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QUANTITATIVE VARIATION OF RHINACANTHIN C CONTENT IN RAW MATERIALS OF Rhinacanthus nasutus

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ABSTRACT:
Rhinacanthus nasutus (L.) Kurz, a plant widely used in Thai traditional medicine for treating skin ailments, contains rhinacanthin C as its primary antifungal compound, predominantly found in leaves and roots. Despite its integration into numerous health products, there is limited scientific data on the variability of rhinacanthin C levels across different raw material sources. This study aimed to quantify rhinacanthin C in R. nasutus raw materials using HPLC methodology. Analysis employed a C18 column with a mobile phase consisting of 0.1% trifluoroacetic acid (TFA) in acetonitrile and 0.1% TFA in water (75:25 v/v) at 1 mL/min flow rate. Detection utilized a photodiode array detector (PDA) set to measure UV absorbance at 254 nm. Results from three sources indicated rhinacanthin C content ranged from 0.01% to 1.27% w/w in leaves and 1.11% to 2.42% w/w in roots. Evaluation of raw materials from four suppliers revealed levels below 0.05% w/w. These findings highlight significant variation in rhinacanthin C content among different sources, underscoring the importance of rigorous content analysis for quality control and selection of optimal raw material sources.

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

INTRODUCTION

Rhinacanthus nasutus (L.) Kurz, known as Thong-Phan-Chang in Thai, is a shrub from the Acanthaceae family commonly found in tropical regions, including Southeast Asia, South China, and India. The leaves and roots of R. nasutus have been traditionally used in Thai medicine to treat skin conditions such as ringworm, tinea versicolor, and eczema [1]. These plant parts are rich in rhinacanthin C, a naphthoquinone recognized for its antifungal properties. Nowadays, R. nasutus extracts are utilized in various health products. However, there is limited documentation regarding the quality of raw materials from different sources.
Previous studies have shown that the rhinacanthin C content in the roots of R. nasutus from various regions in Thailand ranges from 0.22% to 2.00% w/w [4]. Despite this, there is a lack of scientific data on the rhinacanthin C levels in the leaves of R. nasutus from different sources. This study aimed to determine the rhinacanthin C content in the raw materials of R. nasutus using HPLC, reporting the levels found in leaves and roots from various sources, as well as raw materials from different suppliers.[2]

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

MATERIALS AND METHODS

Collection of Plant Materials

The leaves and roots of R. nasutus were collected from three locations in Ratchaburi Province, Thailand. Botanists at the Medicinal Plant Research Institute authenticated the plants. Voucher specimens were deposited at the Department of Medical Sciences Herbarium, Nonthaburi, Thailand, with specimen numbers DMSC.: 5351, DMSC.: 5352, and DMSC.: 5353 for 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[3].

Preparation of Extracts

Fresh leaves and roots of R. nasutus were thoroughly washed and air-dried in the shade. The dried plants were ground and sieved through a 10-mesh sieve. The ground plant material was extracted with ethanol (Merck, Germany) (1g: 20 mL, twice) at room temperature. The extracts were concentrated under reduced pressure using a rotary evaporator (Hei-VAP Advantage, Heidolph, Germany) and freeze dryer (DC801, Yamato, Japan) to yield the ethanolic extracts. The extraction process was duplicated for each source[4].

Preparation of Rhinacanthin C Standard Solution and Extract Samples

Ten milligrams of rhinacanthin C (isolated in-house) or the ethanolic extracts were accurately weighed using a microbalance (XP2U, Mettler Toledo, 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 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 the determination of rhinacanthin C content.

Determination of Rhinacanthin C Content

HPLC grade solvents were used to analyze the rhinacanthin C content in the ethanolic extracts of raw materials. Quantification was performed using the 1260 Infinity II LC system (Agilent Technology, USA). A C18 column (VertiSeTM 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 (75:25 v/v) were used at a

flow rate of 1 mL/min, with detection at 254 nm. The injection volume for each solution was 50 µL, and the total run time was 20 minutes per injection.

RESULTS

The leaves and roots of R. nasutus from three different origins were extracted with ethanol, yielding ethanolic extracts ranging from 4.20-6.75% w/w for leaves and 5.67-6.91% w/w for roots. Extraction of raw materials from four suppliers resulted in ethanolic extracts ranging from 3.77-4.32% w/w. The rhinacanthin C content in the leaf and root extracts was found to be between 0.01-1.27% w/w and 1.11-2.42% w/w, respectively. Additionally, the rhinacanthin C content in raw materials from various sources was below 0.05% w/w. The detailed results are presented in Tables 1 and 2.

Table 1: Extraction yield and rhinacanthin C contents of the leaves and roots of R. nasutus from different sources

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

Results are represented as mean ± standard deviation

Table 2: Extraction yield and rhinacanthin C contents of the raw materials of R. nasutus from different suppliers

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

Results are represented as mean ± standard deviation

DISCUSSION

The analysis of rhinacanthin C content in the leaves and roots of R. nasutus revealed significant variations among plants from different origins, even those in close proximity. Specimens from Ratchaburi I showed higher levels of rhinacanthin C in both leaves and roots

compared to those from Ratchaburi II. In contrast, while specimens from Ratchaburi III had high rhinacanthin C levels in the roots, the leaves contained very low levels.

Genetic, ontogenic, morphogenetic, and environmental factors play crucial roles in the biosynthesis and accumulation of secondary metabolites. These diverse factors influence the synthesis of plant secondary metabolites, and even slight changes in one factor can affect the secondary metabolite content, even if other factors remain constant. In this study, all specimens of R. nasutus were collected during the same period and stage of plant development and were cultivated in close proximity. Therefore, the observed variations in rhinacanthin C content may be attributed to genetic factors. Additionally, analysis of rhinacanthin C levels in raw materials from various suppliers consistently showed very low concentrations of the active compound. This finding aligns with previous research indicating that the aerial part powders of R. nasutus contain very low total rhinacanthins , with rhinacanthin C primarily found in the roots and leaves, and very low levels in the stems and twigs . Since the aerial parts contain a significant amount of stem and twig, this results in a low level of rhinacanthin C.

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