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Comparative anthelmintic potential of different extracts of Tubiflora acaulis Kuntze against selected helminth worms: An in vitro study

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Abstract
Helminthiasis is a major parasitic disease, and increasing resistance to conventional anthelmintics has encouraged the search for plant-based alternatives. This study evaluated the *in-vitro* anthelmintic activity of petroleum ether, chloroform, and methanolic extracts of Tubiflora acaulis Kuntze against Eudrilus eugeniae (Roundworm) and Haemonchus contortus (Tapeworm) at 50, 75, and 100 mg/mL. Albendazole was used as the standard drug and 1% gum acacia as the control. Paralysis and death times were recorded and analyzed statistically. All extracts showed concentration-dependent anthelmintic activity, with the methanolic extract exhibiting the highest activity. At 100 mg/mL, it produced paralysis and death times of 23.53 ± 1.32 and 41.05 ± 0.52 min against E. eugeniae and 21.05 ± 0.58 and 41.35 ± 0.42 min against H. contortus, respectively. The activity may be attributed to phytoconstituents such as alkaloids, flavonoids, tannins, and phenolics. The findings suggest that T. acaulis, particularly its methanolic extract, has promising anthelmintic potential and warrants further phytochemical and *in-vivo* investigation.

Keywords: Tubiflora acaulis Kuntze, anthelmintic activity, albendazole

Introduction

Nature has offered a vast storehouse of medicines to treat all of humanity's ills and disorders. Plants were utilised to make practically all medications in the past. For millennia, it has served as man's chemist. Bioactive compounds found in medicinal plants include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids, all of which have a physiological effect on the human body [1].

Helminthiasis

Helminthiasis, or worm infection, is one of the world's most common diseases. Helminthiasis affects over half of the world's population, and the disease is on the rise. Because of poor sanitation, poor family hygiene, hunger, and crowded living situations, it is not just limited to tropical and subtropical countries, but is also endemic in many locations [2].

Intestinal nematodes infect an estimated 2 billion individuals worldwide. Anthelmintic medications are used to treat parasitic infections caused by helminths. The demand for novel and effective anthelmintics is enormous, as the chemical medications currently used to control helminths are costly, and most of them lose their potency within 20 years due to resistance. Because of the strong link between these diseases and poverty, eradicating helminthiases is particularly difficult [3]. Metazoa, which includes roundworms (nematodes) and two forms of flatworms, flukes (trematodes) and tapeworms, are pathogenic for humans (cestodes). These physiologically distinct eukaryotes differ in terms of life cycle, bodily structure, development, physiology, host location, and chemotherapeutic susceptibility. Immature forms infect humans through the skin or gastrointestinal tract, developing into well-differentiated adult worms with distinct tissue distribution.

Pharmacology of anthelmintics [4]

The parasitic helmintic infection is increasing death and morbidity all over the world. Intestinal nematodes (roundworms), trematodes (flukes), and cestodes are examples (tapeworms). Because it is a major source of environmental contamination and transmission, it is an unevenly distributed disease in low-income nations that affects the worst and highest risk of morbidity. Due to the persistence of periodic emergence of resistance, albendazole, mebendazole, and praziquantel are the most regularly used anthelmintic medicines with broad spectrum activity and high cure rates.

- **Roundworms**

Migraine, eosinophilia, and pulmonary difficulties are caused by the migration of larval forms and egg transfer by skin contact in moist soil and tropical locations. With the household aggregation of infection, *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenal* are the most prevalent intestinal worm infections. Due to self-infection, the eggs are deposited on the perianal area. Infections can also be spread via contaminated surfaces such as carpets and drapes. Because humans are the unintentional hosts, the airborne and inhalation of a limited number of eggs is transmitted by ingestion of infected food. Immunological lungs, liver, and central nervous system damage occur after intake of infected items.

Fig 1: Roundworms

- **Tapeworms**

With the creation of tissue cysts, humans are the intermediary host for *Taenia solium*. It produces cysts and causes moderate gastrointestinal symptoms after intake of raw beef (*T. saginata*) or pork. Neurocysticercosis is an infection of the central nervous system caused by the swine tapeworm or flukes, which is treated with albendazole and praziquantel.

