QUALITY ASSESSMENT OF *RHINACANTHUS NASUTUS*

Tanasorn Tunsaringkarn[1], Chanida Palanuvej[1], Somchai Issaravanich[1], Niran Vipunngeun[1], Anusorn Rungsiyothin[1], Anchalee Chuthaputti[2] and Nijsiri Ruangrungsi[1,3,*]

[1]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[2]Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health, Nonthaburi 11000, Thailand [3]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT: Dried whole plants of *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae) were collected from 15 Thai traditional drugstores located around four regions of Thailand. The pharmacognostic specifications were performed according to the World Health Organization (WHO) guidelines for herbal standardization. Microscopic investigation revealed multicellular and glandular trichomes as well as lithocyst cells occured on both leaf surfaces. Calcium carbonate and raphide crystal were found on the epidermal and parenchyma cell of the stem. The powdered crude drug displayed microscopic identification of both leaves and stems. The mean contents of foreign matter, acid insoluble ash, total ash, moisture content, ethanol-soluble extractive, water-soluble extractive and loss on drying were 0.2, 6.5, 10.7, 8.7, 1.8, 16.9 and 9.5 % of dry weight respectively. TLC fingerprint of the methanolic extracts were demonstrated.

Keywords: *Rhinacanthus nasutus*, pharmacognostic specification

INTRODUCTION: *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae family) is widely distributed in some parts of the sub-continent India, China and in the region of Southeast Asia including Thailand. *R. nasutus* have been used for various illness treatments. The literatures reported of traditional medicine preparations from roots and leaves of this shrub were widely advocated for prickly heat and scurf and whole plant for ringworm, various skin problems such as eczema[1,2] as well as pulmonary tuberculosis[3]. The active ingredients of extracts have been reported for antifungal[1-3], anti-bacterial[3,4], antivirus[5,6], cytotoxic[7], anti platelet[8], antitumor[9,10] and antiproliferative[11] activities. This study aimed to examine the pharmacognostic identification of *R. nasutus* crude drug distributed among traditional drug stores in Thailand.

MATERIALS: The dried whole plants of *R. nasutus* were collected from 15 Thai traditional drugstores located at 4 regions of Thailand as follow : Bangkok (3 stores), Chon Buri, Nakorn Pathom, Kamphaeng Phet, Pichit, Nakorn Sawan, Chiang Mai, Nakorn Ratchasima, Ubon Ratchathani, Roi et, Songkhla, Trang and Nakorn Si Thammarat. The samples were identified by one of the authors (N.R.). The voucher and numbers of specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand.

METHODS: Size, colour and other visual inspections were examined for macroscopic characteristics. Transverse sections and ground powders were inspected under microscope (Olympus BX41) for anatomical and histological characteristics. The foreign matter was excluded and recorded the percentage amount. The total and acid-insoluble ashes remained after burning at 500-600°C were determined for inorganic components part. The loss on drying test was performed to measure the amount of water and volatile matters by drying the sample at 105°C until constant weight. In addition, the moisture content was also quantitated using Azeotropic distillation method. Extractable matters in absolute ethanol as well as water were determined by maceration to represent the amount of active constituents. Chemical fingerprint was performed by thin-layer chromatography after reflux in methanol. The details of methodology were described elsewhere[12,13].

*To whom correspondence should be addressed.
E-mail: nijsiri.r@chula.ac.th, Tel./fax. +66 2218 8158

