

Effects of Aqueous Extracts of Siamese Cassia's Root on the Death and the Tegumental Surface of the Trematode Using Scanning Electron Microscopy

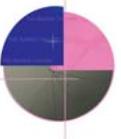
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ABSTRACT

In vitro effects of aqueous extracts of Siamese cassia's Root (*Cassia siamea* Britt.) were studied on tegumental surface of trematode (*Stellantchasmus falcatus*). Findings an appropriated concentration and time which effect on trematode were investigated by light microscope. The worms were examined the death with aqueous extract 6.25%, 12.5%, 25% and 50% in Tyrode' s solution at 1, 6, 12 and 24 hours respectively. Worms in Tyrode's solution were served as controls. The result showed that extract of 6.25%, 12.5% and 25% can kill all the worms within 6 hours, while 50% extract concentration can kill worms 92.5 % within 1 hour. The SEM (Scanning Electron Microscopy) observation of the death trematode focused mainly on the tegumental surface of the worm's body. It was found that the trematode in the extract of 6.25% solution showed loss of the scales at 6 hours and curving at the edge of scales at 12 hours. The trematode in the extract of 12.5% solution showed loss of the scales and curving at the edge of scales at 6 hours. Whereas the trematode in the extract of 25% and 50% solution had the same variations of the tegument as the trematode in the extract of 12.5% but more apparently scale loosing at 1 hour. It was also found that rupturing and the tegument tearing off from a tegumental surface were evident at 12 hours. This findings suggest that Siamese cassia's root is an effective anthelmintic drug.

Keywords : Siamese cassia's Root Anthelmintic plants Trematode SEM

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ผลของสารสกัดด้วยน้ำจากรากขี้เหล็กต่อการตายและการเปลี่ยนแปลงพื้นผิวพยาธิใบไม้ด้วยเทคโนโลยีคุณธรรมคุณนิลเล็กต่อนแบบสแกน

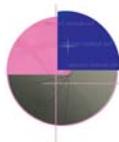
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บทคัดย่อ

การทดสอบในห้องปฏิบัติการหาความเข้มข้น และระยะเวลาการออกฤทธิ์ที่เหมาะสมของสารสกัดด้วยน้ำจากรากขี้เหล็ก (*Cassia siamea* Britt.) ต่อการเคลื่อนไหว และการตายของพยาธิในหลอดทดลองภายใต้กล้องจุลทรรศน์แบบใช้แสง และผลที่มีต่อการเปลี่ยนแปลงพื้นผิวของพยาธิใบไม้ *Stellantchasmus falcatus* ด้วยกล้องจุลทรรศน์อิเล็กตอรอนแบบสแกน ทำการทดลองโดยตรวจสอบการตายของพยาธิใบไม้ในสารสกัดด้วยน้ำจากรากขี้เหล็กความเข้มข้น 6.25%, 12.5%, 25% และ 50% โดยเปรียบเทียบกับกลุ่มควบคุมคือ Tyrode's solution ที่ระยะเวลา 1, 6, 12 และ 24 ชั่วโมง ตามลำดับพบว่าสารสกัดที่ความเข้มข้น 50% สามารถฆ่าพยาธิใบไม้ได้ 92.5% ภายในระยะเวลา 1 ชั่วโมง ส่วนสารสกัดที่ความเข้มข้น 6.25%, 12.5% และ 25% สามารถฆ่าพยาธิใบไม้ได้หมดภายในระยะเวลา 6 ชั่วโมง จากนั้นนำตัวอย่างของพยาธิใบไม้ที่ตายมาตรวจสอบสภาพพื้นผิวของลำตัวภายใต้กล้องจุลทรรศน์อิเล็กตอรอนแบบสแกน (SEM) พยาธิที่แข็งในสารสกัดที่ความเข้มข้น 6.25% พบร่องรอยหลุดออกที่ระยะเวลาตั้งแต่ 6 ชั่วโมง ที่ระยะเวลา 12 ชั่วโมง ตรงส่วนปลายของหัวมันผนังลำตัวมีลักษณะรุ่ม起 ลักษณะรุ่มของส่วนปลายหัวมันที่ระยะเวลา 6 ชั่วโมง พยาธิที่แข็งในสารสกัดที่ความเข้มข้น 12.5% หัวมันผนังลำตัวเริ่มหลุดออก และเกิดการรุ่มของบริเวณส่วนปลายหัวมันที่ระยะเวลา 6 ชั่วโมง พยาธิที่แข็งในสารสกัดที่ความเข้มข้น 25% และ 50% พบร่องรอยหลุดออกที่ตัวหัวมันที่ระยะเวลา 6 ชั่วโมง พยาธิที่แข็งในสารสกัดที่ความเข้มข้น 12.5% แต่มีความรุนแรงมากกว่า จนกระหั้นแรง เห็นส่วนหัวมันผนังลำตัวหัวมันหลุดออกที่ระยะเวลา 1 ชั่วโมง และเริ่มเกิดเป็นรอยแตก และหลุดลอกของพื้นผิวที่ระยะเวลาตั้งแต่ 12 ชั่วโมง ผลจากการศึกษานี้สรุปได้ว่ารากขี้เหล็กสามารถใช้เป็นยากำจัดหนอนพยาธิใบไม้ได้อย่างมีประสิทธิภาพ

