INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTHELMINTIC ACTIVITY OF *MILLINGTONIA HORTENSIS* LINN

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ABSTRACT

The present study was designed to evaluate the anthelmintic potential of *Millingtonia hortensis* Linn. (*M. hortensis*) is an important medicinal tree in Indian traditional system of medicine. In this study, the various extract of stems of *M. hortensis* was investigated for their anthelmintic activity against the earthworms, *Pheritima posthuma*. Each extract was studied in the bioassay at 20 mg/ml, which involved determination of time of paralysis and time of death of the worms. The stems extracts of plant, *M. hortensis* showed significant activity against worms and exhibited considerable anthelmintic activity. Albendazole (20 mg/ml) and distilled water were included in the assay as a standard reference drug and control respectively. Present investigation indicated that among the various extract, ethyl acetate soluble fraction of methanolic extracts of plant, *M. hortensis* showed more prominent activity. These findings will be useful toward the better acceptability of this plant in therapeutics.

Keywords: *Millingtonia hortensis* Linn, Preliminary Phytochemical testing, *Pheritima posthuma*.

INTRODUCTION

A very tall tree, *Millingtonia hortensis* Linn (*M. hortensis*) belongs to family "*Bignoniaceae*" commonly known as 'Akas Nim' has been a great medicinal value. in Southern Asia, ranging from India, Burma, Thailand and Southern China. In folklore medicine, the leaves of *M. hortensis* are used as antipyretic, sinusitis, cholagogue and tonic. Dried flower is a good lung tonic and used in the cough diseases. Bark is used to produce yellow dye.[1, 2]. Literature reports suggested that leaves contains hispidulin, scarotene, dinatin, rutinoside. Bark having bitter substances and tannins. Flowers showed presence of hispidulin[3], scutellarein, scutellarein-5-galactoside[4], hortensin[5], Cornoside, recimic rengyolone, rengyoside B, rengyol, rengyoside A and iso rengyol[6], Millingtonine[7]. Plant has reported for pharmacological actions like apoptosis and antipoliferation activity[8, 9], mutaginicity and antimutaginicity[10], antifungal[11], anticonvulsant[12] and antioxidant activity[13]. Flowers & leaves showed antiasthmatic and larvicidal activity respectively[14, 15]. Flowers and leaves both reported for antimicrobial activity[16, 17]. Although the plant has great medicinal value in the treatment of various diseases, still preliminary reports was found on this plant. Therefore, present investigation was planned to find out scientific evidences and data for evaluation of anthelmintic potential of stems of *M. hortensis.*

MATERIALS AND METHODS

Plant materials

The stems of the plant, *M. hortensis* were collected at Rajapur area, Sangamner, Maharashtra in May 2014. The plant was authenticated and herbarium deposited at the Department of Botany, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Ahmednagar, Maharashtra, India under voucher specimen number HRS/143. The stems of the plant were dried, powdered and passed through

40 mesh sieve and stored in an airtight container for further use.

Preparation of extracts

The air-dried stems of *M. hortensis* were made into a coarse powder. The powdered material was defatted with petroleum ether. The defatted material was extracted with methanol and distilled water using a Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate to get ethyl acetate soluble and ethyl acetate insoluble fractions. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried[18].

Preliminary phytochemical analysis

The preliminary phytochemical screening of various extracts of *M. hortensis* stems was carried out by performing qualitative chemical test[19].

Procurement of animals

Indian adult earthworms (*Pheretima posthuma*) was collected from moist soil and authenticated at Department of Horticulture, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Ahmednagar, Maharashtra. The earthworm of 3-5 cm in length and 0.1-0.2 cm in width was washed with normal saline to remove all the earthy and foreign matter and were used for the experimental protocol of anthelmintic activity because of due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being[20, 21].

DRUGS AND CHEMICALS

Albendazole was procured from Mankind Pharma Ltd., New Delhi. All chemicals such as methanol, dimethyl formamide (DMF) and saline water were purchased from Modern Chemicals, Nashik.

Anthelmintic activity

The various extracts of *M. hortensis* stems were dissolved in minimum amount of dimethyl formamide (DMF) and the volume was adjusted to 10 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment.

In each case, six earthworms were released into 10 ml of desired formulations such as vehicles (5% DMF in normal saline), Albendazole (20 mg/ml), petroleum ether extract, methanolic extract, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extracts of stems of *M. hortensis* (20 mg/ml, each) in normal saline containing 5% DMF. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors[22].

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. The values are expressed as mean ± SEM and $P<0.05$ was considered significant.

RESULTS

Present investigation is indicated the extractive values of petroleum ether, methanolic, ethyl acetate soluble, ethyl acetate insoluble and aqueous extract were found to be 3.75%, 8.0%, 3.8%, 4.2%, 8.44% w/w respectively (Table 1).

