# Establishment of Pharmacognostic and Chemical Informations of *Rhinacanthus nasutus* (Linn.) Kurz

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Master of Pharmacy Thesis in Pharmaceutical Sciences

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| ชื่อวิทยานิพนธ์ | การสร้างรายละเอียดทางเภสัชเวทและเคมีของทองพันชั่ง |
| ผู้เขียน | นางสาวอุบล ชาติกระพันธุ์ |
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## บทคัดย่อ


การศึกษารายละเอียดทางเภสัชเวทและทางเคมีของใบและรากของทองพันชั่ง ใช้วิธีการตามที่ระบุใน Thai Herbal Pharmacopoeia โดยทำการวิเคราะห์ตัวอย่างของทองพันชั่ง ได้แก่ ใบ 15 ตัวอย่าง และราก 13 ตัวอย่าง เพื่อสร้างเป็นข้อมูลในหัวข้อต่าง ๆ ได้แก่ ลักษณะทางมหภาคและจุลภาคของพืชและผงยา การตรวจสอบเอกลักษณ์ ค่าความชื้น ปริมาณเถ้า ค่าการสกัดด้วยตัวทำละลาย และปริมาณ total rhinacanthins ซึ่งคำนวณในรูป rhinacanthin-C

ลักษณะทางจุลภาคของใบทองพันชั่งประกอบด้วย lithocyst cell เซลล์ปากใบชนิด diacytic และเซลล์ขนชนิด uniseriate multicellular trichome, glandular trichome และ collapsed trichome ลักษณะเฉพาะทางจุลภาคของผงรากทองพันชั่ง ประกอบด้วย pitted vessel, cork cell, scleried, stone cell และเซลล์ที่มีสารสีน้ำตาลแดง

จากการศึกษาพบว่ารายละเอียดค่ามาตรฐานของใบทองพันชั่ง คือ ปริมาณเถ้ารวมไม่เกิน 17.9% w/w ปริมาณเถ้าที่ไม่ละลายในกรดไม่เกิน 1.1% w/w ปริมาณสารสกัดด้วยเอธานอลไม่น้อยกว่า 2.2% w/w ปริมาณสารสกัดด้วยน้ำ ไม่น้อยกว่า 19.5% w/w และปริมาณ total rhinacanthins คำนวณในรูป rhinacanthin-C ไม่น้อยกว่า 1.1% w/w รายละเอียดค่ามาตรฐานในรากทองพันชั่ง คือ ปริมาณเถ้ารวม ไม่เกิน 24.7% w/w ปริมาณเถ้าที่ไม่ละลายในกรด ไม่เกิน 1.5% w/w ปริมาณสารสกัดด้วยเอธานอล ไม่น้อยกว่า 3.9% w/w ปริมาณสารสกัดด้วยน้ำ ไม่น้อยกว่า 22.9% w/w ปริมาณ total rhinacanthins คำนวณในรูป rhinacanthin-C ไม่น้อยกว่า 2.1% w/w ซึ่งข้อมูลเหล่านี้สามารถใช้เป็นแนวทางในการสร้างข้อกำหนดมาตรฐานของทองพันชั่ง

การศึกษาถึงการกระจายของปริมาณ rhinacanthins และผลของช่วงเวลาในการเก็บเกี่ยวต่อปริมาณ rhinacanthins พบว่า rhinacanthins จะเก็บสะสมในปริมาณสูงที่รากและใบตามลำดับ ในขณะที่ในลำต้นมีปริมาณต่ำมาก นอกจากนี้การเก็บเกี่ยวใบและรากทองพันชั่ง ในช่วงเดือนกรกฎาคมหรือช่วงที่กำลังออกดอก จะทำให้ได้ปริมาณสารสำคัญ rhinacanthins สูงสุดทั้งในใบและราก

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| Academic Year | 2003 |

## ABSTRACT

Pharmacognostic and chemical specifications of *Rhinacanthus nasutus* leaves and roots were studied using the method according to Thai Herbal Pharmacopoeia. The samples of *R. nasutus* leaves (15 samples) and roots (13 samples) were analyzed for the establishment of the informations including macroscopic and microscopic of the plant and powder drug, identification, loss on drying, ash content, extractive values, and total rhinacanthins calculated as rhinacanthin-C.

The microscopic characteristic of *R. nasutus* leaf powder was lithocyst cell, diacytic stomata and trichomes, which were uniseriate multicellular trichome, glandular trichome and collapsed trichome. The microscopic characteristic of the root powder drug was pitted vessel, cork cell, sclereid, stone cell and cell with reddish brown mass.

In this study, the informations of standard values of the leaves are as follow; total ash not more than 17.9% w/w, acid insoluble ash not more than 1.1% w/w, ethanol-soluble extractive value not less than 2.2% w/w, water-soluble extractive value not less than 19.5% w/w and the total rhinacanthins calculated as rhinacanthin-C not less than 1.1% w/w. The informations of standard values of the roots are as follow; total ash not more than 24.7% w/w, acid insoluble ash not more than 1.5% w/w, ethanol-soluble extractive value not less than 3.9% w/w, water-soluble extractive value not less than 22.9% w/w and the total rhinacanthins calculated as rhinacanthin-C not less than 2.1% w/w. The informations from this study can be used as guidelines for establishment of *R. nasutus* monograph.

Study on the distribution of rhinacanthins and the effect of harvesting period on the accumulation of rhinacanthins found that rhinacanthins were higher accumulated in the roots and leaves, respectively, while the accumulation in the stems is very low. In addition, harvesting in July or in blossom lead to the highest content of rhinacanthins in both roots and leaves.

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Ubon Chatkrapunt

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# ABBREVIATIONS AND SYMBOLS

| | | |
|---|---|---|
| cm | = | centimetre |
| $EC_{50}$ | = | effective concentration at 50% of test subject |
| $IC_{50}$ | = | inhibitory concentration at 50% of test subject |
| g | = | gram |
| $hR_f$ | = | relative retention factor |
| KOH | = | potassium hydroxide |
| $LD_{50}$ | = | lethal dose at 50% of test sample |
| mg | = | milligram |
| MIC | = | minimum inhibitory concentration |
| ml | = | millilitre |
| µg | = | microgram |
| µl | = | microlitre |
| ng | = | nanogram |
| No. | = | number |
| $\lambda_{max}$ | = | maximum wavelength |
| TLC | = | thin layer chromatography |
| UV | = | ultraviolet |
| Vis | = | visible |

# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 General informations

Despite the availability of a number of modern efficacious medicines nowadays, the herbal medicines are still widely used and their importance is increasing. However, one of the main problems of phytotherapy is the standardization of herbal preparations. The use of preparations inadequately standardized involves a considerable risk of distortion and produces a false negative overall result. Thus, reaching to a good therapeutic efficacy of an herbal medicine, its quality regarding origin, cleanliness and the content of therapeutically active constituents should be considered. The content of an active constituent in a medicinal plant depends on various factors including varieties, cultivation, harvesting, post-harvesting, packing and storage. Thus, many researchers were interested to study on pharmacognostic and the factors affecting the content of the active constituents in herbs. For example, It has been reported on chemical specification of Thai herbal drugs (Dechatiwongse Na Ayudhya *et al.*, 1993) and distribution of hydroxyanthracene derivatives in *Cassia alata* and the factors affecting the quality of the raw material (Panichayupakaranant and Intaraksa, 2003). It will be beneficial for the public consumers if an appropriate quality of pharmacognostic and chemical specifications or the monograph of crude drugs are established.

