Indian J Microbiol (October 2010) 50(Suppl 1):S62–S69
DOI: 10.1007/s12088-010-0004-2

ORIGINAL ARTICLE

Solanum melongena: A potential source of antifungal agent

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Received: 4 July 2008 / Accepted: 8 November 2008

Abstract The antifungal activity of *Solanum melongena* leaf, extracted with petroleum ether, chloroform, methanol and water was evaluated against three human pathogenic dermatophytes namely *Trichophyton mentagrophytes*, *T. rubrum* and *T. tonsurans* and two opportunistic fungi *Candida albicans* and *Trichosporon beigelii*. Maximum yield of plant components was 4.32 g, extracted in water and minimum 1.07 g in petroleum ether from 150 g of dry plant material. Except water extract, all the extracts possessed significant antifungal property. All the test pathogens showed highest sensitivity towards chloroform extract, exhibiting maximum inhibition zone diameter of 50.0 mm in *T. mentagrophytes* and minimum 30.0 mm in *C. albicans* at 2×10^5 µg/ml concentration. Chloroform extract at lower concentration 2.5 × 104 µg/ml was inhibitory for all the test pathogens, exhibiting inhibition zone diameter 21.0 mm against *T. tonsurans* and 15.0 mm against *C. albicans* and *T. beigelii*. The activity of the different solvent extracts against the test pathogens in terms of inhibition zone diameter in decreasing order was as follows:

Chloroform extract > Petroleum ether extract > Methanol extract *for T. mentagrophytes*, *T. rubrum* and *T. tonsurans*.

Chloroform extract > Methanol extract > Petroleum ether extract for *C. albicans* and *T. beigelii*.

Keywords *Solanum melongena* · Solvent extract · Antifungal activity

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Introduction

Infectious fungal skin diseases are most common throughout the tropical and subtropical region of the world. The incidence of fungal diseases predominated in North-East India, accounting for almost 50% of the total infectious skin diseases [1]. Among these dermatophytoses, also known as 'ringworm' or 'tinea', caused by a group of keratinophilic fungi 'dermatophytes' pose a serious concern. It involves superficial infections of the keratinized tissue of the skin, hair and nails of animals and human beings. The importance of opportunistic fungal pathogens is also increasing because of the expansion of the immunocompromised patient population. *Candida albicans*, an opportunistic pathogen causing local and systemic infections in predisposed persons, commonly affects immunologically compromised patients and those undergoing prolonged antibiotic treatment. Another important opportunistic pathogen *Trichosporon beigelii* causes white piedra, superficial and deep infections in humans.

Owing to limited antimicrobial spectrum of most of the drugs, emergence of multidrug resistant strains and serious ill effects of the current drugs, treatment of such cutaneous infections is quite challenging. Plants are considered as a major source of medicinal constituents. The efficacy and safety of herbal medicines have turned the scientific community towards medicinal plant's research. To check the microbial diseases, extensive research on herbs, carried out throughout the world has revealed that some plants possess antimicrobial ingredients in them [2–5]. The inhibitory effect of *Eclipta alba* (whole plant) extracted in different organic solvents against *Candida tropicalis*, *Rhodotorula glutanis* and *C. albicans* was found very similar to those of standard antibiotics [6]. It was reported that antifungal activity of four essential oils extracted from the leaves of *Cympopogon martini* (Roxb.) Watson, *C. nardus* (Linn.)

Rendle of poaceae, *Mentha arvensis* Linn. of Lemiaceae and bark of *Eucalyptus citriodora* Hook of myrtaceae against six keratinophilic fungi viz. *T. mentagrophytes*, *T. rubrum, T. simii, T. tonsurans* and *Microsporum gypseum* were varying in the degree of their activity depending on the nature of their effective compounds and capacity of diffusion into agar medium [7].

North-East India, being one of the biodiversity hotspot has immense potential in developing effective herbal remedy for such skin infections. The scope of developing effective herbal products from the indigenous resources for combating fungal diseases is immense in this part of country.

Solanum melongena Linn. is commonly known as brinjal, widely cultivated in India and other parts of the World. In traditional Chinese medicine, all parts of the plant are used to stop intestinal bleeding. The fruit of the plant is used as an antidote in cases of mushroom poisoning. In Indonesia, parts of the plant are used as a purgative. For traditional Malay medicine, the ashes of the fruit are used in dry, hot poultices to treat hemorrhoids. However, information regarding its effectiveness against human pathogenic microorganisms, particularly dermatophytes and other associated opportunistic pathogens has not been reported so far.