RESULTS AND DISCUSSION: *R. nasutus* is an erect, branched shrub, tall up to 2 m. Its stem is obtusely quadrangular. The leaves are opposite, simple, ovate to lanceolate or elliptical, 3-10 cm long and 1-5 cm wide. The young stem and leaves are puberulent. The flowers are white with red markings at base (Figure 1). Dried whole plants have been used as crude drug in Thai traditional drug stores (Figure 2). Anatomical and histological characteristics of *R. nasutus* were presented (Figure 3-6). Multicellular and glandular trichomes as well as lithocyst cells occured on both leaf surfaces (upper and lower epidermis). The microscopic characters of *R. nasutus* dried leaves observed in this study were in agreement with the previous report of fresh leaves by Phisutthanan S. and Phisutthanan N.[14]. In addition to the leaves, this study explored the stems microscopically and found multicellular and glandular trichomes as well. The epidermal cell containing calcium carbonate and parenchyma cell containing raphide crystals were also presented. The powdered crude drug displayed microscopic identification of both leaves and stems, for example, stomata, trichomes, calcium carbonate, raphide crystals, fibers, vessels and sclereids (Figure 6).



Figure 1 Twig of *R. nasutus* with flowers

Table 1 showed the quality characters of *R. nasutus* crude drug. The contents of foreign matter, acid-insoluble ash, total ash, moisture content and loss on drying were found to be 0.2 ± 0.2, 6.5 ± 2.1, 10.7 ± 2.6, 8.7 ± 1.2 and 9.5 ± 0.9 % of dry weight respectively whereas the determination of ethanol- and water- extractive values were found to be 1.8 ± 1.2 and 16.9 ± 3.8 % of dry weight respectively. From these data, the requirements of the specification could be set up (in integer) using the criteria of either mean+10% or mean-10%[15] (Table 2). Phisutthanan S. and Phisutthanan N.[14] reported the contents of ethanol-, water-extractives and loss on drying, of *R. nasutus* fresh leaves from 3 sources in the Northern provinces of Thailand. Their results were 5.6, 4.6 and 5.6 % of ethanol-extractive, 29.0, 27.7 and 26.1 % of water-extractives and 23.3, 13.4 and 13.0 % of loss on drying. The three content values of this study were likely lower than the earlier report especially ethanol-extractive value[14]. This was understandable for the fresh samples collected from the wilds *versus* the dried samples obtained from drugstores.

Table 1 The quality characters of *R. nasutus* crude drug

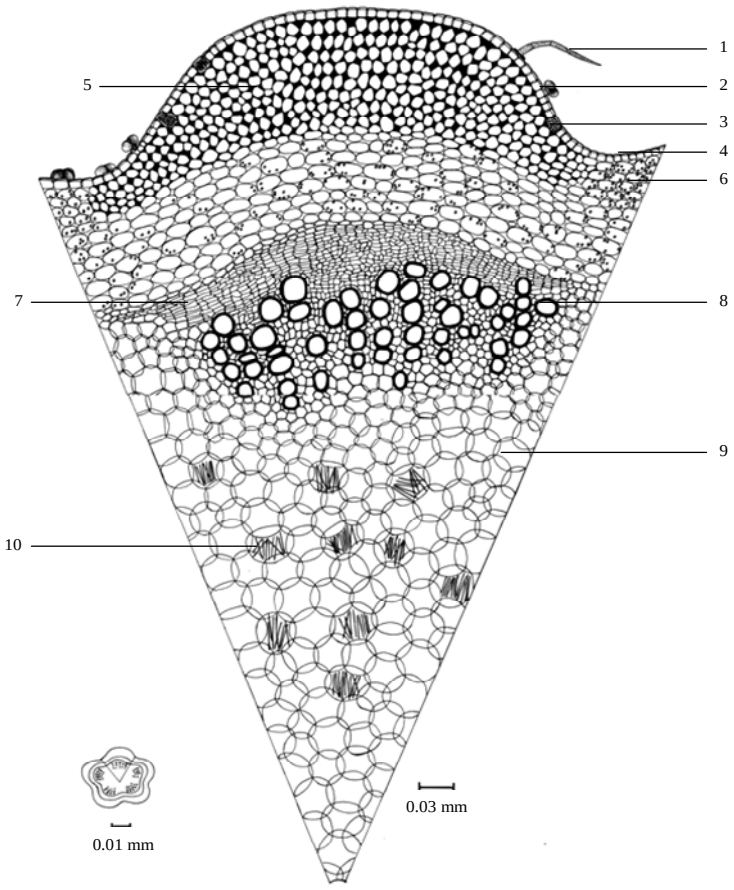
Specification	Mean ± SD*	Min – Max*
Foreign matter	0.20 ± 0.15	0.06 - 0.74
Acid-insoluble ash	6.53 ± 2.10	3.52 – 11.98
Total ash	10.68 ± 2.62	6.46 – 15.03
Moisture content	8.74 ± 1.15	6.00 - 10.80
Volatile oil content	Not found	Not found
Loss on drying	9.53 ± 0.90	6.82 - 10.98
Ethanol- extractive	1.84 ± 1.18	0.30 - 5.50
Water- extractive	16.90 ± 3.80	9.40 - 27.08

