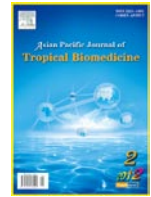




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## Evaluation of antidermatophytic activity of *Ranunculus sceleratus* and *Pongamia pinnata* available in North Eastern Region of India

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### ABSTRACT

**Objective:** To investigate the *in vitro* antifungal activity of *Ranunculus sceleratus* (*R. sceleratus*) and *Pongamia pinnata* (*P. pinnata*), collected from North East India. Chloroform, Methanol and Water extracts of the plants were evaluated for anti-ringworm activity against five strains of dermatophytes viz., *Trichophyton rubrum* (*T. rubrum*), *Trichophyton mentagrophytes* (*T. mentagrophytes*), *Trichophyton tonsurans* (*T. tonsurans*), *Microsporium gypseum* (*M. gypseum*) and *Microsporium fulvum* (*M. fulvum*). **Methods:** Agar well diffusion method was used to determine the inhibition zone of the different extracts. The minimum inhibitory concentrations of the extracts were determined by broth macro dilution method. **Results:** When compared, the chloroform extracts of both the plants were found to be highly active against all the tested fungi followed by the methanol extract. The water extract showed the least activity. The minimum inhibitory concentration (MIC) of the extracts was ranging from 1.25–10.00 mg/mL for chloroform and methanol extracts and greater than 10 mg/mL for the water extracts. **Conclusion:** The results obtained from the experiments suggest the anti-dermatophytic nature of the plants.

## 1. Introduction

Dermatomycoses or ringworm are common superficial cutaneous fungal infections caused by filamentous fungi such as *Trichophyton*, *Microsporium* or *Epidermophyton* species, which have the capacity to invade keratinous tissues, such as hair, skin or nails, of humans and other animals[1]. The incidences of fungal infections have been severely increasing in recent years because of the increase in number of immuno compromised hosts[2]. Although, a large number of synthetic allopathic drugs are available in the market the majority of these clinically used antifungals suffer from various drawbacks in terms of toxicity, lack of fungicidal efficacy, cost and emergence of resistant strains caused by the frequent use of some of them. Therefore, the development of an antifungal agent from local raw material is still necessary[3–5].

Medicinal plants are the oldest known health-care products. Natural products have served as a major source of drugs for centuries, and about half of the pharmaceuticals

in use today are derived from natural products[6]. Microbiologists and natural-product chemists are trying to discover more about phytochemicals, which could be developed for treatment of infectious diseases[7]. As opposed to synthetic drugs, antimicrobials of plant origin are not associated with many adverse effects and have an enormous therapeutic potential to heal many infectious diseases[8].

*Ranunculus sceleratus* (*R. sceleratus*) Linn. belonging to the family Ranunculaceae is an annual plant, growing along the edge of water in ponds and stream banks[9]. This plant is considered somewhat toxic and can cause skin irritation to people allergic to it. However the toxic properties of the plant get destroyed on boiling. It is also consumed after boiling. The plant is considered stimulant and diuretic. Leaf juice is used in sciatica, rheumatism, dysuria, asthma, pneumonia etc. It is also used against various cutaneous disorders. Ethno-medico reports suggest that its seeds are prescribed to cure kidney troubles[10–11].

*Pongamia pinnata* (*P. pinnata*) (L.) Pierre belonging to the family Fabaceae is a medium sized glabrous, perennial tree grows in the littoral regions of South Eastern Asia and Australia[12]. Root, bark, leaves, flower and seeds of this plant have several medicinal properties. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers[13]. In the

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traditional system of medicines *P. pinnata* is used for anti-inflammatory, anti-plasmodial, anti-noniceptive, anti-hyperglycemic, anti-lipidperoxidative, anti-diarrhoeal, anti-ulcer, anti-hyperammonic and antioxidant activity<sup>[14]</sup>. Based on the local use against some common diseases, an attempt has been made to assess the antidermatophytic properties of these two plants, *R. sceleratus* and *P. pinnata* readily available in the state of Assam, against five species of dermatophytes viz. *Trichophyton mentagrophytes* (*T. mentagrophytes*), *Trichophyton rubrum* (*T. rubrum*), *Trichophyton tonsurans* (*T. tonsurans*), *Microsporum gypseum* (*M. gypseum*) under laboratory conditions.

