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Research Article

In vitro study of antifungal activity of *Entada spiralis* Ridl. crude extract against dermatophytes of superficial skin disease

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Abstract

The antifungal activity of crude extracts from the stem bark of *Entada spiralis* was evaluated *in vitro* against human dermatophytes by disc diffusion method. Three types of human dermatophytes, known as *Trichophyton mentagrophytes, Microsporum gypseum, Trichophyton tonsurans* and one non-dermatophyte *Candida glabrata,* were tested against petroleum ether, ethyl acetate and methanol crude extracts of the *E. spiralis*. Results revealed that all dermatophytes were susceptible towards all tested crude extracts, whereas, the non-dermatophyte showed resistance to all the extracts. *M. gypseum* was found to be most susceptible towards petroleum ether extract (400mg/ml), with a zone of inhibition of 16 mm. The ethyl acetate and methanol crude extracts (400mg/ml each) exhibited highest activity against *T. tonsurans* with inhibition zones of 12.7 mm and 11.5 mm, respectively. Nystatin was used as the standard antifungal drug in all experiments and served as the positive control. All these results suggested that the petroleum ether crude extract was the most active extract against all tested dermatophytes except for *C. glabrata.* Based on these current findings, it can be concluded that the stem bark extracts of *E. spiralis* have promising antifungal activities and can be used as a potent antifungal drug against certain dermatophytes.

Keywords: Leguminoceae, *Entada spiralis,* antifungal, dermatophytes

Abstrak

Aktiviti antifungus dalam ekstrak mentah yang diambil daripada goresan kulit *Entada spiralis* telah dinilai dan diuji secara *in-vitro* terhadap dermatologi manusia dengan menggunakan kaedah penyerakan dalam piring. Tiga jenis dermatofit manusia yang dikenali sebagai *Trichophyton mentagrophytes, Microsporum gypseum Trichophyton tonsurans* dan bukan-dermatofit *Candida glabrata ekstrak* telah diuji terhadap petroleum ether, ethyl acetate dan ekstrak mentah methanol yang mengandungi *E. spiralis.* Keputusan menunjukkan bahawa semua dermatofit mudah dipengaruhi oleh kesemua ekstrak mentah yang diuji, manakala bahan bukan dermatofit menunjukkan ketahanan terhadap semua ekstrak. *M. gypseum* adalah yang paling mudah dipengaruhi oleh ekstrak petroleum ether (400 mg/ml), dengan zon daya tahan pada takat 16 mm. Ethyl acetate dan ekstrak mentah methanol (400 mg/ml setiap satu) menunjukkan keaktifan yang tinggi terhadap *T. tonsurans* dengan zon daya tahan pada tahap 12.77 mm dan 11.5 mm mengikut urutan. Nystatin telah digunakan sebagai ubat antifungus piawai dalam semua eksperimen dan bahan ini juga bertindak sebagai bahan kawalan positif. Semua keputusan membuktikan bahawa ekstrak mentah petroleum ether merupakan ekstrak yang paling aktif bertindak terhadap semua dermatofit yang diuji kecuali *C. glabrata*. Berdasarkan dapatan-dapatan semasa, satu rumusan dapat dibuat iaitu

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ekstrak goresan kulit *E. spiralis* mempunyai potensi dalam aktiviti-aktiviti antifungus dan boleh digunakan sebagai ubat antifungus yang mujarab terhadap dermatofit-dermatofit yang tertentu.

Kata kunci: Leguminoceae, *Entada spiralis*, antifungus, dermatofit.

Introduction

A dermatophyte is a parasitic fungus that causes infections of the skin in animals and humans and includes the imperfect fungi of the genera *Epidermophyton, Microsporum* and *Trichophyton.* The infections are due to their ability to obtain nutrients from keratinized material. Dermatophytes colonize the keratin tissues and inflammation is caused by the host immune response to the metabolic byproducts. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissues of an immunocompetent host. The dermatophytes usually do not invade living tissues but colonize the outer layer of the skin. The types of skin infections caused by *Trichophyton tonsurans, Trichophyton mentagrophytes* and *Microsporum gypseum* are tinea capitis (scalp and hair), tinea corporis (glabrous skin), tinea unguium (nails), tinea manuum (hand), etc.

