Antifungal effect of *Senna tora* (L.) Roxb. seed extract against *Microsporum gypseum*

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Abstract

Objective: This study tested the antifungal activity of *Senna tora* (L.) Roxb. (*S. tora*) seed extract against dermatophytes. Seed powders of *S tora* were extracted by sonication method with 80%ethanol and dried to obtain crude extract. Thin layer chromatography (TLC) was used to identify anthraquinone constituents of crude ethanolic extract. *In vitro* antifungal effect of the extract was determined against the microorganisms which cause dermatophytic disease in both human and animals including *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis* and *Microsporum gypseum*. The ethanol extract of *S. tora* seed was tested for antifungal effect by broth microdilution method. The antifungal drug, ketoconazole was used as positive control. The result showed the antifungal activity of crude ethanolic extract and the anthraquinone constituent. The minimal inhibition concentration (MIC) of crude ethanolic extract and physcion on *Microsporum gypseum* was 1000 and 200 µg/ml, respectively. The ethanolic extract of *S. tora* seed was found anthraquinones constituents such as chrysophanol and physcion and showed inhibition on *Microsporum gypseum*. This data could be used to develop an antifungal product from *S. tora* extract.

***Keywords*:** *Senna tora, anthraquinones, antifungal, Microsporum gypseum*

บทคัดย่อ

วัตถุประสงค์ เพื่อทดสอบฤทธิ์ยับยั้งเชื้อราของสารสกัดจากเมล็ดชุมเห็ดไทย (*Senna tora* (L.) Roxb.) สกัดสารสำคัญจากผงแห้งของเมล็ดชุมเห็ดไทยด้วยวิธีโซนิเคชั่นโดยใช้สารละลายเอทานอลความเข้มข้นร้อยละ 80 เป็นตัวทำละลายและทำให้แห้งได้สารสกัดหยาบ ตรวจสอบสารแอนทราควิโนนที่เป็นส่วนประกอบในสารสกัดหยาบด้วยวิธีทินเลเยอร์โครมาโตกราฟี การทดสอบฤทธิ์ต้านเชื้อราของสารสกัด ทดสอบกับเชื้อจุลินทรีย์ก่อโรคในมนุษย์และสัตว์ ได้แก่ เชื้อ *Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis* และ *Microsporum gypseum* โดยวิธี broth microdilution method โดยใช้ยาต้านเชื้อราคีโตโคนาโซลเป็นตัวควบคุมเชิงบวก พบว่าสารสกัดเอทานอลจากเมล็ดชุมเห็ดไทยและ physcion (อนุพันธ์แอนทราควิโนนที่พบในสารสกัดเมล็ดชุมเห็ดไทย) สามารถยับยั้งการเจริญของเชื้อรา *Microsporum gypseum* โดยแสดงค่าความเข้มข้นน้อยสุดที่สามารถยับยั้งเชื้อรา (MIC) เท่ากับ 1,000 และ 200 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ ในสารสกัดเอทานอลของเมล็ดชุมเห็ดไทยมีสารแอนทราควิโนนเป็นส่วนประกอบ เช่น chrysophanol และ physcion สารสกัดดังกล่าวมีฤทธิ์ยับยั้งการเจริญของเชื้อรา *Microsporum gypseum* จากข้อมูลนี้สามารถนำไปพัฒนาผลิตภัณฑ์ต้านเชื้อราจากสารสกัดเมล็ดชุมเห็ดไทย

คำสำคัญ: *ชุมเห็ดไทย แอนทราควิโนน ฤทธิ์ยับยั้งเชื้อรา เชื้อรา Microsporum gypseum*

1. Introduction

Senna tora (L.) Roxb. (Chumhet Thai) is one of the medicinal plants in the Fabaceae and included in Thai Herbal Pharmacopeia (Thai Pharmacopoeia Committee, 2009), Chinese Pharmacopeia (Pharmacopoeia Commission China, 2000) and Indian Medicinal Plants (Khare, 2008). The seed and leave of *S. tora* were used as traditional medicine (Khare, 2008; Pawar & D'mello, 2011; Sarwa, Rudrapal, Debnath, Kumar, & Verma, 2014). The traditional uses of *S. tora* were vision disease, liver disease, intestine disease, and skin disease such as leprosy and ringworm (Jain & Patil, 2010; Zhu, Yu, Zeng, Fu, & Zhao, 2008). The anthraquinone compounds were reported the antifungal activity against *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum*, *Geoetrichum candidum*, *Epidermophyton floccosum*, *Candida albican*, *Cryptococcus neoformans*, and *Sporotrichum schenckii* (Acharya & Chatterjee, 1974; Agarwal, Singh, Verma, & Kumar, 2000; Sakunpak, Sirikatitham, & Panichayupakaranant, 2009; Wuthi-Udomlert, Kupittayanant, & Gritsanapan, 2010). The seeds of *S. tora* composed of anthraquinone constituents including rhein, emodin, physcion and chrysophanol. (Khare,

2008; Thai Pharmacopoeia Committee, 2009; Zhu, Yu, Zeng, Fu, & Zhao, 2008) and the structures of these compounds were shown in Figure 1.

