

# ANTIFUNGAL ACTIVITY AND LOCAL TOXICITY STUDY OF *ALANGIUM SALVIIFOLIUM* SUBSP *HEXAPETALUM*

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**Abstract.** *Alangium salviifolium* subsp *hexapetalum* is a medicinal plant which has been traditionally used for tonic and treatment of hemorrhoid. This plant showed promising antimicrobial activity in our preliminary experiments, this study was, therefore, conducted to investigate its inhibitory effect against dermatomycotic organisms and its toxicity. The lyophilized powder extract (4.59%) of pulverized wood was tested for its inhibitory effect by agar disc diffusion test. The extract gave inhibitory zone diameters of 25.23 and 14.78 mm against 26 and 14 isolates of dermatophytes and *Candida albicans*, respectively. Ketoconazole, used as a reference antifungal agent, had inhibitory zone diameters of 33.15 and 27.93 mm against dermatophytes and *C. albicans*, respectively. There was no significant difference between the extract and ketoconazole in their inhibition against dermatophytes ( $p > 0.01$ ), but their difference was significant against *C. albicans* ( $p < 0.01$ ). Using Buehler's method, different amounts of extract (3, 6, and 9 mg/inch<sup>2</sup> gauze pad) were tested in five male New Zealand white rabbits. All tested amounts of extract did not induce dermatitis among those rabbits within 1 week. The results demonstrated the inhibitory effect of *Alangium salviifolium* subsp *hexapetalum* against fungi without any local toxicity; a tendency to further develop a herbal preparation for the treatment of some dermatomycotic infections.

## INTRODUCTION

Plant extracts were widely screened for its medicinal usage. The increasing incidence of infections by fungi in systemic, central nervous system or dermal infections, emphasizes the search for new antifungal substances. Healthcare properties of *Alangium salviifolium* subsp *hexapetalum* were reported in many Asian countries: India, China, Philippines as well as Thailand. *A. salviifolium* subsp *hexapetalum* traditionally called in Thai as Proo, is a shrub or a small tree found in forests, uplands and plains. Its white flowers frequently bloom in March. In old Thai texts, bark of Proo was used to treat diarrhea and as an expectorant in patients with asthma. Its fruits have anthelmintic and carminative properties while its wood was used as tonic and treatment of hemorrhoid. This plant is used externally in Ayurvedic soap: Tulsi-Neem commercialized by an USA company. In India, a study in mice indicated that anti-fertility activities were found in methanol aqueous, and ethyl acetate extracts of stem bark (Murugan *et al*, 2000). The present investigation demonstrates inhibitory effects of an aqueous lyophilized extract of *A. salviifolium*

subsp *hexapetalum* against dermatomycotic organisms and its local toxicity using Buehler method (1995).

## MATERIALS AND METHODS

### Plant material

Commercial *A. salviifolium* subsp *hexapetalum* was purchased from a local herbal supplier.

The ground wood of *A. salviifolium* subsp *hexapetalum* was macerated (1/4) in sterile distilled water. After 24 hours, the filtrate was lyophilized and kept in tightly closed container. The maceration of *A. salviifolium* subsp *hexapetalum* yielded 4.59% yellowish brown sticky powder.

### Tested organisms

Dermatophytes and *Candida albicans* were obtained from the Department of Microbiology, Faculty of Pharmacy, Mahidol University. Actively growing dermatophytes and *C. albicans* were suspended in sterile distilled water and the density was adjusted to be equivalent to McFarland No. 1.

### Disc

The extract was dissolved in distilled water, ketoconazole was diluted in a mixture of ethanol and water (1:3). The test material was dropped onto sterile blank discs (6 mm in diameter) (Schleicher & Schuell, Germany) at the amount of 500 µg/disc and 20 µg/disc for ketoconazole.

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**Reference antifungal**

Ketoconazole was kindly provided by Unison Co Ltd; Bangkok, Thailand.

