Research Article

A Study on *in vitro* Anthelmintic Activity of Aqueous and Alcoholic Extracts of *Memecyclon malabaricum* Leaves against *Pheritima posthuma*

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ABSTRACT

Aqueous and ethanol extracts from the leaves of *Memecyclon malabaricum* 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 *Memecyclon malabaricum* has therefore been demonstrated for the first time.

Keywords: *Memecyclon malabaricum*, anthelmintic, *Pheritima posthuma* and Piperazine citrate.

INTRODUCTION

Helminthic infections are now being recognised as cause of much chronic ill health and sluggishness amongst the tropical people. More than half of the population in the world suffers from worm infection of one or other.[1] As per WHO, only few drugs are frequently used in the treatment of helminthes in human being.[2] Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver or other organs. Helminthiasis can have immunomodulatory effects on the host, with implications for any co infecting pathogens.[3] The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases.[4] Hence there is an increasing demand towards natural anthelmintics.[5]

Memecylon is genus of shrubs or small tree distributed throughout the world. *Memecylon malabaricum,* cogn is a tree up to 5 m tall, widely distributed in Western Ghats (Dakshina kannada and Udupi districts of Karnataka, Kerala, and Tamilnadu) of India, China, Srilanka, Asia, Africa, Australia, Madagascar, Pacific Islands, belongs to the family Melastomataceae**.** Up to this 300 species were identified. The Melastomataceae family is a vast source of pharmacologically active tannins, flavonoids, alkaloids, resins, waiting for experimentation and observed for anti-microbial, anti-inflammatory, anti-HIV, anti-hypertensive, for treatment of skin disorders, diarrheal, bleeding, and scavenging of free radicals, inhibition of monoamine oxidase inhibitors (MAO-B), post partum invigoration and astringent activities. *Memecylon malabaricum* has considerable reputation for its traditional use in the treatment of diabetes, various bacterial infections, inflammatory and skin disorders including herpes, chicken pox. It is also used as a root ecbolic like ergot. Although this herb has many useful claims, no specific scientific study has been carried out to examine the antihelmintic activity of the plant, that's why the current study was designed.[6]

MATERIALS AND METHODS

Plant material

The leaves of *Memecyclon malabaricum* was collected from the local area of udupi and authenticated by botanist Prof. Ramakrishna Marati Pilikula Nisargadhama, Vamanjoor, Mangalore, Karnataka.

Preparation of plant extract

Aqueous extract preparation

The crude aqueous extract of the leaves of *Memecyclon malabaricum* 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 40ºC 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.[7]

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 4ºC until used. The extract yield (% w/w) from the plant material was recorded.[8]

Both extracts were dried at 40-60ºC.

Phytochemical analysis

Various Phytochemical examinations were conducted for both aqueous and ethanol extracts of *Memecyclon malabaricum* leaves and they show the presence of the tannins, saponins, carbohydrates and glycosides.

Worms Collection and Authentication

Indian earthworm *Pheritima posthuma* (Annelida) were collected from the water logged areas of soils from udupi, Karnataka. Indian earthworms are identified at Department of Zoology,St. Raymonds science college Vamanjoor, Mangalore, Karnataka.

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[9].

Table 1: Phytochemical analysis for both aqueous and ethanol extracts of *Memecyclon malabaricum*

S. No	Constituent	Observation	
		AMC	EMC
1	Glycosides	+	+
2	Tannins	+	+
3	Saponins	+	+
4	Sterols	+	+
5	Phenol	+	+
6	Flavonoids	+	+

AMM= Aqueous extract of *Memecyclon malabaricum*
EMM= Ethanol extract of *Memecyclon malabaricum*

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 40 and 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[10]. The aqueous extract of leaves of *Memecyclon malabaricum* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 40 and 60 mg/ml, in shorter time as compared to reference drug. 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[11]. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[12]. It is possible that tannins presence in both aqueous and ethanol extract of *Memecyclon malabaricum* produced similar effects. Another possible anthelmintic effect is that they can bind to free proteins in the gastrointestinal tracts of host animal or glycoprotein on the cuticle of the parasite and may cause death.[13,14]

In conclusion, both aqueous and ethanol extract of leaves of *Memecyclon malabaricum* 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 leaves as anthelmintic drug and isolate active constituent from extracts to establish(s) mechanism of action.