Recently, various Thai herbal plants are used widely for preparing of herbal medicines. Unfortunately, vast of commonly used herbal plants are still lacks of monograph for their quality control. In Thailand, the monographs that described the quality aspect of Thai crude drugs have been approved in the Thai Herbal Pharmacopoeia (THP). Nowadays, there are only two volumes of Thai herbal Pharmacopoeia, which contain 19 medicinal plants (Subcommittee on the establishment of the Thai Herbal Pharmacopoeia, 1995; 2000). Moreover, the monograph of crude

drugs in the other pharmacopoeias e.g. the United Stated Pharmacopoeia (USP), The British Pharmacopoeia (BP) and the European Pharmacopoeia (EP), etc. are mostly available for western crude drugs.

The plants that belong to a family of Acanthaceae are widely used as a traditional medicine. One of which is *Rhinacanthus nasutus* (Linn.) Kurz (so called "Thong phan chang" in Thai). This plant has long been widely used in Southeast Asia, South China and India for a treatment of dermatomycosis such as tinea vesicolor and ringworm (Farnsworth and Bunyapraphatsara, 1992). It has been reported that *R. nasutus* possessed several interesting biological activities, e.g. antifungal (Achararith, 1983; Wu *et al.*, 1998[a]; Panichayupakaranant *et al.*, 2000; Kongchai and Panichayupakaranant, 2002), antiviral (Kernan *et al.*, 1997), antitumour (Thirumurugan *et al.*, 2000) anti-platelate aggregation (Wu *et al.*, 1998[b]) and hypotension activities (วรรณดี แต้โสตถิกุล, 2528). 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 (มาโนช วามานนท์ และ เพ็ญนภา ทรัพย์เจริญ, 2537; ปัจจุบัน เหมหงษา, 2541). In addition *R. nasutus* is a medicinal plant that has been used as the active ingredient in several Thai herbal medicines, for example Ya-Kheaw, Ya-Kheaw Benjakhun (สุวัตร์ ตั้งจิตตรง, 2523). The roots of *R. nasutus* are categorized in Pikat Benjaloha and Navaloha, which are used for the treatment of anti-dyspepsia, flatulent, vertigo, thirsty, cold fever, skin disease, gonorrhea, abnormal menstruation, diabetes, asthma and ringworm (อรุณพร อิฐรัตน์ และ เพชรน้อย สิงห์ช่างชัย, 2533; สุวัตร์ ตั้งจิตตรง, 2523). Nowadays, there is no monograph that described the quality aspect of *R. nasutus*. We, therefore, study on an establishment of the pharmacognostic and chemical specification of *R. nasutus* leaves and roots. The pharmacognostic and chemical specification contains the information of definition, macroscopic and microscopic characteristic, chemical constituent, identification and quality specification of the plant. In this study, the valuable information was obtained from various literatures as well as our experiments.

The aims of the present study are therefore as follows:

1. To establish the pharmacognostic and chemical informations of *R. nasutus* in order to used as a guideline for an establishment of the monograph of *R. nasutus*.
2. Study on the factor affected the quality of *R. nasutus* raw materials including a distribution of rhinacanthins in plant parts and a period of plant harvesting.

## 1.2 Literature review

### 1.2.1 Botanical aspect of *Rhinacanthus nasutus*

*Rhinacanthus nasutus* (Linn.) Kurz (*Rhinacanthus communis* Nees) is a plant of the family Acanthaceae. It is so called in Thai "Thong phan chang" or "Yaa man kai". It widely distributes in Southeast Asia, South China and India (Farnsworth and Bunyapraphatsara, 1992).

The plant is a small shrub up to 1.5 m high. The stem is obtusely quadrangular, when young it is covered with fine, up curved hairs. Leaves are simple opposite. The leaf shapes are elliptic to lanceolate with the size of 4 - 6 by 2 - 3 cm, entire, light green; shortly pubescent and having acute base and apex. Flowers are white color, in short axillary clusters; densely appressed pubescent and bisexual. The calyx is divided into 5 deeply acute parted, light green, 5 - 6 mm long. The corolla tube is about 2 cm, having brownish purple spots at the throat of the tube, bilabiate, upper lip erect, bifid, lower lip 3 lobed; 4 stamens, insert in the throat; ovary 2-loculed. Capsule is loculicidally 2-valved (Farnsworth and Bunyapraphatsara, 1992) (Figure 1.1).

### 1.2.2 Ecology and propagation of *Rhinacanthus nasutus*

*R. nasutus* widely distributed in tropical countries. It scatters along the edges of evergreen forests. This plants usually grown as ornamentals and requires sandy and well-drained soil. Seed or cutting can propagate them.

**Figure 1.1** *Rhinacanthus nasutus* (Linn.) Kurz

### 1.2.3 Ethnomedical uses of *Rhinacanthus nasutus*

The whole plants of *R. nasutus* have been used for the treatment of skin diseases, oozing eczema due to lymphatic disorder, tinea vesicolor, ringworm, pruritic rash, yaws, cancer, inguinal hernia, amputating necrosis of penis and disorder of urination (นันทวัน บุณยะประภัศร, 2530). The leaves of *R. nasutus* have been used for the treatment of skin disease such as ringworm, rash, cancer, falling hair, abscess pain. In addition, the leaves have also been used as an antipyretic and anti-hypertension (วรรณดี แค้โสตถิกุล, 2528; Noriko *et al.*, 1997), health promotion, antitoxin, anti-inflammatory, antiflatulent and antihaemorroids. The roots of *R. nasutus* have been used for a treatment of skin diseases such as tinea vesicolor, ringworm, and cancer. In addition, the roots have also been used as an antipyretic and antisnake venom. The leaves and stems of *R. nasutus* have been used for health promotion (นันทวัน บุณยะประภัศร, 2530).

### 1.2.4 Fundamental use of *Rhinacanthus nasutus* in primary health cares

*R. nasutus* is an herb in the list of Thai medicinal plants that recommended for primary health care system (มาโนช วามานนท์ และ เพ็ญนภา ทรัพย์เจริญ, 2537; Farnsworth and Bunyapraphatsara, 1992). The leaves and roots of *R. nasutus* are used as an antifungal agent. Fresh or dried leaves and roots are prepared as a tincture by soaking the leaves or roots in alcohol for 7 days. The tincture is applied over the infected area 3 - 4 times a day until the infected area is completely healed, and then continuously applied for another one week.

