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Variation of Rhinacanthin C Content in the Raw Materials of Rhinacanthus nasutus

Patamaporn Pruksakorn[1], Chattraporn Jaima[2], Parnuphan Panyajai[2], Sakwichai Onthong[1], Sangtawan Sriboran[1]

[1]*Medicinal Plant Research Institute, Department of Medical Sciences, Nonthaburi 11000 Thailand*
[2]*Medical Life Sciences Institute, Department of Medical Sciences, Nonthaburi, Thailand*
Corresponding Author: Patamaporn Pruksakorn

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ABSTRACT: Rhinacanthus nasutus (L.) Kurz has been utilized in Thai traditional medicine for the treatment of skin diseases. This plantcontains rhinacanthin C as a principal antifungal constituent, predominantly present in its leaves and roots.Currently, R. nasutus extracts have been used as an ingredient in the production of numerous health products. Nevertheless, there are limited scientificreportsregarding the levels of rhinacanthin C in diverse sources of raw materials. The objective ofthis study was to determine rhinacanthin C contents in theraw materials of R. nasutususingthe HPLCmethod.The quantification of rhinacanthin C was performed by using a C_{18} column and a mobile phase comprising 0.1% trifluoroacetic acid (TFA) in acetonitrile and 0.1% TFA in water (75:25 v/v) at a flow rate of 1 mL/min. The detection was carried out using a photodiode array detector (PDA), measuring UV absorbance at 254 nm.Analysis of rhinacanthin C in the leaves and the roots ofR. nasutus from three sources revealed content in the range of 0.01-1.27% w/w and 1.11-2.42% w/w, respectively. In addition, determination of rhinacanthin C in raw materials from four suppliers showed levels below 0.05% w/w.These resultsindicated that there isa very high variation in rhinacanthin C content among different sources. Therefore, theanalysis of rhinacanthin C content inR. nasutusraw materialis crucial for quality control and selectingthe optimalmaterial sources.

KEYWORDS: Rhinacanthus nasutus, Rhinacanthin C, Quantitative analysis, Leaf extract, Root extract

I. INTRODUCTION

Rhinacanthus nasutus (L.) Kurz, or Thong-Phan-Chang in Thai, is a shrub belonging to the Acanthaceae family and is widely distributed in tropical areas including Southeast Asia, South China, and India. Theleaves and roots ofR. nasutus have been used in Thai traditional medicine for the treatment of skin diseases, especially ringworm, tinea versicolor, and eczema[1-2].Boththe leaves and roots of this plant contain a naphthoquinone, rhinacanthin C as a major bioactive compound for antifungal activity[3].Presently, R. nasutus extracts have been used in the production of many health products. However,there are limited documentsregarding the quality of raw materials from diverse sources.

It was reported that the roots of R. nasutusindifferent regions of Thailand showed variations in rhinacanthin C contentsin the range of 0.22-2.00% w/w[4].Nevertheless, there is a lack of scientific reports regarding the levels of rhinacanthin C in the leaves of R. nasutusfrom diverse sources.This study aimed to determine the rhinacanthin C content in the raw materials of R. nasutususing the HPLC method, reporting on the levels of rhinacanthin C found in leaves and roots from various sources as well as raw materials from different suppliers.

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Figure 1: Morphology of Rhinacanthus nasutus (L.) Kurz, dried leaves, and dried roots.

II. MATERIAL AND METHODS

Collection of Plant Materials

The leaves and roots of R. nasutus were collected from three areas in Ratchaburi Province, Thailand. All plants were authenticated by botanistsat theMedicinal Plant Research Institute. The voucher specimens were deposited at theDepartment of Medical Sciences Herbarium, Nonthaburi, Thailand, with herbarium specimen numbersDMSC.: 5351, DMSC.: 5352, and DMSC.: 5353for R. nasutus from Ratchaburi I(13°38' 20.828"N,100°1' 19.0042" E), Ratchaburi II (13°38' 25.0955" N, 100°1' 24.5176" E), and Ratchaburi III (13°38' 13.3483" N, 100°1' 20.6238" E), respectively.

Preparation of Extracts

Fresh leaves and roots of R. nasutus were washed thoroughly and air-dried in the shade.The dried plants were ground and sieved through a 10-mesh sieve. The ground plants were extracted with ethanol (Merck, Germany) (1g: 20 mL, twice) at room temperature. The extracts were concentrated under reduced pressure using rotary evaporator (Hei-VAP Advantage, Heidolph, Germany) and freeze dryer (DC801, Yamato, Japan) to yield the ethanolic extracts. The extracts were prepared in duplicate for each source.

Preparation of Rhinacanthin C StandardSolution and Extracts Samples

Ten milligrams of rhinacanthin C (in house isolation) or the ethanolicextracts were accurately weighed using a microbalance (XP2U, Mettler Teledo, USA)and dissolved in 10 mL of HPLC grade methanol (J.T Baker, USA) in a 10 mL volumetric flask. The resulting rhinacanthin C solution was subsequently diluted to concentrations ranging from 10-100 µg/mL with HPLC grade methanol to serve as calibration standards. The extract solutions were diluted to a concentration of 100 µg/mL with HPLC grade methanol for thedetermination of rhinacanthin C content.

