ฤทธิ์ต้านเชื้อรา *Sclerotium rolfsii* ของสารสกัดหยาบในชั้นเฮกเซนจากรากพาหมี

Antifungal Efficiency of the Crude Hexane extract from the Roots of *Linostoma pauciflorum* Griff. on *Sclerotium rolfsii*

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บทคัดย่อ

นำสารสกัดหยาบในชั้นเฮกเซนและสารแดปโนรีตินที่แยกได้จากรากพาหมีมาทดสอบประสิทธิภาพในการยับยั้งการเจริญเติบโตของเส้นใยของเชื้อรา *Sclerotium rolfsii* ซึ่งเป็นเชื้อราก่อโรคในพืชที่มากับดิน โดยการนำเส้นใยของเชื้อรามาเพาะเลี้ยงในอาหารเลี้ยงเชื้อ PDA ที่มีการเติม สารสกัดหยาบในชั้นเฮกเซน และสารแดปโนรีตินที่ความเข้มข้น 0 (ควบคุม), 2 และ 100 มก/ล ตามลำดับ พบว่า ทั้งสารสกัดหยาบในชั้นเฮกเซนและสารแดปโนรีตินสามารถยับยั้งการเจริญเติบโตของเส้นใยของเชื้อรา *S. rolfsii* ได้ที่ 79.25 และ 79.25% (ที่ความเข้มข้น 2 มก/ล) และเพิ่มขึ้นเป็น 83.46% และ 94.56% (ที่ความเข้มข้น 100 มก./ล.) ตามลำดับ

คำสำคัญ : พาหมี สารแดปโนรีติน เชื้อรา

Abstract

The crude hexane extract and daphnoretin obtained from the roots of *Linostoma pauciflorum* was investigated for its inhibitory potential against the mycelial growth of *Sclerotium rolfsii*, a causal agent of soil-borne plant disease. The mycelium of the pathogen was cultured on potato dextrose agar amended with the crude hexane extract or daphnoretin at 0 (control), 2 and 100 mg/L respectively. The crude hexane extract and daphnoretin were able to inhibit the mycelial growth of *S. rolfsii* up to 79.25 and 79.25%, (at 2 mg/L) and 83.46 and 94.56% (at 100 mg/L), respectively.

Keywords: *Linostoma pauciflorum* Griff., Daphnoretin, *Sclerotium rolfsii*

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INTRODUCTION

Linostoma pauciflorum Griff. belongs to the family Thymelaeaceae, which comprises more than 898 species. This scandent shrub tree is in the southern part of Thailand and is traditionally used for several purposes. For example, whole plant is used as fish poison and insecticide, and a dried mixture of its grounded root is smoked as a cigarette for relieving nasal polyps [1]. In 2011, daphnoretin, a well-known bis-coumarin derivative, has been isolated from the roots and the vines of this plant [2]. Daphnoretin has showed interestingly diverse biological properties including antineoplastic activity against Ehrlich ascites carcinoma in mice and the inhibition of the tyrosine-specific protein kinase activity of human epidermal growth factor reporters [3]. Moreover, this compound also showed strong suppressive effects on the expression of the hepatitis B surface antigen (HBsAg) in human hepatoma Hep 3B cells [4] and anti fungal activity against phytopathogenic fungi *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium solani*. [5]. The pictures of *L. pauciflorum* are shown in Figure 1.

A B C

Figure 1. *L. pauciflorum* showing leaves: A, flowers: B and roots: C

Sclerotium rolfsii, soil-borne fungal pathogen, causes disease on a wide variety of crop and forage plants including cocoyam, tomato, chilli, lentil, turmeric, ground nut, cereals and betel vine resulting in significant crop loss [6]. Its wide host range, prolific growth, and the ability to produce-persistant sclerotia that lives with the soil for years, makes it impossible to manage this pathogen chemically [7]. Therefore, plant extracts that contain mixture of different bioactive components and which are also eco-friendly, may be used as an alternative source for controlling soil-borne diseases [8]. Plant extracts and plant-based formulations have shown to possess marked inhibitory activity against *S. rolfsii* [9]. However, the plant extract of *L. pauciflorum* has not been tested against *S. rolfsii*. Therefore, we assessed their against mycelia growth of the soil-borne plant pathogen, *S. rolfsii*. This paper describes about the findings.