With this background, the present investigation was carried out to evaluate different solvent extracts from *S. melongena* leaves for their antifungal activity against some of the most important human pathogenic dermatophytes namely *T. mentagrophytes, T. rubrum* and *T. tonsurans* and two opportunistic fungi *C. albicans* and *T. beigelii* under laboratory conditions.

Materials and methods

Maintenance of pure culture of test fungi: Pure cultures of the test pathogens were maintained at 4°C on sabouraud dextrose agar (SDA) slants and subcultured at regular intervals.

Collection and identification of plants: Fresh leaves of *S. melongena* were collected from agricultural farm located at Tezpur (Assam) during the month of February to March 2008. Identification of the plant species was done on the basis of morphological characters.

Extraction of plant materials: Plant materials (leaves) were thoroughly washed and dried under shade and crushed into powder and extracted successively with different solvents as per protocol (Fig. 1) with some modification [8]. The crude solvent extracts were used as stock.

Preparation of test extract: Test extracts of different concentrations, i.e. 2×10^5 µg/ml, 1×10^5 µg/ml, 5×10^4 µg/ml, 2.5×10^4 µg/ml and 1.25×10^4 µg/ml were prepared by dissolving the crude extract in desired amount of dimethylsulphoxide (DMSO) and finally sterilized by filtering through millipore filter (0.2 mm pore size).

Preparation of inoculum: The inoculum was prepared by adding one loop full of the stock culture in 50 ml of sabouraud dextrose broth and incubated at 28 ± 2°C for 10 days.

***In vitro* assay for antifungal activity:** The antifungal activity of the test extracts so obtained was determined by employing agar well diffusion method [9]. With a sterile cotton swab, 0.4 ml of broth inoculum was spread evenly on the surface of the petri plate containing solidified SDA. Well of 7 mm diameter was made in the center of the agar plate with a sterile cork borer. The well was then filled with the respective test extracts (0.3 ml) and allowed to diffuse at room temperature for 2 h. A control set was maintained with DMSO. Clotrimazole was used as a reference standard. The plates were then incubated at 28 ± 2°C for 24 h to 3 weeks depending on the growth rate of the test pathogens. The experiment was replicated thrice and the average results were recorded. The antifungal activity of the extracts was determined by measuring the diameter of the inhibition zone around the well that was filled with the extract.

Activity index determination: The activity index of the extracts was determined by the following formula [10]:

$$\text{Activity index} = \frac{\text{Inhibition zone of extracts}}{\text{Inhibition zone of standard}}$$

Percentage inhibition: Percentage of inhibition was calculated according to the following formula [11]:

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

*Growth zone is equal to plate diameter i.e. 80 mm as growth occurs all over the agar plate.

Results and discussion

Despite the stunning success of pharmaceutical industries in developing new antifungal drugs, finding novel and consumer friendly antifungal agents for treatment of wide range of fungal diseases, is still a top priority because of emerging multidrug resistant pathogens. One novel approach is to search for effective antimicrobial agents from natural resources, particularly the plant resources. In pharmaceuticals use, plant preparations show a wide range of effects, with antimicrobial effect being one of the