Preliminary Phytochemical screening including qualitative chemical examination of various extracts of stems of *M. hortensis* reveals the presence of carbohydrates, glycosides, flavonoids tannins and phenolic compounds in methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract. Aqueous extracts also showed presence of proteins. Petroleum ether extract shows positive result for the phytosterol (Table 2).

In the present investigation, the various extracts of stems of *M. hortensis* were evaluated for its anthelmintic potential. It is evident from the experimental data that, the various extracts of stems of *M. hortensis* showed significant ($P<0.01$) anthelmintic activity at 20 mg/ml when were comparable with the standard drugs, Albendazole at same concentration. It reveals that ethyl acetate soluble fraction of methanolic extract of stem of *M. hortensis* group (V) showed the significant paralysis at 3.28 ± 0.085 and death at 3.37 ± 0.045 when compared with standard Albendazole drug group (II) showed the paralysis at 3.21 ± 0.046 and death at 3.39 ± 0.012 (Table 3).

DISCUSSION

Present investigation has evident that, among various extracts of *M. hortensis* stems, ethyl acetate soluble fraction of methanolic extract showed least time required for paralysis as well as death of earthworm rather than the other extracts. Hence it observed that the stems of plant, *M. hortensis* having prominent significant anthelmintic activity when compared with standard, Albendazole. The function of the anthelmintic drugs like Albendazole is to cause

paralysis of worms so that they are expelled in the feaces of man and animals. The ethyl acetate soluble fraction of methanolic extracts of *M. hortensis* stems demonstrated intrinsic anthelmintic properties especially at 20 mg/ml giving a shortest time of paralysis and death of earthworm, *Pheretima posthuma* when compared with Albendazole (Figure 1 & 2).

Therefore this may conclude that the traditional medicinal plants, *M. hortensis* have been scientifically confirmed to display anthelmintic potential and anticipated for acceptable in therapeutics. Present study has supported to traditional medicinal potential of this plant and has keen attention toward profound potential of phytoconstituents which is responsible for the anthelmintic potential of this plant.

ACKNOWLEDGMENTS

The authors express sincere thanks to Principal and Management, Amrutvahini Sheti & Shikshan Vikas Sansthas, Sangamner, Maharashtra for providing valuable support for this work.

Table 1: Extractive values of *M. hortensis* stem extracts

S. No.	Extract	Yield (% w/w)	Color of extract
1.	Petroleum Ether	2.7	Yellow
2.	Methanolic	6.8	Reddish brown
3.	Ethyl acetate soluble fraction	3.8	Reddish brown
4.	Ethyl acetate insoluble fraction	2.2	Brown
5.	Aqueous	4.4	Brown

Table 2: Preliminary Phytochemical analysis of *M. hortensis* stems extracts

Chemical Constituents	Chemical tests	Petroleum ether extract	Methanol extract	Ethyl acetate soluble	Ethyl acetate insoluble	Aqueous extract
Alkaloids	Dragendorff's test	_	_	_	_	_
	Mayer's reagent	_	_	_	_	_
Carbohydrates	Molisch's test	_	+	+	_	+
	Barfoed's test	_	+	+	_	+
Glycosides	Borntrager's test	_	+	+	+	+
	Keller-killianin test	_	_	_	_	_
Saponin glycosides	Foam test	_	_	_	_	_
Flavonoids	Shinoda Test	_	+	+	+	+
	Sodium hydroxide test	_	+	+	+	+
	Lead acetate test	_	+	+	+	+
Tannins	Ferric chloride test	_	+	+	+	+
	Phenazone test	_	+	+	+	+
Steroids	Salkowaski test	+	_	_	_	_
	Libermann-burchard test	+	_	_	_	_
Proteins	Biuret test	_	_	_	_	+

+: Present, - : Absent

Table 3: *In vitro* anthelmintic activity of *M. hortensis* stems at 20 mg/ml

Group	Treatment	Time taken for the paralysis (min)	Time taken for the death (min)
I	Control (in normal saline)	-	-
II	Albendazole (in 5% DMF)	3.21 ± 0.046	3.39 ± 0.012
III	Petroleum ether extract (PEE)	4.40 ± 0.157	6.02 ± 0.057*
IV	Methanolic extract (ME)	3.42 ± 0.103	3.94 ± 0.022**
V	Ethyl acetate soluble fraction (ESF)	3.28 ± 0.085	3.37 ± 0.045**
VI	Ethyl acetate insoluble fraction (EIF)	3.98 ± 0.122	4.26 ± 0.026*
VII	Aqueous extract (AE)	4.03 ± 0.031	4.78 ± 0.049*

(-) no any mortality observed in control worm group (I) during 24 hr observation.
Values are expressed as mean ± SEM, n=6.
When Group (III, IV, V, VI, VII, VIII) are compared with Group (II)
*P<0.05, **P<0.01, ***P<0.001