Entada spiralis Ridl. (Leguminoceae) which is locally known as 'Beluru' or 'Sintok' is a woody climber that grows wildly in Malaysia. For generations, the stem bark of *E. spiralis* has been used traditionally as shampoo, soap and also been used to treat syphilis and insect bites. Many reports showed that *Entada sp* has an antimicrobial and antifungal activity against various kinds of fungal pathogens (Aboaba et al., 2006). The traditional uses of other *Entada sp* such as *Entada abyssinica* has been used to treat bronchitis, coughs, miscarriage, fever and abdominal pain (Olajide and Alada, 2001). *Entada africana* is used traditionally to treat hepatitis, wound-healing, sore, skin-eruption, rheumatism, cataract, fevers and dysentery (Burkill, 1995). The application of the juice from the crushed stem of *Entada rhedeii* Spreng has been found to be important in the treatment skin diseases such as eczema, itches and scabies (Ram et al., 2004).

Since there are no reports on the antifungal activity of the *E. spiralis,* against dermatophytes, therefore, this study was undertaken to evaluate the anti fungal activity of the petroleum ether, chloroform and methanol extracts of stem bark of *E. spiralis* against different dermatophytes.

Materials and Methods

Plant materials and extraction

Stem bark of *E. spiralis* were collected from the forest in Tasik Chini, Pahang. It was autenthicated by a plant botanist from Universiti Kebangsaan Malaysia. A voucher specimen (KMS -5228) was deposited at Universiti Kebangsaan Malaysia, Bangi. About 3 kg of finely powdered dry stem bark was successively soaked in petroleum ether or chloroform or methanol, respectively. The soaking process was repeated 3 times for each solvent. The soaked powders were filtered and the filtrates were evaporated under vacuum at 55^0C yielding respective crude extracts. The crude extracts were transferred into sample bottles and kept in a refrigerator until used.

Microorganisms

The microorganisms used in this study were dermatophytes (*Trichophyton mentagrophytes* ATCC 9533, *Trichophyton tonsurans* ATCC 28942, *Microsporum gypseum* ATCC 24102) and a non dermatophyte (*Candida glabrata,* ATCC 66032). The stock cultures were maintained through monthly subculturing on Saubaroud Dextrose Agar (SDA) medium at room temperature in the dark.

Preparation of discs from crude extracts

Stock solution of 400 mg/ml of crude extract was prepared by dissolving 0.4 g of extracts in 1 ml of suitable diluents (depending on the polarity of the extracts). Serial two-fold dilution of the stock solution was made to get 200 mg/ml, 100 mg/ml and 50 mg/ml, respectively. Twenty µL of each dilution was impregnated onto a sterile paper disk (Whatman AA disk, 6mm) using micropipette and allowed the solvent to dry at room temperature (Chandrasekaran and Venkatesalu, 2004; Prasad et al., 2004). All discs were stored at -5^0C prior to use.

Chemicals for antifungal assay

Nystatin (Oxoid, England) and tetracycline (Oxoid, England) discs were used as reference antifungal drugs against yeasts and fungas. These drugs were used as positive controls, while methanol (Merck, Germany) and empty discs as

negative controls. Sauboraud Dextrose Agar (SDA) and Sauboraud Dextrose Broth (SDB) were purchased from Merck, Germany.

Microbial suspension

Stock cultures were maintained at 4ºC in SDB cultures. The dermatopyhtes were subcultured on SDA slants and incubated at 35ºC for 7-14 days. The mycelial growth was scraped aseptically and suspended thoroughly in sterile distilled water. The suspension was standardized spectrophotometrically to an absorbance (OD) of 0.600 at 450 nm. These adjusted suspensions approximately corresponded to 0.5 - 2.5 x 10^3 cells/ml and were used as inocula for antifungal susceptibility testing (Chandarasekaran and Venkatesalu, 2004; Pankajalakhsmi and Taralakshmi, 1995; Prasad et al., 2004).

