as standard Anthelmintic drug and Normal saline solution was used as control. The comparative results of present study indicated that the aqueous and ethanolic extract of *Tridax procumbens*, linn and *Piper nigrum*, linn shows significantly dose depending pharmacological activity on the earthworms.

**Keywords:** Anthelmintic activity, *Piper nigrum*, *Pheritima posthuma*, *Tridax procumbens*.

### INTRODUCTION

Anthelmintics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them. They may also be called vermifuge (stunning) or vermicides (killing). This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. Helminthes infection is the most common infection in man which effects the large proportion of world's population. In the treatment of parasitic diseases Anthelmintics are used accidentally.<sup>1</sup> Some Anthelmintic drugs, such as praziquantel and Albendazole, are contraindicated for certain groups of patients like pregnant and lactating woman. Keeping this in view, the present communication deals with the evaluation of the Anthelmintic activity of *Tridax procumbens* and *Piper nigrum*.<sup>2</sup>

*Tridax procumbens* linn is commonly known as 'Ghamra' in Hindi and 'Dagadi Pala' in Marathi. It is a weed found throughout India. A hispid, procumbent herb with woody base sometime rooting at the node, up to 60 cm high.<sup>3</sup> Leaves are ovate-lanceolate 2 to 7 cm and lamina pinnatisect, sometimes three lobed, flowers in small, long peduncled heads. It is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, in bronchial catarrh, diarrhea and dysentery. Moreover it possesses wound healing activity and promotes hair growth. The leaf gel possesses antiseptic, insecticidal and parasiticidal properties.<sup>4</sup>

*Piper nigrum* linn is commonly known as 'Kalimiri' in Hindi. Black pepper is a flowering vine, cultivated for its fruit, which is usually dried and used as a spice and seasoning. The fruit, known as a peppercorn when dried, is approximately 5 millimeters (0.20 cm) in diameter, dark

red when fully mature, and, like all drupes, contains a single seed. Black pepper is native to south India, and is extensively cultivated there and elsewhere in tropical regions. Black pepper is the world's most traded spice. The spiciness of black pepper is due to the chemical piperine. In traditional medicine, black pepper has been used for digestive disorders, such as indigestion, vomiting, diarrhea, and flatulence. Suggested modern medicinal applications of black pepper have included the treatment of cigarette withdrawal symptoms, postural instability in older adults, and swallowing difficulties in post-stroke and neurological disorder patients.<sup>5</sup>

Literature survey reveals that till date no comparative reports were found on the anthelmintic activity of *Tridax procumbens* and *Piper nigrum*.

### MATERIALS AND METHODS

#### Collection and Authentication of Plant material

##### *Tridax procumbens* and *Piper nigrum*

Fresh whole plant (leaves, stems, roots and flowers) of *Tridax procumbens* were collected from R.K.Nagar, Kolhapur, premises and authenticated by Dr. Miss. K.R. Datar (Head of Dept of botany) Deccan Education Society Willingdon college, Sangli. After authentication, fresh plant was collected in bulk, washed under running tap water, dried under shade for a period of 7 days and then pulverized in mechanical grinder to obtain coarse powder. The dried powder was stored in airtight bottles.

Black pepper (*Piper nigrum*) seeds were collected from the local market of Kolhapur. After authentication, the seeds were pulverized in mechanical grinder to obtain



coarse powder. The dried powder was stored in airtight bottles.

### Extraction methodology

#### Aqueous of *Tridax procumbens*

The coarse powdered material (each 100 gm) was soaked in distilled water (500ml) by Maceration technique for continuous 72 hours and then strained and the concentrate was evaporated on water bath until concentrate (syrupy consistency) is left and then evaporated to dryness.

#### Ethanol extract of *Tridax procumbens*

The coarse powdered material (each 100 gm) was soaked in 95% ethanol (500ml) by Soxhlet technique for continuous 72 hours. The extract was evaporated to dryness until dry mass is obtained. The yield obtained was 0.483%.<sup>6</sup>

#### Aqueous extract of *Piper nigrum* (Black pepper)

The coarse powdered material (each 25 gm) was soaked in distilled water (100ml) by Maceration technique for continuous 24 hours and then strained and the concentrate was evaporated on water bath until concentrate (syrupy consistency) is left and then evaporated to dryness.

#### Ethanol extract of *Piper nigrum* (Black pepper)

Place 15 g of coarse black pepper powder in 250 ml round bottomed flask add 150 ml of 150ml ethanol, by reflux by using Soxhlet extractor for 2 hours, filter the mixture by suction filtration and evaporate the concentrate until dry mass is obtained. The yield obtained is 0.6 g piperine.<sup>7</sup>

### Worms Collection

Indian earthworm *Pheretima posthuma* (Annelida) were used to study Anthelmintic activity. The earthworms were collected from the moist soil from the near region of Jaysingpur, Kolhapur, Maharashtra, and washed with water. The earthworms in 6-8 cm in length were used for all experimental protocol. The worms resemble both anatomically and physiologically characters to the intestinal roundworm parasites of human beings, hence can be used to study Anthelmintic activity.