The aim of the present study to analyze the ethanolic extract of *S. tora* seed and to investigate the MIC value of crude ethanolic extract against dermatophytes including *T. rubrum*, *T. mentagrophytes*, *M. canis*, and *M. gypseum*. The crude extract was prepared by sonication method; it is an easy and quick extraction. The antifungal activity was tested by broth microdilution method and plate method for determining the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the sample, respectively.

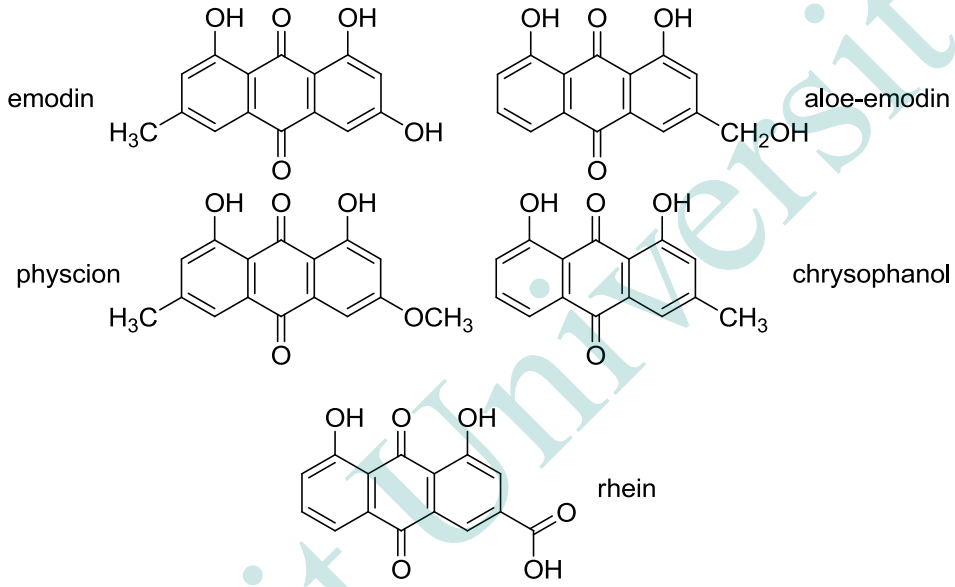

Figure 1 Structure of anthraquinone constituents of *S. tora* (L.) Roxb.

2. Objective

This study tested the antifungal activity of *S. tora* (L.) Roxb. seed extract against *T. rubrum*, *T. mentagrophytes*, *M. canis* and *M. gypseum.*

3. Materials and methods

Materials

Plant sample and chemicals

S. tora seed was purchased from a herbal drugstore in Nakhon Pathom, Thailand. Ketoconazole was obtained from Himedia, India. Aloe-emodin and physcion were purchased from Chengdu Biopurify Phytochemicals, China. Chrysophanol, emodin, and rhein were purchased from Sigma-Aldrich Chem, USA. Sabouraud dextrose agar and broth were purchased from Difco™, USA. Dimethyl Sulfoxide (DMSO) and methanol were purchased from Carlo Erba Reagents, Spain. Commercial ethanol was purchased from Samchai Chemical, Thailand.

Methods

Plant extraction

Plant sample was cleaned and dried by hot air oven at 60°C for 12 hours. Then milled and sieved by sieve no. 60. The dried powder was stored in a tight container and protected from light. Ten grams of *S. tora* seed powders were extracted with 500 ml of 80% ethanol by sonication method for 60 minutes, for 5 times extracted. All extracts were filtered through filter paper (Whatman no.1) and pooled. Then the solvent was evaporated by using rotary evaporator and dried on a water bath.

Identification of anthraquinones by TLC analysis

The crude ethanolic extract was investigated for anthraquinone compounds by TLC and compared with reference standards of aloe-emodin, chrysophanol, emodin, physcion and rhein. TLC plate that coated with silica gel 60 F_{254} (Merck, Germany) was used as stationary phase and the developing system composed of hexane: ethyl acetate: acetic acid, in a ratio of 7.5: 2.5: 0.1 (v/v/v). The crude extract and standard solution were prepared by dissolved with methanol and spotted on TLC plate. The plate was dipped in TLC tank and developed for about 15 minutes. The developed TLC was observed at 254 and 366 nm and detected with 10% potassium hydroxide in methanol. The anthraquinone compounds showed pink to orange color spot after being sprayed with alkaline solution at Rf 0.78 for physcion and 0.83 for chrysophanol.