### 1.2.5 Chemical constituents of *Rhinacanthus nasutus*

Chemical studies of *R. nasutus* reported on many compounds isolated from different parts of the plant. List of the compounds found in *R. nasutus* is shown in Table 1.1.

**Table 1.1** Chemical constituents of *Rhinacanthus nasutus*

| Chemical compound | Plant part | References |
|---|---|---|
| **1. Naphthoquinones** | | |
| 1.1 rhinacanthin-A | Roots | Wu *et al.*, 1988; Wu *et al.*, 1998[a]; Wu, *et al.* 1998[b]; Singh *et al.*, 1992 |
| 1.2 rhinacanthin-B | Roots | Wu *et al.*, 1988; Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.3 rhinacanthin-C | Whole plants and roots | Sendl *et al.*, 1996; Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.4 rhinacanthin-D | Whole plants and roots | Sendl *et al.*, 1996; Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.5 rhinacanthin-G | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |

**Table 1.1** (continued)

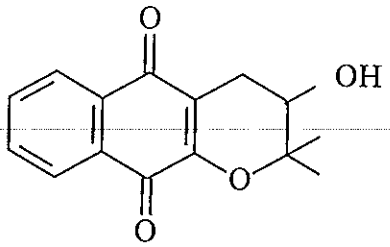
| Chemical compound | Plant part | References |
|---|---|---|
| 1.6 rhinacanthin-H | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.7 rhinacanthin-I | Leaves and roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.8 rhinacanthin-J | Leaves and root | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.9 rhinacanthin-K | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.10 rhinacanthin-L | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.11 rhinacanthin-M | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.12 rhinacanthin-N | Leaves and roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.13 rhinacanthin-O | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.14 rhinacanthin-P | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| 1.15 rhinacanthin-Q | Roots | Wu *et al.*, 1998[b] |
| 1.16 rhinacanthone | Leaves and stems | Kodama *et al.*, 1993; Kuwahara *et al.*, 1995 |
| 1.17 dehydro-α-lapachone | Roots | Wu *et al.*, 1998[a]; Wu *et al.*, 1998[b] |
| **2. Lignans** | | |
| 2.1 rhinacanthin-E | Aerial parts | Kernan *et al.*, 1997 |
| 2.2 rhinacanthin-F | Aerial parts | Kernan *et al.*, 1997 |

**Table 1.1** (continued)

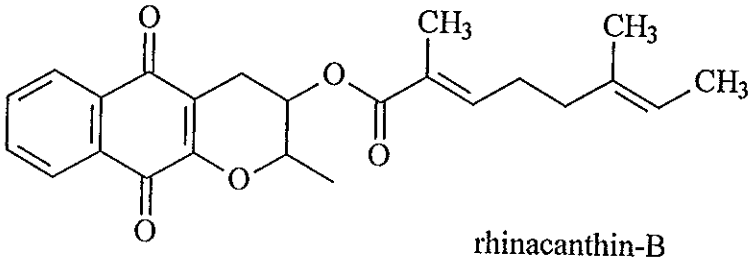
| Chemical compound | Plant part | References |
|---|---|---|
| **3. Benzenoids** | | |
| 3.1 *p*-hydroxy-benzaldehyde | Roots | Wu *et al.*, 1998[b] |
| 3.2 vanillic acid | Leaves and stems | Wu *et al.*, 1995 |
| 3.3 syringic acid | Leaves and stems | Wu *et al.*, 1995 |
| 3.4 2-methoxy-4-propionylphenol | Leaves and stems | Wu *et al.*, 1995 |
| 3.5 methyl vanilate | Roots | Wu *et al.*, 1998[b] |
| 3.6 syringaldehyde | Roots | Wu *et al.*, 1998[b] |
| **4. Anthraquinone** | | |
| 4.1 2-methyl anthraquinone | Leaves and stems | Wu *et al.*, 1995 |
| **5. Triterpenoid** | | |
| 5.1 β-amyrin | Roots | Wu *et al.*, 1995 |
| 5.2 glutinol | Roots | Wu *et al.*, 1995 |
| 5.3 lupeol | Roots | Wu *et al.*, 1988, Wu *et al.*, 1995; Wu *et al.*, 1998[b] |
| **6. Flavonoids** | | |
| 6.1 wogonin | Roots | Wu *et al.*, 1998[b] |
| 6.2 oroxylin A | Roots | Wu *et al.*, 1998[b] |
| 6.3 rutin | Flowers | Subramanian and Nagarajan, 1981 |
| **7. Sterols** | | |
| 7.1 stigmasterol | Roots | Wu *et al.*, 1988 |
| 7.2 β-sitosterol | Roots | Wu *et al.*, 1988 |
| **8. Chlorophyll** | | |
| 8.1 methylpheophorbide-A | Leaves and stems | Wu *et al.*, 1995 |

Table 1.1 (continued)

| Chemical compound | Plant part | References |
|---|---|---|
| **9. Coumarin** | | |
| 9.1 (+)-praeruptorin | Roots | Wu *et al.*, 1998[b] |
| 9.2 umbelliferone | Leaves and stems | Wu *et al.*, 1995 |
| **10. Amide** | | |
| 10.1 allantoin | Roots | Wu *et al.*, 1998[b] |
| **11. Carbohydrate** | | |
| 11.1 methyl-α-D-galactopyranoside | Leaves and stems | Wu *et al.*, 1995 |
| **12. Quinol** | | |
| 12.1 4-acetonyl–3, 5-dimethoxy-*p*-quinol | Leaves and stems | Wu *et al.*, 1995 |
| **13. Benzoquinone** | | |
| 13.1 2,6-dimethoxy benzoquinone | Leaves and stems | Wu *et al.*, 1995 |
| **14. Glycosides** | | |
| 14.1 sitosterol-β-D-glucopyranoside | Leaves and stems | Wu *et al.*, 1995 |
| 14.2 stigmasterol-β-D glucopyranoside | Leaves and stems | Wu *et al.*, 1995 |
| 14.3 3,4-dimethylphenol-β-D-glucopyranoside | Leaves and stems | Wu *et al.*, 1995 |
| 14.4 3,4,5-trimethylphenol-β-D-glucopyranoside | Leaves and stems | Wu *et al.*, 1995 |