*% of dry weight

Table 2 The requirement specification of *R. nasutus* crude drug

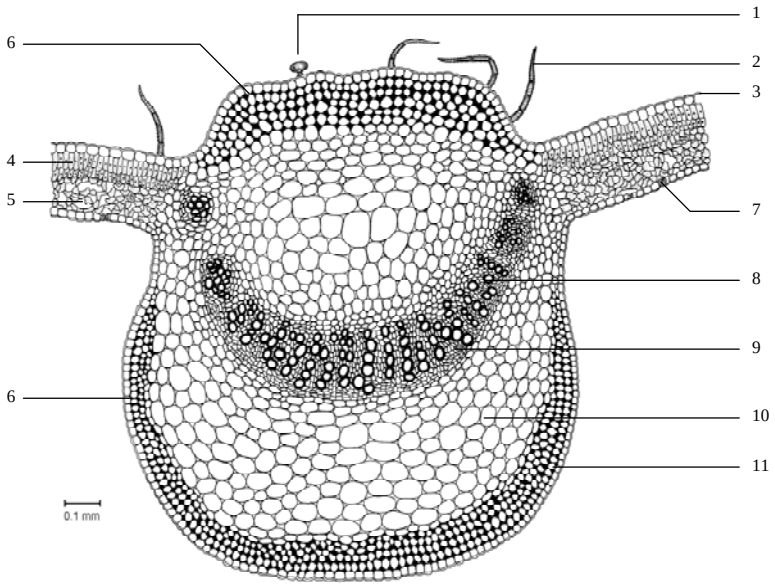
	Specification*		
Foreign matter	Not more than	1	%
Acid-insoluble ash	Not more than	7	%
Total ash	Not more than	12	%
Moisture content	Not more than	10	%
Loss on drying	Not more than	11	%
Ethanol- extractive	Not less than	1	%
Water- extractive	Not less than	15	%

*% of dry weight



1. multicellular trichome
2. glandular trichome
3. epidermal cell containing calcium carbonate
4. epidermis layer
5. collenchymas
6. chlorenchyma
7. phloem tissue
8. xylem vessel
9. parenchyma
10. parenchyma containing raphide crystals

Figure 3 Microscopic character of the transverse section of *R. nasutus* stem



1. glandular trichome
2. multicellular trichome
3. upper epidermis
4. palisade cell
5. spongy cell
6. collenchymas
7. stomata
8. xylem vessel
9. phloem tissue
10. parenchyma
11. lower epidermis