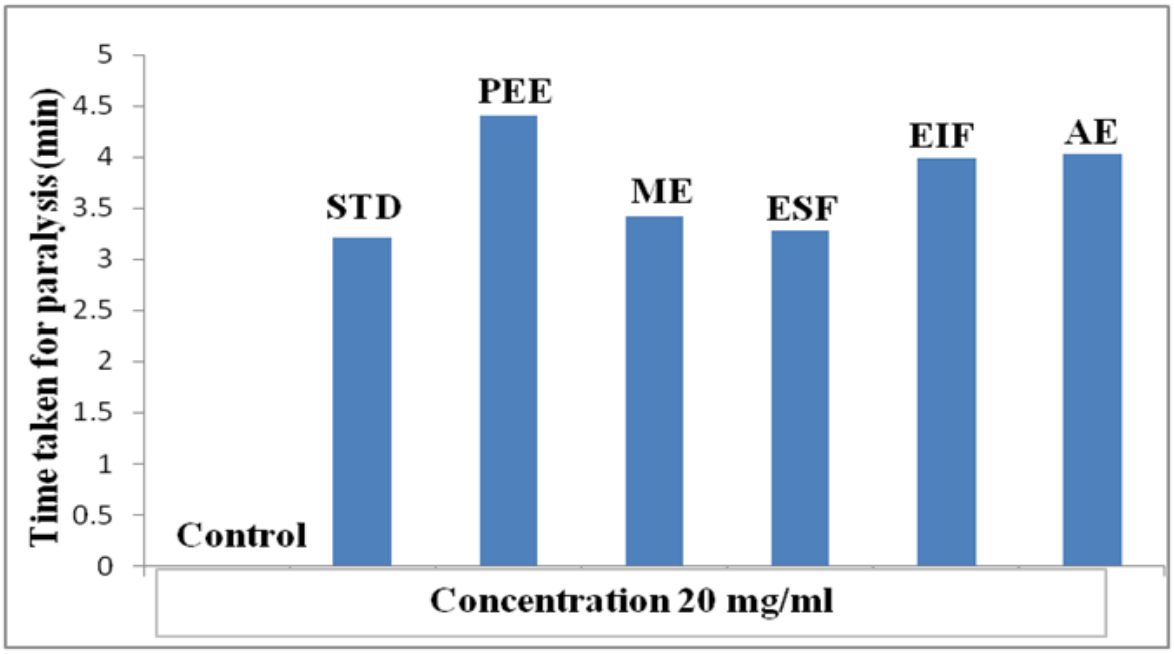

Fig. 1: Graph showing time taken for paralysis (min) (Concentration Vs Time)

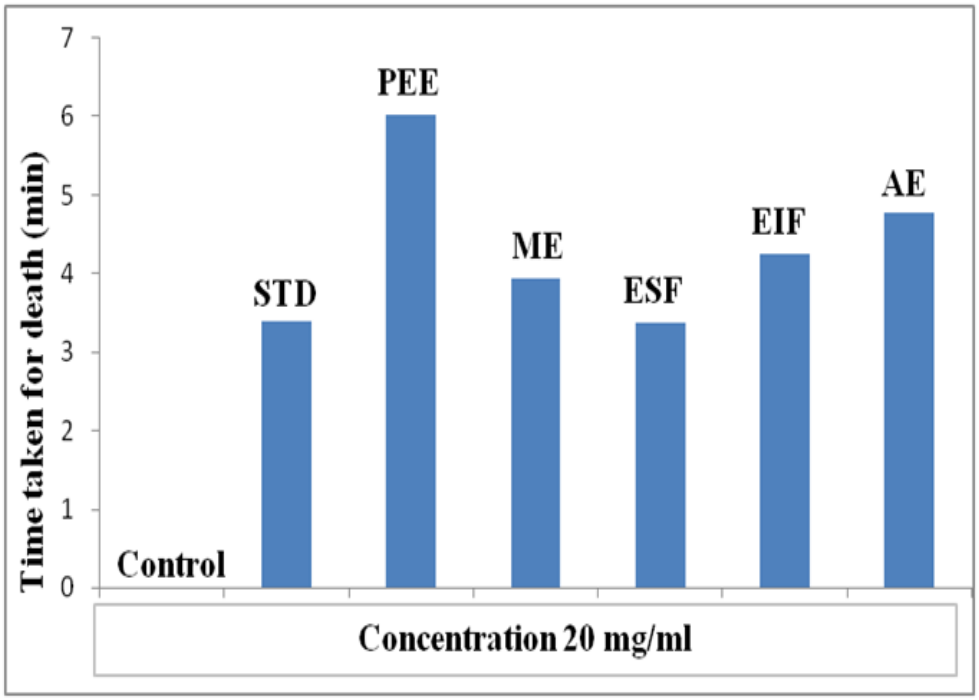

Fig. 2: Graph showing time taken for death (min) (Concentration Vs Time)

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