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In-vitro anthelminthic activity of Adina cordifolia, Nyctanthes arbor tristis and Thevetia peruviana on Phertima postuma and Ascaris gallis

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Abstract

In Indian medicinal plant literature, leaves of *Thevetia peruviana, Nyctanthes arbor-tristis* (Linn.), *Adina cardifolia* (Roxb.) have been traditionally reported to be used as anthelmintics. The scientific authentication about traditional claim of anthelmintic activity of the aqueous and ethanolic leaf extracts of *Thevetia peruviana, Nyctanthes arbor-tristis* (Linn), *Adina cardifolia* (Roxb.) on Indian earthworms *Pheretima postuma* and nematode *Ascaris gallis* not yet available. Hence we conducted a comparative study to examine these plant species for anthelmintic potential to support traditional claim which can prove beneficial to the suffering people.

The aqueous and ethanolic extracts of the three plant parts were investigated for anthelmintic activity using Indian earthworms *Pheretima postuma* and nematode *Ascaris gallis* showed significant result on both worms as compared to piperazine citrate. Determination of anthelmintic activity was done by recording the paralysis time (P) and death time (T) of the worms. Anthelmintic treatment is an important in inducing immunomodulation, subsequently useful in treatment of various diseases.

Keywords: Anthelmintic activity, ethanolic extracts, helminthiasis, phytochemicals. *Thevetia peruviana* Nyctanthes arbor-tristis Linn Adina cordifolia

Introduction

There is a close relation between immune system and parasite infection where the response of the immune system could result in cancer development and protection of parasites as per earlier reports ^[1]. Parasite is considered to be significant regulator of the host immune system as they can restrain pathways of immune activation as T cell cytokine, B cell antibody production and other immune mechanism. Infection due to parasites could augment carcinogenesis through the discharge of procarcinogenic factors, or suppression ^[1, 2].

These infections caused by helminth are common health problems in developing countries can affect most population in endemic areas with major economic and social consequences. Currently there are large number of available anthelmintic drugs treatment helminthes diseases but there are evidence of resistance amongst gastro-intestinal helminthes towards such drugs shown tremendous side effect as nausea and vomiting, abdominal pain, appetite loss, headache and diarrhea ^[3, 4]. Hence there is an increasing demand towards natural anthelmintics as there easy to obtain and safe and moreover they have holistic approach.

Thevetia peruviana (Yellow Oleander) belonging to family Apocynaceae plant is native of Central & South America, but found in the tropical and sub-tropical. The plant shows diverse medicinal properties for the treatment of gastrointestinal and inflammatory diseases, heart failures and skin tumor ^[5, 6].

Nyctanthes arbortristis (Linn.) (Night Jasmine) belonging to family Oleaceae is an important drug used in use in Ayurveda, Sidha and Unani systems used for treatment of rheumatic joint pain, malaria, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic ^[7]. *Adina cordifolia* (Roxb.) (*Haldina cordifolia*; Kelikodom) belonging to the family Rubiaceae, native to East Asia and Southeast Asia and grows in Bangladesh, India, and Thailand. It has been used for the treatment of cholera, cold cough, fever, headache, rheumatism etc. ^[8].

Selection of this activity is based on the fact that the selected medicinal plant has been used to expel worms in traditional text and which can be correlated to its influence the immunological balance. Indian adult earthworms (*Pheretima posthuma*) obtained from soil and nematode (*Ascaridia galli*) obtained from the infected intestine of freshly slaughtered fowls were used due to its anatomical resemblance with the intestinal roundworm parasites present in human beings are used for the evaluation of anthelmintic activity. The phytoconstituents from all the selected plant has shown to have the presence of flavonoids, tannins, terpenes alkaloids ^[7, 8].

Methods

Plant material

The selected plant leaves of *Adina cordifolia* (Roxb.) and *Thevetia peruviana* L. were collected locally from Ramling Mudgad located in Latur district in state of Maharashtra and the leaves of *Nyctanthes arbortristis* (Linn.) was collected locally from the place of Jule Solapur. The authenticities of the selected plants were confirmed by Dr. M. N. Jagtap Coordinator P. G. Depatment of Botany and Research Centre D. B. F. Dayanand College of Arts and Science, Solapur, India.

Preparation of extracts

The selected plant leaves of *Thevetia peruviana, Nyctanthes arbor-tristis* (Linn.), *Adina cardifolia* (Roxb) were air dried at room temperature (25-30 °C) and powdered with the help of electrical grinder. The material was extracted successively with ethanol (60-80%) in soxhlet extractor. Finally the plant marc was macerated with chloroform-water. Extracts were dried by using rotatory evaporator. The extracts obtained were used for the present study.

Earthworms and nematode

Indian adult earthworms (*Pheretima posthuma*) were collected from water logged areas and *Ascardia galli* (nematode) worm were obtained from freshly slaughtered fowls (*Gallus gallus*). Both worm types were identified and confirmed at the Department of Zoology, Research Centre D. B. F. Dayanand College of Arts and Science, Solapur, India.