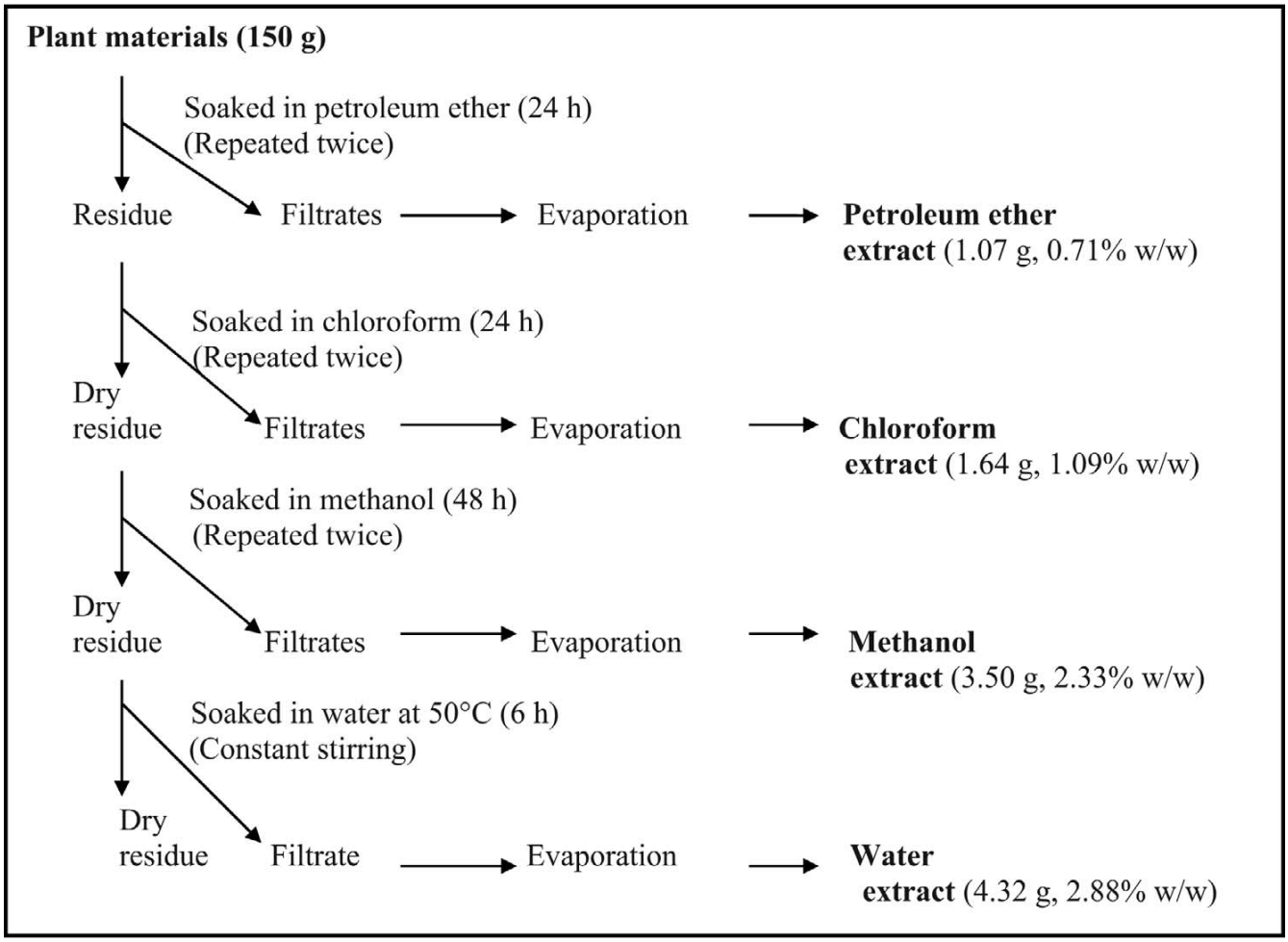

Fig. 1 Extraction of *S. melongena* leaves.

most important. Effect on different microorganisms is the subject of many recent scientific studies.

The plant *S. melongena*, studied in the present investigation, is widely used as traditional medicines. Traditional use of this plant suggests that there is no undesirable effect to human. Antimicrobial properties of some other species of have been reported. Acetone extract of *Solanum indicam* (fruit) was shown to have antibacterial activity towards *Bacillus cereus* and *Staphylococcus aureus* [12]. The aqueous extract of *Solanum nigrum* was found effective against *T. mentagrophytes* and *T. violaceum* and *Microsporum canis* [13]. Antileishmanial activity of Solanum americanum and Solanum concinnum has been reported. Methanol extract of this plant species was seen effective against *Leishmania amezonensis* and *Leishmania chagasi*. *S. americanum* extract also inhibit the growth of *C. albicans* [14].

The activity of plant extract may vary according to their nature of active ingredients in the plant. Successful prediction of bioactive components from plant materials is largely dependent on type of solvents used in the extraction procedure. In the present investigation, maximum yield of 4.32 g of crude extract in water and minimum 1.07 g in petroleum ether was obtained from 150 g of dry leaves of dry plant materials (Fig. 1). The observation can be rationalized in terms of the polarity of compounds being extracted by each solvent and in addition to their intrinsic bioactivity, ability to dissolve or diffuse in culture media used in the study. The results indicated that, except water extract, all the solvent extracts suppressed the growth of the test fungi in a concentration dependent manner (Fig. 2). However, the variation of susceptibility from one species to another is evident. The details of the results are shown in Tables 1–3. Highest sensitivity was shown by all the test pathogens towards chloroform extract, which exhibited maximum inhibition zone diameter of 50.0 mm in *T. mentagrophytes* at 2×10^5 µg/ml concentration. The inhibition zone formed by this extracts at the same concentration were 44.8, 37.8,

