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In vitro anthelmintic activity of *Millettia auriculata* leaves and stems

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ABSTRACT

Objective: To study the anthelmintic activity of phytochemically charaterized chloroform extracts of *Millettia auriculata* leaves and stems.

Methods: Chloroform extracts of leaves and stems were prepared. Phytochemical characterisation involved preliminary screening, optimisation of solvent system in thin layer chromatography (TLC) and UV absorption measurement of each bright yellow (in view of a common physical property of flavonoids) TLC fraction for each extract. Anthelmintic activity of each extract was carried out for different concentrations (10, 20 and 40 mg/mL) using *Pheretima posthuma*.

Results: Phytochemical characterisation of the extracts revealed the presence of steroids/triterpenes, phenolics/flavonoids and carbohydrates with optimum TLC-separation in chloroform-hexane, 19:1. All the investigated extracts possessed significant anthelmintic activity ($P<0.001$) at a minimal dose of 10 mg/mL when compared with the corresponding concentration of reference drug albendazole. Leaf extract was found to have more efficacy and potency than that of stem and albendazole.

Conclusions: The results of this study indicated that *Millettia auriculata* can be used as anthelmintic drug. It would be also interesting to find out any novel or existing chemical entities showing anthelmintic activity with mechanism of action.

1. Introduction

After decades of mass application of synthetic benzimidazole carbamates in the control of gastrointestinal nematodes in livestock, resistant strains have emerged all over the world. With the recent emphasis of the World Health Organization on the development of novel antifilarial agents from natural products, extracts derived from both terrestrial plants and marine flora/fauna were involved in the screening programs. Ivermectin, a macrolide antibiotic from natural source (*Streptomyces avermitilis*) is widely used in the treatment of onchocerciasis in humans and filariasis in livestock. Anthelmintic activities of several isolates (papaverine and protopine from *Papaver somniferum*; allocryptopine from *Glaucium arabicum*; dehydrocorydaline from *Corydalis yanhusuo*; acaciaside A and B from *Acacia auriculiformis*; solasodine, solakhasanin and solamargine from *Solanum myriacanthum*; vasicine and vasicinone from *Adhatoda vasica*; piplartines and piperine from *Piper tuberculatum*; quercetin-3-glucuronide, linalool, camphor, geraniol and coumarins from *Coriandrum sativum*) from higher plants represent the significance of natural products for control of parasitic helminths[1]. The use of medicinal plants for helminthiasis also has the advantage of sustainable supply and ecological acceptance[2].

A literature survey on *Millettia auriculata* (*M. auriculata*) revealed its traditional uses mainly as insecticide, vermifuge and fishing poison[3-5]. Other species *viz.*

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Millettia barteri, *Millettia demeusei*, *Millettia gentilii* and *Millettia urophylla* are also traditionally used as vermifuge[6]. Scientific evaluations on antischistosomal activity of *Millettia thonningii* and anthelmintic activity of *Millettia pachycarpa* mediated by apoptosis in *Raillietina echinobothrida* were reported in the recent past[7,8]. Keeping the folkloric claims on *Millettia* species in view, the authors initiated the evaluation of anthelmintic activity of chloroform extract of *M. auriculata* leaves (CEMAL) and stems (CEMAS).

2. Materials and methods

2.1. Plant material

The plant material *M. auriculata* was collected from the forest Pilak, an archeological spot. It was authenticated by scientist Dr. P.V. Prasanna at BSI, Deccan Regional Centre, Hyderabad. A voucher specimen (SD002) was deposited at Herbarium of the University, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

2.2. Preparation of extracts

Around 0.4 kg of dried powdered leaf of *M. auriculata* was extracted at room temperature for 24 h with chloroform (3×0.6 L). The combined extract was concentrated under reduced pressure and yielded 7.8 g thick green residue (1.95% w/w). Similarly, 0.5 kg of dried coarsely powdered stem yielded 7.4 g thick brown residue (1.48% w/w). The extracts were stored at 20 °C until used.

2.3. Drugs and chemicals

Albendazole suspension (Glaxo SmithKline Pharmaceuticals Ltd., Bangalore), sodium caroboxymethyl cellulose (SCMC, Loba Chemie Pvt. Ltd., Mumbai) and chloroform (Merck, Mumbai) were used during the experimental protocol.

2.4. Phytochemical characterisation of the extracts

Preliminary phytochemical screening of CEMAL and CEMAS were carried out to evaluate the presence of various bioactive principles. An attempt to optimise the solvent system in thin layer chromatography (TLC) for the extracts was made to record the colours of the separated spots with their corresponding R_f values. UV spectrum for ethanolic solution of each bright yellow (in view of a common physical property of flavonoids) TLC fraction of the CEMAL and CEMAS was measured at 120 nm/min scan speed on Jasco V-650 spectrophotometer.