Determination of Rhinacanthin C Content

HPLC grade solvents were used toanalyse the rhinacanthin C content within the ethanolic extracts of raw materials. The quantification of rhinacanthin C was conducted using the1260 Infinity II LC system (Agilent Technology, USA). The analysis was performed by using a C_{18}column (VertiSe^{TM} UPS 4.6 × 250 mm, 5 µm, Thailand)and a mobile phase consisting of 0.1% trifluoroacetic acid (TFA) (Sigma-Aldrich, USA) in acetonitrile (J.T. Baker, USA) and 0.1% TFA in water (in a ratio of 75:25 v/v) at a flow rate of 1 mL/min, with a detection wavelength of 254 nm. The injection volume was 50 µL of each solution. The total run time was 20 minutes for each injection.

III. RESULTS

The leaves and roots ofR. nasutus from three origins were extracted with ethanol to yield ethanolic extracts in the range of 4.20-6.75% w/w and 5.67-6.91% w/w, respectively.The extraction of raw materials from four suppliersyielded ethanolic extracts in the range of 3.77-4.32% w/w.The determination of rhinacanthin C in the leaf and root extracts revealed contents in the range of 0.01-1.27% w/w and 1.11-2.42% w/w, respectively. Additionally, analysis of rhinacanthin C in raw materials exhibited levels below 0.05% w/w. The results were presented in Tables 1 and 2.'

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Table 1 Extraction yield and rhinacanthin C contents of the leaves and roots of R. nasutus from different sources.Results are represented as mean ± standard deviation.

Sources of Specimens(Location)	Part Used	Ethanolic Extracts (% Yield, w/w)	% Rhinacanthin C Contents (w/w)
Ratchaburi I (13° 38' 20.828" N, 100° 1' 19.0042" E)	Leaves	6.75±1.00	1.27±0.05
	Roots	5.67±0.42	1.58±0.05
Ratchaburi II (13° 38' 25.0955" N, 100° 1' 24.5176" E)	Leaves	5.04±0.06	0.58±0.02
	Roots	5.72±1.29	1.11±0.16
Ratchaburi III (13° 38' 13.3483" N, 100° 1' 20.6238" E)	Leaves	4.20±0.33	0.01±0.00
	Roots	6.91±0.42	2.42±0.17

Table 2 Extraction yield and rhinacanthin C contents of the raw materials of R. nasutus from different suppliers.Results are represented as mean ± standard deviation.

Sources of Raw Material	Appearance	Ethanolic Extracts (% Yield, w/w)	% Rhinacanthin C Contents (w/w)
Supplier I	Powders	4.32±0.09	0.05±0.01
Supplier II	Powders	3.77±0.13	0.04±0.00
Supplier III	Dried arial parts	4.27±0.31	0.03±0.00
Supplier IV	Dried arial parts	4.00±0.15	0.00±0.00

IV. DISCUSSION

Analysis of rhinacanthin C contents in the leaves and roots of R. nasutusindicated variations in the content of major active compoundsamong plants sourced from different origins, even in proximity. Specifically, specimens from Ratchaburi I exhibited higher rhinacanthin C levels compared to specimens from Ratchaburi II, evident in both leaves and roots. Conversely, while specimens from Ratchaburi III demonstrated a high level of rhinacanthin C in the roots, the leaves exhibited a verylow level.

Genetic, ontogenic, morphogenetic, and environmental factors play crucial roles in the biosynthesis and accumulation of secondary metabolites. The synthesis of plant secondary metabolites is influenced by these diverse factors, and even a slight alteration in one factor can lead to changes in secondary metabolite content,even if the other factors remain unchanged[5]. In this study, all specimens of R. nasutus were collected during the same period and stage of plant development, and all plants were cultivated in close proximity. Therefore, the variations in rhinacanthin C content observed may be attributed to genetic factors.

Analysis of rhinacanthin C levels in raw materials from various suppliers consistently revealed very low concentrations of active compounds. This finding is in line with prior research indicating that the aerial part powders of R. nasutus contain a very low content of total rhinacanthins[6], with rhinacanthin C primarily found in the roots and leaves, while it is very low in the stem and twig [3, 6]. The aerial parts contain a significant amount of stem and twig, resulting in a low level of rhinacanthin C.

V. CONCLUSION

In summary, R. nasutusholds medicinal value in Thai traditional medicine for treating fungal skin conditions. The presence of rhinacanthin C, a major antifungalcompound, in its leaves and roots supports its therapeutic potential. However, our investigation revealed notable variations in rhinacanthin C levels among plants sourced from different origins, even when grown in

close proximity under similar conditions. These variations may be attributed to genetic factors. In addition, our analysis of raw materials from various suppliers showed low levels of rhinacanthin C in both arial parts and powders. These findingsemphasize the importance ofanalysing rhinacanthin C content in R. nasutus raw materials to ensure quality control and to identify optimal source for material selection.

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