

Antifungal Activities of *Senna alata* Extracts Using Different Methods of Extraction

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Abstract

Many Thai medicinal plants have been used for the treatment of dermatomycotic infections, as recorded in Thai traditional texts. Some have been selected and recommended for the primary health care system in Thailand. *Senna alata* (L.) Roxb. has been used for a long time for the treatment of tinea versicolor and ringworm infections. Extracts were prepared in order to study the active ingredients and find scientific evidence for the herbal activities. Conventional methods using various solvents and soxhlet extraction yielded fractions with different properties. Lyophilization of the aqueous extract might limit the ingredients obtained. However, both methods differed in their yields: in the obtained percentage, appearance, properties and time and cost consumed. In addition, sonication was an alternative to acquire the active ingredients from the plants. With those different manipulations, it was of interest to demonstrate the different antifungal activities of *S. alata*.

In this investigation, *S. alata* leaves were extracted by three different methods. Using a soxhlet apparatus, an 80 % ethanol extract (26.4 %) (A) was obtained. The extract was treated with HCl, which after further purification gave 7.3 % of crude anthraquinones (B). Lyophilization of water macerates yielded 10.1 % (C). In the third method, a 100-mg amount of pulverised leaves was sonicated with either 95 % ethanol (D) or water (E). After filtration, the residue was extracted for another two times in the same way. The filtrate was volume adjusted to 25 ml.

All the extracts were investigated for their antifungal activities. On the basis of inhibitory zone, activities against dermatophytes and *Candida albicans* 36 and 26 clinical isolates, respectively, were established by an agar diffusion method. The extracts A, B and C (20 mg, each), D and E (80 µg, each) inhibited the dermatophytes by 13.8, 9.9, 21.9, 8.2 and 7.5 mm and *C. albicans* by 18.8, 10.7, 14.1, 10.1 and 7.2 mm, respectively.

From TLC, the crude ethanol and ethanol sonicated extracts (A and D) of *S. alata* were shown to contain rhein (anthraquinone aglycone), while the lyophilized water extract (C), contained some polar compounds, which might be anthraquinone glycosides.

INTRODUCTION

Senna alata is a pantropical shrub, cultivated throughout Thailand. The leaves contain anthraquinones such as emodin, aloe-emodin, and rhein¹ and possess laxative activity². In Thai Traditional Medicine, *S. alata* leaves are used for the treatment of tinea versicolor and ringworm infections by crushing fresh leaves with or without alcohol. The Thai Ministry of Public Health recommends the plant for the use in the primary health care system for skin disease treatment³.

This study was performed in order to compare different methods used to extract

the required ingredients from *S. alata*. Leaves were extracted by continuous extraction, maceration and lyophilization, and sonication using ethanol or water as solvents. Crude anthraquinones were further purified from the ethanolic extract. The antifungal activities of each extract were compared by means of inhibitory efficacy using an agar diffusion method against clinical fungal isolates.

MATERIALS AND METHODS

Plant Materials

S. alata leaves were purchased from a local herbal supplier and authenticated by comparison with herbarium specimens at the department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

Extraction

1. Continuous Extraction Using a Soxhlet Apparatus Dry powdered leaves were extracted using a Soxhlet apparatus and 90 % ethanol as solvent. After evaporation, an ethanolic extract (A) was collected.

2. Extraction of Crude Anthraquinones The extract A was hydrolyzed with 5% hydrochloric acid and extracted with chloroform. After filtration, the volume was reduced using a rotary vacuum evaporator; a crude anthraquinones extract (B) was obtained.

3. Maceration and Lyophilization Dry leaves were pulverized and macerated in sterile distilled water for 24 hours. After filtration, the filtrate was lyophilized. The lyophilized extract (C) was kept in a tightly closed container.

4. Sonication A 100-mg amount of pulverized leaves was sonicated with either 95 % ethanol (D) or water (E). After filtration, the residue was extracted another two times in the same way. The filtrate was volume adjusted to 25 ml.

