



Preliminary anti-onychomycosis efficacy study of cream from selected medicinal plant

Giwanon R^{1*}, Soradech S¹, Limsiriwong P¹, Ruengsri S¹, Peungsumrong S¹, Panapong K¹, and Thubtimthed S¹

¹ Pharmaceutical and Natural Product Department (PNPD), Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani 12120, Thailand

Keywords: onychomycosis, *Piper betle* L., *T. mentagrophytes*, *T. rubrum*

Objectives: Our study aims to investigate anti-dermatophyte activities against 3 dermatogens relevant to onychomycosis (*T. mentagrophytes* DMST 19735, *T. mentagrophytes* ATCC 9533, and *T. rubrum* ATCC 10218) of 8 herbal extracts (*P. betle* L.(A), *A. nervosa* (Burm.f.) Bojer (B), *P. granatum* L. (C), *P. sarmentosum* Roxb.(D), *R. nasutus* (L.) Kurz (E), *P. obtusa* L.(F), *M. elliptica* Ridl. (G), and *C. adansonii* DC. subsp. *trifoliata* (Roxb.) Jacobs (H)), to compare anti-dermatogen potency of the selected one with 5 antifungal agents (econazole, miconazole, ketoconazole, metronidazole, and voriconazole) against the tested strains, to determine MIC of the selected one, and to evaluate anti-onychomycosis potency of cream from selected plant against the 3 dermatogens.

Methods: The anti-dermatogen activities of the 8 extracts against the tested strains were evaluated by disk diffusion method. Plant extracts that possessed strong antifungal effect would be selected to compare its potency with 5 antifungal agents by the same protocol. The MIC of the selected one was also determined by agar dilution method. Cream containing the selected plant extract was formulated and evaluated antifungal efficacy by using hole diffusion method.

Results: Among the 8 extracts, A possessed the significantly strongest antifungal action with its inhibition zones ranging from 35.77-73.33 mm. It exhibited the significantly higher anti-dermatophytic activity than 5 antifungal agents did. The best MIC value of A was 100 µg/ml. Formulated cream containing 0.1 % (w/w) of A had antifungal potency against all tested strains with its inhibition zone ranging from 22.08 - 23.86 mm., respectively.

Conclusion: These findings indicate the possibility to use the formulated cream from *P. betle* Linn. extract as antimycotic agent for onychomycosis treatment.

* Corresponding author: Pharmaceutical and Natural Product Department (PNPD), Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani 12120, Thailand ; Tel. +66(025779123) ; Fax. +66(025779124)

E-mail address: rattanasiri@tistr.or.th

Introduction

Onychomycosis is superficial fungal infection of the nail. An incidence of this disorder is found more than 40 % in elderly. The causative fungal were anthropophilic dermatophytes, in particularly by *Trichophyton mentagrophytes* and *T. rubrum*.¹ Nowadays, medicinal plant extracts are widely used to substitute instead of using antifungal agents for onychomycosis treatment owing to their enhancing resistance and side effects. The finding out anti-dermatogen efficacies against dermatophytes from medicinal plants to use as an anti-nail infection product has been accomplished. Thus, the aims of our study were (1) to investigate anti-dermatophyte activities against three dermatomycoses (*Trichophyton mentagrophytes* DMST 19735 (TM1), *T. mentagrophytes* ATCC (American Type Culture Collection) 9533 (TM2), and *T. rubrum* ATCC 10218 (TR)) of eight herbal extracts (*Piper betle* L.(A), *Argyrea nervosa* (Burm.f.) Bojer (B), *Punica granatum* Linn. (C), *Piper sarmentosum* Roxb.(D), *Rhinacanthus nasutus* (L.) Kurz (E), *Plumeria obtusa* L.(F), *Morinda elliptica* Ridl. (G), and *Crateva adansonii* DC. subsp. *trifoliata* (Roxb.) Jacobs (H)), (2) to compare anti-dermatogen potency of the selected one with five antifungal drugs against the tested strains, (3) to determine the Minimal Inhibitory Concentration (MIC) of the selected one, and (4) to evaluate anti-onychomycosis potency of cream from selected plant against the three dermatogens.

Methods

Preparation and isolation of extract:

The A to H were extracted with 95% ethanol three times for 48 hr and was evaporated. The yield of the extracts (A to H) (w/w) are shown in Table 1.

