Efficacy and active components of herbal extracts on the treatment of tinea pedis

KM Lau 劉潔雯
LH Fu 傅麗杭
YL Wong 黃妍麗
CP Lau 劉清浦
CW Wong 黃俊維
L Cheng 鄭　玲
CBS Lau 劉碧珊
VEC Ooi 黃榮春
PKS Chan 陳基湘
KP Fung 馮國培
M Hui 許明媚
PC Leung 梁秉中

Key Messages

1. Ethanolic extracts of Fructus Psoraleae (補骨脂), Rhizoma Curcumae Longae (姜黃), and Folium Eucalypti Globuli (藍桉葉) possess *in vitro* antifungal activities (against *Trichophyton mentagrophytes* and *Trichophyton rubrum)*.

2. An herbal formula, comprising these ethanolic extracts in the ratio of 1:1:1, could effectively alleviate tinea pedis caused by *T mentagrophytes* in guinea pigs (P<0.01).

Hong Kong Med J 2011;17(Suppl 2):S44-7

The Chinese University of Hong Kong: Institute of Chinese Medicine
KM Lau, YL Wong, CP Lau, CW Wong, L Cheng, CBS Lau, VEC Ooi, KP Fung, PC Leung
Department of Microbiology
LH Fu, PKS Chan, M Hui
School of Biomedical Sciences
KP Fung

RFCID project number: 05050212

Principal applicant and corresponding author:
Prof Ping Chung Leung
Institute of Chinese Medicine, 5/F, School of Public Health Building, Prince of Wales Hospital,
Shatin, NT, Hong Kong SAR, China
Tel: (852) 2252 8868
Fax: (852) 2632 5441
Email: pingcleung@cuhk.edu.hk

Introduction

Tinea pedis (also known as athlete's foot) is a superficial fungal infection of the feet. In a prospective epidemiological study conducted in Hong Kong in 1999, its prevalence was 20.4% in 1014 subjects.[1] Tinea pedis is more commonly found in males, the middle-aged or elderly, and in those with diabetic or other underlying medical (particularly immune system) problems that favour fungal growth. The most common causal organisms are the dermatophytes: *Trichophyton mentagrophytes, Trichophyton rubrum* and *Epidermophyton floccosum*.

Treatment for tinea pedis involves the use of topical antifungals (such as ketoconazole, terbinafine, econazole, and cicloprox creams) and oral agents (such as griseofulvin, itraconazole, fluconazole, and terbinafine). Inadequate spectrum of activity, drug resistance, toxicities, and drug-drug interactions limit successful results, for which reason alternative medicines may be desirable. In a review on the antifungal activities of over 1000 species of traditional Chinese medicines (TCMs) recorded in Pharmacopoeia of the People's Republic of China (2005 edition), 83 have been demonstrated to possess antifungal activity of which 11 are efficacious against *Trichophyton* strains. These include Bulbus Allii (大蒜), Fructus Galangae (紅豆蔻), Semen Cassiae (決明子), Herba Cichorii (菊苣), Rhizoma Curcumae Longae (姜黃) [RCL], Semen Juglandis (核桃仁), Herba Portulacae (馬齒莧), Cortex Pseudolaricis (土荊皮), Fructus Psoraleae (補骨脂) [FP], Fructus Chebulae (訶子), and Folium Eucalypti Globuli (藍桉葉) [FEG].

Methods

This study was conducted from December 2006 to December 2008. Aqueous and ethanolic extracts of the 11 TCMs were yielded by extracting in water or 95% ethanol under reflux. The antifungal efficacies of these extracts were compared using an *in vitro* antifungal test. Three most potent extracts were selected and subjected to activity-guided fractionation. A guinea pig model was used to evaluate the tinea pedis–treating effects of the active extracts and fractions. The *in vitro* assay was performed in duplicates and the *in vivo* assay was on at least four samples.

Results

In vitro antifungal susceptibility test

The aqueous extracts of Fructus Galangae, Herba Portulacae, Semen Juglandis, Semen Cassiae, and FP promoted fungal growth, whereas those of Fructus Chebulae and FEG were effective in inhibiting dermatophyte (*T mentagrophytes* and *T rubrum*) growth at 3.91 and 7.81 μg/mL concentrations, respectively (Table 1). Among ethanolic extracts, those of FP, RCL, and FEG possessed the most potent antifungal activity. Their minimum inhibitory concentration (MIC) values of 0 (for 100% inhibition) of both dermatophytes were low. After comparing the antifungal effects of all 22 extracts, ethanolic extracts of FP, RCL, and FEG were the most effective and selected for further fractionation.

