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IN VITRO ANTHELMINTIC ACTIVITY OF STEM BARK OF *MILLINGTONIA HORTENSIS* LINN.

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ABSTRACT

The present study was undertaken to evaluate anthelmintic activity of different extracts (petroleum ether, benzene, chloroform, methanol and aqueous extracts) of stem bark of *Millingtonia hortensis* (Bignoniaceae) against adult earthworm *Pheretima posthuma*. Piperazine citrate was used as standard reference drug. Among all the extract tested, methanol showed dose dependent anthelmintic and better activity in comparison with reference standard. Chloroform and benzene extracts at 20 mg/ml concentration also showed similar activity in comparison with piperazine citrate at dose of 60 mg/ml. Aqueous extract was not at all active. Preliminary phytochemical screening revealed the presence of steroids, flavonoids and tannins in different extracts.

KEY WORDS

Millingtonia hortensis Linn, *Pheretima posthuma*, anthelmitic activity; piperazine citrate; methanol extract; stem bark; phytochemical screening.

INTRODUCTION

Millingtonia hortensis Linn (Bignoniceae) commonly known as Cork tree is important medicinal plant in Southern Asia ranging from India, Burma, Thailand and South China. The stem bark is used traditionally as mainly lung tonic, anti asthmatic and antimicrobial[1]. The scientific activities reported so far from the plants are antifungal[2], larvicidal[3,4], antimicrobial[5],antioxidant[6] and antiproliferative[7, 8] activities. However there is no report on anthelmintic activity of this plant. In the light of the above information, the present investigation was under taken to evaluate the anthelmintic potential of different extracts of stem bark of *Millingtonia hortensis* Linn.

MATERIALS AND METHODS

Plant material

The fresh stem bark of *Millingtonia hortensis* Linn were collected from Bengaluru region, identified and authenticated by Dr Shiddamallayya N (SMPU/NADRI/BNG/ 2010-11/304) at National Ayurveda Dietetics Research Institute, Bengaluru, Karnataka.A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

Drugs and chemicals

The drug, Piperazine citrate was procured from SD Fine Chemicals Ltd., Mumbai. All organic solvents and chemicals were purchased From SD Fine Chemical Ltd., Mumbai and were of analytical grade.

Preparation of extracts

The dried stem bark powder were coarsely powdered and subjected to successive extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, methanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 1.44, 0.52, 0.61, 15.91 and 2.33 % w/w respectively with reference to the air dried plant material.

Preliminary phytochemical screening

The coarse powder stem bark of *Millingtonia hortensis* (20 g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether, benzene, chloroform, methanol and distilled water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents[9].

Earthworms collection and authentication

Healthy adult Indian earthworm (*Pheretima posthuma*; Annelida; Megascolecidae) were collected from Microbial Resources Division, Gandhi Krushi Vijnana Kendra (GKVK), Government of Karnataka, Bengaluru. Earthworms in moist soil were washed with normal saline and used for the study. The earthworm of 3 -5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[10,11].

Anthelmintic activity

The anthelmintic activity of stem bark extracts of *Millingtonia hortensis* Linn was evaluated as per the method reported by Dash et al[12]. The extracts were suspended in Tween 80 (0.1 %) in normal saline. All the drugs and extracts were freshly before starting the experiment. Thirteen groups of six earthworms each were released into 20 ml of desired formulation as follows; vehicle (0.1 % Tween 80 in normal saline), piperazine citrate (40, 60 mg/ml), petroleum ether (20, 40 mg/ml), benzene extract (20 mg/ml), chloroform extract (20 mg/ml), methanol extract (20, 40, 60, 80 mg/ml) and aqueous extract (20, 40 mg/ml). As the percentage yield of petroleum ether, benzene and chloroform extract were less, so only few concentrations could be prepared. Observation were made for the time (in minutes) taken to paralysis and death of individual worms up to 4 hrs of the test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body color[13].

RESULTS AND DISCUSSION

The results of the anthelmintic activity are given in the Table 1 and Fig 1.