Antifungal method:

The anti-dermal pathogen activities of the eight extracts against three fungal strains relevant to onychomycosis (TM1, TM2, and TR) were evaluated by disk diffusion method². Plant extracts that possessed strong antifungal effect would be selected to compare its potency with five antifungal agents by the same protocol. The MIC of the selected one was also determined by agar dilution method³. The anti-dermatogen of cream containing the selected plant extract as an

active ingredient was formulated and evaluated against the tested dermal pathogens by using hole diffusion method². All experiments were carried out in triplicate. One way's ANOVA was used for comparison among groups. Statistical significance was defined as $p < 0.05$.

Disk diffusion testing:

According to modified Clinical and Laboratory Standards Institute (CLSI) standards 2008 guidelines (CLSI Document M2-A8,2006)², the antimycotic activities of the eight extracts against three tested fungal strains were performed by disk diffusion method² (16 mg/disc) using Sabouraud Dextrose Agar (SDA). TM 1 was provided from DMST Culture Collection (National Institute of Health; NIH) and the others (TM 2 and TR) were obtained from ATCC Culture Collection. The result was evaluated by measuring diameter of the inhibition zones of fungal growth. Plant extracts that possessed strong antifungal effect would be selected to compare its potency with five antifungal drugs (econazole: EZ (10 µg/disc), miconazole: MCZ (10 µg/disc), ketoconazole: KCA (15 µg/disc), metronidazole: MTZ (50 µg/disc), and voriconazole: VCZ (1 µg/disc)) against the three dermatogens by the same protocol.

Agar dilution testing:

According to modified CLSI standards 2006 guidelines (CLSI Document M7-A7,2006)³, MIC of the selected plant extract was determined by agar dilution method³ using SDA.

Hole diffusion testing:

According to modified CLSI standards 2008 guidelines (CLSI Document M2-A8,2006)², the diameters of inhibition zones (mm) of cream against the tested strains were evaluated by hole diffusion method (0.30 g/hole) using SDA. The antifungal potency of cream result was evaluated by measuring diameter of the inhibition zones of fungal growth.

Results

Among the extracts from eight selected medicinal plants, the antimycotic effects against all tested fungal strains revealed that A possessed the significantly strongest antifungal action with its inhibition zones ranging from 35.77-73.33 mm. The antifungal efficacy of D, G, C and E demonstrated moderate activities with their inhibition zones ranging from 9.51-40.75 mm., respectively. Weak antimycotic actions against the tested strains were found in B and H. They could inhibit only TR with their inhibition zone were 7.86 and 7.43 mm., respectively figure 1. Therefore, A was selected for the further study. The comparison of antifungal efficacy of A with five antifungal agents (EZ, MCZ, KCA, MTZ, and VCZ) revealed that the A possessed the significantly higher anti-dermatophytic activity than five antifungal agents did. The four antifungal agents (EZ, MCZ, KTZ, and VCZ) showed moderate activities with their inhibition zone ranging from 22.78-61.52 mm. There was no inhibitory effect of MTZ against the three tested strains (figure 2). The best MIC value of A against the three dermatoses caused nail infection was 100 µg/ml. The cream using 0.1 % (w/w) of A as active ingredient was formulated. Formulated cream from A exhibited antifungal potency against the three dermatogens relevant to onychomycosis. Its inhibition zone against TM1, TM2 and TR was 24.41 ± 0.05 , 23.86 ± 0.60 , and 22.08 ± 0.79 mm., respectively.

Table 1 Part of use and yield of plant extracts

Medicinal plants	Part of use	Yield of extracts (%) (w/w)
A	Leave	18.49
B	Leave	9.10
C	Peel	8.41
D	Leave	4.92
E	Leave	5.36
F	Leave	3.64
G	Root	21.31
H	Leave	8.07