## 2. Materials and methods

### 2.1. Collection, identification and preparation of plant materials

Plant materials (leaves) of the two plants were collected from Kamrup district, (25.43° and 26.51° North Latitude and between 90.36° and 92.12° East Longitude) of Assam, India in the month of April, 2009. The plant materials were authenticated by Dr. G. C. Sarma, taxonomist, Dept. of Botany, Gauhati University, Assam (India). Herbarium voucher specimen (*P. pinnata* (L.) Pierre (IASST/MEP/H No. 22/2009), *R. sceleratus* Linn. (IASST/MEP/H No. 33/2009) were prepared and deposited in the Life Sciences Division of IASST, Guwahati, Assam, India for future reference.

### 2.2. Collection of fungal strains

The tested human pathogenic fungal strains include *T. rubrum* (MTCC 8477), *T. mentagrophytes* (MTCC 8476), *T. tonsurans* (MTCC 8475), *M. gypseum* (MTCC 8469) and *M. fulvum* (MTCC 8478) procured from Institute of Microbial Technology (IMTECH), Chandigarh–160036 (India). The procured samples were sub-cultured and maintained in Sabouraud Dextrose Agar slants at 4 °C.

### 2.3. Preparation of plant extracts

Freshly collected plant materials were washed with distilled water to remove the sand and dirt. Plant materials were then dried under shade in a well ventilated room and were finely powdered in a mixture grinder. The powdered materials were exhaustively extracted with chloroform and methanol<sup>[15]</sup>. The filtrate is then concentrated under reduced pressure using a rotary evaporator (Buchi R–124) at low temperature (< 40 °C). Finally vacuum desiccators were used to completely remove the solvent. For the aqueous extract, 100 g of powdered plant materials were heated in 1000 ml water for one hour in a water bath at 40 °C, filtered and finally lyophilized to dryness. The extracted samples were kept in refrigerator at 4 °C prior to use.

### 2.4. In vitro antifungal screening

The antifungal screening of the test extracts was

determined by employing agar well diffusion technique<sup>[16]</sup>. A control set was maintained with DMSO. Clotrimazole was used as a reference standard. The plates were incubated at (28 ± 2) °C for 96 h to 2 weeks depending on the growth rate of the test pathogens. The experiment was replicated thrice and the average results were recorded. The antifungal activities of the extracts were determined by measuring the diameter of the inhibition zone around the well that was filled with the extract.

### 2.5. Determination of minimum inhibitory concentration

MICs were performed by the visual broth macrodilution method<sup>[17]</sup>. Fungal suspensions were diluted in Sabouraud Dextrose Broth to give a final concentration of 10<sup>6</sup> cfu/mL. The extract was serially diluted to give concentrations in the range, 100.0–0.156 mg/mL. The tubes were then incubated at (28±2) °C for 4–14 days depending upon the growth rate of the pathogen. MIC was defined as the lowest concentration that did not yield visual growth after the incubation period. All experiments were performed in triplicate.

### 2.6. Determination of the percentage inhibition of diameter growth

Percentage inhibition: Percentage of inhibition was calculated according to the following formula<sup>[18]</sup>:

$$\% \text{ inhibition} = \frac{\text{Inhibition zone in mm}}{\text{Control}^*} \times 100$$

\*Growth zone is equal to plate diameter i.e. 90 mm as growth occurs all over the agar plate.