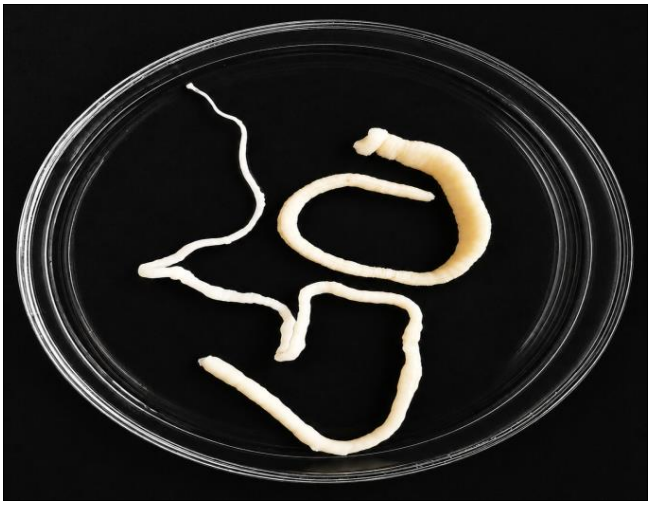

Fig 2: Tapeworms

Mechanism of Action

It prevents the development of microtubules. As a result, the parasite's cytoskeleton and motility are lost, and it dies. It also reduces glucose absorption and ATP production. Other modes of action for the Benzimidazole, aside from tubulin, have been described, including disruption of the host's energy metabolism. In fact, early research of Benzimidazole's method of action focused on their role in carbohydrate metabolism, as these chemicals have been found to impede glucose uptake in numerous helminth species both in vitro and *in vivo*. Albendazole has been demonstrated to impede glucose intake by sensitive parasite larval and adult stages, depleting their glycogen reserves and lowering ATP production, resulting in parasite death [3].

Treatment

Early and consistent administration of WHO-recommended anthelmintic drugs such as albendazole, mebendazole, diethylcarbamazine (citrate), ivermectin, levamisole, praziquantel, and pyrantel reduces the occurrence, extent, severity, and long-term consequences of morbidity, and contributes to a sustained reduction in transmission in certain epidemiological conditions [5]. Anthelmintics are made up of a variety of chemical compounds that are classed as follows [6].

- **Benzimidazole:** Mebendazole, Albendazole, Thiabendazole
- **Quinolines and Isoquinolines:** Oxamniquine, Praziquentel
- **Piperazine:** Piperazine citrates, Diethyl carbamazine
- **Vinyl pyrimidines:** Pyrantel Pamoate
- **Amides:** Niclosamide
- **Imidazothiazoles:** Levamisol
- Organophophanes Metrifonate

Plants having anthelmintic activity

Various medicinal plants possess anthelmintic activity. The list of medicinal plants having anthelmintic activity is given below [8]