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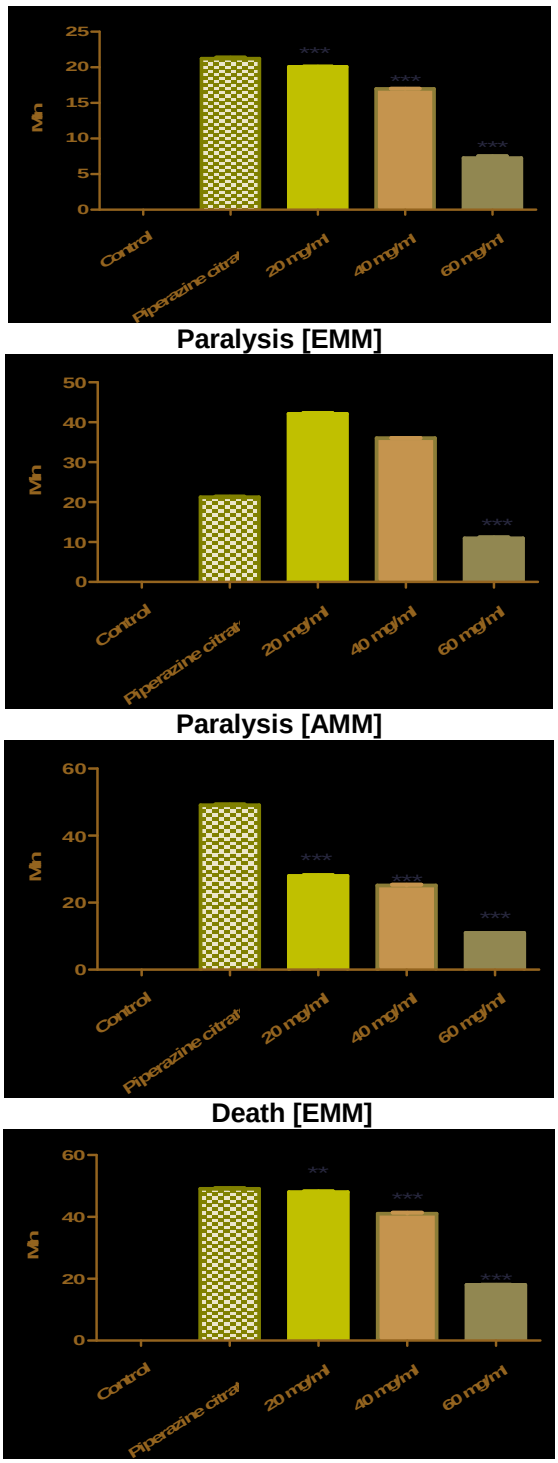

All the values are expressed as mean±SEM, n=6, ***p<0.001 (One way Analysis of Variance [ANOVA] followed by multiple comparison Dunnett's test) as compared to standard group. AMC= Aqueous extract of *Memecyclon malabaricum*, EMC= Ethanol extract of *Memecyclon malabaricum*, PC= Piperazine citrate.

Fig. 1: Anthelmintic activity of aqueous and ethanol extracts of *Memecyclon malabaricum* against *Pheritima posthuma*

Table 2: Anthelmintic activity of aqueous and ethanol extracts of M*emecyclon malabaricum* against *Pheritima posthuma*

Treatment	Concentration used (mg/ml)	Time taken for para-lysis (min)	Time taken for death (min.)
Control [NS]	9	-	-
AMC	20	42.10±0.6325***	48.02±0.55831***
	40	36±0.1134***	41.02±0.8281***
	60	10.93±0.6439***	18.05±0.1332***
EMC	20	20.07±0.1366***	27.98±0.6047***
	40	16.97±0.1033***	25.12±0.6911***
	60	7.25±0.6892***	10.97±0.5164***
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 ofx, EMC= Ethanol extract ofx, NS= Normal Saline.

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