

ANTHELMINTIC ACTIVITY OF *NYCTANTHES ARBORTRISTIS* LEAVES ON INDIAN EARTH WORMS

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<p>*For Correspondence: Jijamata College of Pharmacy, Nandurbar, Dist. Nandurbar, 425412, Maharashtra State, India</p>	<p>ABSTRACT The present study was undertaken to evaluate Anthelmintic activity of aqueous, chloroform and acetone extract of <i>Nyctanthes arbortristis</i>, against Indian earth worm <i>Phereima posthuma</i>. The result revealed that all tested extract of <i>Nyctanthes arbortristis</i> shown Anthelmintic activity in a dose dependant manner. The potency of test sample was found to be inversely proportional to time taken for paralysis or death of worms. The activities were comparable with reference drug Piperazine Citrate. Among the tested extract, the Acetone extract was found to possess promising Anthelmintic activity in comparison with other extract and standard. The present study therefore justifies its use in folklore remedies as Anthelmintic drug of natural origin.</p> <p>KEY WORDS: <i>Nyctanthes arbortristis</i>, Anthelmintic, Acetone extract, Piperazine Citrate.</p>
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INTRODUCTION

Nyctanthes arbor-tristis L. (Oleaceae) is widely distributed along subtropical, tropical to sub-Himalayan regions in the South East Asia. It has been extensively used as a therapeutic agent in the Ayurvedic healing traditions of South Asia. (Satyal, et al, 2012.) *Nyctanthes arbortristis* Linn. commonly known as Harsinghar or Night Jasmine is one of the well known medicinal plants. Different parts of *N. arbortristis* are known to possess various ailments by rural mainly tribal people of India (Orissa and Bihar) along with its use in Ayurveda, Sidha and Unani systems of medicines (Vats, et al, 2009). It is a common wild hardy large shrub upto 10m or small tree with gray or greenish white rough bark¹⁹. *N. Arbortristis* is a shrub or small tree up to 10m heights with gray or greenish rough bark with stiff whitish hairs; young branches sharply quadrangular. Leaves are opposite, 5 -10 by 2.5 – 6.3 cm, ovate, acute or acuminate, entire or with a few large distant teeth, short bulbous hairs rounded or slightcuneate; main nerves few, conspicuous beneath; petiol 6cm long, hairy (Abhyankar, et al, 2009).

CHEMICALS CONSTITUENTS:

A variety of constituents belonging to different chemical classes such as terpenes, steroids, glycosides, flavonoids, alkaloids and aliphatic compounds have been isolated and characterized from different parts of *N. arbortristis*. The bark contains a glycoside and two alkaloids, one soluble in water and the other soluble in chloroform. Its roots are composed of alkaloids, tannins and glucosides. (Suri, et al 2015) Leaves of the plant contain tannic acid, methyl salicylate, an amorphous

glycoside 1%, mannitol 0.3%, and amorphous resin 1.2%, trace of volatile oil. They also contain ascorbic acid (30 mg/100g) and carotene, the ascorbic acid content increases on frying the leaves in oil. The bark contains glycoside (m.p. 86-88°C) and two alkaloids, one soluble in water and soluble in chloroform. The glycoside increases the amplitude of frog's heart in small doses, but in large doses diastolic period is decreases till the heart stops with auriculoventricular block; it also depresses the CNS. The water soluble alkaloids stimulate the ciliary movements of esophagus; the chloroform soluble alkaloids have no such action. The alkaloid and glycoside have little action on blood pressure or respiration. Leaves juice can be used as antipyretic it can also use in obstinate fever, rheumatism, sciatica, diuretic, laxative, anthelmintic ,given in children as remedy for intestinal worms . Bark oil use in pain of eye, it can also use in bronchitis, as expectorant, given in excessive diuresis, in enlargement of spleen, stimulate ciliary movement of oesophagus. Flowers can be used as stomachic, carminative, astringent, as hair tonic. Seeds are useful in piles and skin disease, affection of scalp. ^(The Wealth of India, 1997)



Parijataka twinge with leaves, Flower and Seeds

Introduction to Helminthiasis:

Helminthiasis or worm infection is one of the most prevalent diseases. Many worms are parasitic in humans and causes serious complication. It is estimated that one fourth of the world population may be infected by worms. In Helminthiasis this organism multiply outside of the definitive host and have the unique ability to evade host immune defenses, for reasons that are not fully understood. Helminthiasis tends to be chronic, possibly lasting an entire lifetime of the host. Infected host humans are divided in to two categories or phyla.

- Platy helminthes(flatworms):-

In which a.Cestodes (tapeworms), b.Trematode (flukes) is included.

- Nematodes (roundworms):-

In which a. Roundworm, b.Hookwor, c.Pinworm, d.Whipworm is included.^{(Perry,et al, 1999),(Goodman and Gilman's, 2001)}

Synthetic Drugs used in Helminthiasis:

- Benzimidazoles :-Ex-mebendazole, thiabendazole, albendazole, oxybendazole, parbendazole.
- Diethyl carbamazine.