MATERIALS AND METHODS

Plant material

The roots of *L. pauciflorum* were collected in April 2013 from Khounkanun district, Phuttalung province, Thailand, and identified as a *Linostoma pauciflorum* Griff. by Asst. Prof. Ubonwan Upho (Taksin University, Thailand). A voucher specimen has been deposited in the Forest Herbarium:BKF, Bangkok, Thailand (BKF. 125572).

Extraction and Isolation

Extraction procedures and isolation of pure compounds were carried out as described by Navarat *et al*. [2]. The air-dried roots (1.90 Kg) were chopped and grounded into powder then extracted with 10 L of MeOH. The residue was treated with CH_2Cl_2. After CH_2Cl_2 was added, the insoluble part was collected by filtration to obtain daphnoreitn (2.43 g) as a pale yellow powder. ^1H and ^{13}C NMR data for daphnoretin are shown in Table 1. MS (CI +ve) *m/z* 352 (M^+, 100%). HREIMS: *m/z* = 352.0474 $[M]^+$ (Calc. for $C_{19}H_{12}O_7$: 352.0474). The air-dried roots (170.0 g) were chopped and grounded into powder then extracted with 1.6 L of hexane. The hexane extract was concentrated in *vacuo* afforded the crude hexane extract (8.05 g) as a brown powder.

Microorganism

The root rot and stem rot fungus *S. rolfsii* was used to determine its susceptibility to extracts of selected plants. The fungus was isolated from the rotten root and stem of yard long beans. Infected sample of yard long beans displaying rot symptom was cut in to small pieces of about 3-5 mm thick and sterilized with 1% sodium hypochlorite solution for two minutes, rinsed thrice in sterilized distilled water (SDW) and dried on sterilized filter paper at room temperature. The plant sections were then transferred aseptically on sterile Potato Dextrose Agar (HiMedia, Mumbai) and incubated at 26-32 °C for 7 days. Initially the colonies were rapidly growing with silky-white hyphae covering much portion of the plate in about 7 days. Sclerotia (brown colored, spherical, 1-2 mm in size) production was observed after 4 days of incubation.

Bioassay

Inhibitory effect of plant extracts (crude hexane extract or daphnoretin) at 0 (control), 2 and 100 mg/L was assessed using poisoned food technique. A volume of 1 mL of each plant extract was aseptically poured into the sterile petri plates followed by the addition of Potato dextrose agar medium (10 mL) and allowed to solidify. An agar plug of *S. rolfsii* was placed at the center of the plate to determine its effect in inhibiting mycelial growth of *S. rolfsii*. Treatment consisted of *S. rolfsii* cultured on PDA incorporated with the plant extracts of *L. pauciflorum*, while a culture of *S. rolfsii* on PDA with sterile distilled water incorporated was used as a control. Each treatment consisted of 6 replications. The percentage of mycelial inhibition was assessed after incubation for 5 days at 26-32 °C [10] and was calculated by the following equation:

$$\% \text{ mycelial inhibition} = 100 - [(r^2/R^2) \times 100]$$

Where r it the colony radius in poisoned and R is colony radius in control plate.

Data were subjected to one way analysis of variance and compared with Duncan's Multiple Range Test (DMRT) at $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

After dichloromethane was added, the insoluble part was collected by filtration to give a known biscoumarin ether, daphnoretin (4.92 g) as a pale brown powder. This compound was identified by comparisons of its NMR and MS data with those reported in the literature [11].