rhinacanthin-A

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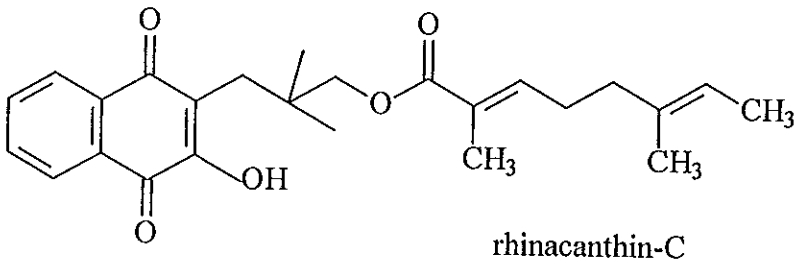
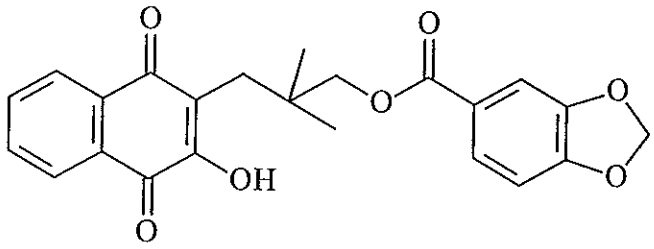
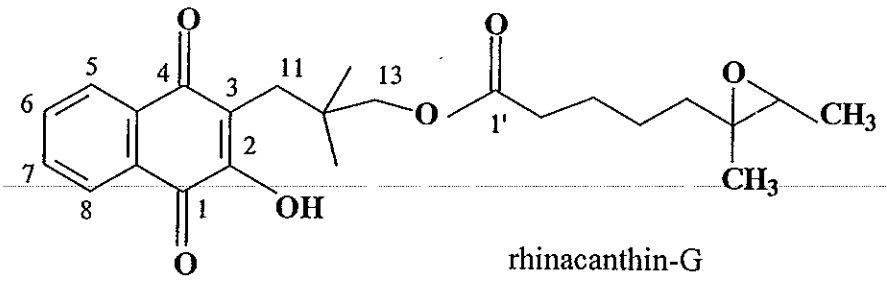



rhinacanthin-C

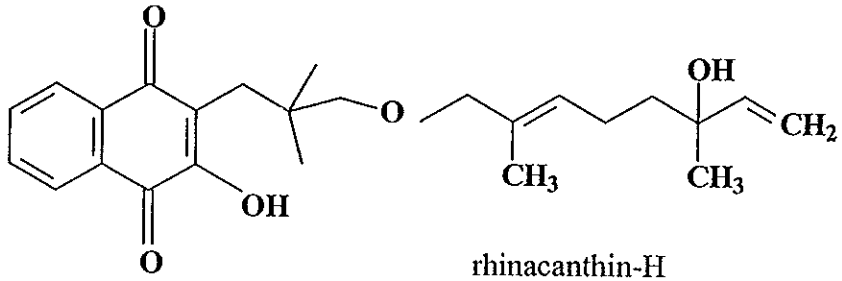



rhinacanthin-D

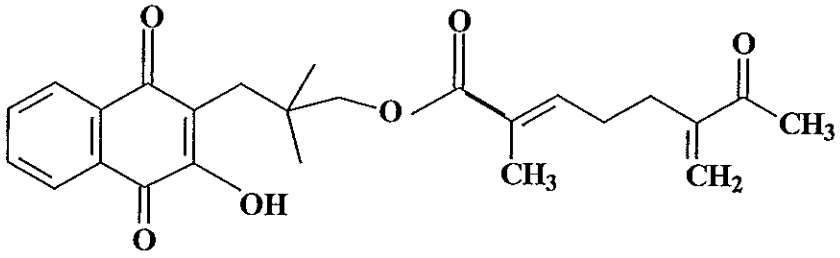
**Figure 1.2** Structure of rhinacanthins



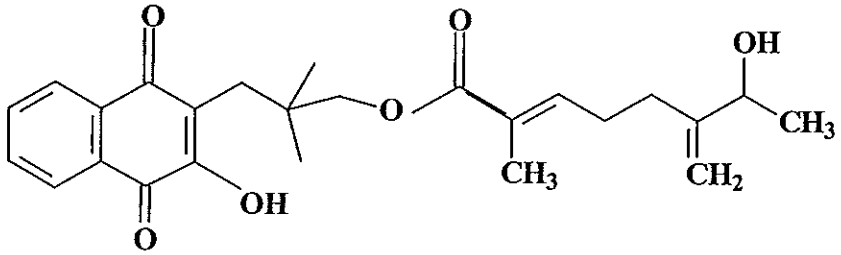

rhinacanthin-G




rhinacanthin-H

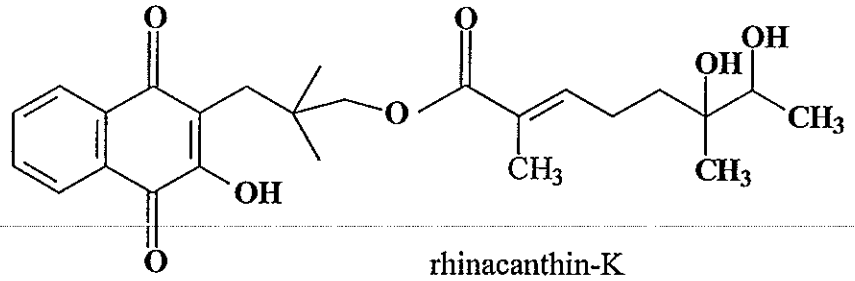



rhinacanthin-J

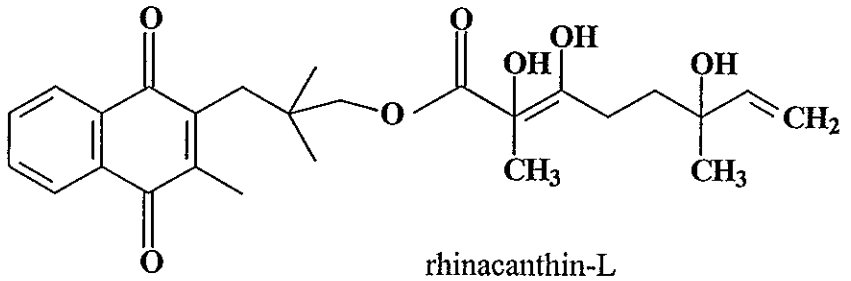



rhinacanthin-I

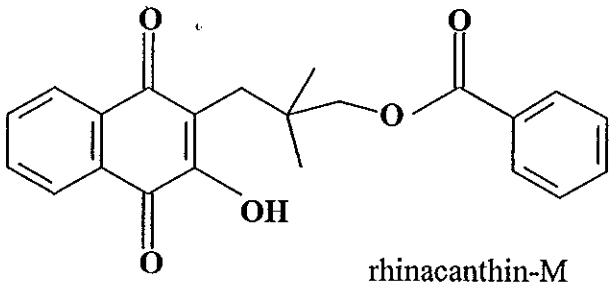
**Figure 1.2** Structure of rhinacanthins (continued)