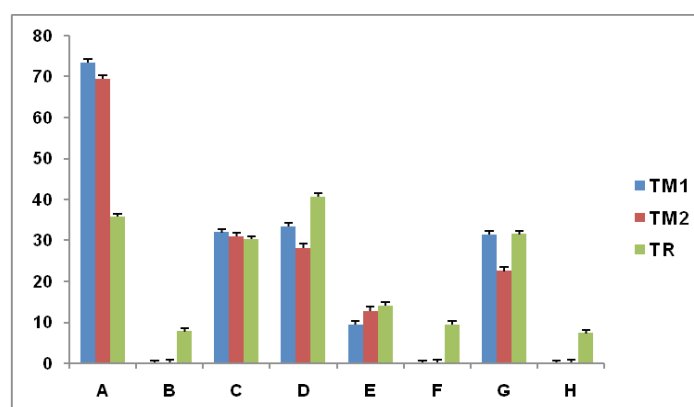


Figure 1 The comparison of inhibition zones of 8 medicinal plant extracts (A-H) against the 3 dermatogens relevant to onychomycosis using disk diffusion method (16 mg/disc) * $p < 0.05$ level

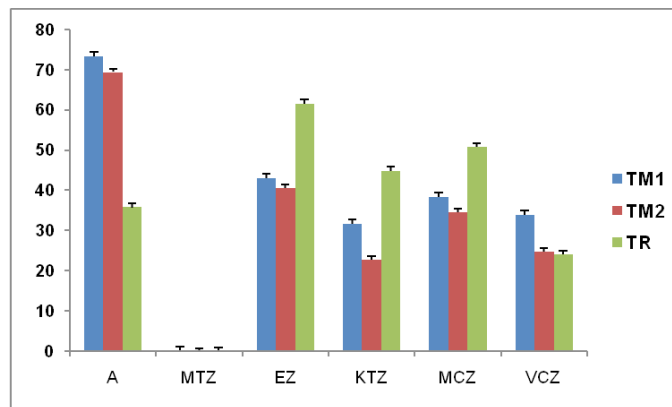


Figure 2 The comparison of inhibition zones of A (16 mg/disc) with 5 antifungal agents (EZ, MCZ, KCA, MTZ, and VCZ) against the 3 dermatoses using disk diffusion method * $p < 0.05$ level

Discussion

Among the tested extracts, extract from leave of *P.betle* L. or beetle possessed the significantly strongest antifungal effect against all the three fungal strains associated with nail infection. Previous studies showed beetle leaf extract having a wide variety of bioactive compounds especially phenolic compounds which play important roles in antifungal effect against dermatophytes⁴. The comparison of antifungal effect of the beetle leaf extract with five antifungal agents against the three dermatogens displayed that the extract was able to express antifungal activity against onychomycosis caused pathogens much more than five antifungal drugs did. Preliminary study of antifungal potency of cream from 0.1 % (w/w) of beetle leaf extract against the dermatoses was performed. The result showed the cream had antifungal efficacy against all the tested fungal strains. Thus, *P.betle* L. may potentially to be an alternative anti-nail infection agent. Nowadays, current topical treatments for fungal nail infection have limited effectiveness because of minimal antifungal agents permeability through the nail plate limitation⁵. For this reason, our further studies would be focus on formula improvement from beetle leaf extract with greater efficacy and more enhance adsorption. Moreover, identification of its active constituents, study on anti-inflammatory potency and on toxicological effect of the formula would be of interest and await further investigation.

Conclusion

These findings indicate the possibility to use the formulated cream from *P.betle* L. extract as antimycotic agent for onychomycosis treatment caused by species of the genera *Trichophyton*.

Acknowledgements

We are grateful to Thailand Institute of Scientific and Technological Research (TISTR) for financial supporting.

References

1. Piraccini BM, Alessandrini A. Onychomycosis: A Review. *J. Fungi*. 2015;1:30-43.
2. Clinical and Laboratory Standards Institute (CLSI). 9th ed. Vol.26(1). M2-A8: Performance standards for antimicrobial disk susceptibility tests: Approved Standard. Wayne: CLSI; Jan2006. 37 p.
3. Clinical and Laboratory Standards Institute (CLSI). 7th ed. Vol.26(2). M7-A7: Performance standards for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard. Wayne: CLSI; Jan2006. 49 p.
4. Bhalerao S, Verma DR, Gavankar RV, Teli NC, Rane YY, Didwana VS, Trikanad A. Phytochemistry, Pharmacological profile and therapeutic uses of *Piper betle* Linn.- an overview. *RRJPP*. 2013;1:10-19.
5. Kumar KJR, Muralidharan S, Dhanraj SA. Anti-fungal activity of microemulsion based fluconazole gel for onychomycosis against *Aspergillus Niger*. *Int.J. Pharm.Pharm.Sci*. 2012;5(1):96-102.