

Komunikasi Pendek/Short Communications

Antimycotic Activity of the Extracts of *Stichopus chloronotus* Brandt in the Treatment of Experimental Dermatophytosis

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ABSTRAK

Ekstrak akueus dan etanol *Stichopus chloronotus* Brandt telah diuji keberkesanannya terhadap dermatofitosis pada argus menggunakan teknik folikel rambut. Ekstrak etanol pada 10 mg/ml menunjukkan keberkesanan 82.8% terhadap *Trichophyton mentagrophytes* manakala ekstrak akueus pada kepekatan yang sama pula menunjukkan keberkesanan 84.8% terhadap jangkitan *Microsporum canis*, berbanding dengan Econazole yang mempamerkan keberkesanan 100 % terhadap kedua-dua jenis jangkitan. Tiada kesan sampingan dikesan pada kulit argus yang diberi rawatan ekstrak kajian. Kesimpulannya, ekstrak akueus dan etanol *S. chloronotus* menunjukkan aktiviti antimikotik yang tinggi terhadap dermatofitosis yang diaruhkan secara eksperimen pada argus kajian.

ABSTRACT

The aqueous and ethanol extracts of *Stichopus chloronotus* Brandt were investigated for their effectiveness against guinea pig dermatophytosis caused by *Microsporum canis* and *Trichophyton mentagrophytes* using the hair root invasion test. The ethanol extract at 10 mg/ml showed 82.8 % efficacy against *T. mentagrophytes* while the aqueous extract at similar concentration showed 84.8% efficacy against *M. canis* infection, as compared to econazole which showed 100% efficacy against both infections. No adverse effect on the skin was observed in the treated animals. In conclusion, aqueous and ethanol extracts of *S. chloronotus* showed high antimycotic activity against experimentally induced dermatophytosis in guinea pigs.

Dermatophytosis is among the most common skin diseases worldwide. Although mycotic infections caused by dermatophytes are rarely fatal, nevertheless they are prevalent in the environment and stubborn to control (Odom 1993). In humans, *Trichophyton mentagrophytes* is an important source of inflammatory fungal infection of the feet whereas *Microsporum canis* is responsible for a large number of cases of classic body ringworm infections (Ally 1998). The antifungal activities of sea cucumbers have been widely investigated. The *in vitro* antimycotic activities of five Malaysian sea cucumber species; *Stichopus chloronotus*, *S. badionotus*, *S. variegatus*, *Holothuria atra* and *H. edulis* have been reported (Dayang Fredalina et al. 1997). However, not much is known about the effectiveness of sea cucumbers on fungal infections induced in experimental animals. This paper described the *in vivo* efficacy of extracts from *S. chloronotus* against experimental dermatophytosis in guinea pigs. In this study, the ethanol extract was used against *T. mentagrophytes* while the aqueous extract was tested against *M. canis*.

Evidence of infection was visible 3 days after inoculation of the dermatophyte with the appearance of scaly lesions on the skin of the guinea pigs. After 7 days of treatment, notable improvement in the skin condition in view of absence of scaly lesions was seen in the animals given either the ethanol and aqueous sea cucumber extracts or econazole as compared to the untreated animals. The hair root invasion test at day 3 post-treatment revealed a higher number of infected hairs for the untreated animals ( $p < 0.05$ ) while econazole treated animals showed no evidence of infection. The animals treated with 10 mg/ml each of the aqueous and ethanol extracts exhibited a reduction of 84.8% and 82.8% of the infection caused by *M. canis* and *T. mentagrophytes*, respectively (Table 1 and 2). The sea cucumber extracts did not appear to cause any side effects on the skin and neither was there any allergic reaction visible in the 2 hr after application of treatment. There appeared to be no visible delayed reaction 24 hr and 48 hr after application of treatment and normal hair growth had resumed.

Our results indicated that both the ethanol and aqueous extracts of *S. chloronotus* have a high treatment efficacy rate against experimental dermatophyte infections. This efficacy rate may be improved with longer treatment duration or a higher treatment dose. Several triterpene oligoglycosides known

as stichoposides and telenosides have been isolated from *S. chloronotus* and these compounds were found to exhibit high antifungal activity (Matsushima 1981). They exert their antifungal effect by interaction with ergosterol leading to disruption of cell membrane and eventually cell lysis (Verbist 1993). Thus the antimycotic activity of the *S. chloronotus* extracts against the experimental dermatophytosis in guinea pigs was possibly due to the presence of these saponin glycosides. Toxicity studies showed that there was no overall incidence of local, adverse effects such as redness or swelling and hence, the extracts were well-tolerated.