Figure 4 Microscopic character of the transverse section of *R. nasutus* midrib

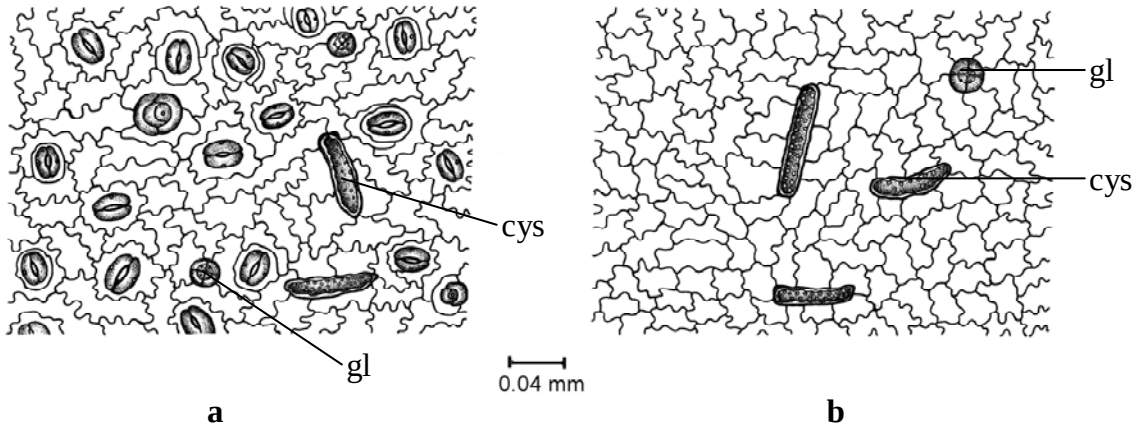


Figure 5 Microscopic character of the surface views of *R. nasutus* lamina
a. lower epidermis with stomata, lithocyst cells (cys) and glandular trichome (gl)
b. upper epidermis with lithocyst cells (cys) and glandular trichome (gl)