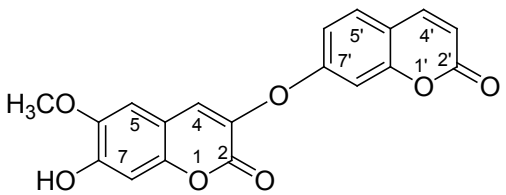

Figure 2. The structure of daphnoretin

Table 1. ^{1}H (*J*, Hz) and ^{13}C NMR data for daphnoretin in DMSO-d_6.

position	δ_H (J in Hz)	δ_c	δ_c (ref.*)	position	δ_H (J in Hz)	δ_c	δ_c (ref.*)
2		159.8	157.0	3′	6.37 d (*J* = 9.55)	114.6	113.9
3		136.0	135.7	4′	8.02 d (*J* = 9.55)	144.6	144.1
				4′a		115.6	114.4
4	7.87 s	131.6	131.1	5′	7.70 d (*J* = 8.60)	130.4	129.9
4a		110.6	110.2	6′	7.10 dd (*J* = 2.22,8.60)	113.9	113.4
5	7.21 s	109.7	109.3	7′		160.1	159.7
6		146.1	145.7	8′	7.15 d (*J* = 2.22)	104.3	103.9
7		150.8	150.4	8′a		155.4	155.0
8	6.86 s	103.2	102.7	6-OCH_3	3.80 s	56.5	56.0
8a		147.9	147.5				

ref.* [11]

The effect of both the crude hexane extract and daphnoretin on mycelial growth of *S. rolfsii* were significant. The plant extracts were significantly more effective in inhibiting the mycelial growth of *S. rolfsii* under *in vitro* conditions (Table 2) comparison with the control. The maximum inhibition was recorded by daphnoretin (Figure 3) followed by the crude hexane extract. The significant decrease is also possible that the extracts inhibited or altered the mode of action of pathogen's biological chemicals. Inhibitory effects of the plant extracts have also been observed on soil fungi [9]. Fungal pesticides from natural sources are effective, selective, biodegradable, and less toxic, would possibly help to decrease the negative impact of synthetic agents, such as high cost, residues in food, resistance development in fungal pathogens and environmental pollution.

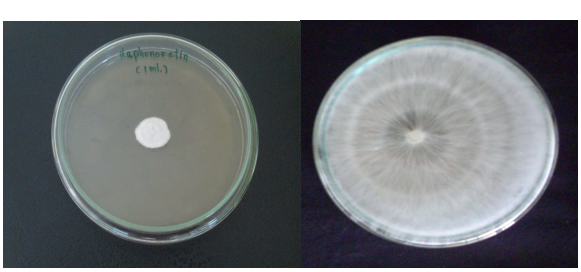

Figure 3. Inhibition of *S. rolfsii* mycelial growth test on PDA, incorporated with Daphnoretin (left) compared with control (right).

Table 2. Efficacy of the plant extracts (crude hexane extract or daphnoretin) of *L. pauciflorum* on inhibition of mycelial growth *S. rolfsii*

Plant extracts	Dose (mg/L)	
	2	100
Crude hexane extract	79.25± 0.45[a]	83.46± 0.53[a]
Daphnoretin	79.25± 0.10[a]	94.56± 0.19[a]
Control(sterile water)	00.00± 0.00[b]	00.00± 0.00[b]
F-test	**	**
C.V. (%)	1.93	2.62

** Means followed by the same letter are not significantly different by Duncan's Multiple Range Test at $p<0.01$.

CONCLUSION

In this study we reported that the dose of 2 mg/L of the crude hexane extract and daphnoretin were able to inhibit the mycelia growth of *S. rolfsii* at 79.25 and 79.25%, respectively. At the concentration 100 mg/L, the crude hexane extract and daphnoretin were able to inhibit the mycelia growth of pathogen by 83.46 and 94.56%, respectively. Thus this study can be very helpful for the development of new fungal pesticides from new natural source.

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