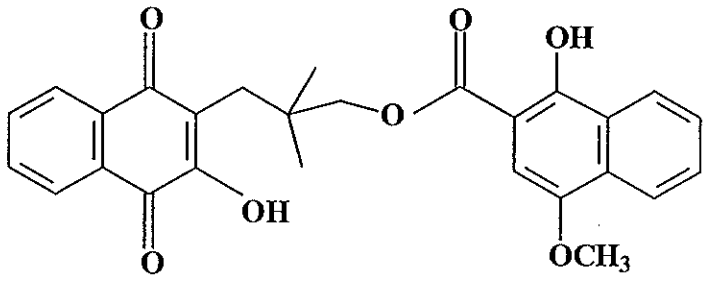
rhinacanthin-K



rhinacanthin-L

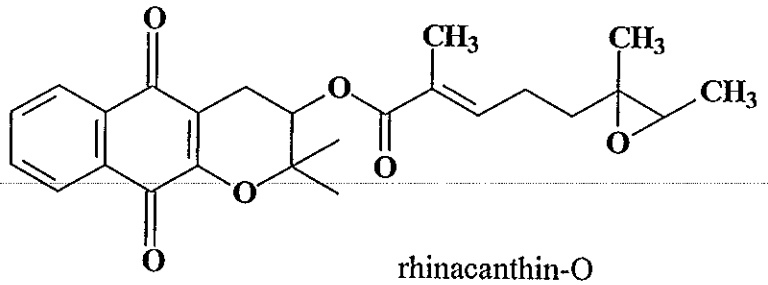


rhinacanthin-M

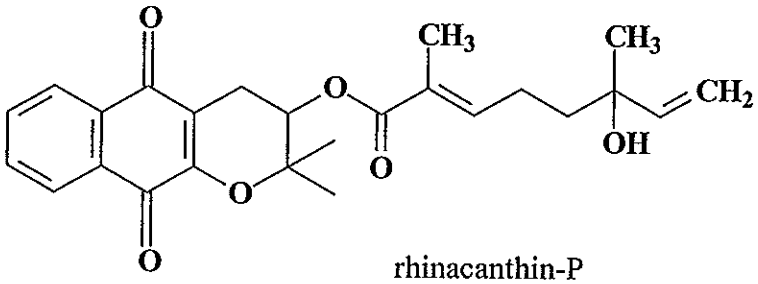


rhinacanthin-N

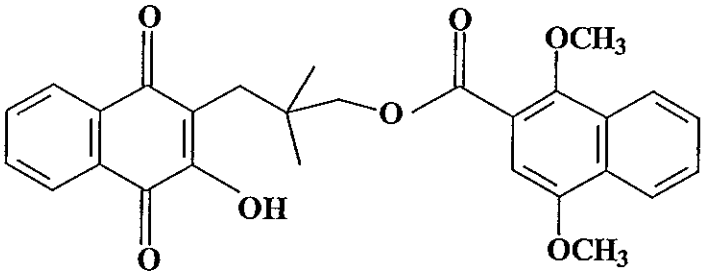
**Figure 1.2** Structure of rhinacanthins (continued)




rhinacanthin-O

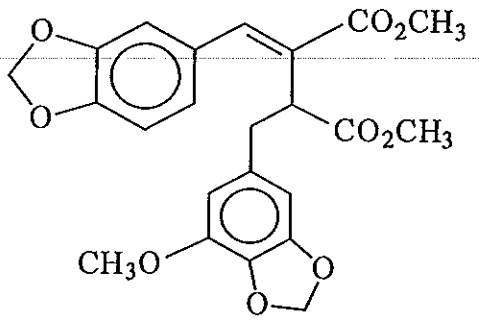



rhinacanthin-P




rhinacanthin-Q

**Figure 1.2** Structure of rhinacanthins (continued)




rhinacanthin-E: Δ7E

rhinacanthin-F: Δ7Z

**Figure 1.3** Structure of lignans

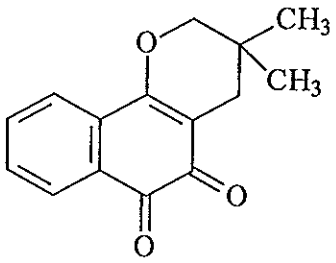



**Figure 1.4** Structure of rhinacanthone

### 1.2.6 Biological activity of *Rhinacanthus nasutus* and rhinacanthins

It has been reported that the extract of *Rhinacanthus nasutus* and the compounds isolated from this plant exhibited the interesting biological activities as follows:

**Antifungal activity**

The extracts from *R. nasutus* possessed an antifungal activity against *Microsporum gypseum*, *M. canis*, *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*, *Candida albicans*, and *Cryptococcus neoformans Saccharomyces* spp. (Farnsworth and Bunyapraphatsara, 1992; Kodama *et al.*, 1993; Akatsuka *et al.*, 1994; Panichayupakaranant *et al.*, 2000; Darah and Jain, 2001). It has been demonstrated that the water extract of *R. nasutus* leaves and stems exhibited the lowest antifungal activity, while the chloroform and 95% ethanol extracts showed similarly inhibitory activity against filamentous fungi (Farnsworth and Bunyapraphatsara, 1992).

The minimum inhibitory concentration (MIC) values of the leaf extract against *T. mentagrophytes* var. *mentagrophytes*, *T. mentagrophytes* var. *interdigitate*, *T. rubrum*, *Microsporum gypseum* and *M. canis* was reported at 13.6 mg/ml. The leaf extract exhibited fungistatic activity at lower concentration (<13.6 mg/ml or below the MIC value) and fungicidal activity at higher concentration (>13.6 mg/ml or above the MIC value). Moreover, it has been suggested that the extract of *R. nasutus* leaves acted on the cell wall of the dermatophytes which subsequently leading to the formation of cytoplasm and membrane structural degeneration and finally leading to cell lysis and death (Darah and Jain, 2001).

In addition, rhinacanthone has been demonstrated to be an antifungal active compound, which exhibited inhibitory action on spore germination of *Pyricularia oryzae* (Kodama *et al.*, 1993).

**Antibacterial activity**

The leaf and stem extract of *R. nasutus* exhibited inhibitory activity against oral *Streptococcus* spp. (22 isolates strains), which were isolated from dental plaque of 25 patient cases. It was found that the MIC of the extract was 3.8 ng/ml (Apisariyakul *et al.*, 1991).

**Antiviral activity**

The naphthoquinone esters, rhinacanthin-C and rhinacanthin-D, which were isolated from the aerial parts of *R. nasutus* exhibited an antiviral activity against human cytomegalovirus, with the $IC_{50}$ values of 0.02 and 0.22 μg/ml, respectively. However, rhinacanthin-C and rhinacanthin-D were not active against influenza virus type A, herpes simplex virus type 2 and respiratory syncytial virus (Sendl *et al.*, 1996). In addition, the lignans; rhinacanthin-E and rhinacanthin-F, which were also isolated from the aerial parts of *R. nasutus* showed significant antiviral activity against influenza virus type A, with $EC_{50}$ values of 7.4 μg/ml and 3.1 μg/ml, respectively (Kernan *et al.*, 1997).