## 3. Results

The antidermatophytic activities of the extracts obtained in different solvents by well diffusion technique for *R. sceleratus* and *P. pinnata* is given in Table 1. For *R. sceleratus* the chloroform extract exhibited the maximum activity with a halo of 23 mm diameter inhibition against *T. mentagrophytes* followed by *T. rubrum* (22 mm), *M. fulvum* (21 mm), *M. gypseum* (18 mm), and *T. tonsurans* (15 mm). The methanol extracts produced inhibition zone of 21, 16, 17, 17 and 10 mm respectively for *T. mentagrophytes*, *M. gypseum*, *T. rubrum*, *M. fulvum* and *T. tonsurans*. The water extract was completely inactive against *T. rubrum* and *T. tonsurans*, while it showed very weak activity against *M. gypseum* (9 mm), *T. mentagrophytes* (8 mm), *M. fulvum* (8 mm).

Similar type of inhibitory effect was observed in case of *P. pinnata* leaf extracts. Chloroform extract showed marked inhibitory activity against all the tested dermatophytes compared to the methanol and water extracts. The chloroform extract produced zone of 22, 18, 16, 16, and 15 mm for *T. mentagrophytes*, *M. fulvum*, *T. rubrum*, *T. tonsurans* and *M. gypseum*, and methanol extracts produces maximum zones of 17, 16, 12, 12 and 11mm for *M. fulvum*, *T. mentagrophytes*,

**Table 1.**

Antifungal activity (inhibition zones) for *R. sceleratus* and *P. pinnata* extracts (at 10mg/ml) and Clotrimazole (at 0.5mg/ml). (–) = inhibition zone less than 6 mm.

Extracts		Inhibition zones (mm)				
		<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>T. tonsurans</i>	<i>M. gypseum</i>	<i>M. fulvum</i>
<i>R. sceleratus</i>	Chloroform	22.0±0.50	23.0±0.3	15.0±0.70	18.0±0.6	21.0±1.0
	Methanol	17.0±0.23	21.0±0.5	10.0±0.8	16.0±0.3	17.0±0.9
	Water	–	8.0±0.7	–	9.0±0.1	8.0±0.0
<i>P. pinnata</i>	Chloroform	16.0±0.8	22.0±0.6	16.0±0.4	15.0±0.9	18.0±0.4
	Methanol	11.0±0.4	16.0±0.5	12.0±0.7	12.0±1.2	17.0±0.5
	Water	–	–	–	8.0±0.4	10.0±0.7
	Clorimazole	22.0±0.0	19.0±0.1	21.0±0.0	24.0±0.0	20.0±0.0
	DMSO	–	–	–	–	–

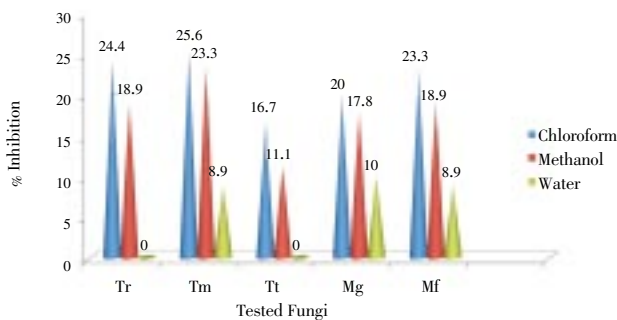
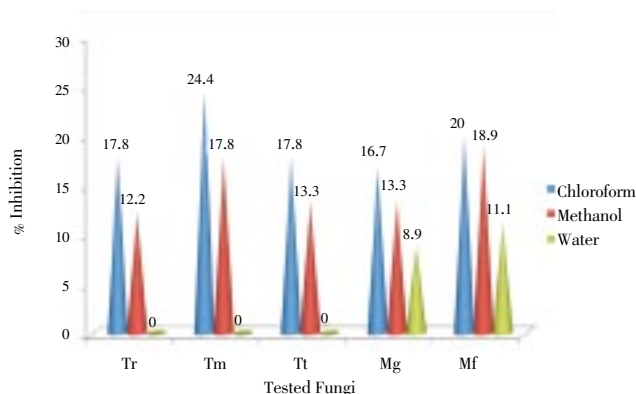
**Table 2.**

Minimum inhibitory concentration for the different extracts (mg/mL).