Table 1: List of plants having anthelmintic activity

Sr. No	Plant Name	Family	Family Plant Part used
1	*Acacia auriculaeformis A. Cunn*	Mimosaceae	Whole Plant
2	*Acacia nilotica*	Fabaceae	Fruit
3	*Acacia suma Roxb*	Fabaceae	Bark
4	*Acalypha fructicosa*	Euphorbiaceae	Whole plant
5	*Acalypha indica Linn*	Euphorbiaceae	Leaves
6	*Achyranthes aspera*	Amaranthaceae	Whole Plant
7	*Acokanthera schimper*	Apocynaceae	Leaves
8	*Aegle marmelos Linn*	Rutaceae	Fruits
9	*Agati gratifola*	Leguminosae	Whole Plant
10	*Agave sisalana Perr*	Agavaceae	Whole Plant
11	*Ailanthus excelsa Roxb*	Simaroubaceae	Bark
12	*Albizia schimperiana*	Mimosaceae	Stem bark
13	*Barringtonia acutangula Gaertn*	Lecythidaceae	Leaves
14	*Bauhinia purpurea Linn*	Fabaceae	Whole Plant
15	*Bauhinia racemosa Linn*	Fabaceae	Whole Plant
16	*Bixa orellana*	Bixaceae	seed
17	*Butea monosperma*	Fabaceae	Seeds
18	*Caesalpania pulcherrima Linn*	Leguminaceae	Flowers
19	*Caesalpinia crista L*	Fabaceae	Whole Plant
20	*Carica papaya Linn*	Caricaceae	Seeds
21	*Chenopodium album L.*	Amaranthaceae	Seeds
22	*Cymbapogon schoenanthus Linn*	Poaceae	Leaves
23	*Cymbopogon martinii Roxb*	Poaceae	Leaves
24	*Embelia kilimandschiraca*	Myrsinaceae	Roots
25	*Embelia ribes*	Myrsinaceae	Seeds
26	*Eupatorium triplinerve*	Asteraceae	Flowers
27	*Evodia rutaecarpa*	Rutaceae	Whole Plant
28	*Feruia foetidissima*	Rubiaceae	Whole Plant
29	*Ficus bengalensis Linn*	Moraceae	Fruits
30	*Ficus religiosa*	Moraceae	Whole Plant
31	*Fumaria parviflora*	Fumariaceae	Plant powder
32	*Gloriosa superba Linn*	Liliaceae	Whole Plant
33	*Guazuma ulmifolia Lam*	Sterculiaceae	Whole plant
34	*Hedera Helix L.*	Araliaceae	Fruits

35	*Helleborus niger*	Ranunculaceae	Stem
36	*Jalans regia Linn*	Juglandacaeae	Leaves
37	*Jussiaea hyssopifolia*	Onagraceae	Whole Plant
38	*Khaya senegalensis*	Meliaceae	Bark
39	*Lawsonia inermis Linn*	Lythraceae	Leaves
40	*Leucas martinicensis*	Lamiaceae	Aerial parts
41	*Madhuca indica*	Sapotaceae	Whole plant
42	*Mangifera indica*	Anacardiaceae	Whole Plant
43	*Neolamarckia cadamba Linn*	Rubiaceae	Bark
44	*Nicotiana tabacum Linn*	Solanaceae	Leaves
45	*Ocimum sanctum Linn*	Lamiaceae	Essential Oil and Eugenol
46	*Pandanus fascicularis Linn*	Pandanaceae	Leaves
47	*Paris polyphylla*	Melanthiaceae	Rhizomes
48	*Quisqualis indica*	Combretaceae	Seeds
49	*Randia dumetorum*	Rubiaceae	Seeds
50	*Rapanea melanophloeos*	Myrsinaceae	Fruits
51	*Sapindus trifoliatus Linn*	Sapindaceae	Seeds
52	*Saraca indica Linn*	Caesalpinaceae	Leaves
53	*Trachyspermum ammi Linn*	Apiaceae	Seeds
54	*Uvaria hookeri*	Annonaceae	Root bark
55	*Vernonia amygdalina*	Asterac	Stem bark
56	*Zingiber offcinale*	Zingiberaceae	Rhizomes

Methodology

In-vitro evaluation of anthelmintic activity

Selection of animals

Adult Indian earthworms (*Eudrilus eugeniae*) and tapeworms (*Haemonchus contortus*) were used to test the activity. Earthworms were bought from local suppliers, while tapeworms were taken from the bowel of a recently slaughtered goat's intestine which had been cleansed to eliminate all faeces. The worms were authenticated by Dr. Zuber Shaikh, HOD, Zoology Department, RFNS Senior Science College, Akkalkuwa. The worms were placed in regular saline to offer them with the usual leaving circumstances they require. Earthworms were chosen to examine anthelmintic activity because they were morphologically and physiologically similar to intestinal roundworms.