Evaluation of anthelmintic activity

The anthelmintic assay was carried as per method described by Ajaiyeoba of with minor modifications [9]. The anthelmentic activity was evaluated on adult Indian earthworm Pheretima posthuma worm due to its anatomical and physiological resemblance with the intestinal round worms parasites of human beings [10-15]. Ascardia galli (nematode) worms are easily available in slaughtered fowls and it can be used as a screening model for anthelminthic drugs as advocated earlier. Fifty milliliter of formulation containing three different concentrations, each of crude alcoholic and aqueous extract (25, 50,100 mg/ml in distilled water) was and five worms (same type) were placed in it. This was done for both type of worms Observation were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis (P) in min was noted when noticing the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Piperazine citrate (10mg/ml) was included as reference compound, movement of any sort could be observed, except when the worm was shaken vigorously; time of death (D) in

min was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50 $^{\circ}$ C). Piperazine citrate (10mg/ml) was included as reference compound ^[16].

Results and Discussion

The aqueous and ethanolic extract of all selected plant leaves of *Adina cordifolia*, *Nyctanthes arbortristis* and *Thevetia peruviana* samples were taken for the study. Six different extracts were suspended in 1% (w/v) CMC and concentrations of 25, 50,100 μ g/mL suspension were prepared in normal saline water.

All the extracts exhibit concentration- dependent activity as shown in Table 1. The results in the table clearly depicts that amongst aqueous and ethanolic extract of all selected plant leaves of *Adina cordifolia*, *Nyctanthes arbortristis* and *Thevetia peruviana the* Ethanolic extract nyctanthes arbortrisitis showed significant anthelmintic activity with paralysis time (P) 15 ± 0.28 and death time (D) 40.2 ± 0.52 for Pheritima postuma and paralysis time (P) 12.8 ± 0.33 and death time (d) 36 ± 0.49 for Ascaris gallis as compared to standard piperizine citrate. Ethanolic extract of *Thevetia peruviana* showed good results against both types of worms with death time of 46 ± 1.29 for *Phertima postuma* and 58 ± 0.77 for *Ascaris gallis*. Paralysis Time (P) and Death Time (D) of *Phertima postuma* and *Ascaris galli* are depicted in Figure 1.

The Ethanolic extract *Adina cordifolia* at an conc of 100 μ g/mL paralyzed the worms at17.6±0.36 min and the worms dead at 42±0.28 min for *Pheritima postuma* and death time recorded was 36.2±0.52 min for *Ascaris gallis*. All the extracts showed good anthelmintic activity for both types of worms.

Most of the research groups have estimated that the greater amount of polyphenolics in their respective alcoholic extracts may be responsible for the observed anthelmintic activity.

From the results shown in Table 1, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. The alcoholic extract of all the selected plant *demonstrated* paralysis as well as death of worms in a less time as compared to piperazine citrate especially at higher concentration of 100 mg/ml. The aqueous extract showed the significant activity against selected worms. Phytochemical analysis of the crude extracts revealed presence of phenolics. tannins, flavonoids etc as one of the chemical constituent which could be responsible for the showed activity ^[17].

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	Conc. (µg/ml)	Phertima postuma		Ascaris galli	
Extracts		Paralysis	Death	Paralysis	Death
		(min±SEM)		(min±SEM)	
Aqueous extract Adina cordifolia	25	66.40±0.46	93.60±0.60	54.00±0.49	91.20±0.52
	50	36.20±0.33	62.40±0.46	24.00±0.80	52.60±0.46
	100	20.40±0.36	53.60±0.72	15.60±0.60	35.80±0.77
Ethanolic extract Adina cordifolia (leaf)	25	54.40±0.36	81.40±0.60	53.60±0.46	78.20±0.34
	50	27.20±0.71	53.60±0.46	25.80±0.33	48.00±0.57
	100	17.60±0.36	42.00±0.28	15.40±0.46	36.20±0.52
Aqueous extract Nyctanthes arbortrisitis	25	47.80±0.52	100.20±0.52	42.20±0.33	90.00±0.52
	50	35.60±0.52	70.80±0.33	32.60±0.46	72.20±0.87
	100	17.60±0.21	57.40±0.36	15.40±0.21	54.20±0.33
Ethanolic extract Nvctanthes arbortrisitis	25	34.00±0.63	95.40±0.21	33.00±0.40	78.60±0.46

Table 1: Anthelmintic activity of selected plant extracts against Phertima postuma and Ascaris galli (n=5)

	50	24.20±0.33	53.80±0.33	21.80±0.33	53.20±0.52
	100	15.00±0.28	40.20±0.52	12.80±0.33	36.00±0.49
Aqueous Thevetia peruviana	25	87.60±0.45	127.80±1.21	78.00±0.63	121.80±0.52
	50	64.80±0.52	90.00±0.63	59.80±0.52	82.20±1.03
	100	34.20±0.59	64.00±0.40	32.40±0.46	55.70±0.46
Ethanolic extract Thevetia peruviana	25	79.00±0.63	122.80±0.91	71.20±0.91	100.00±0.63
	50	53.00±0.63	74.20±0.71	58.60±0.92	82.00±1.13
	100	21.00±0.63	46.00±1.29	21.40±0.36	58.00±0.77
Piperazine citrate (Std.)	10	22.20±0.52	74.80 ± 0.87	24.60±0.92	44.20±0.96



Fig 1: Paralysis Time (P) and Death Time (D) of Phertima postuma and Ascaris galli

Conclusion

In conclusion, the traditional use leaf extracts of *Thevetia peruviana*, *Nyctanthes arbor-tristis* (Linn.), *Adina cardifolia* (Roxb) as an anthelmintic have been confirmed and displayed activity against the worms used in the study. Polyphenolic compounds show anthelmintic activity. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that phenolic content in the selected extracts produced similar effects.

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