คำสำคัญ: รากขี้เหล็ก สมุนไพรกำจัดพยาธิ พยาธิใบไม้ กล้องจุลทรรศน์อิเล็กตอรอนแบบสแกน

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Introduction

The minute intestinal fluke, *Stellantchasmus falcatus* is one of the heterophyid trematode distributed with high prevalence in Chiang Mai province, Thailand. The infective stage, metacercaria was found in half-beaked fish, *Dermogenus pusillus* (1). The infection of adult worms was reported in man, chick, mice, rat and cat (1-4). Praziquantel and niclosamide are normal anthelmintic drugs for man but side effects are apparent in some cases. Trends to use anthelmintic plants are investigated in Thailand (5). This research selected the anthelmintic plant, (*Cassia siamea* Britt.) to examine the tegumental surface changing of the death worms using scanning electron microscope, SEM (6). The bark of root was mixed for anthelmintic drug while the leafs for diuretic drug, and the flowers and fruits were used for laxative (7). This investigation serves to compare the pattern of damaging on the surface with the previously heterophyid trematode, *Haplorchis taichui* (8,9) and the results would be useful for optimizing the concentration of this plant's extract to apply as anthelmintic drug in human situation.

Methods

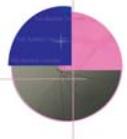
The aqueous extract of Siamese cassia's root (*Cassia siamea* Britt.) was prepared by boiling, filtrated through sieved cloth and freeze drying for powdering.

The powder 100% stock solution was prepared the 2 gm dissolved in Tyrode's solution 50 ml. The various extract concentrations 6.25%, 12.5%, 25% and 50% were used, Tyrode's solution as a control. The chicks were force fed with metacercaria of *Stellantchasmus falcatus* that obtained from the body cavity of the half-beaked fish, *Dermogenus pusillus*. Three days post infection, adult flukes were collected with Baremann's apparatus and washed with 0.85 % NaCl.

Thirty adult worms were incubated in each small Petri-dish containing 5 ml of Tyrode's solution and various concentrations of the aqueous extract. The worms were omitted from the incubating medium at 1, 6, 12 and 24 hours. The death and movement of worms were investigated under compound microscope. The movement was detected with 3 levels, high (movement 5 times/10 seconds to upper, medium (movement 1-4 times/10 seconds) and death (no movement/10 seconds). The death worms were processed for SEM, fixed in 2.5% glutaraldehyde for 24 hours, at 4°C and post fixed with 1% osmium tetroxide, followed by conventional methods and observed with SEM, JEOL JSM-5400LV at 10 KV.

Results

The aqueous extracts concentration of 6.25%, 12.5% and 25% can kill all the worms



(*Stellantchasmus falcatus*) within 6 hours, while 50% the extract concentration can kill worms 92.5 % within 1 hour (Table 1). The concentration of the extract effected to the movement and death at 1 hour respectively low to high concentration. The optimal concentration to indicate the toxicity LD_{50} was estimated 50% of the death worms at the extract concentration 25 % at 1 hour.

The SEM observation of the death trematode was examined the tegumental surface of the body (Figure 1) The examination found that the trematode in the extract of 6.25% solution showed loss of the scales at 6 hours and curving at the edge of

scales at 12 hours, whereas the trematode in the extract of 12.5% solution showed loss of the scales and curving at the edge of scales at 6 hours. The trematode in the extract of 25% and 50% solution had the same variation of tegument's ultrastructural changes as the trematode in the extract of 12.5% at 1 hour at which the rupturing and the tegument tearing off at 12 hours were evident. The patterns of the damaged surface showed rupturing and loss of spines. The blebbing and swelling of the surface was not visible in this study. The body of this trematode was severely ruptured in higher concentration of the root's extract, i.e 12.5% and 25%.