Each potent extract was fractionated using solvent partition into five

fractions (n-hexane, dichloromethane, ethyl acetate, n-butanol, and water residue). Based on the MIC values determined in the *in vitro* antifungal assay, the n-hexane fraction (FP-EtOH-P1) and the dichloromethane fraction (FP-EtOH-P2) from FP, and the n-hexane fraction (FEG-EtOH-P1) from FEG exhibited the most potent inhibitory effect against dermatophytes. They were then subjected to column chromatography to give 10, 9, and 8 subfractions, respectively. Among these 27 samples, subfractions FP-EtOH-P1-C2, FP-EtOH-P2-C1, and FEG-EtOH-P1-C6 were the most active against dermatophytes (Table 2). Subsequent activity-guided fractionation led to two active compounds: bakuchiol from FP and macrocarpal C from FEG. Their antifungal activities have been reported.[2]

Table 1. Minimum inhibitory concentration (MIC) values of 11 traditional Chinese medicines extracted by water or 95% ethanol

Traditional Chinese medicines	Minimum inhibitory concentration (MIC)* values (μg/mL)											
	Aqueous extracts						Ethanolic extracts					
	Trichophyton mentagrophytes (ATCC 9129)			*T rubrum* (ATCC 28191)			*T mentagrophytes* (ATCC 9129)			*T rubrum* (ATCC 28191)		
	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2
Fructus Galangae (紅豆蔻)	PG†	PG	PG	PG	PG	PG	62.5	-	7.81	15.6	-	7.81
Herba Portulacae (馬齒莧)	PG	PG	PG	PG	PG	PG	125	62.5	31.25	125	62.5	-
Fructus Chebulae (訶子)	3.91	0.49	-	31.25	15.6	7.81	3.91	0.98	0.49	62.5	31.25	-
Semen Juglandis (核桃仁)	48	6	0.19	PG	PG	PG	-	-	500	-	250	125
Cortex Pseudolaricis (土荊皮)	7.81	1.95	0.49	31.25	15.6	7.81	3.91	0.98	0.49	62.5	-	-
Semen Cassiae (決明子)	PG	PG	PG	PG	PG	PG	125	31.25	7.81	250	125	62.5
Fructus Psoraleae (補骨脂)	PG	PG	PG	PG	PG	PG	7.81	3.91	-	15.6	7.81	3.91
Rhizoma Curcumae Longae (姜黃)	-	500	250	-	500	125	7.81	-	3.91	15.6	7.81	3.91
Bulbus Allii (大蒜)	>500	-	-	>500	-	-	500	250	-	500	250	-
Herba Cichorii (菊苣)	>500	-	-	>500	-	-		500	-	500	250	-
Folium Eucalypti Globuli (藍桉葉)	7.81	3.91	1.95	7.81	3.91	1.95	3.91	1.95	-	15.6	-	-

* MIC 0 denotes 100% inhibition, MIC 1 75% inhibition, and MIC 2 50% inhibition

† PG denotes promote growth

Table 2. Minimum inhibitory concentration (MIC) values of sub-fractions of the three selected fractions

Sub-fraction	Minimum inhibitory concentration (MIC)* values (μg/mL)					
	Trichophyton mentagrophytes (ATCC 9129)			*T rubrum* (ATCC 28191)		
	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2
Fructus Psoraleae fraction (FP-EtOH-P1)						
C1	-	500.00	-	-	500.00	125.00
C2	7.81	3.91	1.95	31.25	3.91	1.95
C3	125.00	-	62.50	31.25	15.60	7.81
C4	125.00	-	62.50	31.25	15.60	7.81
C5	125.00	-	31.25	62.50	15.60	7.81
C6	125.00	62.50	-	62.50	31.25	15.60
C7	125.00	31.25	-	62.50	31.25	15.60
C8	500.00	125.00	62.50	31.25	15.60	7.81
C9	-	125.00	62.50	31.25	15.60	7.81
C10	-	250.00	125.00	125.00	62.50	15.60
Fructus Psoraleae fraction (FP-EtOH-P2)						
C1	7.81	0.98	0.49	15.60	3.91	1.95
C2	250.00	125.00	-	125.00	62.50	31.25
C3	31.25	15.60	7.81	62.50	31.25	15.60
C4	125.00	62.50	31.25	125.00	-	62.50
C5	62.50	-	31.25	125.00	62.50	31.25
C6	500.00	250.00	125.00	500.00	250.00	125.00
C7	31.25	-	15.60	125.00	31.25	15.60
C8	15.60	-	7.81	250.00	62.50	31.25
C9	-	-	500.00	-	250.00	31.25
Folium Eucalypti Globuli fraction (FEG-EtOH-P1)						
C1	15.60	3.91	1.95	62.50	31.25	7.81
C2	15.60	7.81	3.91	62.50	31.25	15.60
C3	15.60	3.91	1.95	62.50	15.60	7.81
C4	7.81	3.91	1.95	31.25	15.60	3.91
C5	1.95	0.98	0.49	15.60	-	3.91
C6	1.95	0.98	0.49	3.91	1.95	0.98
C7	7.81	3.91	0.98	31.25	15.60	7.81
C8	-	3.91	1.95	-	62.50	15.60