### Anthelmintic Activity Study of *Tridax procumbens* and *Piper nigrum*

For the Anthelmintic activity of *Tridax procumbens* and *Piper nigrum* Indian adult earthworms (*Pheretima posthuma*) in 6-8 cm in length were used. The earthworms were divided in fourteen groups of six earthworms in each group. The ethanol, aqueous extracts (25,50,75 mg/ml conc.) both *Tridax procumbens* and *Piper nigrum* extract was dissolved in sterile distilled water and final volume was adjusted to 50 ml; the extract and standard drug Albendazole (25 mg/ml) were freshly prepared before starting the experiments. The extract of different concentration and standard solution were

poured in different Petri dishes. All the earthworms were washed into normal saline solution before they are released in to Petri dishes. Observation were made for time taken to paralyze (paralysis was said to occur when earthworms didn't revive in normal saline) and death (death was concluded when earthworms lost their motility and followed with their body colors fading away). All the results were expressed as a mean  $\pm$  SEM of six earthworms in each group.

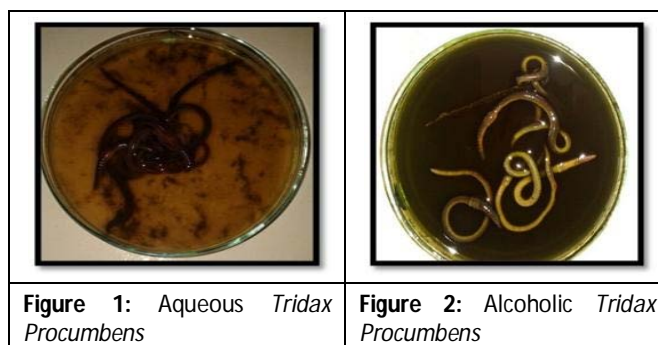


Figure 1: Aqueous *Tridax Procumbens*

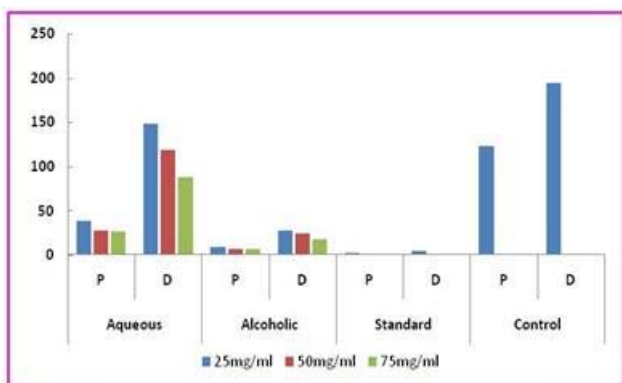
Figure 2: Alcoholic *Tridax Procumbens*

Table 1: Observation of Anthelmintic activity of *Tridax procumbens*

Treatment	Time taken by earthworms for	
	Paralysis (min) mean $\pm$ SEM	Death (min) mean $\pm$ SEM
Normal Saline (Control)	123.333 $\pm$ 0.8819	194 $\pm$ 0.8563
Standard Albendazole (25 mg/ml)	2.333 $\pm$ 0.2108	4.50 $\pm$ 0.2236
<b>Aqueous Extract</b>		
25 mg/ml	38.333 $\pm$ 0.6667	147.50 $\pm$ 0.7638
50 mg/ml	27.50 $\pm$ 0.7638	118.166 $\pm$ 0.7923
75 mg/ml	25.8333 $\pm$ 0.6009	88.166 $\pm$ 0.7923
<b>Ethanol Extract</b>		
25 mg/ml	8.666 $\pm$ 0.4944	27.5 $\pm$ 0.7638
50 mg/ml	7.0 $\pm$ 0.3651	23.666 $\pm$ 0.4944
75 mg/ml	6.666 $\pm$ 0.4216	17.833 $\pm$ 0.6009

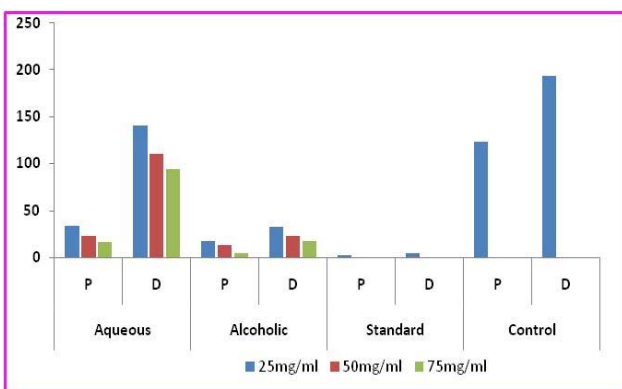
Table 2: Observation of Anthelmintic activity of *Piper nigrum*

