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Evaluation of *In-vitro* Anthelmintic activity of whole plant extracts of *Tridax procumbens* Linn

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Research Article

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ABSTRACT

The aqueous, methanol and chloroform extracts of *Tridax procumbens* Linn belonging to the family Asteraceae were evaluated for anthelmintic activity. The dried powder of whole plant of *Tridax procumbens* was extracted and the anthelmintic activity was studied. Aqueous, methanol and chloroform extracts collected were tested for anthelmintic activity against Indian adult earthworm *Eisenia fetida* and recorded the time taken for induction of paralysis and death. Two concentrations (50 mg/ml and 100 mg/ml) of aqueous, methanol and chloroform extract were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Albendazole (100 mg/ml) was used as reference standard and normal saline solution as a control. Comparative results of present study indicated that the aqueous, methanol and chloroform whole plant extracts of *Tridax procumbens* Linn shows significantly dose depending pharmacological activity on the Indian earthworms.

INTRODUCTION

Helminths parasite infections are global problems with serious social and economic reverberation in the countries. The diseases affect the health status of a large fraction of the human population as well as in animals. To treat parasitic infections anthelmintics are used. Anthelmintics or antihelminthics are group of antiparasitic drugs that expel parasitic worms (helminthes) from body either by stunning or killing them without causing significant damage to the host. They may also be called vermifuges those that stun or vermicides (those that kill). They are used to treat people who are infected by helminthes, a condition called helminthiasis. This includes both flat worms, e.g., flukes and tapeworms and round worms, *i.e.,* nematodes.

They are of immense importance for human tropical and veterinary medicine. Since this drugs used to treat the parasitic infections but they associated with numerous number of side effects hence, the discovery and development of new drugs derived from plant sources is required which cause less harm to human being and animals [1,2].

It is said that nature is enriched with uncountable number of medicinal resource. Long before the existence of medical facilities plant extracts were being used for human well being to prevent themselves from various chronic and infectious diseases. The new era of drug development process is searching for some natural compounds which have great medicinal value that's why the demand of plant based therapeutics seems to be increasing in current scenario due to the growing recognition that they are natural products, having no or less side effects one of the plant that can be used as herbal medicine is *Tridax procumbens*. Commonly known as coat buttons or tridax daisy is a species of flowering plant in the Asteraceae family.

T. procumbens has diverse pharmacological properties such as immunomodulatory, anti oxidant, anti hepatotoxic,

analgesic, anti diabetic, anti inflammatory, antifungal, and antimicrobial activities. The plant contains secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids, saponins etc. The aim of this project is to evaluate the anthelmintic activity of whole plant extracts of *Tridax procumbens* L. as an alternative source of effective remedies for nematodiasis [3,4].

Plant description

Tridax procumbens L. is one of the ancient plants of India. It is commonly known as Coat buttons or Tridax daisy. It is a green perennial plant and is available in all seasons in many parts of India. It is listed as a weed and a pest plant [5]. It has a creeping stem which can reach from to 8-30 inches (20-75) long. The leaves of *tridax procumbens* are opposite, pinnate, oblong to ovate, and 1-2 inches (2.5-5 cm) long with cuneate bases, coarsely serrate margins, and acute apexes. The flowers have white rays and yellow disk flowers. They are about 0.4-0.6 inches (1-1.5 cm) wide, and held on a 4-12 inches (10-30) long stalk. Flowering occurs in spring. Fruits are achenes that are dark brown to black in color, oblong, and 0.08 inches (2 mm) long, each with a head of pappus bristles that vary from 0.12-0.24 inches (3-6 mm) long (Figure 1).

Botanical classification

Botanical name: *Tridax procumbens*
Kingdom: Plantae
Sub kingdom: *Tracheobionta*
Phylum/Division: *Spermatophyta*
Sub-division: Magnoliophyta Class:- *Eudicots*
Order: *Asterales*
Family: *Asteraceae*
Genus: *Tridax L.*
Species: *Tridax procumbens* L.

Vernacular names

English: Coat Buttons or Tridax Daisy Hindi:- Ghamra, Tal muriya, and khal muriya
Sanskrit: Jayanti veda
Marathi: Dagadi Pala, kambarmodi, jakhamjudi, ghavti and tantani
Gujarati: Ghajadvu and Ghaburi
Bengali: Tridhara
Telugu: Gaddi Chemanthi, gayapaaku & balapaaku
Tamil: Thatha poo, vettukaaya poondu or kinatruppasan
Malayalam: Chiravanakku, Thelkuthi, Sanipoovu, Railpoochedi, etc
Japanese/Chinese: kotobukigiku
Oriya: Bisshalya karani
Urdu: Zagh mai hayat

Figure 1. *Tridax procumbens* L. (1) plant, (2) flower, and (3) seed

(1) (2) (3)

Traditional uses

T. procumbens has proclaimed anti-inflammatory and wound healing activity along with this it shows diverse pharmacological properties such as immunomodulatory, anti-oxidant, anti- hepatotoxic, analgesic, antidiabetic, anti-cancer, antifungal, and antimicrobial activity, anti-arthritic, vaso-relaxant etc [6]. It has been used to treat various ailments such as:

- To treat anemia, colds, inflammation, and hepatopathies.
- Used as an antibacterial, antifungal, and antiviral treatment as well as for vaginitis, stomach pain, diarrhea, mucosal inflammations, and skin infections.
- The leaf juice is used to treat wounds and stop bleeding. A study done, showed that lactating pregnant women suffering from anemia could reduce their symptoms by using Tridax.