* MIC 0 denotes 100% inhibition, MIC 1 75% inhibition, and MIC 2 50% inhibition

Besides individual antifungal activity, synergistic effects among herbs (combination of three of the most potent TCM extracts) and among fractions (combination of three of the most potent fractions) were studied using the *in vitro* antifungal susceptibility test. The MIC 0 values of herbal combination on *T mentagrophytes* and *T rubrum* were 250 and 500 μg/mL, respectively, and were much higher than the values of the individual ethanolic extracts. For FP and RCL, their MIC 0 values for *T mentagrophytes* and *T rubrum* were 7.81 and 15.6 μg/mL, respectively, whereas for FEG, they were 3.91 and 15.6 μg/mL, respectively. Therefore, no synergism was evident for the herbal combination. On the contrary, the fractional combination was more effective than its component fractions FP-EtOH-P2 and FEG-EtOH-P1. Nevertheless, when compared to its third component fraction FP-EtOH-P1, enhancement in antifungal activity was not demonstrated in the fractional combination (their MIC 0 values on *T mentagrophytes* and *T rubrum* were equal).

Guinea pig model of tinea pedis–treating effects

Tinea pedis was induced in the right hind feet of guinea pigs by inoculating a fungal suspension of *T mentagrophytes* for 7 days. On day 7 post-infection, the infected feet showed scale formation on the toes and soles. In the negative controls, aqueous cream was applied topically to the infected feet for 12 days. At the end of experiment, the animals were killed and skin from their feet was excised for fungal culture. Among 10 skin blocks from each foot, on average there were eight to nine blocks retrieved for fungal growth on agar slants. On the other hand, the application of positive control terbinafin (Lamisil) cream completely cured the tinea pedis, as reflected by the low fungal burden score. With this successfully established and validated animal model, the tinea pedis–treating effect of ethanolic extracts of FP, RCL, and FEG (the three most potent TCM extracts in the *in vitro* antifungal assay) as well as their active fractions were investigated.

Topical application of ethanolic extracts of FP, RCL, and FEG as 5% cream to the infected guinea pig feet was able to alleviate tinea pedis. Although the results were not significant, the extracts reduced the mean±standard deviation fungal burden to 4.9±4.3, 6.0±3.3, and 7.0±2.8, respectively, compared to 8.5±2.5 in the controls. Although they were not very effective when used alone, synergism was demonstrated when used as a herbal combination. The herbal combination of these three ethanolic extracts (in the ratio of 1:1:1) significantly decreased the fungal burden to 2.5±1.8, compared to 8.0±2.9 in the controls ($P<0.01$, Table 3).

The most potent fractions inhibiting growth of dermatophytes *in vitro* were FP-EtOH-P1, FP-EtOH-P2, and FEG-EtOH-P1. Therefore, their tinea pedis–treating activity was studied in the guinea pig model. These fractions individually or in combination alleviated tinea pedis to some extent; the resulting fungal burdens (5.4-6.1) were lower than that of control group (~8.0), but not significantly.

Discussion

Eleven herbs were shortlisted for antifungal screening, because various aqueous and organic solvent extracts and pure compounds derived from them had been reported to show activity against *Trichophyton* strains. By using a standardised broth dilution method, the antifungal activities of aqueous and ethanol extracts of these 11 TCMs could be compared. The aqueous extracts of Fructus Galangae, Herba Portulacae, Semen Juglandis, Semen Cassiae and FP promoted fungal growth (Table 1). We speculated that the high sugar contents of these extracts provided nutrients for fungal growth. As determined by the anthrone-sulfuric acid test, the total sugar contents in these five extracts were high, and ranged from 19.37% w/w to 68.41% w/w (data not shown). The ethanolic extracts were more effective in inhibiting the dermatophyte. As in all clinical trials using herbs, attention should be paid to the variation in efficacy resulting from different extractions using different solvents.

Among the 11 TCMs, FP, RCL, and FEG extracted by ethanol were the most effective for inhibiting the growth of dermatophytes *in vitro* (Table 1). Fructus Psoraleae are dried ripe fruits of *Psoralea corylifolia* L. and are traditionally used to invigorate kidney function, alleviate asthma and relieve diarrhoea. The antidermatophytic activity of *Psoralea corylifolia* seed extracts have been evaluated using the disc diffusion method.[3] At 250 μg/mL, its ethanolic extract exhibited activity with an inhibition halo diameter of 24 mm and 23 mm against *T mentagrophytes* and *T rubrum*, respectively. Despite different assay methods, our results were in line with those previously documented, and confirmed the antifungal activity of FP.