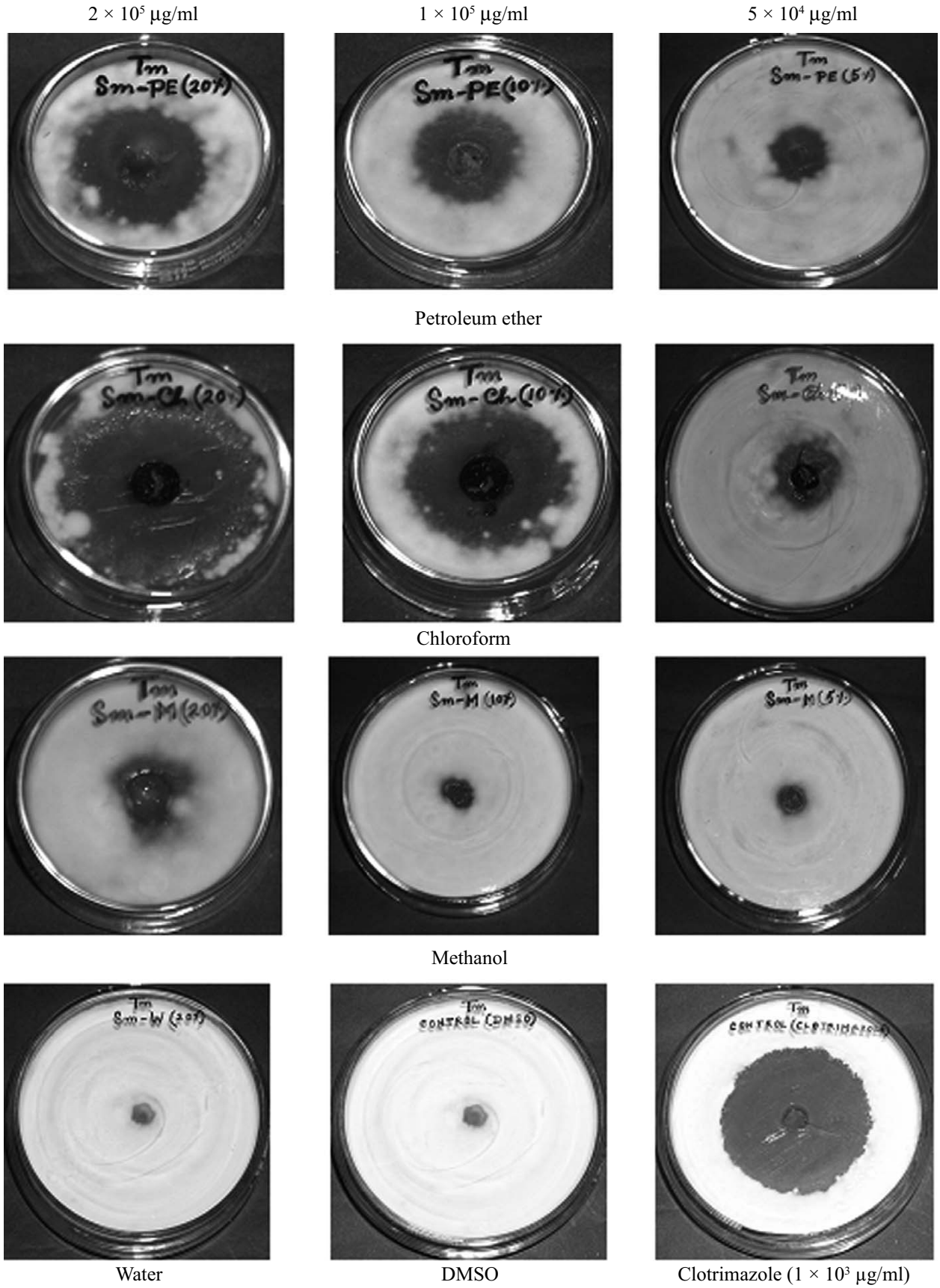

Fig. 2 Inhibition zone of *T. mentagrophytes* caused by *S. melongena* leaf extracts.