Preparation of samples

To obtain different concentrations, required quantities of different plant extracts and a standard medication (Albendazole) were dissolved in DMSO and then diluted with distilled water. (50 mg/ml, 75 mg/ml, and 100 mg/ml)

In-vitro evaluation of anthelmintic activity [2, 9, 10]

Adult Indian earthworms and tapeworms from the intestine of a goat were used to test the antihelmintic activity. Twelve groups of six worms of approximately identical size were given 10 ml of the necessary concentration (five concentrations of total extract and standard). Individual worms were timed to see how long it took them to become paralysed and die. When there was no movement of any kind except when the worms were shaken strongly, the time of paralysis was recorded. After determining that worms did not move when shaken vigorously or dunked in heated water (50 °C), the time it took for them to die was recorded. As a control, 1% gum acacia was employed [2, 9, 10]. The results are presented as a mean standard error of the mean (SEM). Graph Pad Prism 5.0 was used to conduct the statistical analysis, which included one-way analyses of variance (ANOVA) and Dunnett's Multiple Comparison test. The statistical significance of $P<0.05$ was determined.

Results and Discussion

The results of this investigation show that all *Tubiflora acaulis* Kuntze extracts (petroleum ether, chloroform, and methanolic) have anthelmintic action against both worms, which is proportionate to the concentration. In comparison to other extracts, the methanolic extract has high anthelmintic action, according to the findings. (See fig. 3 and 4) (Table 2)

In comparison to other extracts of *Tubiflora acaulis* Kuntze, quantitative phytochemical research showed that methanolic extract contains a higher concentration of flavonoids, tannins, and phenolic components (methanolic extract also contains alkaloids). Alkaloids, flavonoids, tannins, and phenolic chemicals are abundant in chloroform extract. Steroids are found in petroleum ether extract.

The phytoconstituents in the plants, such as phenolic chemicals, flavonoids, tannins, and alkaloids, have anthelmintic activity. They may operate together or independently by inhibiting tubulin polymerization and preventing glucose uptake, causing damage to the muco-polysaccharide membrane of worms, exposing the outer layer, reducing movement, and eventually causing paralysis and parasite death. Tannins may bind to free protein available for larval nutrition, limiting nutritional availability and causing larval mortality. Tannins may also impede oxidative phosphorylation, producing a decrease in gastrointestinal metabolism and hence larval death. Alkaloids may act on the earthworm's central nervous system, causing paralysis, by inhibiting the transfer of sucrose from the stomach to the small intestine, as well as their antioxidant effect, which can reduce nitrate production, interfering with local homeostasis, which is necessary for the development of helminthes [11].

As a result, it is possible to deduce that the anthelmintic action of various plant extracts is related to the presence of phenolic compounds, flavonoids, tannins, and alkaloids components, which may act singly or in combination.

Table 2: Paralysis and death time of Indian earthworm and tape worm for Different extract

Test Sample	Concentration (mg/ml)	Indian Earth worm		Tape Worm	
		For Paralysis	For Death	For Paralysis	For Death
Petroleum ether	50	75.10 ± 0.61	131.57 ± 0.54	72.65 ± 0.75	121.93 ± 0.99
	75	64.53 ± 1.36	105.27 ± 1.48	62.96 ± 0.56	96.13 ± 0.87
	100	50.91 ± 0.91	82.47 ± 0.88	41.25 ± 0.54	76.43 ± 1.13
Chloroform	50	63.67 ± 1.21	109.62 ± 0.86	61.79 ± 0.58	95.06 ± 0.91
	75	52.29 ± 0.50	82.80 ± 1.23	49.39 ± 0.72	77.20 ± 0.53
	100	47.05 ± 0.46	70.48 ± 0.34	37.09 ± 0.39	63.34 ± 0.87
Methanolic	50	51.73 ± 0.62	73.89 ± 0.80	42.37 ± 0.92	72.42 ± 0.47
	75	35.49 ± 0.43	51.88 ± 0.30	33.47 ± 0.87	63.21 ± 0.80
	100	23.53 ± 1.32	41.05 ± 0.52	21.05 ± 0.58	41.35 ± 0.42
Albedazole	50	34.25 ± 1.04	79.64 ± 0.32	31.60 ± 1.18	69.37 ± 0.72
	75	26.34 ± 0.93	45.37 ± 1.11	26.76 ± 0.92	56.68 ± 1.03
	100	19.77 ± 0.53	35.92 ± 0.87	15.20 ± 0.82	38.12 ± 0.66