- Ivermectine.
- Praziquantel.

Diseases caused by helminth parasites in continue to be a major productivity constraint, especially in small ruminants in the tropics and subtropics. (Randolph, et al, 2002). In the developing world, the greatest impact of parasitic diseases is indirect and potential productivity losses. (Perry, et al, 1999). Infections by gastrointestinal helminth parasite of livestock are among the most common and economically important diseases of grazing livestock. (Monteria, et al, 1998). Adulteration of anthelmintics has been found to be common practice. (Dan, et al, 1999). Illiteracy and unfamiliarity with synthetic anthelmintics, resulting in incorrect usage, are also a problem leading to the same consequence. Moreover, these drugs are relatively expensive. As a consequence of these problems and difficulties, pastoralists and small holder farmer have continued to use indigenous plants as livestock dewormers. (Wagho, et al, 2003) Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects. For Example, plants that contain condensed tannins, a class of phenolic secondary metabolite, have this effect. (Randolph, et al, 2002), (Waghom, et al, 2003). The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries and many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed. Since ages, man has been dependent on nature for curing various body diseases. From ancient civilization various parts of different plants were used to eliminate pain, control suffering and counteract disease. Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principal natural source of medicines. (Tripathy, 2003, Gaoodman's and Gimán's, 2001). The plants used, as drugs are fairly innocuous and relatively free from toxic effects or were so toxic that lethal effects were well known. The nature has provided the storehouse of remedies to cure all ailments of mankind. There is no doubt that plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis. (Tripathy, 2003, Gaoodman's and Gimán's, 2001)

Nyctanthes arbortristis Linn. commonly known as Harsinghar or Night Jasmine is one of the well-known medicinal plants. Different parts of *N. arbortristis* are known to possess various ailments by rural mainly tribal people of India (Orissa and Bihar) along with its use in Ayurveda, Sidha and Unani systems of medicines. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. Leaves are also used in the enlargement of spleen. Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant. The claimed traditional medicinal uses have been proved on scientific basis using *in-vitro* and *in-vivo* experiments. The plant has been screened for antihistaminic activity, CNS activities (viz. hypnotic, tranquillizing, local anesthetics), analgesic, anti-inflammatory, antipyretic, antiulcer, amoebicidal, anthelmintic, antitypanosomal to antidepressant, antiviral and immunomodulatory. Leaves were found to have anthelmintic activity traditionally but no report is available on the anthelmintic activity on the leaves extract so the present study is aimed at the screening of the anthelmintic activity in the leaves extracts of the plant *N. arbortristis* Linn. (Gulshan, et al, 2015)

MATERIALS AND METHODS

Plant material and collection: - The leaves of *Nyctanthes arbortristis* have been collected from local area of Jijamata College of Pharmacy Nandurbar Dist-Nandurbar (Maharashtra). The leaves were dried at room temperature (30-40 °C).

Worms Collection: -Indian earthworm *Pheritima posthuma (Annelida)* were collected from the water logged areas of soils Indian earthworms are identified at Jajamata College of Pharmacy Nandurbar Dist-Nandurbar (Maharashtra).

Preparations of Extracts: - The plant material (100 gm., O.D. basis) of *Nyctanthes arbor-tristis* was taken in soxhlet apparatus. The constituents were extracted on the basis of increasing polarity of solvent. Plant material was extracted with following solvents, chloroform, Acetone and hot water in soxhlet apparatus for 8-10 hours. After the completion the extracts were dried under pressure. (Savita, et al, 2013). Each extract was transferred into clean and dried airtight vials until ready for use. (Rangari, 2003)

ANTHELMINTIC ACTIVITY OF *Nyctanthes arbor-tristis* Leaves:

The Anthelmintic activity was carried as per the method of (Ajaiyeoba et al 2009). with necessary modifications. The activity was performed on adult Indian earthworm *Pheritima posthuma*, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Because of easy availability, earth worms have been used widely for initial evaluation of anthelmintic compounds *in vitro*. Nine earthworms, *Pheretima posthuma* of nearly equal size (8±1cm) were placed in each petridishes containing 15ml of normal saline and different dilutions (0.1%, 0.2%, and 0.5%) of the aqueous, chloroform and acetone extracts and the standard piperazine citrate with normal saline. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) (Khandelwal,199) and(Ahirrao, et al, 2011).

