

## Research Article

## Investigation of in Vitro Anthelmintic Activity of *Melochia Carchorifolia* Stem Extract Against *Pheritima Posthuma*

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### ABSTRACT

Aqueous and ethanol extracts from the stems of *Melochia carchorifolia* were investigated for their anthelmintic activity against *Pheritima posthuma*. Three concentrations (20, 40, 60 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worms. Both the extracts exhibited highly significant anthelmintic activity at highest concentration of 60 mg/ml. Piperazine citrate was included as standard reference and normal saline as control. The anthelmintic activity of aqueous and ethanol extracts of *Melochia carchorifolia* has therefore been demonstrated for the first time.

**Keywords:** *Melochia carchorifolia*, anthelmintic, *Pheritima posthuma* and Piperazine citrate.

### INTRODUCTION

Helminthiasis is Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia<sup>1</sup>. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas<sup>2</sup>. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases<sup>3</sup>. Hence there is an increasing demand towards natural anthelmintics. *Melochia carchorifolia* (*M.umbellat* Linn.) L., Malvaceae, is a wild crop and grows in most parts of India as a weed. Some species of the genus *Melochia* have been used in folk medicine, such as *M. corchorifolia* L. (dysentery, abdominal swellings and water-snake bites), *M. umbellata* (Houtt.) Stapf (deobstruent) and *M. pyramidata* L. (bronchitis and cough)<sup>4</sup>.

This plant has a wide reputation among natives of being curative for intestinal-worm infections. This plant is being used

by the tribals of Satpuda hills as an anthelmintic in the form of extract, prepared by boiling the plant material in water for 1 hour. This extract is taken orally once a day for three days to treat intestinal-worm infections. Based on this, an attempt has been made to evaluate the anthelmintic potential of *Melochia carchorifolia*. The leaves are used to poultice ulcers, abdominal swelling and chest pains. A simple decoction of the leaves is used to stop vomiting and as a mixture for treating urinary disorders. A decoction of the roots and leaves is swallowed to treat dysentery. The sap is applied to heal wounds poisoned by Antiaris. The plant is also used to relieve gastralgia and headaches<sup>5</sup>.

### MATERIALS AND METHODS

#### Plant material

The stem of *Melochia carchorifolia* is collected from the local area of surampalem and authenticated by department of Botany, Aditya Institute of science, A.D.B. Road, Aditya Nagar, Surampalem, East Godavari District, Andhra Pradesh.

### Preparation of plant extract

#### Aqueous extract preparation

The crude aqueous extract of the stems of *Melochia carchorifolia* was prepared according to the standard methods. One hundred grams of the powdered plant material was mixed with 500 mL of distilled water in a 1 L flask and boiled for 1.5 h. It was allowed to cool to 40°C and then filtered using whatman No.1 filter paper. The filtrate was then concentrated in a rotary evaporator and the extract stored at 4°C until required<sup>6</sup>.

#### Ethanol extract preparation

Powdered plant material was exhaustively extracted with ethanol in a Soxhlet apparatus. The crude ethanol extract was evaporated to dryness and stored at 4°C until used. The extract yield (% w/w) from the plant material was recorded<sup>7</sup>.

Both extracts were dried at 40-60°C.

#### Phytochemical analysis

Various Phytochemical examinations were conducted for both aqueous and ethanol extracts of *Melochia carchorifolia* stem and they show the presence of the tannins, saponins, carbohydrates and glycosides (Table no1).

#### Worms Collection and Authentication

Indian earthworm *Pheritima posthuma* (Annelida) were collected from the water logged areas of soils Indian earthworms are identified at Department of Zoology, Aditya Institute of science, A.D.B. Road, Aditya Nagar, Surampalem, East Godavari District, Andhra Pradesh.

#### Anthelmintic activity

*Pheritima posthuma* is commonly known as earthworms were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from water Anthelmintic activity was carried out on adult earthworm, *Pherithema posthuma*. Ten groups were made, each containing six adult earthworms of approximately equal size. The solutions of aqueous extract and ethanol extract were made in the concentrations of 20, 40, 60 mg/ml in normal saline and Piperazine citrate were made in concentration of 10 mg/ml.

Groups of earthworms were released into 10 ml of desired formulations as made above, and one group was treating as control in normal saline. The observation was made for the time taken to cause paralysis and death of individual worms. Paralysis was said to occur when the worms did not move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colours<sup>8</sup>.

#### Statistical Analysis

All the data are expressed in mean±SEM. The significance of differences in means between control and treated worms for different parameters was determined by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The minimum level of significance was fixed at  $p < 0.05$ .

### RESULTS AND DISCUSSION

Aqueous and ethanol extracts were used to evaluate anthelmintic activity has shown dose dependant activity. The Mean±SEM. values were calculated for each extracts. The result of anthelmintic activity on earthworm *pheritima posthuma* was given in table 2, reveal that, the 60 mg/ml concentration used for both aqueous and ethanol extracts have shown paralysis and death of earthworms significantly ( $p < 0.001$ ) when compared with Piperazine citrate as reference.