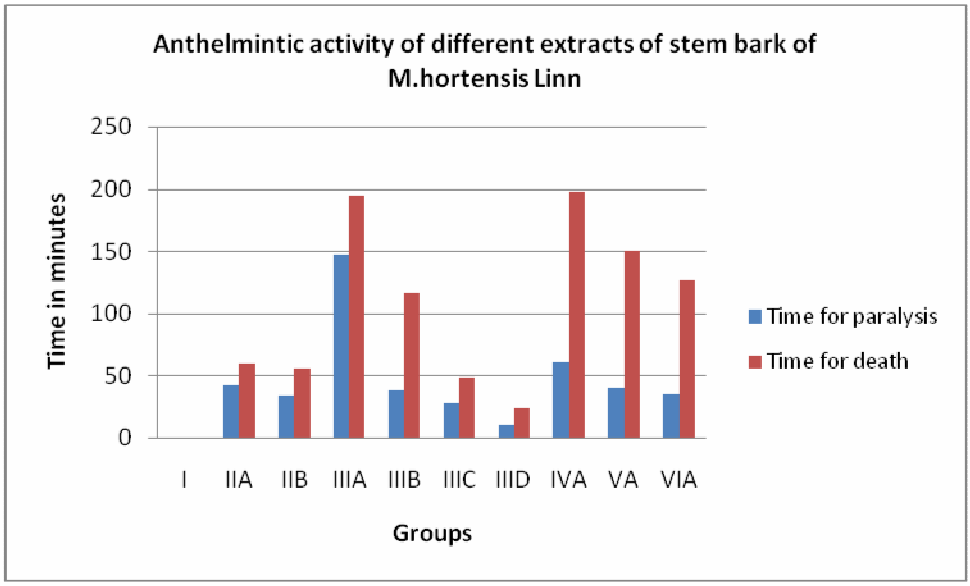
Table1

Anthelmintic activities of different extract of Millingtonia hortensis Linn stem bark

Treatment group	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Vehicle (0.1 % Tween 80 in normal saline	------	------	------
Piperazine citrate	40	42.0 ± 1.26	59.4 ± 0.40
	60	33.5 ± 0.60	55.6 ± 0.24
Methanol extract	20	147.25 ± 0.85	194.5 ± 1.54
	40	38.5 ± 2.02	116.6 ± 0.74
	60	27.3 ± 1.52	48.3 ± 1.36
	80	10.0 ± 0.57	23.6 ± 1.14
Petroleum ether extract	20	160.25 ± 0.73	-----
	40	60.25 ± 0.98	197.7 ± 1.10
Benzene extract	20	39.66 ± 0.31	150 ± 0.31
Chloroform extract	20	34.75 ± 1.67	126.5 ± 3.12
Aqueous extract	20	200 ± 3.12	-----
		173.75 ± 1.12	-----

Results are expressed as mean ± SD of six determinations; vehicle worms were alive up to 24 hrs of observation. Petroleum ether (20 mg/ml) and aqueous extract (20, 40 mg/ml) showed no death but only paralysis. -----: worms were alive.

Fig 1
Anthelmintic activities of different extract of stem bark of *Millingtonia hortensis* Linn on *Pheretima postuma.*



Group I: Vehicle(Normal saline); group IIA,IIB:standard Piperazine citrate at 40 and 60 mg/ml);group IIIA,IIIB,IIIC,IIID: Methanol extract at dose of 20,40,60,80 mg/ ml; group IVA :petroleum ether extract at 40 mg/ml; group VA:benzene extract at 20 mg/ml; group VIA: chloroform extract at 20 mg/ ml respectively.

From the Table 1 and Fig 1, it is very clear that methanol extract at the concentration of 20, 40, 60 and 80 mg/ ml produced anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D). Methanol extract at 80 mg/ml (10.0 ± 0.57 min & 23.6 ± 1.14 min), 60mg/ml (27.3 ± 1.52 min & 48.3 ± 1.36 min) and 40mg/ml (38.5 ± 2.02 min & 116.6 ± 0.74 min) showed shortest time of paralysis (P) and death (D) when compared with piperazine citrate at 40 mg/ml (42.0 ± 1.26 min & 59.4 ± 0.40 min) and 60 mg/ml (33.5 ± 0.60 min & 55.6 ± 0.24 min) concentrations respectively. Benzene extract at 20 mg/ml (39.66 ± 0.31 min & 150 ± 0.31 min) and chloroform extract at 20 mg/ml (34.75 ± 1.67 min & 126.5 ± 3.12 min) showed good anthelmintic activity in comparison to piperazine citrate at 60 mg/ml concentration. Aqueous extract was not at all effective as anthelmintic activity. Whereas, in control group, worms were observed for 24 hours and no paralysis or death was found during that period. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis[14]. The stem bark of *Millingtonia hortensis* extracts not only demonstrated paralysis, but also causes death of worms especially at higher concentration of 40, 60 and 80 mg/ml, in shorter time as compare to reference drug piperazine citrate. Preliminary phytochemical screening of petroleum ether, benzene and chloroform extracts revealed the presence of steroids and methanol extract contains tannins. These phytoconstituents may be responsible for the anthelmintic activity.

CONCLUSION

Among all the extract tested, methanol extract showed dose dependent anthelmintic and

better activity in comparison with reference standard. Chloroform and benzene extracts at 20 mg/ml concentration also showed similar activity in comparison with piperazine citrate at dose of 60 mg/ml. Aqueous extract was not at all active. On the basis of these investigations, we may partially conclude that *Millingtonia hortensis* could be a potent anthelmintic agent for next generation. Further studies are required on phytochemical profiling as well as isolation and identification of bioactive component responsible for anthelmintic activity.

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