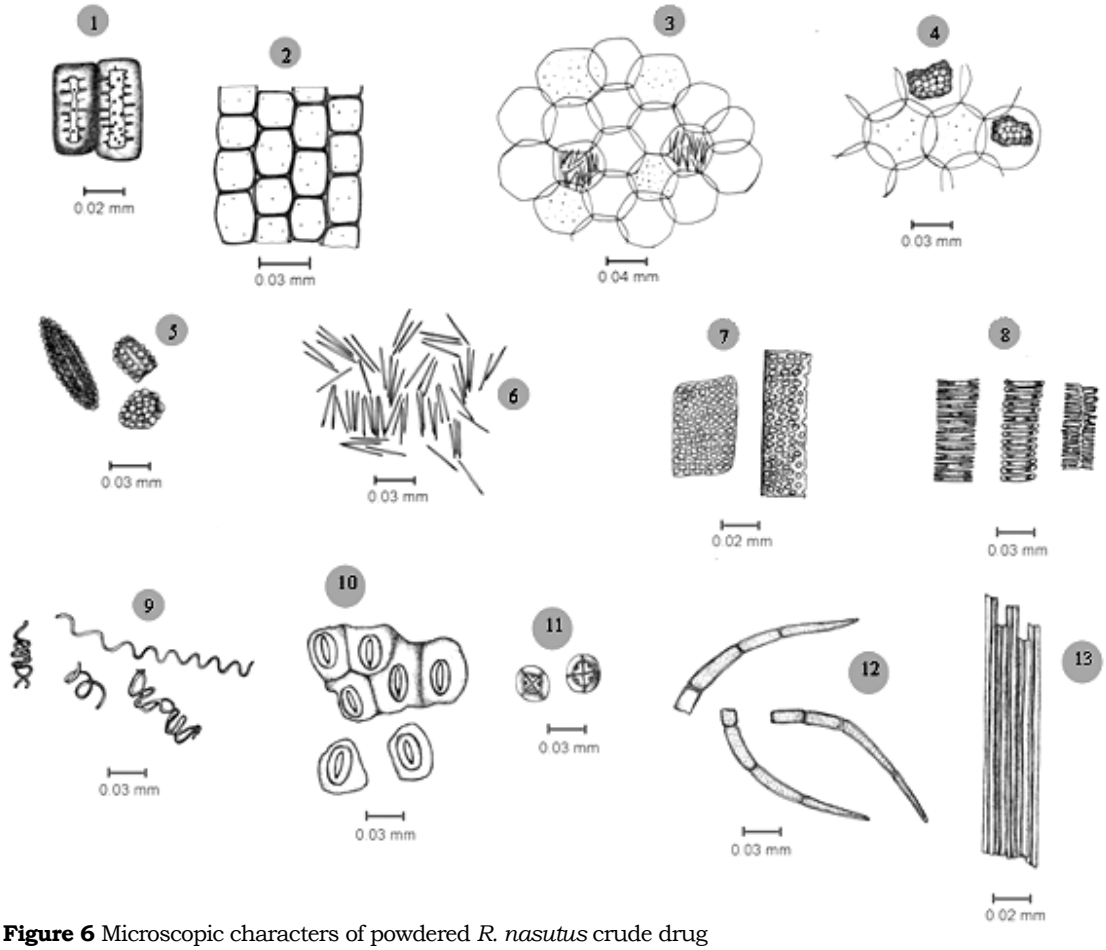
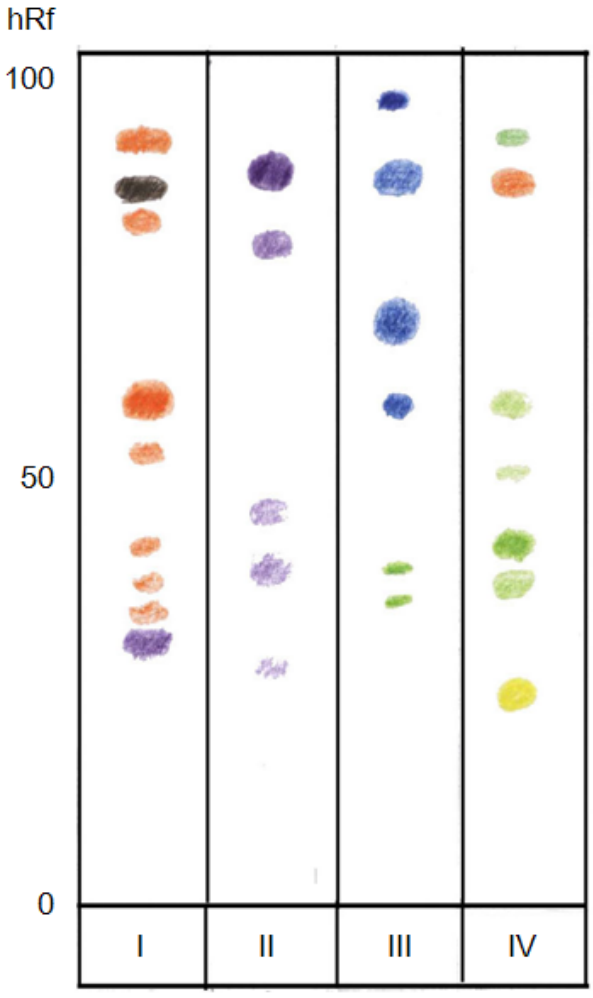


Figure 6 Microscopic characters of powdered *R. nasutus* crude drug

1. sclerenchymatous sclereids vessels
2. parenchyma in longitudinal view
3. parenchyma containing crystals
4. parenchyma containing calcium carbonate
5. calcium carbonate
6. raphide crystals
7. fragment of pitted vessels
8. fragment of reticulated
9. fragment of spiral vessels
10. stomata
11. glandular trichomes
12. multicellular trichomes
13. fragment of fiber



Figure 2 *R. nasutus* dried whole plants



Solvent system:
Toluene : Ethyl acetate 75 : 25

Developing distance:
20 cm

Detections:
I = detection under UV light 366 nm
II = detection under UV light 254 nm
III = detection with vanillin-sulfuric acid
IV = none

Figure 7 TLC fingerprint of methanolic extract of *R. nasutus* dried whole plants

TLC fingerprints of methanolic extracts were shown in Figure 7. This study used Toluene : Ethyl acetate (TE, 75 : 25) as the solvent system instead of Chloroform : Methanol (CM, 19 : 1) in the earlier study[14]. It was found that TLC fingerprints by TE system showed less spots than CM.