Organisms

Dermatophytes and *Candida albicans*, 36 and 26 strains respectively, were obtained from the Department of Microbiology, Faculty of Pharmacy, Mahidol University. The organisms were isolated on Sabouraud dextrose agar plates (SDA, Hispanlab, S.A.) and kept as pure culture on SDA slants.

Inoculum

Actively growing cultures were added with sterile distilled water and the density of the suspensions was made equivalent to McFarland number 1.

Antifungal Activity Test

An inoculum suspension (20 μ l) of dermatophytes was mixed in 5 ml melted SDA and overlaid onto the surface of 10 ml SDA plate while an equal amount of *Candida* inoculum was swabbed. The inoculated plates were punched with a cork borer to make 6-mm diameter wells. 20 ml of test materials and solvent control were added to the wells. The dermatophyte plates were kept at room temperature and the *Candida* ones at 37 °C.

Thin-Layer Chromatography

For TLC determination of rhein in the extracts, silica gel 60 F₂₅₄ layers were used with a mixture of 90% chloroform and 10% methanol as the mobile phase. UV detection was effected at 254 and 366 nm. The detection reagent used was 5% potassium hydroxide.

RESULTS

Extracts with different percentage yields and appearances were obtained, as shown in table 1, by using different types of solvents and extraction methods.

Antifungal Activities

Activities from the ethanolic extracts against *Candida albicans* gave wider zone diameters than those of dermatophytes, while the reverse was the case with the lyophilized extract (Table 2).

TLC Chromatogram

From TLC, the crude ethanolic (A) and ethanolic sonicated extracts (D) of *S. alata* contained rhein (anthraquinone aglycone), and the lyophilized water extract (C) contained some polar compounds, which might be anthraquinone glycosides.

DISCUSSION

On the basis of inhibitory zone diameter, *S. alata* extracts, using different methods, showed varied antifungal activities. The ethanolic extract (A) exhibited higher antifungal activity than that of the aqueous lyophilized extract (C) against *C. albicans*, whereas, dermatophytes were less susceptible to the extract A. It was noticeable that the crude ethanolic extract (A) was superior to the crude anthraquinones (B) for inhibiting both dermatophytes and *C. albicans*. It should be pointed out that some compounds in the ethanolic extract other than anthraquinones also possessed antifungal activities.

Owing to the amount of raw material used, the sonicated extracts showed smaller zones against test fungi. Considering the lesser time consumed, this method could be used for pilot screening of antimicrobial activities of plant material. However, with the measurable zones from that amount of sonicated material, it will be interesting to develop or modify this method further in order to reflect genuine activity.

CONCLUSION

The higher inhibitory effect of *S. alata* aqueous extract against filamentous fungi showed the promising tendency to develop an extract of this plant into an herbal preparation. The ethanolic extract, on the contrary, inhibited unicellular fungi to a greater extent. Both results scientifically support the use of this plant in traditional medicine.

Literature Cited

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Tables

Table 1. Percentage yields and appearances of the extracts

Extract	Extraction method	Solvent	Percent.	Appearance
A	Continuous extraction	Ethanol	26.4	greenish black, sticky
B	Extraction of crude anthraquinones		7.3*	black, sticky
C	Maceration and Lyophilization	Water	10.1	dry powder
D	Sonication	Ethanol	-	Solution
E	Sonication	Water	-	Solution

* Calculated from the ethanolic extract A.

Table 2. Average inhibitory diameters of extracts against test fungi

Extracts	Amount per well	Diameters (mm)	
		Dermatophyts 36 strains	<i>Candida albicans</i> 26 strains
A	20 mg	13.8	18.8
B	20 mg	9.9	10.7
C	20 mg	21.9	14.2
D	80 mcg	8.2	10.1
E	80 mcg	7.5	7.2