In vitro antifungal test

Microorganisms

Two clinical isolates of dermatophyte (*T. mentagrophytes* and *M. canis*) were obtained from Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand. *T. rubrum* and *M. gypseum* were supported from Microbiology unit, Department of Medical Science, Faculty of Science, Rangsit University, Thailand. Characteristics of dermatophytes are showed in Figure 2.

Preparation of the inoculum

All dermatophytes were subcultured on sabouraud dextrose agar (SDA) and incubated at room temperature for 21 days then identified by mycologist before an antifungal test. The characteristic of dermatophytes was showed in figure 2. The spore suspension was prepared with sterile 1%Tween80 in distilled water. Inoculum quantification was determined by counting in hematocytometer. The inoculum suspensions were adjusted, the number in ranged from 2 x 10^4 to 4 x 10^4 CFU/ml by diluting with sabouraud dextrose broth (SDB).

Determination of minimum inhibitory concentration and minimum fungicidal concentration

The crude extract, anthraquinone compounds (chrysophanol and physcion) and ketoconazole were prepared by dissolving with 20%DMSO and diluting to concentration ranging from 2,500 to 40,000µg/ml, 500 to 8,000µg/ml, 25 to 400µg/ml, respectively by two-fold serial dilution method. The antifungal activity of *S. tora* seed extract was tested for MIC using broth microdilution method. The method was performed in a sterile 96 well flat bottom microtiter plate. The 20 µl of each concentration of the crude extract, anthraquinone compounds, and ketoconazole was added to each well in triplicate. Thereafter 80 µl of SDB and 100 µl of inoculum suspensions were added to each well respectively. The solvent control, microbial growth control and media control were tested in all plates. Then all test plates were incubated at room temperature (25 – 30°C) for 3 – 5 days. The MIC was determined by visual inspection of the lowest concentration of sample that showed inhibition growth of the microorganism. The MFC was tested by plating method and considered as the lowest concentration of sample did not grow of hyphal network.

4. Results and Discussion

The crude ethanolic extract of *S. tora* seed was semisolid with dark brown color. The extraction yield was 18.3 %w/w of dried powder. The characteristic of the raw material of sample plant, powder, and crude extract was shown in Figure 3. The TLC fingerprint of crude extract and reference standards were shown in Figure 4. The anthraquinone compound showed pink to orange color spot after being sprayed with alkaline solution at R_f 0.78 for physcion and 0.83 for chrysophanol. The physcion and chrysophanol showed major anthraquinone constituent of ethanolic extract of *S. tora* seed. Thus, physcion and chrysophanol were also tested for antifungal effect and compared the efficacy with the crude extract. The antifungal activity of *S. tora* seed extract was determined by broth microdilution method and reported as MIC value as shown in Table 1. The ethanolic extract showed the highest potency against *M. gypseum* which was similar to the previous study of the methanolic extract of *S. tora* leave (Phongpaichit, Pujenjob, Rukachaisirikul, & Ongsakul, 2004). Our result showed that MIC value of *S. tora* seed extract against *M. gypseum* was a 100-fold lower than that of ketoconazole.

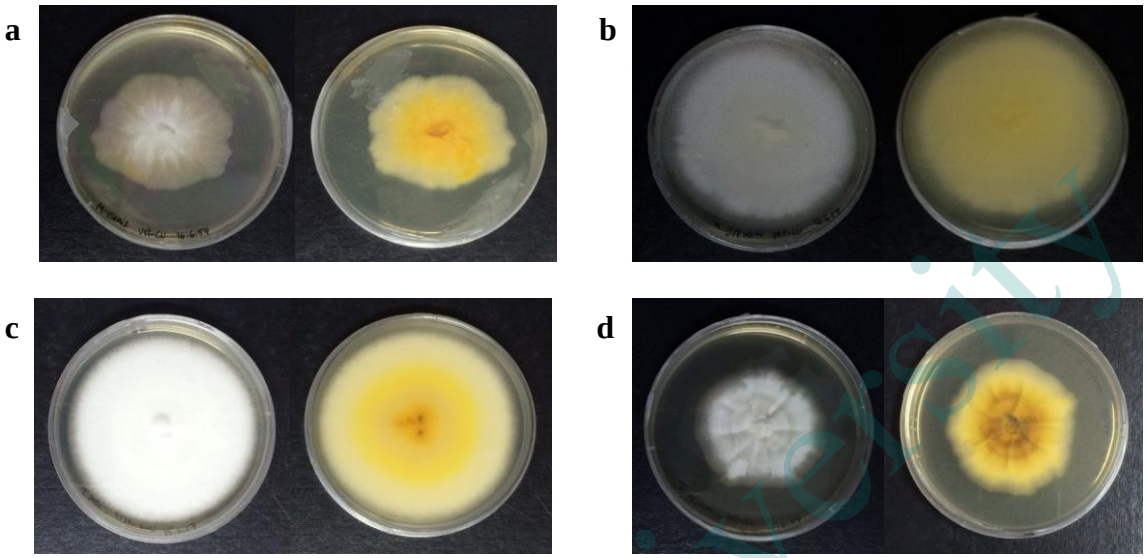

Figure 2 Characteristic of dermatophytes (a) *M. canis*, (b) *M. gypseum*, (c) *T. mentagrophytes* and (d) *T. rubum* at incubated for 14 days