CONCLUSION: The pharmacognostic specification of *R. nasutus* crude drug was firstly illustrated. However, the specific chemical constituents such as Rhinacanthin series should be further investigated.

ACKNOWLEGEMENTS: Thanks are due to the Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health for partial financial support and College of Public Health Sciences, Chulalongkorn University for providing facilities.

REFERENCES:

1. Achararit C. 1983. Study on antifungal activity of Thai medicinal plants extracts, Speacial project for B.Sc. (Pharm.), Faculty of pharmacy, Mahidol University, Bangkok, Thailand.

2. Atsusi K, Yoshioki H. 1993. Isolation and identification of an antifungal naphthopyran derivative from *Rhinacanthus nasutus*. J Nat Prod 56(2): 292–94.

3. Sattar MA, Abdullah NA, Khan AH, Noor AM. 2004. Evaluation of anti-fungal and anti-bacterial activity of a local plant *Rhinacanthus nasutus* (L.). J Biol Sci 4(4): 498-500.

4. Mahady GB. 2005. Medicinal plants for the prevention and treatment of bacterial infections. Curr Pharm Des 11(19): 2405-27.

5. Kernan MR, Send A, Chen JL, Jolad SD, Blanc P, Murphy JT, *et al.* 1997. Two new lignans with activity against influenza virus from the medicinal plant *Rhinacanthus nasutus*. J Nat Prod 60 (6):635-7.

6. Sendl A, Chen JL, Jolad SD, Stoddart CA, Rozhon EJ, Kernan MR, *et al.* 1996. Two new naphthoquinones with antiviral activity from *Rhinacanthus nasutus*. J Nat Prod 59 : 808-11.

7. Wu TS, Tien HJ, Yeh My, Lee KH. 1988. Isolation and cytotoxicity of rhinacanthin - A and - B, two; naphthoquinones, from *Rhinacanthus nasutus*. Phytochemistry 27(12): 3787-8.

8. Wu TS, Hsu HC, Wu PL, T CM, Wu TC. 2004. Rhinacanthin-Q and a naphthaquinones, from *Rhinacanthus nasutus* and its biological activity. J Ethnopharmacol 95(2-3): 183-9

9. Siripong P, Yahuafai J, Shimizu K, Ichikawa K, Yonezawa S, Asai T, *et al.* 2006. Antitumor activity of liposomal naphthoquinone esters isolated from Thai medicinal plant: *Rhinacanthus nasutus* Kurz. Biol Pharm Bull 29(11): 2279-83.

10. Punturee K, Wild CP, Vinitketkumneun U. 2004. Thai medicinal plants modulate nitric oxide and tumor necrosis factor-alpha in J774.2 mouse macrophages. J Ethnopharmacol 95(2-3): 183- 9.

11. Gotoh A, Sakaeda T, Kimura T, Shirakawa T, Wada Y, Wada A, *et al.* 2004. Antiproliferative activity of *Rhinacanthus nasutus* (L.) Kurz extracts and the active moiety, Rhinacanthin C. Biol Pharm Bull 27(7): 1070-4.

12. World Health Organization. 1998. Quality control methods for medicinal plant materials, ISBN 92 415 45100 (NLM Classification QV 766).

13. Issaravanich S, Palanuvej C, Tunsaringkarn T, Rungsiyothin A, Vipunngeun N, Chuthaputti A, *et al.* 2008. Pharmacognostic specification of *Vetiveria zizanioides* roots in Thailand. J Health Res 22(1): 9-14.

14. Phisutthanan S, Phisutthanan N. 1999. Pharmacognostic study of Thai medicinal plants. Part 1: *Barleria lupulina* Lindl. and *Rhinacanthus nasutus* (L.) Kurz. Leaves. Naresuan Univ J 7(2): 88-102.

15. Jirawattanapong W, Thongchin T, Chadchen N, Boonruad T. 2008. Chemical specification of *Gynostemma pentaphyllum* (Thunb.) Makino extract. Bull Dept Med Sci 50(3): 174-84.