

**In –Vitro Anthelmintic Activity of *Adhatoda vasica* Leaves on Indian Adult Earthworm**Savita S. Mali<sup>\*1</sup>, Sujata D. Dongare<sup>1</sup>, Sachin S. Mali<sup>1</sup>, Bhagyashri P. Kumbhar<sup>1</sup>, Prasad V. Patrekar<sup>1</sup><sup>1</sup>Department of Pharmaceutics, Adarsh Institute of Pharmacy, Vita, Maharashtra, India

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**ABSTRACT**

Herbal medicines derived from various plants are being utilized to treat various types of diseases, *Adhatoda vasica* is one of them. After literature survey it was observed that drug has screened for anti-tussive and anti T.B., antiulcer activity. This study highlights on Anthelmintic activity of *Adhatoda vasica* leaves (family: Acanthaceae) against Indian earth worm *Pheretima posthuma*. Helminthiasis is parasitic disease and present in human intestine. Its infection is chronic, debilitating and is major cause of morbidity, produces greater economic and social deprivation. Aqueous and methanolic extract were prepared and paralyzing and death time was recorded. *Adhatoda vasica* leaves have prominent anthelmintic activity against earthworm. The activity was found to be inversely proportional to concentration of extracts. Indian system has great history for ayurveda but researchers don't focus on development herbal formulation. This article also highlight phytochemical screening, physicochemical properties, therapeutic uses of these plant.

**Keywords:** Antihelmintics, phytochemical screening, Acanthaceae, Anthelmintic activity on earthworm.

**INTRODUCTION:**

Herbs contain large number of phytoconstituents such as tannins, alkaloids, resins, glycosides, volatile oils, fixed oil these are separated by various extraction processes and their activity is tested on animal. In previous study it was used as antitussive, abortifacient, anti T.B., anti ulcer, but in the view of its diversity in activity Aqueous and methanolic extract prepared and screened for Anthelmintic activity against Indian earthworm *Pheretima Posthuma* because its anatomical resemblance with intestinal parasite<sup>(1-7)</sup>(*ascaris*).

Helminthiasis is parasitic disease and present in human intestine. Its infection is chronic, debilitating and is major cause of morbidity, produces greater economic and social deprivation<sup>(7)</sup>. Conventional antihelmintics produces resistance, adverse effects hence it is necessary to find

alternative means of treatment so various herbs were screened for its anthelmintic activity.

**Habitat/Description:****Morphological characters:**

Leaves are subjected to study various morphological characters such as colour, odour, taste size, shape, extra features.<sup>(2)</sup>

**Microscopic characters:**

Thin section was taken and subjected to various reagents. It indicate presence of caryophyllaceous stomata, covering and glandular trichomes, 2 layers of palisade cells, 2-3 bi-collateral vascular bundles in midrib, calcium oxalate crystals.

Stomatal index and Palisade ratio of 10.8-18.2 and 5-8.5 respectively.<sup>(1)</sup>



Figure 1: Microscopic view of *Adhatoda vasica* under projection microscope

### Physiochemical evaluation:

As per WHO guideline various physiochemical parameters are calculated to evaluate quality and purity of drug<sup>(7)</sup>.

### Phytochemical investigation:

The different phytoconstituents present in powdered drug is identified by various qualitative chemical tests<sup>(7)</sup>.

### Chemical constituents:

It contain piganine type alkaloids, volatile oils, fat, resin, sugar, mucilage adhatodic acid, steroids, tannins<sup>(5)</sup>

### Medicinal uses:

It is used as an expectorant, bronchodilator, antituberculosis, antiulcer drug. It also shows oxytocic property and aborsificant property same as oxytocin and.

### MATERIAL AND METHOD

#### Collection of leaves:

Leaves of *Adatoda vasica* were collected from our college garden. The plant material was taxonomically identified by Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.).

#### Preparation of extract:

Extract was prepared by soxhlet extraction method. Then filtered, evaporated to dryness and finally dried in electronic water bath<sup>(10,11)</sup>. Extraction was carried out by standard procedure. One kg powder of Leaves of *Adatoda vasica* used for extraction. Sample powder was packed

gently in previously washed and dried cloth bag and solvent was placed from the top with the help of funnel to moisten the drug sample. 3.5 liter of solvent (ethyl acetate, methanol, chloroform water, chloroform, and petroleum ether) was placed in distillation flask and assembly was made air tight with sealing wax. Solvents were selected on the basis of extractive values and with their increasing order of polarity. Extraction was carried out at or slightly above the boiling point of each solvent. Extraction was carried out for 18 hours or on the basis of clarity of dropping solvent (saturation). The solvent was collected every time after completion of the process and powder was dried in hot air oven for 24h at 45°C. The process was repeated for all the next solvents and finally the dried powder was macerated with 3.5 liter of chloroform water IP (0.25% v/v) at room temperature with frequent shaking. All the liquid extracts were subjected for physical analysis and are concentrated in a evaporator (Dolphin, Mumbai) and finally dried under reduced pressure. The residue was weighed, % yield was calculated. All the extracts were further dried over anhydrous calcium chloride and preserved in vacuum desiccators for further studies. Different extracts were abbreviated according to solvent and part of the plant and used throughout the work<sup>14</sup>.



