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Anti-Dermatophyte Activities of "Tea Seed Cake" and "Pegu-catechu"

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Abstract

16 species of Thai medicinal plants and two folk medicines were tested for their antimicrobial activities by the disc agar diffusion method. From the size of inhibition zone against ***Trichophyton mentagrophytes*** *and* ***T. rubrum,*** *the anti-Dermatophyte activity of n-BuOH fraction obtained from tea seed cake (TK) and Pegu-catechu among the tested samples was found at 10 mg/ml. Although these activities were lower by about two orders of magnitude than that of Griseofulvin, these two folk medicines might be effective and valuable in traditional remedies. The aqueous extracts of all tested samples had no antimicrobial activity with other cultures of the microorganisms tested,* ***Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*** *and* ***Candida albicans.***

Key word index : Tea seed cake, Pegu-catechu, *Camellia sinensis*, *Acacia catechu*, Theaceae, Leguminosae, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, anti-Dermatophyte activity, Folk medicine, Thai medicinal plants

Introduction

In our recent papers [1-5], we have reported several biological activities including antimicrobial activity of some Thai medicinal plants. Further studies on the chemical constituents and biological activities of medicinal plants and folk medicines in Thailand led to the finding of anti-Dermatophyte activity in the saponin fraction prepared from the *n*-BuOH extracts of tea seed cake (TK) and in the *n*-BuOH extracts of Pegu-catechu.

Tea seed cake (Chinese name : Tae kow; TK) originated from the defatted seeds of tea (*Camellia sinensis* O. Kuntze :*Thea sinensis* L.; Theaceae) is utilized for skin diseases as a folk medicine and also utilized as a fertilizer traditionally [6]. Pegu-catechu, the xylem aqueous extract of *Acacia catechu* Willd. (Leguminosae), is also widely used as an antidiarrheic and also utilized for skin diseases in traditional remedies.

In this paper, we describe the anti-Dermatophyte activities against *Trichophyton mentagrophytes* and *T. rubrum* of these folk medicines.

Materials and Methods

Plant materials

The plant materials for screening tests, *Abutilon indicum*, *Acacia concinna*, *Allium sativum*, *Alpinia galanga*, *Andrographis paniculata*, *Barleria lupulina*, *Centella asiatica*, *Curcuma longa*, *Entada phaseoloides*, *Eurycoma longifolia*, *Gastrochilus panduratum*, *Leucaena leucocephala*, *Rhinacanthus nasutus*, *Tamarindus indica*, *Tinospora rumphii* and *Zingiber ottensii*, were collected in the vicinity of Chiangmai, Thailand and identified by Assoc. Prof. S. Okonogi, Chiangmai

University. Tea seed cake (TK) and Pegu-catechu were purchased from a local crude drugstore at Chiangmai. The herbarium samples are kept in the herbarium of Faculty of Pharm. Sci., Chiba University, Japan.

Preparation of aqueous extracts and several fractions

Each of the dried plant materials, TK and Pegu-catechu (100 g) was ground into a fine powder and macerated with boiling water (1 l) for about 1 hr. The mixture was filtered through a cotton gauze and the residue was washed with a small amount of hot water. The remaining residue in the filtrate was further removed by centrifugation. The supernatants were combined and lyophilized to make the aqueous extracts. The aqueous extracts of TK and Pegu-catechu were partitioned with AcOEt and *n*-BuOH, respectively, as previously reported [7]. The *n*-BuOH fraction obtained from TK was chromatographed on Diaion HP-20 and eluted with H_2O, 50% MeOH and MeOH, respectively. The 50% MeOH eluate fraction was then rechromatographed on Sephadex LH-20 using a gradient solvent system (H_2O-MeOH) to give crude saccharide, flavonoid and saponin fractions. These were used in biological activity screening tests.

Antimicrobial activity

Aqueous extract and several fractions obtained from test samples were tested for their antimicrobial activities by the disc agar diffusion method as described previously [2]. Test microorganisms were *Trichophyton mentagrophytes, T. rubrum, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans*, purchased from the Institute for Fermi (Osaka, Japan). Heart infusion agar was used as a culture medium. Samples were tested at various concentrations of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} g/ml and 50 ml of each was used per disc (diameter : 8 mm). Incubation was performed at 25 °C for 72 hrs or 7 days against *Trichophyton mentagrophytes* and *T. rubrum*, and at 37°C for 24 or 48 hrs against other microorganisms. Gentamicin (2, 5, 10 mg) and Griseofulvin (0.25, 0.5, 1 mg/ml) were used as a reference standard.

Results and Discussion

The *n*-BuOH extracts of TK and Pegu-catechu displayed anti-Dermatophyte activities at 10 mg/ml against *Trichophyton mentagrophytes* and *T. rubrum* among all tested microorganisms as well as these aqueous extracts, as shown in Table 1. The aqueous extracts of these two folk medicines had no antimicrobial activity with other cultures of the microorganisms tested, *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans*. The crude saponin fractions prepared from *n*-BuOH extract of TK also displayed anti-Dermatophyte activities against *Trichophyton mentagrophytes* and *T. rubrum* at 10 mg/ml, while AcOEt extract, crude saccharide and crude flavonoidal fractions had no activity. Moreover, the aqueous extracts prepared from 16 species of Thai medicinal plants did not show any antimicrobial activity against all tested microorganisms.

Our results in this study indicated that TK might be therapeutically effective for skin diseases such as a dermatophytosis, although the anti-Dermatophyte activity was lower by about two orders of magnitude than that of Griseofulvin. These results also suggested that an active constituent for anti-Dermatophytes in TK may be triterpenoidal saponins [8]. Actually, TK and Pegu-catechu are utilized for skin diseases as a folk medicine in Thailand, traditionally. Pegu-catechu is known to contain a lot of tannins and known to be used as an antidiarrheic. Therefore, these observations suggested that the continuance of this study on these folk medicines should be made to find out the active constituents, which would explain their uses in traditional remedies.

Table 1. Inhibitory effects of aqueous extracts and several fractions obtained from Thai folk medicines on *Trichophyton mentagrophytes* and *T. rubrum*

Sample		Conc. (g / ml)	*Trichophyton mentagrophytes* (mm) *	*T. rubrum* (mm)
Tea seed cake (TK)	aqueous extract	10^{-1}	29, 30	33, 34
		10^{-2}	20, 21	29, 31
		10^{-3}	-	-
	AcOEt extract	10^{-2}	-	-
	n-BuOH extract	10^{-2}	21, 22	30, 30
	saccharide fraction	10^{-2}	-	-
	flavonoid fraction	10^{-2}	-	-
	saponin fraction A	10^{-2}	20, 22	-
	saponin fraction B	10^{-2}	18, 18	25, 27
Pegu-catechu	aqueous extract	10^{-1}	10, 12	20, 22
		10^{-2}	-	17, 18
		10^{-3}	-	-
	n-BuOH extract	10^{-2}	9, 10	18, 18
Griseofulvin		1.0×10^{-3}	25, 25	33, 35
		0.5×10^{-3}	27, 25	33, 34
		0.25×10^{-3}	18, 18	25, 25

* Inhibition zone was measured by the disc agar diffusion method (disc diameter : 8 mm).

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