**Cytotoxic activity**

The methanolic extract of the root of *Rhinacanthus nasutus* showed significant cytotoxicity against human KB tissue culture cell. In addition, rhinacanthin-B, which was isolated from the roots of *R. nasutus* was demonstrated to be the active compound ($ED_{50}$ = 3.0 μg/ml) against human KB tissue culture cell (Wu *et al.*, 1988).

The naphthoquinones and flavonoid including rhinacanthin-A, -B, -C, -G, -H, -I, -K, -M, -N, -Q and wogonin, isolated from the root of *R. nasutus* showed significant cytotoxic activity against P-338, A-549, HT-29 and HL-60 cells with the $ED_{50}$ values as shown in Table 1.2 (Wu *et al.*, 1998[b]).

**Table 1.2** Cytotoxic activity of naphthoquinones and flavonoid isolated from the roots of *Rhinacanthus nasutus*

| Compound | Cell lines $ED_{50}$ (μg/ml) | | | | |
|---|---|---|---|---|---|
| | KB | P-388 | A-549 | HT-29 | HL-60 |
| Rhinacanthin-A | 6.75 | 0.72 | 3.06 | 2.17 | 1.16 |
| Rhinacanthin-B | 8.01 | 0.35 | 6.50 | 3.01 | 2.57 |
| Rhinacanthin-C | 6.26 | 0.26 | 0.35 | 0.68 | 0.68 |
| Rhinacanthin-D | 25.0 | 3.79 | 8.26 | 8.89 | 11.8 |
| Rhinacanthin-G | 4.45 | 0.14 | 0.75 | 0.57 | 1.14 |
| Rhinacanthin-H | 23.8 | 6.43 | 9.97 | 11.5 | 8.87 |
| Rhinacanthin-I | 13.2 | 4.88 | 7.18 | 6.30 | 5.12 |
| Rhinacanthin-K | 17.3 | 3.17 | 16.4 | 7.75 | 6.81 |
| Rhinacanthin-M | 19.2 | 3.95 | 8.90 | 10.1 | 19.9 |
| Rhinacanthin-N | 4.80 | 0.71 | 1.97 | 2.67 | 1.38 |
| Rhinacanthin-Q | >50 | 0.61 | 3.61 | 7.60 | 8.90 |
| Wogonin | 4.46 | 1.70 | 4.14 | 3.35 | 4.66 |

**Antitumour activity**

The antitumour activity of rhinacanthone against Dalton's ascitic lymphoma (DAL) in mice has been reported (Thirumurugan *et al.*, 2000). A significant enhancement of mean survival time of tumor bearing mice and peritoneal cell count in normal mice was observed with respect to the control group.

**Hypotensive activity**

The extract obtained from hot water maceration (decoction) was studied in anesthetized rats for their pharmacological action. The hypotensive activity of *R. nasutus* extract were found to increase with correlation to the amount of the extract (วรรณดี แต้โสตถิกุล, 2528).

**Antiplatelet activity**

The antiplatelet aggregation of naphthoquinones, isolated from the roots of *R. nasutus* including rhinacanthin-A, -B, -C, -G, -H, -I, -K, -M and -Q has been reported. These compounds demonstrated 36 - 100% inhibition of rabbit platelet aggregation induced by arachidonic acid (100 mM). Rhinacanthin-A, -B and -C (10 μg/ml) showed 72 - 100% inhibition of the rabbit platelet aggregation induced by collagen, while rhinacanthin-B (2 ng/ml) inhibited platelet aggregation induced by platelet activation factor (Wu *et al.*, 1998[b]).

**Insect attractant and signaling properties**

An ether extract of the roots of *R. nasutus* exhibited the properties of an insect attractant and signaling to male Mediterranean fruit flies but showed equivocal results on *Aspiculurus tetraptera*, both male and female melon flies and oriental fruit flies (*Dacus dorsalis*) (Farnsworth and Bunyapraphatsara, 1992).

**Juvenile hormone activity**

An ether extract of *Rhinacanthus nasutus* roots, at a dose of 500.0 μg/animal exhibited juvenile hormone activity on *Oncopeltus fasciatus*, but no activity was observed at a dose of 250.0 μg/animal (Farnsworth and Bunyapraphatsara, 1992).

### 1.2.7 Toxicity test of *Rhinacanthus nasutus*

Acute toxicity of *R. nasutus* has been studied in mice. The crude extract of *R. nasutus* was administered by both oral feeding and intradermal injection. The dose of the extract in this study was 3333 times higher than the normal dose. The results showed no toxicity symptoms in mice (นันทวัน บุณยะประภัศร, 2530; 2541).

In addition, the crude extract of *R. nasutus* leaves at the dose of 0.5 - 1 g/kg exhibited no toxic symptoms in mice (พิสิษฐ์ ศรสวัสดิ์, 2540).

### 1.2.8 Thai Herbal Pharmacopoeia

In 1989, the Thai Pharmacopoeia Committee appointed a subcommittee, the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, to establish the Thai Herbal Pharmacopoeia, a companion publication to the existing Thai Pharmacopoeia. The aims of the establishment of the Thai Herbal Pharmacopoeia are to upgrade the status of Thai herbal drug as well as to promote their commercial uses and international recognition. The Thai Herbal Pharmacopoeia is intended to be a legal compendium, which defines and sets standards for all natural products of medicinal value. Despite the majority of the monographs being of plant origin due to its extensive use, various terms described, such as herbal drug or material, are referred not only to plants but also to animals and minerals used for medicinal purposes as well (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1995).

Thai Herbal Pharmacopoeia (THP) has been first established in 1995 and used for the quality assessment of Thai herbal raw material (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1995). Recently, two volumes of Thai Herbal Pharmacopoeia, volume 1 (1995 and 1998) and volume 2 (2000) have been launched. The herbs that have been approved in THP volume 1 (1995) include Boraphet (*Tinospora crispa)*, Chum Het Thate (*Senna alata*), Pha Tha Lai *(Andrographis paniculata),* Kaprao Daeng *(Ocimum tenuiflorum),* Mawaeng Krueo *(Solanum trilobatum),* Khamin Chan *(Curcuma longa),* Phlai (*Zingiber cassumunar*), Phrik Thai Dam; Phrik Thai Lon *(Piper nigrum),* Sawaat (*Ceasalpinia bonduc*), Taan Mon (*Vernonia elliptica*) (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1995). THP volume 1 has been revised in 1998. However, the herbs that have been approved are also the same as in 1995. The herbs that have been approved in THP volume 2 (2000) include Dee-Plee *(Piper retrofractum),* Gratium (*Allium sativa),* Maag Soeng *(Areca catechu)*, Ma-Grood (*Citrus hystrix*), Ma-Kham Pom (*Phyllantus emblica*), Pluu (*Piper betle*), Sa-Maw Phi-Phek (*Terminalia bellirica*), Sa-Maw Thai (*Terminalia chebula*), Waan-Nam *(Acorus calamus)* (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 2000).