Antifungal Assay

An antifungal assay was performed by using the disc diffusion agar method (Bauer et al., 1966). Test plates were prepared with 20 mL of sterile molten SDA. The standardized fungal suspension was applied on the solidified SDA by using sterile cotton swabs and allowed to dry for 5 min. Crude extract impregnated discs were aseptically transferred on the inoculated agar plates and incubated for 48 h to 7 days. Antifungal activity was determined by measuring clear zones of inhibition around the test crude extract discs. The clear zones indicated the fungicidal effect while fungistatic effect referred to the unclear zone of inhibition. Nystatin (100 ug) discs were used as standard reference or positive controls and the solvent or empty discs were used as negative controls. All assays were carried out in triplicate.

Results

The results for the antifungal activity test of different crude solvent extracts of *E.spiralis* are demostrated in Table 1. The growth of *T .mentagrophytes*, *T. tonsurans* and *M. gypseum* were inhibited by all three types of extracts in a concentration dependent manner. However, *C.glabrata* was found to be resistant to all types of extracts. On the other hand, for petroleum ether extract, the inhibition was detected at concentrations of 50-400 mg/ml against all dermatophytes except *C.glabrata*. For ethyl acetate extract, the inhibition zones were detected at 400 mg/ml (*T.mentagrophytes*), 200–400 mg/ml (*T.tonsurans)* and 100–400 mg/ml (*M.gypseum),* respectively. For methanol extract, the inhibition zones were detected at 400 mg/ml (*T. tonsurans*) and 100–400 mg/ml (*M.gypseum* and *T. mentagrophytes*), respectively. The petroleum ether extract was seemed to be the most active extract with an inhibition zone of 16 mm against *M.gypseum*. Among all dermatophytes, *M. gypseum* was found to be most susceptible towards all tested extracts.

All dermatophytes *T. mentagrophytes*, *T. tonsurans* and *M. gypseum* were found to be sensitive to the positive control, nystatin, with inhibition zones of 21.7, 40 and 24 mm, respectively. The negative controls did not affect the growth of dermatophytes. The graphs in Figure 1, 2 and 3 show antifungal activities of all crude extracts against the dermatophytes.

Table 1**:** In vitro antifungal activity of different crude solvent extracts of *E. spiralis* stem bark

Extracts	Concentration (mg/ml)	Inhibition zone (mm)			
		TM	TT	MG	CG
Pet ether	50	-	8.67±0.58	9.50±0.71	-
	100	8.33±0.58	10.3±0.58	10.0±0	-
	200	9.0±0.00	9.00±1.0	13.0±0	-
	400	10.67±0.58	13.0±1.73	16.0±0	-
Nystatin		21.67±0.58	39.67±1.15	24 ±0	24 ±0
Tetracycline		-	-	-	-
Ethyl acetate	50	-	-	-	-
	100	-	-	8.67±0.58	-
	200	-	9.0±0	9.0±0	-
	400	10.5±0.71	12.7±1.2	9.0±0	-
Nystatin		21.33±0.71	39.67±0.58	24±0	25.3±0.58
Tetracycline		-	-	-	-
Methanol	50	-	-	-	-
	100	8.50±0.71	-	9.67±0.58	-
	200	8.50±0.71	-	10.3±0.58	-
	400	10.5±0.71	11.5±0.71	11.0±1.0	-
Nystatin		21.7±0.58	40.0±0.58	24.0±0	24.0±0
Tetracycline		-	-	-	-

-, No activity; TM, *Trichophyton mentagrophytes*; TT, *Trichophyton tonsurans*; MG, *Microsporum gypseum*; CG, *Candida glabrata*, tetracycline and nystatin, control antifungal drug

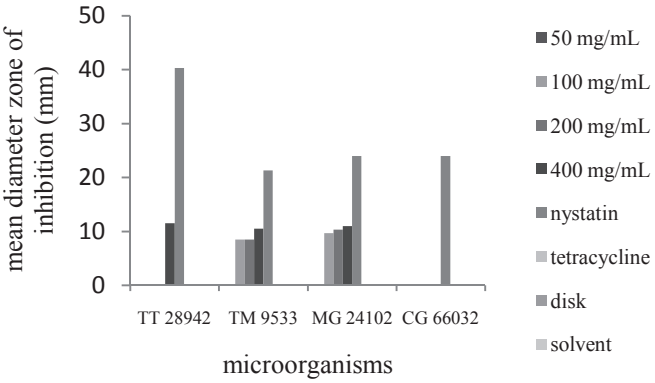

Figure 1: Antifungal activity of methanol extract of *E.spiralis* against dermatophytes. TT, *T. tonsurans*; TM, *T. Mentagrophytes*; MG, *M.gypseum*; CG, *C.glabrata*