- This species is also used in the treatment of gastrointestinal and respiratory infections, high blood pressure, and diabetes.
- The entire plant is used for the treatment of protozoal infections including malaria, leishmaniasis and dysentery.
- Aqueous extracts of *T. procumbens* have strong anti plasmodial activity against chloroquine resistant *P. falciparum* parasites it has activity against *Trypanosoma brucei*, antibacterial and wound healing properties [7].

The plants contain secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids, saponins etc.

MATERIALS AND METHODS

Collection and authentication of plant

Fresh whole plant (leaves, stems, roots and flowers) of *Tridax procumbens* were collected from Thube farm Donwade, Dowade region of Bhagur in the month of February and authenticated by Ayurvedic doctor of S.M.B.T Ayurveda College and Hospital, Dhamangaon, Tal. Igatpuri, Dist. Nashik. After collection, dried leaves and other debries were removed. The plant washed under running tap water to remove soil. It was 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 air tight bottles, and later subjected to extraction process (Figure 2) [8].

Extraction methodology

Figure 2. Soxhlet extraction of *Tridax procumbens*.

Aqueous extract

The coarse powdered material of *Tridax procumbens* (30 g) was subjected to soxhlet extraction with 450 ml of water as solvent for 6 hours and then filtered off. Following extraction process, the extract was concentrated on water bath until syrup consistency and then again evaporated to dryness.

Methanol extract

The coarse powdered material of *Tridax procumbens* (30 g) was subjected to soxhlet extraction with 450 ml of Methanol as solvent for 5 hours and then filtered off. Following extraction process, the extract was concentrated on water bath until syrup consistency and then again evaporated to dryness [9].

Chloroform extract

The coarse powdered material of *Tridax procumbens* (30 g) was subjected to soxhlet extraction with 450 ml of water as solvent for 4 hours and then filtered off. Following extraction process, the extract was concentrated on water bath until syrup consistency and then again evaporated to dryness [10].

Worms collection

Adult Indian earthworms, *Eisenia fetida* (Lumbricidae) were used to evaluate *in vitro* anthelmintic activity.

- Scientific name: *Eisenia fetida*
- Common name: Red worm, Tiger worm, Manure worm, Stink worm, Fish worm, striped worm etc.
- Habitat: Usually found in garden compost but also occurs in wet, decaying leaf litter, organic-rich soils and manure heaps
- Size: Typical size of adult is 6-9 cm.
- Collection: The earthworms were collected from vermicompost/Gandul khat, Adgaon, Nashik. They were washed with water and then by normal saline to remove soil.

The earthworms are 6-9 cm in length was used for the anthelmintic study. The earthworm resembles both anatomically and physiologically to the intestinal roundworms parasites of human being, hence can be used to study anthelmintic

activity (Figure 3) [11].

Figure 3. Earthworms (*Eisenia fetida*).

Drugs and chemicals

Albendazole tablet IP (MANKIND PHARMA LTD) were used as reference drug and for control, normal saline solution (sodium chloride 0.9%w/v and dextrose 5% w/v injection IP) is used. The other chemical used are ethanol, methanol, and chloroform of analytical grade.

Phytochemical analysis

Phytochemical analysis of the extracts was performed according to standard procedures mentioned in trease and evans Pharmacognosy book.

Anthelmintic activity

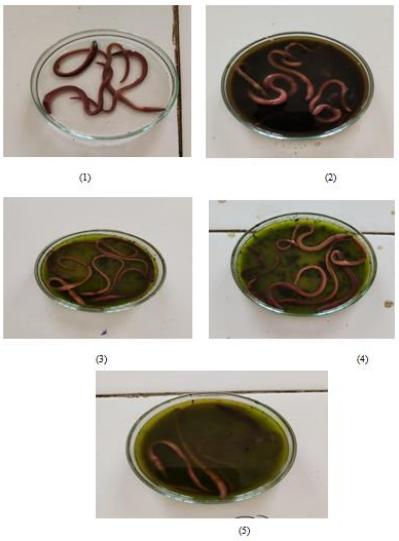
In this the Indian adult earthworms (*Eisenia fetida*) of 6-9 cm length were used. The earthworms are divided into 8 groups, of 4 earthworms in each group. The aqueous, chloroform, and methanol (50,100 mg/ml, each) extracts was dissolved in normal saline solution and final volume was adjusted to 10 ml. similarly the reference drug solution of 10 mg/ml was prepared and adjusted to volume 10 ml. The extracts and standard drug solution were freshly prepared before starting the experiments. Take 8 petri plates, label each plate according to extract:

- Aqueous,
- Methanol,
- Chloroform,
- Standard
- For control.