2.5. Experimental model

Indian adult earthworms *Pheretima posthuma* (*P. posthuma*) were used to carry out the anthelmintic evaluation. The earthworms were collected from the moist soil of water canals of Peddapuram. Worms were washed with saline water (0.9% w/v NaCl) to remove fecal and earthen matters. Worms of about 10 to 11 cm long and 0.3 to 0.4 cm wide were selected for the experiment. Ready availability, anatomical and physiological resemblance of *P. posthuma* with intestinal roundworm parasites made it suitable to be used for *in vitro* study of anthelmintic activity.

2.6. Anthelmintic activity

The anthelmintic evaluation was designed on adult Indian earthworms (*P. posthuma*) of uniform size, six in each group[2,9]. Required aliquot of each of CEMAL, CEMAS and albendazole was suspended in 1% w/v sodium carboxy methyl cellulose solution to obtain the concentrations of 10, 20 and 40 mg/mL. Each worm was placed in a Petri dish accommodating 10 mL sample solution and time for paralysis and death were recorded in average values. Paralysis was said to occur either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water at 50 °C. Death was judged on the basis of the loss of spontaneous movement and complete destruction of the worm in a sequence of secreting white substances followed by shortening, widening, hardening and fading away of colour of the body.

2.7. Statistical analysis

All the results are expressed as mean±SEM. Each group of test data was compared with that (Group II/III/IV, corresponding to the test concentration) of reference data and analysed by Tukey-Kramer multiple comparison test. Values would be considered statistically significant when P value was less than 0.05.

3. Results

3.1. Phytochemical characterisation of the extracts

On preliminary phytochemical screening, the CEMAL and

CEMAS revealed the presence of steroids/triterpenes (+ve Salkowski and Liebermann Burchard's test), phenolics/flavonoids (+ve ferric chloride test) and carbohydrates (+ve Molisch test). TLC examination of CEMAL and CEMAS showed the optimum separation in chloroform-hexane, 19:1. The chromatogram developed on spraying 5% ethanolic sulphuric acid displayed numbers of prominent spots (Figure 1).

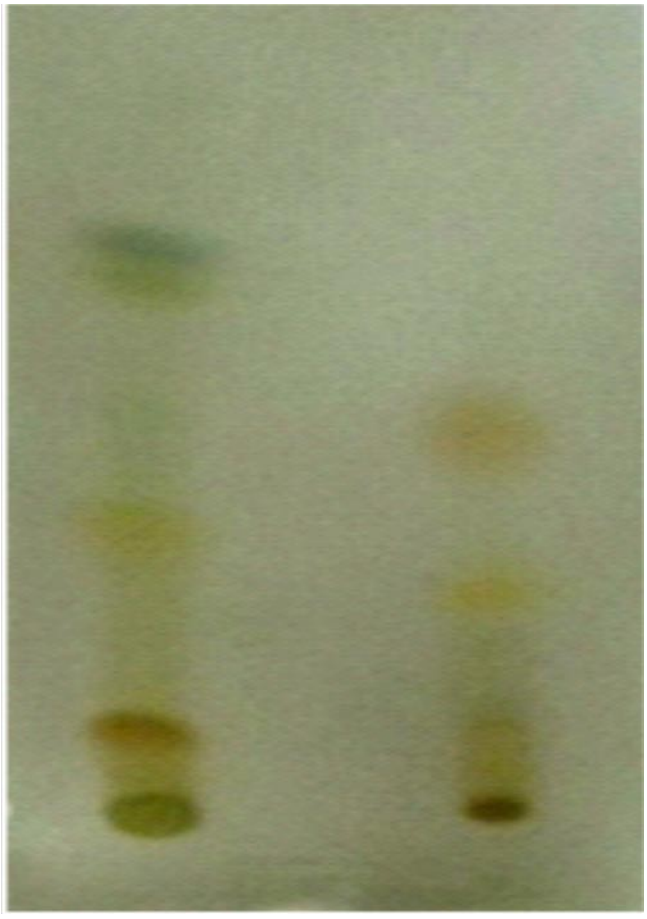

Figure 1. Separations of the CEMAL and CEMAS on a TLC plate from left to right.