Data are mean of 6 replicates ± SEM and are significantly different at $P< 0.05$

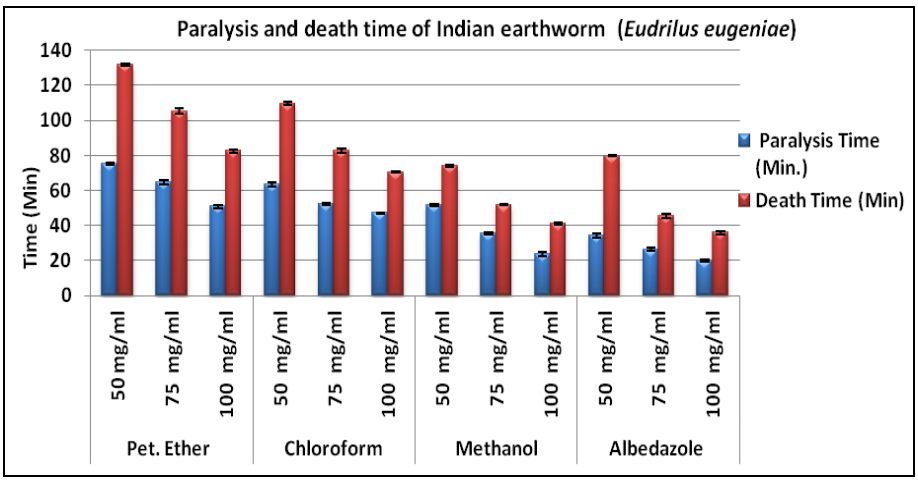

Fig 3: Paralysis and death time of Indian earthworm (*Eudrilus eugeniae)*

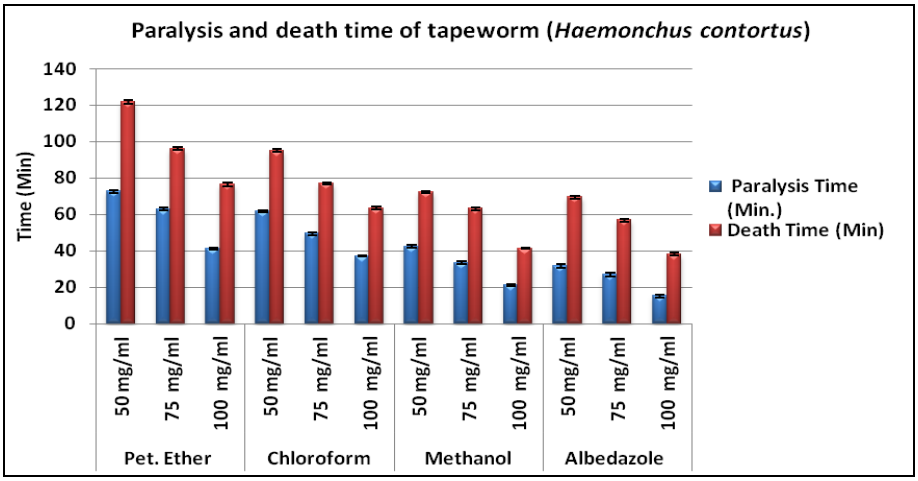

Fig 4: Paralysis and death time of tapeworm (*Haemonchus contortus*)

Conclusion

Adult Indian earthworms (*Eudrilus eugeniae*) and tapeworms were used to test the anthelmintic activity (*Haemonchus contortus*). The results of this investigation show that all *Tubiflora acaulis* extracts (petroleum ether, chloroform, and methanolic) have anthelmintic action against both worms that is proportionate to the concentration. In comparison to other extracts, the methanolic extract has high anthelmintic action, according to the findings. In comparison to other extracts, methanolic extract has a higher concentration of alkaloids, flavonoids, tannins, and phenolic chemicals, which may explain the activity.

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