In conclusion, the extracts of *S. chloronotus*, applied topically once a day throughout one-week course of experimental dermatophyte infections presented a high therapeutic efficacy and good tolerability.

## MATERIALS AND METHODS

Male Harlan guinea pigs (250-50 g each) were housed in the Animal Unit, Universiti Kebangsaan Malaysia (UKM) at the Institute for Medical Research, Kuala Lumpur, Malaysia. The animals were divided into 4 groups of 8 animals each for each experiment. They were housed in appropriate cages and fed with guinea-pig pellets and water *ad libitum*. All animal experiments were carried out with approval from UKM Animal Ethics Committee (UKMAEC). Thirteen fresh samples of *S. chloronotus* (100 g-120 g each) were collected around Kapas Island, Terengganu in the east coast of Peninsular Malaysia and the extracts were prepared based on modification of Yasumoto (1967) and Shimada (1969) respectively. The dermatophyte stock cultures were maintained on potato dextrose agar (PDA, Merck, Darmstadt, W. Germany) slants, stored at 4°C. The fungal inoculum was prepared by harvesting the fungal conidia using an aseptic wire loop after addition of 10 ml sterile saline containing 0.1 % Tween 80 to the culture plates. About 7 ml of the fungal suspension was then centrifuged at 270 g for 20 min at 4°C to remove hyphal fragments. The concentration of the conidial suspension was

adjusted to a concentration of  $1.0 \times 10^6$  conidia per ml by counting with a hemocytometer. This suspension was used as the fungal inoculum in the experiments.

#### INOCULATION PROCEDURE

The method of infection employed was according to Petranyi et al. (1981). Prior to inoculation, the hair was shorn from the backs of the guinea-pigs and about 0.1 ml of the inoculum containing  $10^5$  infective particles, was abraded onto the portion of the skin encircled by the cylinder using a roughened glass rod. The inoculation procedure was performed on all the animals in the test groups 1, 2 and 3. The animals in group 4 was not subjected to inoculation but was used for assessment of side effects of the extract used. Each test and control group consisted of 8 animals. All inoculated animals which showed presence of hyphal fragments at the hair root 3 days post-inoculation, indicated fungal infection. Dermatophytosis was confirmed by subculturing the infected hair root onto selective mycosel agar plates and growth of dermatophytes were observed after 48h - 72h incubation at 27°C (Petranyi 1982). Treatment commenced once daily for 7 consecutive days beginning 3 days post inoculation. The animals in groups 1 and 2 were treated with 10 mg/ml (0.4 ml) of the tested extracts and econazole (Sigma Chemical Co, USA), respectively prepared by dissolving the test compounds in sterile distilled water and 5 % polyethylene glycol (Analar Ltd.). The animals in group 3, however were left untreated and were maintained alongside the treated animal groups to serve as non-treated infected control.

#### ASSESSMENT OF ANTIMYCOTIC ACTIVITY

Mycological status was assessed 3 days after treatment was ended using the hair root invasion test (Petranyi 1982) in which, the incidence of infection in the skin foci of the individual animals was evaluated and expressed as a percentage cure. The mycotic focus of each animal was divided into 2 parts whereby a hair sample (consisting of 10 individual hairs) was taken from each part of the infected skin area. Dermatophyte test medium plates, similarly divided into 2 parts, were inoculated with the hair samples from the corresponding part of the skin. The plates were incubated at 27°C for 7 days and were then examined under a light microscope for signs of fungal growth at the hair root.

#### EVALUATION OF MYCOLOGICAL EFFICACY

The effectiveness of a test compound in reducing the number of mycologically positive hair samples per treated group was expressed as a percentage of the corresponding untreated control group of animals as follows :

where T is the mean number of hairs per animal that are mycologically positive in the test group and K is the same for group 3 (infected, untreated control).

#### EXAMINATION OF TOPICAL SIDE EFFECTS

Guinea pigs from group 4 were only subjected to topical application of the sea cucumber extracts without prior inoculation of fungus to observe any untoward effects on the skin of the animals by the extracts. About 0.4 ml of the extracts (10 mg/ml) was gently rubbed onto the left ear while the right ear was left untreated. Physical examination was observed every 15 min throughout a period of 2 hr to detect any changes such as swelling, redness or other adverse effects associated with allergic reactions in comparison with the right ear.

#### ACKNOWLEDGEMENT

The authors wish to thank Ridzwan Hashim of Islamic International University, Malaysia, for his help in the collection and identification of *S. chloronotus* species used in this study. This work was funded by Universiti Kebangsaan Malaysia - UKM grant NN-006-2003.

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