The extracts of different concentration, Standard and control solutions were poured in respective labelled petri plate. All the earthworms first, washed by tap water than by normal saline solution before they released in to petri plate. Observations, were made for the time taken to paralyze (paralysis was said to occur when earthworm didn't revive in normal saline solution) and time of death (death was concluded when earthworms lost their motility and followed with their body color fading away). All the results were expressed as a mean ± SEM of 4 earthworms in each group (Figures 4 and 5).

Figure 4. Experimental setup for evaluation of antihelmintic activity.

Figure 5. Anthelmintic activity: (1) control group, (2) Aqueous extract group, (3) Methanolic extract group (50 mg/ml), (4) Methanolic extract (100 mg/ml), and (5) Chloroform extract group.



RESULTS AND DISCUSSION

The preliminary phytochemical investigation of all extracts of *Tridax procumbens* shows presence of alkaloids, flavonoids, tannins, steroids, carbohydrates, glycosides, saponins etc. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity (Table 1).

Table 1. The Phytochemical investigation of various chemical constituents in *Tridax procumbens* L extracts. (+ =Present, - =Absent, C.E.=Chloroform Extract, M.E.=Methanolic Extract, A.E.=Aqueous Extract).

S.NO	phytochemicals	C.E.	M.E.	A.E.
1	Alkaloids	Present	Present	Present
1.1	Dragendroff's test	+	+	+
1.2	Wagner's test	+	+	+
2	Saponins	Present	Present	Present
2.1	Foam test	+	+	+
3	Glycosides	Present	Present	Present
3.1	Borntrager's test	+	+	+
4	Flavonoids	Present	Present	Present
4.1	Lead acetate test	+	+	+
4.2	NaOH+Dil.acid	+	+	+
5	Steroids	Present	Present	Present
5.1	Salkowaski test	+	+	+
6	Tannins	Absent		
6.1	$FeCl_3$ test	-	+	+
7	Amino acids	Present	Present	Present
7.1	Ninhydrin test	+	+	+
8	Cardiac	Absent	Absent	Absent
	Glycosides			
8.1	Kellar-Killiani test	-	-	-
9	Phenols	+	+	+
10	Anthocyanin	-	+	+
11	Proteins	-	-	-

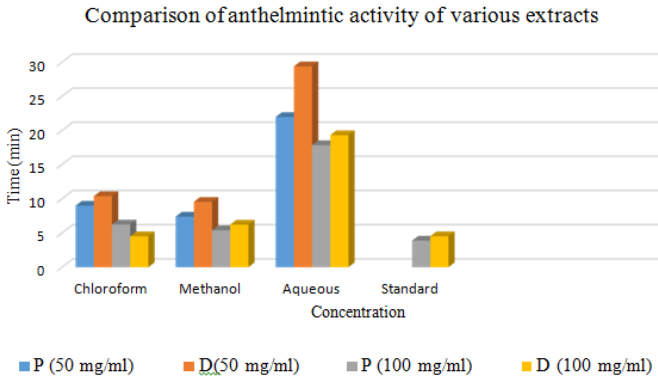
The perusal of the data reveals that as the concentration increases, paralysis and death time decreases also from the

observation made, that higher concentration of extract produced paralytic effect much earlier and time of death was shorter for methanol and chloroform extracts of *Tridax procumbens* L (Table 2 and Figure 6).

Table 2. Comparison of anthelmintic activity of various extracts of *Tridax procumbens*.

S.NO	Treatment	Time taken by earthworms for	
		Paralysis (min)	Death (min)
1	Control	-	-
2	Standard	3.89 ± 0.172	4.525 ± 0.195
3	Chloroform extract		
3.1	50 mg/ml	8.99 ± 0.124	10.38 ± 0.128
3.2	100 mg/ml	6.247 ±0.235	7.64 ± 0.264
4	Methanol extract		
4.1	50 mg/ml	7.387 ± 0.015	9.547 ± 0.054
4.2	100 mg/ml	5.40 ± 0.073	6.22 ± 0.078
5	Aqueous extract		
5.1	50 mg/ml	21.975 ± 0.403	29.4 ± 0.039
5.2	100 mg/ml	17.835 ± 0.137	19.31 ± 0.121

Figure 6. Graph showing comparison of different extracts of *Tridax procumbens* Linn.



CONCLUSION

On the basis of the results obtained at the end of the study we can conclude that the methanol and chloroform extract of *Tridax procumbens* Linn. Shows potent anthelmintic activity to that of standard anthelmintic drug *i.e* Albendazole. Further studies using *in vitro* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Tridax procumbens* Linn as an anthelmintic drug. The drug can be further explored for the isolation and characterization of the active constituents responsible for anthelmintic activity.

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