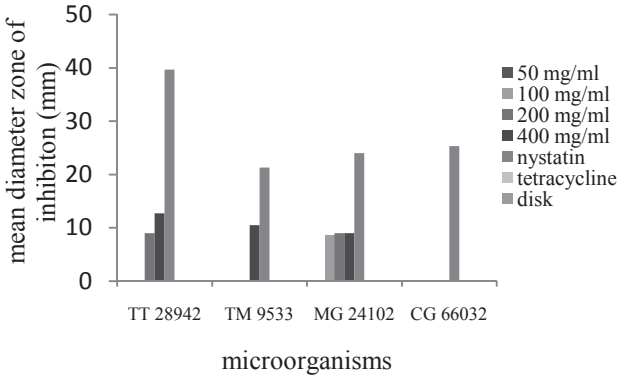

Figure 2: Antifungal activity of ethyl acetate extract of *E spiralis* against dermatophytes. TT, *T tonsurans;* TM, *T.mentagrophytes*; MG, *M.gypseum*; CG, *C.glabrata*

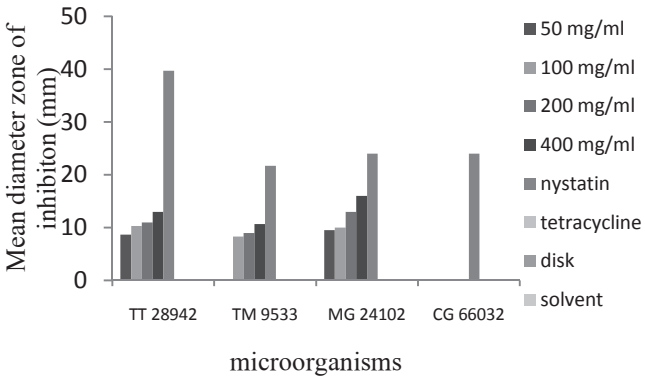

Figure 3: Antifungal activity of petroleum ether extract of *E spiralis* against dermatophytes. TT, *T tonsurans;* TM, *T.mentagrophytes*; MG, *M.gypseum*; CG, *C.glabrata*

Discussion

The results of this study indicated antifungal activities of the extracts of *E.spiralis* stem bark against certain dermatophytes that causes ringworm and skin diseases. Traditionally in Malaysia, this plant has been used to treat hair scalp and as a body soap. Therefore, the results of this current study revealed the scientific basis of the traditional usage of *E. spiralis*. Moreover, these current findings were consistent with some previous reports, where antifungal activity of crude extract from *Cassia alata* (Leguminoceae) were demonstrated against *Trycophyton rubrum*, *Trycophytom mentagrophytes* and *Microsporum gypseum* (Ibrahim and Osman, 1995; Webster et al., 2008).

The larger inhibition zones, detected at higher concentrations of all the extracts, could be due to the higher concentrations of active compounds found in the extracts except for *C. glabrata*. The reason why all dermatophytes except *C. glabrata,* sensitive towards extracts was most probably due to the interference by the active compounds of the extracts, which consequently leaked into the cell wall of the microconidia. As a result, the microconidia lost its rigidity and caused the death of the cell (Ibrahim and Osman, 1995). The resistance of *C. glabrata* towards all extracts at all concentrations was probably due to its cell wall was strong enough to overcome the effect of interference and remained resistant.

All dermatophytes were most sensitive towards petroleum ether extracts followed by methanol and lastly ethyl acetate extracts. These results could be due to the synergistic action of different groups of active compounds contained in the extracts (Kuiate et al., 2006). The antifungal activity of *E.spiralis* stem bark was not as effective as commercial antifungal drug nystatin (21.7 – 40 mm). Nevertheless, future studies with same concentration or higher extract concentration may be good enough to evaluate the actual antifungal properties of *E.spiralis* stem bark.

The present study indicates that the crude extracts of *E. spiralis* stem bark possess some antifungal activities against certain dermatophytes. However, further phytochemical studies are required to identify the active compounds for the bioactivity.

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