Pnb 23
Induction Of Rhinacanthin Formation In *Rhinacanthus Nasutus In Vitro* Cultures

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INTRODUCTION

Rhinacanthus nasutus (Linn.) Kurz (*Rhinacanthus communis* Nees) is a plant in Acanthaceae family. It widely distributes in Southeast Asia, South China and India. In Thailand, the Thai Foundation Health Committee, Ministry of Public Health has recommended the leaves and roots of *R. nasutus* for the treatment of tinea and ringworm (Farnsworth and Bunyapraphatsara, 1992). Moreover, this plant is used for the treatment of hepatitis, diabetes, pruritic rash, abscess pain and hypertension (Wu *et al.*, 1988). Naphthoquinones found in the leaves and roots of this plant are rhinacanthin and rhinacanthone. Rhinacanthin-C is the major naphthoquinone of *R. nasutus* and plays the interesting pharmacological activities, such as antimicrobial and antitumor activities (Kodama *et al.*, 1993; Panichayupakaranant *et al.*, 2000; Darah and Jain, 2001).

Recently, the study on rhinacanthin production by *R. nasutus in vitro* cultures is rarely reported. There is only one report on establishment of *R. nasutus* in shoot cultures and their rhinacanthin production (Plodpai and Kawprajan, 2001). However, the shoot cultures produced small amount of rhinacanthin-C. We therefore study on establishment of *R. nasutus* root cultures and their rhinacanthin-C production.

MATERIALS AND METHODS

Plant materials and explant preparation

R. nasutus leaves were collected from the Botanical Garden, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla. The young leaves of *R. nasutus* were washed in running tab water for 2 hrs and subjected to surface sterilization with 70% (v/v) ethanol for 5 s, 20% (v/v) Clorox® solution for 15 min and rinsing 3 times with sterile double distilled water, respectively.

Establishment of the root cultures of *R. nasutus*

Two types of the leaf explants (four-side excised leaves and the whole leaves) were cultured on solid B5 medium supplemented with 0.1 mg/l 3-indolebutyric acid (IBA), 0.8% plant agar, 2% sucrose and incubated at 25 ± 2°C under light and dark conditions for root initiation. After 4 weeks, the root cultures were transferred to the same B5 liquid medium in 250 ml flask and cultured on a rotary shaker at 80 rpm 25 ± 2° C, under light and dark conditions. The root cultures were harvested when they were 4-week old and subjected to determination of dried weight and rhinacanthin production.

Preparation of *R. nasutus* root extracts

The root cultures of *R. nasutus* (4-week old) and the intact roots were harvested and dried in a hot air oven at 50°C for 24 hrs. The dried *R. nasutus* roots were ground and the dried powder were extracted with 20 ml ethyl acetate under ultrasonic conditions for an hour.

The extracts were filtered and concentrated under reduced pressure. The sample was reconstituted and adjusted to 1 ml with methanol and subjected to determination of rhinacanthin production by HPLC.

Determination of rhinacanthin production by HPLC

HPLC analysis was carried out using Agilent 1100 series equipped with photodiode-array detector (PDA) and autosampler. Data analysis was performed using Agilent software (Agilent, U.S.A). Separation was achieved isocratically at 25°C on a TSK-gel ODS-80Ts column (150 mm x 4.6 mm i.d.). The mobile phase consisted of methanol and water (containing 5% acetic acid) in a ratio of 80:20 (v/v). The flow rate was 1 ml/min and the injection volume was 20 µl. The quantitative wavelength was set at 254 nm (Charoonratana, 2007). The quantitative analysis of rhinacanthin-C was carried out using the calibration curve of standard rhinacanthin-C (Y=1789.2X-44.763, R^2 = 0.9999).

RESULTS AND DISCUSSIONS

Effect of type of the leaf explants and light on root initiation of *R. nasutus*

The four-side excised leaves and the whole leaves were initiated on solid B5 medium supplemented with 0.1 mg/l IBA and cultured under light and dark conditions. It was found that only the explants that initiated under the dark conditions were capable of producing roots. However, the whole leaf explants produced higher amount of roots than the four-side excised (Table 1). In contrast, neither the whole leaf explants nor the four-side excised leaf explants produced the roots.

Table 1. Effect of type of the leaf explants and light on root initiation of *R. nasutus*

Type of leaf explants	Culture conditions	Number of root per explant (Mean ± S.D.)
Four-side excised leaves	Light (n = 10)	0
	Dark (n = 10)	0.4 ± 0.84
Whole leaves	Light (n = 10)	0
	Dark (n = 10)	4 ± 2.05*

*significant difference ($p < 0.01$)

Effect of light on rhinacanthin-C production in the root cultures of *R. nasutus*

The root cultures of *R. nasutus* that produced from the whole leaf explants under dark conditions. It was found that under both conditions, the root cultures were capable of producing rhinacanthin-C. However, the root cultures that cultured under the dark conditions produced higher amount of rhinacanthin-C than that of the root cultured under the light conditions (Table 2). In addition, under the dark conditions, the root cultured produced higher amount of biomass than that cultured under light conditions (Table 2).

Table 2. Effect of light on rhinacanthin-C production in the root cultures of *R. nasutus*

Culture conditions	Dry Biomass (mg/250 ml-flask)	Rhinacanthin-C content (% w/w)
Light	3.2	0.68
Dark	7.3	2.82

CONCLUSIONS

In this study, we found that the type of the leaf explants and light conditions play an important role on root initiation and rhinacanthin-C production. The whole leaf explants were suitable for root initiation of *R. nasutus* root cultures. In addition, the root cultures of *R. nasutus* should be cultured in the dark conditions in order to improve their growth and rhinacanthin-C production.

REFERENCES

Charoonratana, T. (2007). "Establishment of standard information of rhinacanthins extract from *Rhinacanthus nasutus* leaves", *Master of Pharmacy Thesis in Pharmaceutical Sciences.* Prince of Songkla University. Thailand.

Darah, I. and Jain, K. (2001). Efficacy of the *Rhinacanthus nasutus* Nees leaf extract on dermatophytes with special reference to *Trichophyton mentagrophytes* var. *mentagrophytes* and *Microsporum canis. Natural Product Sciences.* 7 (4): 114- 119.

Farnsworth, N.R. and Bunyapraphatsara, N. (1992), Thai Medicinal Plants: *Plant Recommended for Primary Health Care System.* Prachachon, Bangkok: 216-217.

Kodama, O., Ichikawa, H., Akatsuka, T., Santisopasri, V., Kata, A. and Hayashi, Y. (1993). Isolation and identification of antifungal naphthopyran derivative from *Rhinacanthus nasutus. Journal of Natural Products.* 56 (2): 292-294.

Plodpai, P. and Kawprajan, S. (2001). Study on naphoquinone formation in *Rhinacanthus nasutus* tissue cultures. *Bachelor of Pharmacy Senior Project in plant tissue culture.* Faculty of Pharmaceucical Sciences. Prince of Songkla University. Thailand.

Panichayupakaranant, P., Ebizuka, Y., Kaewnopparat, S. and Sungkarak, S.(2000). Antifungal and antibacterial activity of naphthoquinones from *Rhinacanthus nasutus*leaves. *The Fifth Joint Seminar on Natural Medicines.* Bangkok. Thailand: 42.

Wu, T. S., Tien, H. J., Yeh, M. Y. and Lee, K. H. (1988). "Isolation and cytotoxicity of rhinacanthin-A and –B, Two naphthoquinones from *Rhinacanthus nasutus*", *Phytochemistry.* 27: 3787-3792.