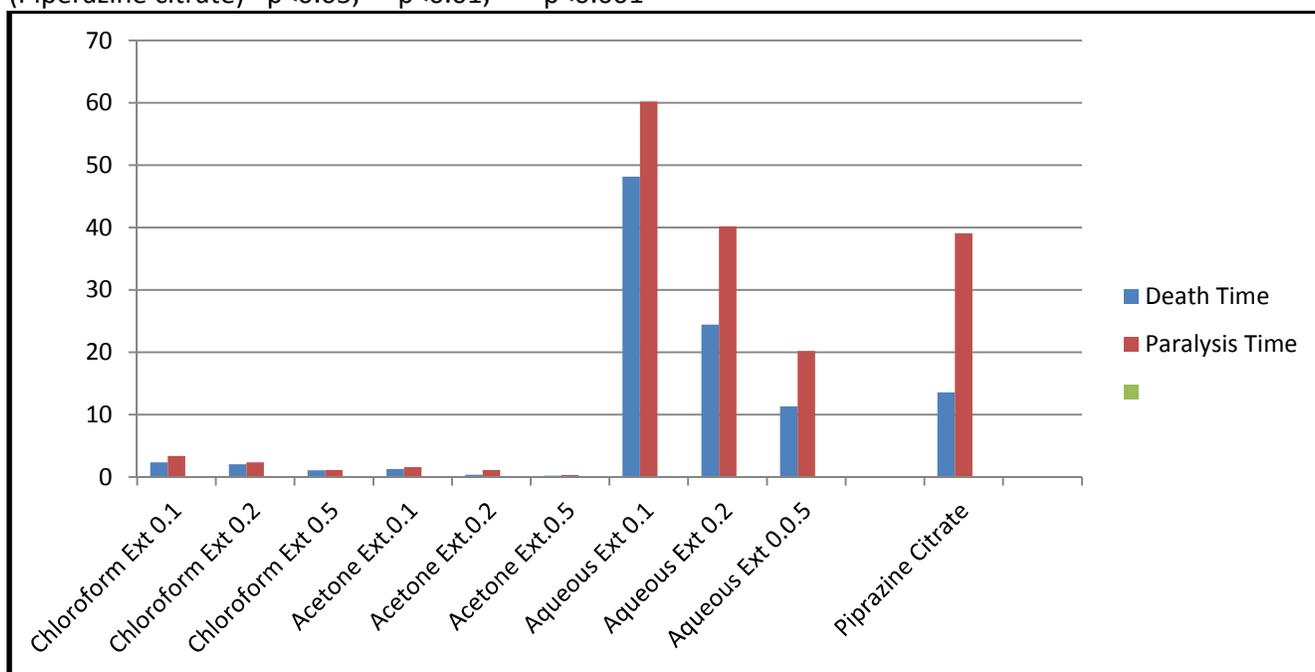
RESULT

The results are shown in table for aqueous, chloroform and acetone extracts of Parijataka leaves. The chloroform extract at conc. 0.1mg/ml, 0.2mg/ml, 0.5mg/ml causes paralysis at 2.38, 2.03, 1.10 min. and the time of death at 3.38, 2.36, 1.11 min. respectively. The Acetone extract at conc. 0.1mg/ml, 0.2mg/ml, 0.5mg/ml causes paralysis at 1.30, 0.37, 0.20 min. and the time of death at 1.56, 1.12, 0.32 min. respectively. The Aqueous extract at conc. 0.1mg/ml, 0.2mg/ml, 0.5mg/ml causes paralysis at 48.16, 24.47, 11.36 min. and the time of death at 60.21, 40.23, 20.20 min. respectively. The Piperazine citrate at conc. 0.1mg/ml causes paralysis at 13.56 min and death at 39.10 min.

Table: Anthelmintic activity of *Nyctanthes arbor-tristis* extract of Leaves.

Sr.No.	Test Substance	Concentration in mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute	
			Paralysis time(min) P	Death time(min) D
1	Chloroform extract	0.1	2.38	3.38
		0.2	2.03	2.36
		0.5	1.10	1.11
2	Acetone extract	0.1	1.30	1.59
		0.2	0.37	1.12
		0.5	0.20	0.32
3	Aqueous extract	0.1	48.16	60.21
		0.2	24.47	40.23
		0.5	11.36	20.20
4	Piperazine citrate	0.5	13.56	39.10

All values are Mean \pm SEM; n=3 in each group. Values are significantly different from reference standard (Piperazine citrate) *p<0.05; **p<0.01; ***p<0.001



Anthelmintic Activity of Different Extract

DISCUSSION

The Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produce hyperpolarization and reduced excitability that lead to muscle relaxation and flaccid paralysis. The leaves extract of *Pheretima posthuma* not only demonstrated paralysis, but also cause death of worm especially at higher concentration of 0.1mg /ml in shorter time as compare to standard drug piperazine citrate. The reported anthelmintics effects of tannins is that they can bind to free proteins in g.i.t. of post animal or glycoprotein on cuticale of parasite and may cause death.

Among the all extract acetone extract at 0.5mg/ml conc. have shown most promising anthelmintic activity as compare to standard.

CONCLUSION

The study has shown that chloroform, acetone, and aqeous extract of *Nyctanthes arbortristis* leaves have significantly determined anthelmintic activity. But the acetone extract of *Nyctanthes arbortristis* leaves shown most significant anthelmintic activity as compared to chloroform and aqeous extract. Further studies can be done to identify the possible phytoconstituents responsible for anthelmintic action. In conclusion the traditional use of plant *Nyctanthes arbortristis* having anthelmintic properties have been confirmed, on the different extracts displayed anthelmintic action on *Pheritima posthuma* (*Annelida*) worm.

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