Table 1 The numbers of death worms in various times.

Group	Time incubation (hours)											
	1			6			1			24		
	The level of movement											
	***	**	*	***	**	*	***	**	*	***	**	*
Control	40	-	-	40	-	-	16	24	-	8	32	-
6.25%	2	30	8	-	-	40	-	-	40	-	-	40
12.5%	-	22	18	-	-	40	-	-	40	-	-	40
25%	-	15	25	-	-	40	-	-	40	-	-	40
50%	-	3	37	-	-	40	-	-	40	-	-	40

***High movement **Medium movement *No movement or death

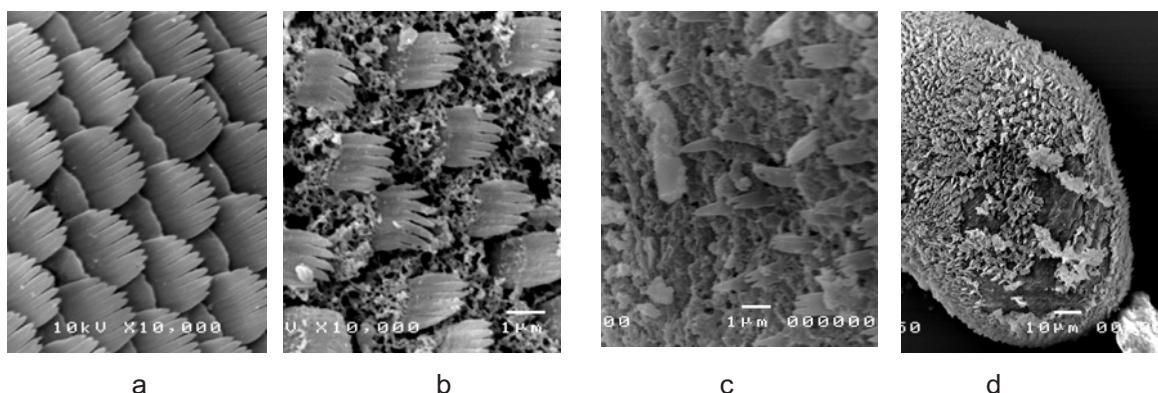
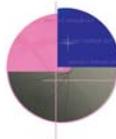


Figure 1 The damaged patterns of the tegumental surface. (a) control (b) curving at the edge of spines (c) loss of spines (d) rupturing of the body.

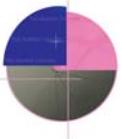
Discussion

All extract concentrations from this study at 6 hours can killed 100% worms, while the previous reports of 3 anthelmintic plants, *Artocarpus lakoocha* Roxb., *Murraya paniculata* Jack. and *Phyllanthus embica* Linn. can killed the trematode, *Haplorchis taichui* 100% at 12 hours (9). The result from this study showed the extracts of Siamese cassia's Root can killed the worms at the lower time than the previously 3 anthelmintic plants. But all of these plants would be studied the effectiveness in the same species of the trematode.

The SEM examination found that the trematode in the extract of 25% and 50% solution shown the same variation of the tegument as the trematode in the extract of 12.5% but more effectively as loss of the scale trematode tegumental changing were

apparently like the other trematode alterations. The patterns of the damaged surface such as rupturing and loss of spines were similar to that found in the trematode, *Haplorchis taichui* affected by *Artocarpus lakoocha* Roxb. and *Murraya paniculata* Jack. but curving at the edge of spines was identical to that caused by *Phyllanthus embica* Linn(9). Although the blebbing and swelling of the surface were not remarked, the body rupturing in higher concentration seen in this study was also described by Lee (10). However, the patterns of the damaged surface of another trematode, *H. taichui* by this plant and the mechanism of changes caused by this anthelmintic drug would be the area of fruitful investigation.

The results from this study illustrated that the aqueous extracts of Siamese cassia's root (*Cassia siamea* Britt.) can kill



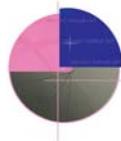
the trematode with optimal concentration and time. Nevertheless, the proper concentration and time for practical usage of this herbal plant's extract need to be verified.

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