The informations given in the monograph of the Thai Herbal Pharmacopoeia are as follows:

1. Monograph nomenclature

Thai name is adopted as "main title" of each pharmacopoieal substance. It is transcripted to English following the Royal Institute's official transliteration system and printed with capital letters. Subsidiary title are Latin genitives of plant and where available, common English names.

2. Category

The statements given under "Category" are provided only for information on the drug's main pharmacological action which are presumeably based on its use in traditional medicine. It should not be assumed that the substance has no other action.

3. Definition of the herbal includes parts used and sciencetific name.

4. Constituents

The compounds which have been found in the plants.

5. Description of the plant

In addition to macroscopical and microscopical descriptions of crude drug, the morphological and anatomical descriptions of plants are provided for the botanical identification of the samples.

Macroscopical description in the monographs refer to features which can be seen by the unaided eye or with aid of a hand lens. Statements of the characteristic microscopial descriptions of the whole drug are included in the monograph as a means for determining, identity, quality or purity.

6. Packaging and storage

The suitable process of drying and preservation should be done to prevent the deterioration of plant materials.

7. Identification

Identification tests of active/main constituents are performed by preliminary test and confirmatory test (Chromatographic analysis).

Preliminary test is the procedure to chemically detect the group of active constituents by color reaction, formulation of precipitate or any others chemical

reactions that are useful tools for more sophisticated testing of the chemical component further.

Confirmatory test is the chemical procedures or techniques to determine the composition of the group of active constituents previously identified in the preliminary testing. Thin layer chromatography is used as one of the principle means of identification of herbal drugs. In some cases were isolated constituents of herbal drugs are commercially available, chromagraphically-separated constituents are related to known constituents as markers. For purposes of evaluation, an $hR_f$ value is in place of an $R_f$ value in order to preclude the use of decimal fractions. The $hR_f$ value is $R_f$ value multiplied by the factor 100, resulting in value of 0 - 100. In the monograph, the $hR_f$ value of known and unknown constituents are listed in the table, accompanied by the corresponding thin layer chromatograms. The illustration of thin-layer chromatograms are provided in color drawing.

8. Moisture content

The presence of excessive water in herbal drugs will promote the growth of microbials, fungi or insects and the hydrolysis of constituents leading to deterioration of the drug. Therefore, limits of water should be prescribed for herbal drugs, especially absorb moisture or in which deterioration is promoted in the presence of excessive water. The pharmacopoeial limits of water for herbal drugs are usually 8-14% (e.g. Digitalis leaf, 6%).

Two methods may be used for determining the water content of herbal drugs, *viz* the gravimetric (or drying) method and azeotropic (or toluene distillation) method. The gravimetric method determines the moisture content by heating the plant material in the oven until completely dry and the "loss on drying" weight of the plant material is determined as the moisture content. This method is easier to use but not applicable to plant which contain volatile substances other than water. Azeotropic distillation method determines moisture content by measuring the water content obtain by distillation. It is suitable for plant materials containing volatile substances other than water, e.g. herbal materials containing volatile oils.

9. Ash content

The determination of ash is a method to measure the residual substance not volatized when the drug sample is ignite by the method described. Ash may be

derived from plant tissue itself and is usually called "physiological ash". Ash may also be come from extraneous substances. The methods for determination of ash content include determination of total ash and acid insoluble ash. The limits of ash wherever possible drafting the specific monograph for herbal drugs.

Total ash which is the sum of the amount of physiological ash derived from plant tissue and non-physiological ash derived from other foreign matters e.g. rock, soil, sand, etc. Generally, the amount of total ash should be between 1 - 20%. Acid-insoluble ash is used to determine the amount of inorganic foreign matters, e.g. gravel, soil, sand, contaminated in the plant material. In general, the amount of acid insoluble ash should be between 1 - 10% (Trease and Evans, 1983).

10. Extractives

The determination of extractive is a method designed to measure the amount of constituents which are extractable by the appropriate solvent under the specific condition. The determination of water-soluble or ethanol-soluble extractive is used as a means of evaluating drug constituents of which are not ready estimated by others means. But as suitable assays become available (e.g. with the anthraquinone containing-drugs), the extractive test are no longer required as pharmacopoeial standards. In certain cases extraction of the drug is by maceration, in others by a continuous process.

11. Quantitative determination

Quantitative determination is a method for determination of the content of an active ingredient. Unless otherwise specified, all quantitative determinations prescribed in the monograph are carried out on materials which have not have specially dried and calculations are made accordingly.

12. Other information

Other pieces of information that will be useful for the safe and effective utilization of herbal medicine and should be give to the consumers are indication, toxicity, contraindication, warning, precaution, dosage form, strength and dosage.

### 1.2.9 Factors affected the quality of medicinal plants

The quality of the medicinal plant raw materials is inevitably inconstant, depending on a variety of factors, including age and origin, harvesting period, the

specific parts of the plant to be process, the drying and storage, etc (Capasso *et al.*, 2003).

The effect of harvesting factor and post-harvesting on the quality of *Cassia alata* raw material have been reported (Panichayupakaranant and Intaraksa, 2003). It was found that when *C. alata* leaves had been harvested in March, June or September, the hydroxyanthracene delivatives were accumulated more in the leaf position 1 - 3 (1.82, 1.25, 1.63% w/w, respectively) and 4 - 6 (1.39, 1.58, 1.09% w/w, respectively). In December (flowering and fruiting season), hydroxyanthracene derivatives were accumulated more in the flowers (2.21% w/w) and the pods (1.82% w/w), respectively. In addition, the method and temperature of drying also markedly affected the hydroxyanthracene derivative content. It was found that drying of the leaves in a hot air oven at 50°C gave a higher hydroxyanthracene derivativescontent (1.43% w/w) than drying in a hot air oven at 80 °C (0.44% w/w) or drying in the sun (0.95% w/w).

The effect of harvesting factor on the quality of *Andrographis paniculata* raw material has also been reported (Dechatiwongse Na Ayudhya *et al.*, 1993). It was found that the active constituents, the lactone compounds were more accumulated in the leaves and the most suitable time for harvesting is before or during the beginning of blossom period because the plant gave a high yield of the lactone content.

# CHAPTER 2

# EXPERIMENTALS

## 2.1 Plant materials

*Rhinacanthus nasutus* leaves (15 samples) and roots (13 samples), each sample were collected in 2002 - 2004 from various provinces of Thailand, including Songkhla ( amphur Hat-Yai and amphur Jana), Narathiwat, Phetchabun, Phang-nga, Pattani, Phattalung, Nakhonpathom, Chanthaburi, Chiang Mai and Surin. Some samples were purchased from drug stores. The entire samples were harvested from 1-year-old plants, during blooming. The fresh leaf and root samples from Songkhla and Narathiwat Provinces were directly harvested from the field, while those from Phetchabun, Phang-nga, Pattani, Phattalung, Nakhonpathom, Chanthaburi, Surin, Chiang Mai and the drug stores were purchased as dried samples.