Figure 3 Characteristic of *S. tora* seed (a) raw material, (b) powders and (c) crude extracts

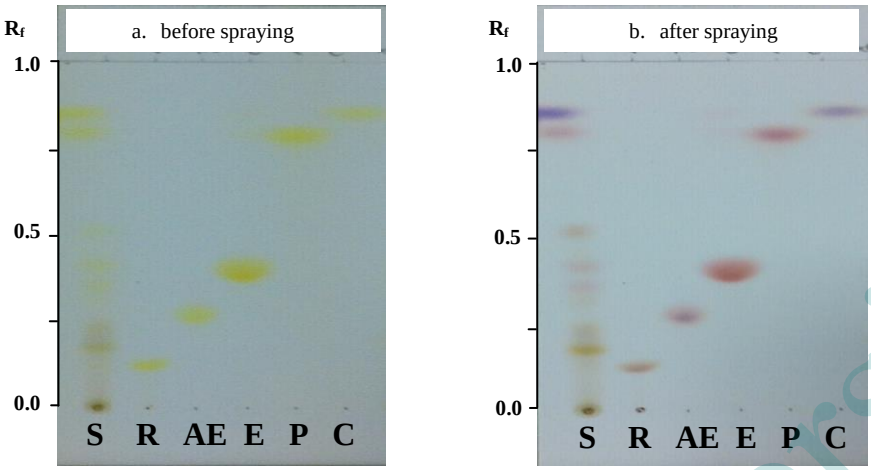

Figure 4 TLC fingerprints of *S. tora* seed (a) before being sprayed with alkaline solution, (b) after being spayed with alkaline solution; (S) extract, (R) rhein, (AE) aloe-emodin, (E) emodin, (P) physcion and (C) chrysophanol

In addition, the extract presented antifungal activity against *T. mentagrophytes* and *T. rubrum* with MIC values of 2,000 and 4000 µg/ml, respectively. The positive control antifungal drug, ketoconazole showed inhibitory effect on all selected microorganisms with MIC value range from 5-10 µg/ml. The MFC of the extract was tested with only the concentration showed an antifungal activity and the result of MFC was shown in Table 2. Furthermore, the anthraquinone compounds (physcion and chrysophanol) were tested for MIC by broth microdilution on *M. gypseum*. The result was showed in Table 3, physcion showed high potency antifungal but chrysophanol showed inactivity against *M. gypseum* at the highest tested concentration of 800 µg/ml which was similar to the previous study (Sakunpak, Sirikatitham, & Panichayupakaranant, 2009). Thus, the antifungal effect of crude extract may be resulted from physcion.

Table 1 MIC of *S. tora* seed extract and ketoconazole

Dermatophytes	Sources	Minimum inhibitory concentrations (MIC) (µg/ml)	
		Ethanol extract	Ketoconazole
M. canis	CU	>4,000	10
M. gypseum	RSU	1,000	10
T. mentagrophytes	CU	2,000	10
T. rubrum	RSU	4,000	5

Table 2 MFC of *S. tora* seed extract and ketoconazole

Dermatophytes	Sources	Minimum fungicidal concentrations (MFC) (µg/ml)	
		Ethanol extract	Ketoconazole
M. gypseum	RSU	4,000	>40
T. mentagrophytes	CU	4,000	40
T. rubrum	RSU	4,000	10

Table 3 MIC of *S. tora* seed extract and anthraquinone derivatives on *M. gypseum*

Antifungal test	Test sample			
	crude extract	physcion	chrysophanol	Ketoconazole
MIC (µg/ml)	1,000	200	NA	10

Comment; NA=Not active at concentration of 800 µg/ml

5. Conclusion

From TLC results, the ethanolic extract of *S. tora* seed showed the anthraquinone compounds mainly with physcion and chrysophanol. The ethanolic extract showed antifungal activity. Since physcion exhibited highly antifungal effect against *M.gypseum* and it was the main anthraquinone found in *S. tora* extract, it may be responsible for the antifungal effect in *S. tora* extract. This data could be used to develope an antifungal product from *S. tora* extract.

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