Treatment	Time taken by earthworms for	
	Paralysis (min) mean $\pm$ SEM	Death (min) mean $\pm$ SEM
Normal Saline (Control)	123.333 $\pm$ 0.8819	194 $\pm$ 0.8563
Standard Albendazole (25 mg/ml)	2.333 $\pm$ 0.2108	4.50 $\pm$ 0.2236
<b>Aqueous Extract</b>		
25 mg/ml	33.166 $\pm$ 0.9098	140.166 $\pm$ 0.9458
50 mg/ml	22.5 $\pm$ 0.7638	110.833 $\pm$ 0.6009
75 mg/ml	16.666 $\pm$ 0.8819	94.166 $\pm$ 0.6009
<b>Ethanol Extract</b>		
25 mg/ml	16.833 $\pm$ 0.6009	32.0 $\pm$ 0.5774
50 mg/ml	12.50 $\pm$ 0.7638	22.5 $\pm$ 0.7638
75 mg/ml	3.833 $\pm$ 0.6009	17.33 $\pm$ 0.6667



X-axis: Concentration (mg/mL), Y-axis: Time in minutes

Figure 3: Anthelmintic activity of Aqueous, Alcoholic Extract of Tridax Procumbens, Standard drug and Control



X-axis: Concentration (mg/mL), Y-axis: Time in minutes

Figure 4: Anthelmintic activity of Aqueous, Alcoholic Extract of Piper nigrum, Standard drug and Control

Table 3: Phytochemical Evaluation of *Tridax procumbens*, Linn (Alcoholic and Aqueous extract)<sup>8</sup>

Chemical Tests	Result	Chemical tests	Result
<b>Test For Carbohydrates</b>		<b>Test For Tannins</b>	
A. Benedicts Test	Positive	A.5% Ferric chloride	Positive
B. Fehling's Test	Positive	B. Acetic acid test	Positive
C.Molisch's Test	Positive	C. Dil. KMnO <sub>4</sub> Test	Positive
<b>Test For Steroids</b>		<b>Test For Flavonoids</b>	
Salkowski test	Positive	A. Lead acetate test	Positive
		B. NaOH + Dil.acid	Positive
<b>Test For Alkaloids</b>		<b>Test for Glycosides</b>	
Dragendroff's test	Positive	Borntrager's test	Positive
Wagner's test	Positive		
Mayer's test	Positive		

**RESULTS AND DISCUSSION**

The perusal of the data reveals that as the concentration increases paralysis and death time decreases. Table 1 of *Tridax procumbens* ethanolic extract at the concentration of 75 mg/ml showed both paralysis and death in 6.66 min and 17.83 mins respectively as compared to Aqueous extract. While Table 2 of *Piper nigrum* ethanolic extract at the concentration of 75 mg/ml showed both paralysis and

death in 3.83 min and 17.33 mins respectively as compared to Aqueous extract.

This possesses dose dependent and significant Anthelmintic activity as compared with standard drug Albendazole on earthworms. Among all extract ethanolic extract of both *Tridax procumbens* and *Piper nigrum* required least time to cause paralysis and death of the earthworms followed by aqueous extracts.

Table 4: Phytochemical Evaluation of *Piper nigrum*, Linn (Alcoholic and Aqueous extract)<sup>9</sup>

Chemical tests	Result	Chemical tests	Result
<b>Test for Saponins</b>		<b>Test For Tannins</b>	
A. Foam test	Positive	A. 5% Ferric chloride	Positive
		B. Acetic acid test	Positive
		C. Dil. KMnO <sub>4</sub> Test	Positive
<b>Test For Steroids</b>		<b>Test For Flavonoids</b>	
Salkowski test	Positive	A. Lead acetate test	Positive
		B. NaOH + Dil.acid	Positive
<b>Test For Alkaloids</b>		<b>Test for Glycosides</b>	
Dragendroff's test	Positive	Borntrager's test	Positive
Wagner's test	Positive		
Mayer's test	Positive		

**CONCLUSION**

From the above results, it is concluded that the ethanolic extract of *Tridax procumbens* Linn and *Piper nigrum* Linn shows potent Anthelmintic activity to standard Anthelmintic drug. Some of these phytoconstituents may be responsible to show a potent Anthelmintic activity. It is also confirmed that these drugs triggers natural immune system to fight against various parasites and helminthes. This comparative study reveals that *Piper nigrum* Linn shows potent Anthelmintic activity as compared to *Tridax procumbens* Linn.

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