**Agar disc diffusion test**

Twenty microliters of dermatophyte inoculum was added into 5 ml of melted Sabouraud Dextrose Agar (SDA) (Hispalab, SA, Spain) before overlaying on 10 ml-SDA plate and an inoculum of *C. albicans* was swabbed on the surface after the solidification of the agar.

Discs of test material and the reference drug were placed onto the surface of inoculated plates. Plates were incubated at room temperature for 3-5 days and 37 °C for 24-48 hours for dermatophytes and *C.albicans* respectively.

**Contact dermatitis induction**

The extract was diluted with DMSO and sterile distilled water (1 : 1); 0.5 ml (3, 6 and 9 mg extract each) was absorbed on 1 X 1 inch gauze pad. These gauze pads and the control pads (DMSO: sterile distilled water and normal saline solution) were tightly

overlaid on a lateral shaved area of 5 male New Zealand white rabbits, weighted 2 kg each. After 24, 48, 72 hours and one week after the contact, responses of redness, wheal or inflammation were observed and graded as 0-4 as the degree of dermatitis: +, slightly irritation: redness; ++, moderately irritation : redness, wheal; + + +, irritation presence : redness and wheal.

**RESULTS**

Inhibitory-zone diameters were measured using a ruler and the zone was regarded as antifungal activity of the tested materials against 26 dermatophytes and 14 *C. albicans*, as shown in Table 1.

Dermatitis induction after 24, 48, 72 hours. and 1 week of contact was observed. There was no signs of dermatitis occurred at the site of contact in all rabbits (Table 2).

**DISCUSSION**

The extract of *A. salviifolium* subsp *hexapetatum*

Table 1  
Average diameters of *A. salviifolium* subsp *hexapetalum* extract's activities against tested dermatophytes.

Organism	Number of isolates	Diameters (mm)	
		<i>A. salviifolium</i> extract (500 µg)	ketoconazole (20 µg)
Dermatophytes	26	25.23	33.15
<i>C.albicans</i>	14	14.78	27.93

Table 2  
Contact dermatitis induction of extract at observed interval on 5 rabbits.

Duration of contact (hour)	Degree of response in 5 rabbits				
	Extract /test site (mg)			Control	
	3	6	9	DMSO:H <sub>2</sub> O	NSS
24	-	-	-	-	-
48	-	-	-	-	-
72	-	-	-	-	-
1 week	-	-	-	-	-

-, no irritation

yielded 4.59 % in the form of freeze-dried powder. A previous study by Murugan *et al* (2000) reported a yield of 4.20% of aqueous extract by Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, methanol and water as solvents and revealed that alkaloids, steroids, saponin and flavonoids were ingredients present in stem bark of *A. salviifolium* subsp *hexapetalum*. Methanolic extract contained tannins and phenolic compounds.

The inhibitory effect of lyophilized aqueous extract of *A. salviifolium* subsp *hexapetalum* on dermatophytes was not significantly different from that of the reference drug, ketoconazole, while the activity against *C.albicans* differed. In addition, lyophilized extract, up to 9 mg per test site, demonstrated no induction of dermal irritability in rabbits. However, the exact ingredient responsible for dermatitis was not investigated. It was noted that, lyophilized powder was obtained by maceration of ground stem bark, without an exposure to any heat. The freeze-dry procedure did not alter the ingredient's configuration. In the experiment of Murugan *et al* (2000), successively aqueous extract by Soxhlet apparatus with polarity order of solvents, gave slight score of 2 on rats. Therefore, the source of different local wood, the

amount of the extract, animal model used and its age, sex and weight, as well as procedures used to extract the ingredients might be responsible for the different outcome in dermal dermatitis.

From our results, *A. salviifolium* subsp *hexapetalum* showed no toxicity, its active components can be further developed into naturally based cosmetic, externally used products or even herbal drug for treatment of dermatomycotic infections.

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