Table 1 Antifungal activity (inhibition zone) of leaf extract of *S. melongena*

Solvent used	Plant extract Conc. (µg/ml)	Test pathogen/inhibition zone (dia in mm)				
		T. mentagrophytes (mean ± SD)	*T. rubrum* (mean ± SD)	*T. tonsuran* (mean ± SD)	*C. albican* (mean ± SD)	*T. beigelii* (mean ± SD)
PE	2×10^5	38.0 ± 0.41*	39.3 ± 0.21	35.0 ± 0.21	23.0 ± 0.40	27.0 ± 0.50
	1×10^5	26.0 ± 0.82	33.0 ± 0.71	33.0 ± 1.47	17.5 ± 0.40	22.0 ± 0.33
	5×10^4	20.8 ± 0.54	25.3 ± 0.74	30.3 ± 0.22	14.0 ± 0.21	19.5 ± 0.14
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
CH	2×10^5	50.0 ± 1.42	44.8 ± 0.52	37.8 ± 0.22	30.0 ± 0.70	35.5 ± 0.21
	1×10^5	34.8 ± 0.65	34.8 ± 0.14	35.0 ± 0.37	25.0 ± 0.08	30.0 ± 0.16
	5×10^4	31.8 ± 0.56	21.5 ± 0.35	33.8 ± 0.75	19.0 ± 0.70	25.0 ± 0.35
	2.5×10^4	20.8 ± 0.59	19.0 ± 0.35	21.0 ± 0.82	15.0 ± 0.49	15.0 ± 0.08
	1.25×10^4	0	0	0	0	0
ME	2×10^5	20.3 ± 0.24	25.3 ± 0.22	37.89 ± 0.14	28.0 ± 0.28	30.0 ± 0.08
	1×10^5	17.0 ± 0.35	23.0 ± 0.72	35.0 ± 0.00	22.0 ± 0.29	24.5 ± 0.14
	5×10^4	14.5 ± 0.29	19.0 ± 0.35	29.8 ± 0.22	18.0 ± 0.08	20.0 ± 0.00
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
Water	2×10^5	0	0	0	0	0
	1×10^5	0	0	0	0	0
	5×10^4	0	0	0	0	0
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
CL	1×10^3					
	49.0 ± 0.35	55.0 ± 0.37	35.0 ± 0.82	38.0 ± 0.14	46.0 ± 0.35	
DMSO		0	0	0	0	0

PE: Petroleum ether; CH: Chloroform; ME: Methanol; CL: Clotrimazole.

SD = Standard deviation is calculated by using formula $\sqrt{1/n\sum (Xi - \acute{X})^2}$ where $\acute{X}$ = arithmetic mean of the values.

*Mean value of three replications.

Table 2 Antifungal activity (activity index) of leaf extract of *S. melongena*

Solvent used	Plant extract Conc. (µg/ml)	Test pathogen/activity index				
		T. mentagrophytes	*T. rubrum*	*T. tonsurans*	*C. albicans*	*T. beigelii*
PE	2×10^5	0.78	0.71	1.00	0.60	0.59
	1×10^5	0.53	0.60	0.94	0.46	0.48
	5×10^4	0.42	0.46	0.87	0.37	0.42
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0

Table 2 *(Contd.) on next page*

Table 2 Antifungal activity (activity index) of leaf extract of *S. melongena (Contd.)*

CH	2×10^5	1.02	0.81	1.08	0.79	0.77
	1×10^5	0.71	0.63	1.00	0.66	0.65
	5×10^4	0.65	0.39	0.97	0.50	0.54
	2.5×10^4	0.42	0.35	0.60	0.39	0.33
	1.25×10^4	0	0	0	0	0
ME	2×10^5	0.41	0.46	1.08	0.74	0.65
	1×10^5	0.35	0.42	1.00	0.58	0.53
	5×10^4	0.30	0.35	0.85	0.47	0.43
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
Water	2×10^5	0	0	0	0	0
	1×10^5	0	0	0	0	0
	5×10^4	0	0	0	0	0
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
DMSO		0	0	0	0	0

PE: Petroleum ether; CH: Chloroform; ME: Methanol.
Inhibition zones of standard clotrimazole (1×10^3 µg/ml) against *T. mentagrophytes* = 49.0 mm, *T. rubrum* = 55.0 mm, *T. tonsurans* = 35.0 mm, *C. albicans* = 38.0 mm and *T. beigelii* = 46.0 mm.