Extract type		MIC (mg/mL)				
		<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>T. tonsurans</i>	<i>M. gypseum</i>	<i>M. fulvum</i>
<i>R. sceleratus</i>	Chloroform	2.5–5.0	1.25–2.50	2.5–5.0	2.5–5.0	1.25–2.50
	Methanol	2.5–5.0	2.5–5.0	5.0–10.0	5.0–10.0	2.5–5.0
	Water	>10.0	>10.0	>10.0	>10.0	>10.0
<i>P. pinnata</i>	Chloroform	2.5–5.0	2.5–5.0	2.5–5.0	2.5–5.0	2.5–5.0
	Methanol	2.5–5.0	2.5–5.0	2.5–5.0	2.5–5.0	2.5–5.0
	Water	>10.0	>10.0	>10.0	>10.0	>10.0

*T. tonsurans*, *M. gypseum* and *T. rubrum* respectively. Water extract produced inhibition zones for *M. fulvum* (10 mm) and *M. gypseum* (8 mm) while no inhibition was found against the tested Trichophyton species.

*pinnata*, maximum respective inhibition percentage of 25.6% and 24.4% was shown by the chloroform extracts against *T. mentagrophytes*. Antidermatophytic activity of the crude extract was compared to the standard antifungal clotrimazole (0.5 mg/mL) and DMSO 2% (Table 1). The minimum inhibitory concentrations of the extracts was also determined (Table 2).

**Figure 1.** Antifungal activity (% inhibition) by *R. sceleratus*.**Figure 2.** Antifungal activity (% inhibition) by *P. pinnata*.

The inhibition percentage (%) of the crude extracts were also evaluated (Figure 1 and 2). For both *R. sceleratus* and *P.*

#### 4. Discussion

With the rise in the emergence of various multi drug resistant microorganisms and the scenario worsening through the indiscriminate use of antibiotics, new and/or alternative antimicrobial compounds must be developed to treat common infections. The changing patterns of susceptibility and the availability of new antimicrobial agents, demands continuous updating of knowledge concerning treatment of diseases caused by such pathogens[8].

In the present study, the antifungal activities of the Chloroform and Methanol and Water extracts of the two plants *Ranunculus sceleratus* and *Pongamia pinnata* against the selected five dermatophytic strains were evaluated and the activities of the chloroform extracts were found to be superior as compared to the other extracts. The activities of different extracts can be stated as Chloroform > Methanol > Water. The standard drug Clotrimazole was found effective at a much lower concentration than the crude extracts. However, as these plants are commonly used by the traditional practitioners against a large number of diseases, it is expected to have a very less toxicity and one might conclude that the use of these plants would probably produce also less side-effects and toxicity compared with conventional chemotherapeutic agents.

Recently many medicinal plants extracts and essential oils have been investigated for their antifungal properties against

dermatophytes<sup>[19–23]</sup>. Investigation of the anti-dermatophytic potential of crude extracts and essential oil of the seeds and leaves of *Moringa oleifera* Lam. ethanol extracts showed anti-fungal activities *in vitro* against dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis*<sup>[24]</sup>. Similarly, the methanolic and butanolic fractions of *Hymenaea martiana* were found to be most active against *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum canis* with very low MICs<sup>[25]</sup>. Other reports include the study of crude leaf extract of *Senna alata* Linn. against various species of *Trichophyton*, *Microsporum* and *Epidermophyton*<sup>[26]</sup>. However, no reports on the antidermatophytic activity of the studied plants from North East India have been so far come across in the literature. The results of the present study indicated the presence of active anti-microbial agents in the two studied plants and support the folklore usage of the studied plants.

### Conflict of interest statement

We declare that we have no conflict of interest.

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