Figure 2: Soxhlet extraction apparatus and electronic water bath

### Selection of Indian earthworms:

Earthworms required for experiment were collected from moist soil and washed with distilled water to clean the worms. Healthy earthworms were selected and two worms were placed in each petri plates i.e. standard, control, test solution.<sup>(7)</sup>

### Preparation of standard solution:<sup>(7)</sup>

Albendazole (20mg/ml) was prepared using 2% v/v tween 80 as suspending agent.

### Preparation of extract for activity:<sup>(7)</sup>

The suspension of Methanolic and aqueous extract of leaves of *Adhatoda vasica* of different concentration (10, 15, 20 mg/ml) were prepared using 2% v/v of tween 80 as

suspending agent and final volume was made up to 10 ml of normal saline for respective concentration.

### PROCEDURE FOR ANTHELMINTIC ACTIVITY:<sup>(7)</sup>

Group of two equal sized worm released into 10 ml of standard drug and extract solution in Petri dish and was observed for paralyzing and death time. Time for paralysis was noted when no movement of any sort, except when the worm was shaken vigorously. Then death time was recorded after ascertaining that worms' might not move when shaken nor when given external stimuli. The fading away of body colour were noted. Hemorrhagic and Necrotic spots were observed<sup>(9)</sup>. The test result was compared with standard Albendazole.

## RESULT:

Table 1: Morphological characteristic <sup>(5)</sup>

Sr. No.	Organoleptic Characteristics	Observations
1	Color	Light green
2	Odour	characteristic
3	Taste	bitter
4	Height	10-30cm
5	Width	4-10cm
6	Shape	Ovate -lanceolate
7	Texture	Thin and leathery
8	Fracture Inner surface and outer	Glagbrous slightly pubesant
9	Margin	Crenate with acuminate apex
10	Venation	pinnate, prominent midrib and 8-12 lateral veins

Table 2: Physiochemical parameters <sup>(12,13)</sup>

Sr.No.	PARAMETERS	Results obtained (%w\w)
1	Foreign organic matter	Not more than 2%
2	Moisture content	Not more than 7.8%
3	Total ash value	12.5%
4	Acid insoluble ash value	1.5%
5	Water soluble ash value	3.5%
6	Water soluble extractive value	31.6%
7	Alcohol soluble extractive value	7.4%

Table 3: Phytochemical screening <sup>(12,13)</sup>

Sr. No.	Constituents	Methanol	Water
1	Volatile oil	+	+
2	Alkaloids	+	+
3	carbohydrate	+	-
4	Steroids	+	+
5	Glycoside	+	-
6	Tannins	+	+
7	resins	+	+
8	Gums and mucilage	+	+
9	saponins	+	+
10	flavonoids	+	+

Table 4: -% yield of methanolic and aqueous extraction process

Name of Extract	Part of plant	Nature and colour	% yield
Methanol	leaf	Sticky, greenish	7.4%
aqueous	leaf	Sticky, dark brown	31.5%

Table 5:-Anthelmintics potency of methanolic and aqueous extracts of Adhatoda vasica leaves

	Concentration (mg/ml)	Pheretimaposthuma	
		Paralise time	Death time
Methanol	10	20±0.51	25±1.45
	15	14±0.70	22±0.76
	20	11±0.49	15±1.54
Aqueous	10	32±0.89	40±.34
	15	29±2.78	36±.78
	20	24±0.67	31±1.23
Standard	10	18±2.22	22±0.69
	15	12±0.98	20±1.32
	20	10±0.34	15±0.79
Control	-	-	-



Figure 3: Photographic representation of anthelmintic activity of Adathoda vasica by soxhelt extraction (methanolic extract) a) Test solution (Plant Extract 20mg/ml), b) Standard solution (Albendazole 20 mg/ml), c) Control ( Normal saline)

#### DISCUSSION:

Morphological characterization, microscopic study, essential for identification and proper authentication of drug and also helpful to avoid adulteration. Physiochemical study also support for identification and proper authentication of drug. Foreign organic matter, ash value, Moisture content, extractive value constant for a drug. Ash value is major judge for identity and purity of drug, extractive value majors chemical constituents present in drug. Phytochemical screening reveals that Adhatoda vasica leaves contains secondary metabolites like Volatile oil, Alkaloids, Carbohydrate, Steroids, Glycoside, Tannins, Resin, Saponins, flavonoids. Adhatoda vasica contains major chemical constituent vacinone which incorporate anthelmintic activity to extract. Alkaloidal 'N' atom helps to enhance penetration of active constituents in earthworm. Hence from results it clear that methanolic extract has an equivalent activity as

slandered Albendazole. Earthworms in both solutions died within 15min. But aqueous extract showed less activity due to less solubility of extract in water.

#### CONCLUSION:

From the result it is clear that methanolic extract of, Adhatoda vasica leaves has prominent Anthelmintic activity against earthworm. The activity was found to be inversely proportional to concentration of extracts. Indian system has great history for Ayurveda but researchers don't focus on development herbal formulation.

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