3.2. Anthelmintic activity

The mode of treatment and observations concerning experimental annelid are displayed in a dose-dependent manner (Table 1). The durations for CEMAL to induce paralysis were (2.50±0.22), (1.50±0.22) and (1.16±0.16) min and death were (4.50±0.22), (3.33±0.20) and (2.66±0.21) min corresponding to 10, 20 and 40 mg/mL suspensions respectively. Similarly the durations for CEMAS to induce paralysis were (9.66±0.42), (7.00±0.26) and (5.33±0.21) min and death were (31.66±0.67), (17.50±0.43) and (15.33±0.21) min corresponding to 10, 20 and 40 mg/mL suspensions respectively. However, in case of albendazole, durations to induce paralysis were (4.43±0.22), (3.25±0.16) and (2.33±0.21) min and death were (25.13±0.28), (12.93±0.58) and (10.83±0.47) min respectively. In control, no paralysis or death was found.

Table 1

Anthelmintic activity of *M. auriculata* leaves and stems on *P. posthuma*.

Groups	Drug treatment	Concentration (mg/mL)	Time for paralysis (min)	Time for death (min)
I	Control	–	–	–
II	Albendazole	10	4.43±0.22	25.13±0.28
III	Albendazole	20	3.25±0.16	12.93±0.58
IV	Albendazole	40	2.33±0.21	10.83±0.47
V	CEMAL	10	2.50±0.22***	4.50±0.22***
VI	CEMAL	20	1.50±0.22**	3.33±0.20***
VII	CEMAL	40	1.16±0.16**	2.66±0.21***
VIII	CEMAS	10	9.66±0.42***	31.66±0.67***
IX	CEMAS	20	7.00±0.26***	17.50±0.43***
X	CEMAS	40	5.33±0.21***	15.33±0.21**

Values are expressed as mean±SEM, n=6. The results were analysed by analysis of variance followed by Tukey-Kramer multiple comparison test. **P<0.01 and ***P<0.001 when compared with Group II/III/IV corresponding to the test concentration.

4. Discussion

The time taken for paralysis and death showed an orderly decline with the increasing concentration of the test extracts. The mean±SEM values were calculated for each extract. Result of anthelmintic activity on *P. posthuma* against each concentration of each extract was compared with that of corresponding concentration of albendazole as reference drug. Albendazole acts by inhibiting the polymerization of helmintic β-tubulin, and thus interferes with microtubule dependent functions like glucose uptake and glycogen depletion[10].

Among the test extracts, CEMAL showed more efficacy and potency of anthelmintic activity than that of stems and albendazole. On preliminary phytochemical screening, the extracts revealed the presence of steroids/triterpenes, phenolics/flavonoids and carbohydrates. The UV spectrum displayed characteristic absorption at 288.0 nm for the bright yellow TLC fraction of CEMAL and 288.5 nm for the same of CEMAS. The UV absorption maxima at 288.0 and 288.5 nm were probably the characteristic UV bands II arising from ring A of flavonoids[11,12]. The possible mechanism of anthelmintic effect due to the presence of triterpenes and phenolics/flavonoids is that they can bind with the free gastrointestinal proteins of host or glycoprotein on the cuticle of the parasite and may cause death[13]. In addition, phenolics/flavonoids and their metabolites may have a direct effect on the viability of the pre-parasitic stages of the helminths. This speculation was supported by the varying rates of anthelmintic efficacy of *M. auriculata*.

Ethanolic extract of entire plant of *Evolvulus alsinoides* Linn. (Convolvulaceae) at higher concentration of 100 mg/mL was found more potent than reference control piperazine citrate. Anacardic acid extracted from oil of nuts of *Semecarpus anacardium* Linn. (Anacardiaceae) and its sodium salts were also found to be potent anthelmintic

agents than piperazine citrate[14]. Similarly, crude aqueous methanol extract of *Verbascum thapsus* Linn. was observed to be more potent wormicidal agent than albendazole[15]. Ethanolic and aqueous extracts of whole plant of *Ventilago denticulata* Wild. (Rhamnaceae) were found to have better anthelmintic activity than reference drug albendazole[16]. Aqueous and hydroalcoholic extracts of *Caesalpinia pulcherrima* L. (Fabaceae) bark were also found to have more anthelmintic efficacy than reference drug albendazole[17]. Better anthelmintic efficacy of the CEMAL than reference drug albendazole found in the present study also agrees to the aforementioned evidence-based findings of the literature.

The experimental evidence obtained in the laboratory model could provide a rationale for the folkloric use of *M. auriculata* as anthelmintic drug. A comprehensive chemical analysis on nutritive values of *M. auriculata* suggested its leaves and twigs to be lopped for cattle fodder[18]. Henceforth, the anthelmintic potential of this plant strongly signifies its use as a dietary supplement in cattle with an additional advantage of chemotherapeutic prevention from helminthiasis.

Further studies on *in vivo* anthelmintic activity to substantiate the folk claim, standardization of the plant extract and development of the best herbal formulation to replace synthetic drugs could be carried out. It would be also interesting to find out any novel or existing chemical entities responsible for the anthelmintic activity and their mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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