Table 3 Antifungal activity (inhibition %) of leaf extract of *S. melongena*

Solvent used	Plant extract Conc. (µg/ml)	Test pathogen/inhibition %				
		T. mentagrophytes	*T. rubrum*	*T. tonsurans*	*C. albicans*	*T. beigelii*
PE	2×10^5	47.5	49.1	43.8	28.8	33.8
	1×10^5	32.5	41.3	41.3	21.9	27.5
	5×10^4	26.0	31.6	37.9	17.5	24.4
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
CH	2×10^5	62.5	56.0	47.3	37.5	44.4
	1×10^5	43.5	43.5	43.8	31.3	37.5
	5×10^4	39.8	26.9	42.3	23.8	31.3
	2.5×10^4	26.0	23.8	26.3	18.8	18.8
	1.25×10^4	0	0	0	0	0
ME	2×10^5	25.4	31.6	47.3	35.0	37.5
	1×10^5	21.3	28.8	43.8	27.5	30.6
	5×10^4	18.1	23.8	37.3	22.5	25.0
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0

Table 3 *(Contd.) on next page*

Table 3 Antifungal activity (inhibition %) of leaf extract of *S. melongena (Contd.)*

Water	2×10^5	0	0	0	0	0
	1×10^5	0	0	0	0	0
	5×10^4	0	0	0	0	0
	2.5×10^4	0	0	0	0	0
	1.25×10^4	0	0	0	0	0
CL	1×10^3	61.2	68.8	43.8	47.5	57.5
DMSO		0	0	0	0	0

PE: Petroleum ether; CH: Chloroform; ME: Methanol; CL: Clotrimazole.

30.0 and 35.5 mm against *T. rubrum*, *T. tonsurans*, *C. albicans* and *T. beigelii*, respectively. The inhibition zone range formed by 2×10^5 µg/ml petroleum ether extract was 39.3 to 23.0 mm against *T. rubrum* and *C. ablicans* whereas methanol extract (2×10^5 µg/ml) formed inhibition zone of 37.8 mm in *T. tonsurans* and 30.0 mm in *C. albicans*. The antifungal activity of chloroform extract was recorded as highest followed by petroleum ether and methanol extract against *T. mentagrophytes*, *T. rubrum* and *T. tonsurans*.

The opportunistic fungi *C. albicans* and *T. beigelii* also exhibited highest sensitivity towards chloroform extract. However, methanol extract was found more active than petroleum ether extract in inhibiting the growth of *C. albicans* and *T. beigelii*. Minimum inhibitory concentration (MIC) values for each of the potent extracts were recorded as the lowest concentration of the test extract, which causes visible inhibition effect. The MIC values were 2.5×10^4 µg/ml for chloroform extract and 5×10^4 µg/ml for petroleum ether and methanol extract for all the test fungi. In control set no inhibition zone was observed. The activity of standard antibiotic clotrimazole (1×10^3 µg/ml) is slightly higher than that of the plant extracts. The activity of different solvent extracts in term of inhibition zone diameter in decreasing order was as follows:

- Chloroform extract > Petroleum ether extract > Methanol extract for *T. mentagrophytes*, *T. rubrum* and *T. tonsurans*.
- Chloroform extract > Methanol extract > Petroleum ether extract for *C. albicans* and *T. beigelii*.

It can be concluded that the most active components against the dermatophytes tested, found in the leaves of *S. melongena* are most likely to be non polar, as these components dissolve in chloroform. More or less similar observations were also made by other workers when tested different solvent extracts from Thai medicinal plants against opportunistic fungal pathogens associated with AIDS and recorded the chloroform extracts of *Alpinia galanga*, *Bosenbergia pandurata*, *Eclipta prostrata*, *Piper betle* and *Zingiber zerumbent* have more pronounce antifungal activity against *C. albicans*, *C. neoformance* and *M. gypseum* than the methanol extract, whereas, water extract was found inactive against all the pathogens [8]. On the other hand, petroleum ether, chloroform, acetone and ethanol extracts of *Eclipta alba* (whole plant), showed significant growth inhibition of *C. tropicalis*, *Rhodotorula glutanis* and *C. albicans* and their degree of inhibition was comparable with that produced by standard antibiotics like amphotericin B and nystatin [6]. Difference between studies may be due to the extraction procedures and other factors.

Conclusion

The results of the present investigation indicated that *S. melongena* Linn. is a potential antifungal plant, with broad spectrum of activity. The organic solvents like petroleum ether, chloroform and methanol are much more suitable and powerful for extraction of the antifungal plant components than water, which is mostly used by traditional practioners as a solvent. The results of the present study is highly promising and highlights a new way for further phytochemical analysis to ascertain the maximum potential of plant extract as drug with minimum effective dose. The bioactivity guided fractionation of the plant extracts to isolate and identify the antifungal component(s) is in progress.

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