Piperazine citrate shows its action by increasing chloride ion conductance in worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis<sup>9</sup>. The aqueous extract of stems of *Melochia carchorifolia* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 60 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts revealed the presence of tannins among the other chemical constituent within them. Tannins were shown to produce anthelmintic activities<sup>10</sup>. Chemically tannins are polyphenolic compounds<sup>11</sup>. Some synthetic phenolic anthelmintics e.g. niclosamide,

oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation<sup>12</sup>. It is possible that tannins presence in both aqueous and ethanol extract of stems of *Melochia carchorifolia* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tracts of host animal<sup>13</sup> or glycoprotein on the cuticle of the parasite<sup>14</sup> and may cause death. The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in developing countries, including in India. It is in this context that the people consumed several plants or plant derived preparation to cure helminthic infections<sup>15</sup>. The origin of many effective drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the folklore

medicinal plants for their proclaimed anthelmintic efficacy.

### CONCLUSION

In conclusion, both aqueous and ethanol extract of stems of *Melochia carchorifolia* have a potent anthelmintic activity when compared with conventionally used drug like Piperazine citrate. Further studies using in vivo model are required to find out and to establish effectiveness and pharmacological rationale for the use of stems as anthelmintic drug and isolate active constituent from extracts to establish(s) mechanism of action.

### ACKNOWLEDGEMENT

Authors are thankful to Dr. V B Narayanaswamy, Principal, Karavali College of Pharmacy, Mangalore for providing all facilities for research purposed.

**Table 1: Phytochemical analysis for both aqueous and ethanol extracts of *Melochia carchorifolia***

SL. No	Constituent	Tests	Observation	
			AMC	EMC
1	Carbohydrates and Glycosides	Molisch's test	+	+
		Benedicts test	+	+
		Keller killani test	+	+
		Fehling's test	+	+
2	Tannins	Ferric chloride test	+	+
		Lead acetate test	+	+
3	Saponins	Foam test	+	+

AMC= Aqueous extract of *Melochia Carchorifolia*

EMC= Ethanol extract of *Melochia Carchorifolia*

**Table 2: Anthelmintic activity of aqueous and ethanol extracts of *Melochia carchorifolia* against *Pheritima posthuma***

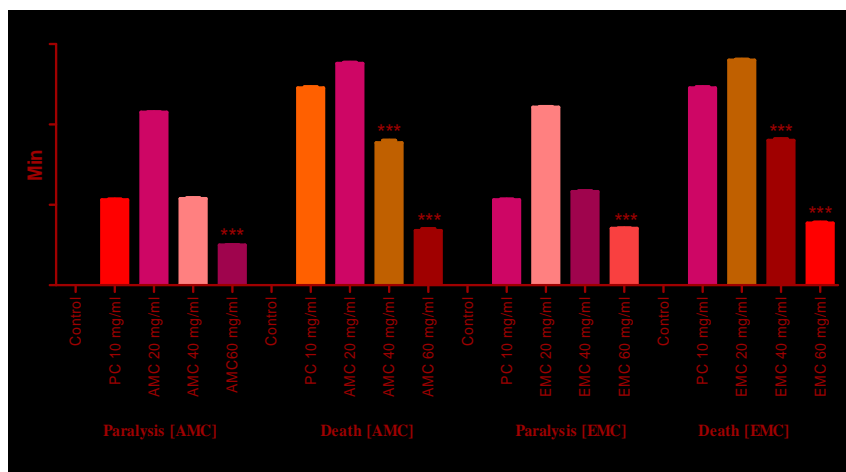
Treatment	Concentration used (mg/ml)	Time taken for paralysis (min)	Time taken for death (min.)
Control [NS]	9	00±00	00±00
AMC	20	43.1±0.0837	55.16±0.6823
	40	21.5±0.453	35.42±1.429***
	60	9.97±0.106***	13.57±1.168***
EMC	20	44.27±0.4082	56±0.563
	40	23.3±0.4382	36±0.957***
	60	14.4±0.1866***	15.4±0.488***
Piperazine citrate	10	21.2±0.404	49.07±0.6377

All the values are expressed as mean±SEM, n=6, \*\*\*p<0.001

(One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test)

as compared to standard group. AMC= Aqueous extract of *Melochia Carchorifolia*,

EMC= Ethanol extract of *Melochia Carchorifolia*, NS= Normal Saline.



**Fig. 1: Anthelmintic activity of aqueous and ethanol extracts of *Melochia carchorifolia* against *Pheritima posthuma***

All the values are expressed as mean $\pm$ SEM, n=6, \*\*\*p<0.001 (One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test) as compared to standard group. AMC= Aqueous extract of *Melochia Carchorifolia*, EMC= Ethanol extract of *Melochia Carchorifolia*, PC= Piperazine citrate.

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