Rhizoma Curcumae Longae are the dried rhizomes of

Table 3. Therapeutic efficacy of the herbal combination of Fructus Psoraleae, Rhizoma Curcumae Longae and Folium Eucalypti Globuli ethanolic extracts (1:1:1) by topical application for 12 days on tinea pedis of guinea pigs infected with *Trichophyton mentagrophytes*

Treatment	No. of animals	No. of culture-positive skin blocks/total no. of skin blocks	Fungal burden
Aqueous cream (control group)	7	56/70	8.0±2.9
Terbinafine cream	4	0/40	0*
Herbal combination	8	20/80	2.5±1.8*

* $P<0.01$, versus control group

Curcuma longa L. and are traditionally used to eliminate blood stasis, promote the flow of qi, stimulate menstrual discharge, and relieve pain. The ethanolic extract of RCL could exert 65% inhibition on *T longifusus*. We reported the inhibitory effect of RCL on *T mentagrophytes* and *T rubrum*. Turmeric oil and curcumin are two active components of RCL. Curcumin has no antifungal activity, whereas turmeric oil could inhibit dermatophytes, but at a relatively high MIC (>200 μg/mL).[4] Our results also showed that fractions of RCL were less effective than the crude ethanolic extract, indicating that the latter might be a more effective treatment against dermatophytes.

Folium Eucalypti Globuli are the fresh leaves of *Eucalyptus globules* Labill and are used to treat influenza, headache, cough, eczema and dermatomycosis. Its methanol-dichloromethane (1:1) extract was demonstrated to inhibit *T mentagrophytes*, with a MIC of 31 μg/mL.[5] Its ethanolic extract also had potent activity against this dermatophyte.

In TCM practice, treatment of tinea pedis involves the use of TCM decoctions of four to eight herbs. We investigated whether a formula composing of FP, RCL, and FEG could exert synergism and, thus, better antidermatophytic activity. Using an *in vitro* antifungal assay, this herbal formula showed only weak inhibitory effects. The combination might be antagonistic, instead of synergistic. Nonetheless, this formula could significantly reduce the fungal burden in the guinea pig model of tinea pedis. We speculate that involvement of host's immune response might play a role in the effectiveness of this herbal formula.

The herbal formula (FP, RCL, and FEG ethanolic extracts in the ratio of 1:1:1) used as a topical agent is efficacious for alleviating tinea pedis (Table 3). However, when compared with existing western medications, such as terbinafine (Lamisil) cream, it was much less effective, probably because the herbal extracts contain macromolecules that the presumed antifungal activity cannot penetrate effectively into the epidermis and thus their dermatophyte-killing actions remain superficial. To increase the efficacy of the herbal formula, the therapeutic concentration/dosage form/ratio of the three extracts needs refinement, or nano-technology could be applied to enhance transdermal absorption. The use of simple chemicals could also enhance the transcutaneous absorption and efficacy. At this point, the herbal formula was not powerful enough to replace terbinafine for treating tinea pedis. Nonetheless, it may be considered as an alternative/supplementary medicine for terbinafine-resistant cases.

Besides efficacy, safety issues should also be considered when developing the herbal formula. Liver injury associated with the oral intake of FP has been reported. Skin toxicity of these three herbs is rarely reported. Therefore, hypersensitivity and allergic responses following topical application of the herbal formula need evaluation. Our laboratory has done sensitivity tests for topical agents used for wound healing. The same platform may serve this purpose.

Acknowledgement

This study was supported by the Research Fund for the Control of Infectious Diseases, Food and Health Bureau, Hong Kong SAR Government (#05050212).

References

1. Cheng S, Chong L. A prospective epidemiological study on tinea pedis and onychomycosis in Hong Kong. Chin Med J (Engl) 2002;115:860-5.
2. Lau KM, Fu LH, Cheng L, et al. Two antifungal components isolated from Fructus Psoraleae and Folium Eucalypti Globuli by bioassay-guided purification. Am J Chin Med 2010;38:1005-14.
3. Rajendra Prasad N, Anandi C, Balasubramanian S, Pugalendi KV. Antidermatophytic activity of extracts from Psoralea corylifolia (Fabaceae) correlated with the presence of a flavonoid compound. J Ethnopharmacol 2004;91:21-4.
4. Apisariyakul A, Vanittanakom N, Buddhasukh D. Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). J Ethnopharmacol 1995;49:163-9.
5. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from Eucalyptus maculata. Lett Appl Microbiol 2004;39:60-4.