## 2.2 Chemicals

- Absolute ethanol, AR grade (Merck® Germany)
- Chloroform, AR grade (Merck® Germany)
- Ethyl acetate, AR grade (Lab-SCAN®, Ireland)
- Methanol, AR grade (Lab-SCAN®, Ireland)
- Potassium hydroxide AR grade (UNIVAR®, Australia)
- Standard rhinacanthin-C, was isolated from the leaves of *Rhinacanthus nasutus* (Panichayupakaranant *et al.*, 2000)
- Silica gel 60 $F_{254}$ precoated plate (Merck®, Germany)
- Filter paper (Whatman® No. 4)
- Ashless filter paper (Whatman® No. 42)

## 2.3 Instruments

- Digital camera (Camera, USA)
- Hot air oven (Thailand)
- Microscopic camera (Nikon® Microscope, Wild® MPS 12 Camera, Switzerland)
- Microscope (Nikon®, Japan)
- Muffle furnace (NEYTECH® 85P, USA)
- Analytical balance (OHAUS® E02140, USA)
- Spectrophotometer UV-Vis RS (Digital Spectrophotometer Labomed®, USA)
- Sartorious moisture analyzer (Sartorious® Model MA100/MA 50 Electronic, Germany)

## 2.4 Methods

### 2.4.1 Preparation of the leaf and root samples

The fresh leaves and roots harvested from Songkhla and Narathiwat Provinces were washed and dried in hot air oven at 50 - 60°C. The samples that received as dried materials were subjected to removal of foreign matter and dried in hot air oven at temperature 50°C, 24 hours for leaves and 60°C, 48 hours for roots. The dried samples were ground to the fine powder and passed through the sieve number 45. The dried leaf and root powders were kept in a well-closed container and in desiccator.

### 2.4.2 Macroscopic characterization

The macroscopic characteristic of *Rhinacanthus nasutus* was studied using both fresh and dried plants collected from the botanical garden of Faculty of Pharmaceutical Sciences, Prince of Songkla University. The morphological data of the plant was recorded and photographed. A Voucher specimen collection No. 001 18 14 of *R. nasutus* was kept at Southern Center of Traditional Medicine, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

### 2.4.3 Microscopic characterization

Microscopic characteristic of *Rhinacanthus nasutus* was studied on histological informations of the fresh leaves and roots and their powdered drugs using a microscope with camera.

### 2.4.4 Identification test

#### 2.4.4.1 Preliminary test

The preliminary test of the powder drugs was performed on the basic of the Borntrager's reaction (Trease and Evans, 1989) with a little modification. The dried powders of Rhinacanthus nasutus leaves or roots were accurately weighed about 100 mg and extracted by sonication with ethyl acetate (10 ml) for 30 minutes. The extracts were then filtered through filter paper (Whatman No. 4). A solution of 20% potassium hydroxide (KOH) in water (3 ml) was added into the obtained extract (3 ml) to produce orange–red color in an ethyl acetate layer.

#### 2.4.4.2 Confirmatory test

Thin layer chromatography (TLC) was used for confirmatory test of *Rhinacanthus nasutus* powder drugs. The procedure is as follow;

- Silica gel 60 $F_{254}$ precoated plate was used as a stationary phase and chloroform : hexane (8 : 2) was used as a mobile phase. Rhinacanthin-C was used as the standard marker.
- The dried powders (500 mg) were extracted with methanol (10 ml) under reflux condition for 10 minutes. The extracts were allowed to cool and filtered through a plug of cotton wool.
- Preparation of the standard solution of rninacanthin-C. The standard rhinacanthin-C was accurately weighed about 10 mg and dissolved with methanol, and the volume adjusted to 10 ml.
- The standard and test solutions (10 µl) were spotted on the TLC plate. The TLC plate was then developed in a TLC tank, using chloroform : hexane (8 : 2) as developing solvent, and allowed

the solvent front to 10 cm. After that the plate was dried to remove the solvent, and detected the chromatogram under UV 254 and 366 nm and sprayed with 20% KOH in methanol. The $hR_f$ values of the entire spots and the color of the spots positive to 20% potassium hydroxide solution were recorded.

### 2.4.5 Determination of the moisture content

Moisture content of the powdered drug of *Rhinacanthus nasutus* leaves and roots were performed by loss on drying method. The powdered drugs were accurately weighed about 2 g in the pan of the Sartorious Moisture Analyzer and dried at 105 °C for 4 - 8 minutes until the weight was constant. The percentage loss on drying of the test sample was automatically recorded. The analyses in all samples were in triplicate.

### 2.4.6 Determination of the ash content

#### 2.4.6.1 Total ash

The powdered drugs were accurately weighed about 2 g and placed in a tared crucible, which was previously ignited, cooled and weighed. The sample was incinerated by gradually increasing the temperature not exceeding 450 °C in muffle furnace until free from carbon, then cooled and weighed. The percentage of the total ash was calculated with reference to the weight of the dried powdered drug.

#### 2.4.6.2 Acid insoluble ash

The total ash obtained from section 2.4.6.1 was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. The mixture was then filtered through an ashless filter paper. The filter paper was then washed with hot water until the filtrate is neutral. After that, the filter paper was ignited at about 500 °C in muffle furnace, then cooled and weighed. The percentage of the acid insoluble ash was calculated with reference to the weight of the dried powdered drug.

### 2.4.7 Determination of the extractive values

#### 2.4.7.1 Water-soluble extractive

The powdered drug was accurately weighed about 5 g and macerated in chloroform water (0.25% v/v chloroform) 100.0 ml in 250-ml Erlenmeyer flask for 24 hours. The flask was frequently shaken during the first 6 hours, and allowed to stand for 18 hours. The extract was then rapidly filtered. An adequate of the filtrate (20.0 ml) was evaporated to dryness and dried at 105°C to constant weight. The percentage of water-soluble extractive was calculated with reference to the weight of the dried powdered drug.

#### 2.4.7.2 Ethanol-soluble extractive

The powdered drug was accurately weighed 5.0 g and macerated in ethanol 100.0 ml in 250-ml Erlenmeyer flask for 24 hours. The flask was frequently shaken during the first 6 hours, and allowed to stand for 18 hours. The extract was then rapidly filtered. An adequate of the filtrate (20.0 ml) was evaporated to dryness and dried at 105°C to constant weight. The percentage of ethanol-soluble extractive was calculated with reference to the weight of the dried powdered drug.

### 2.4.8 Quantitative determination of the total rhinacanthins

The total rhinacanthin content calculated as rhinacanthin-C was determined on the basis of spectrophotometric method (Panichayupakaranant and Kongchai, 2002)

#### 2.4.8.1 Standard curve of rhinacanthin-C

A stock solution of the authentic rhinacanthin-C was prepared by dissolving rhinacanthin-C (10.0 mg) in methanol (25.0 ml) to give a concentration of rhinacanthin-C 0.4 mg/ml. Adequate portions of the stock solution were diluted with methanol to give the standard solutions of 160, 128, 96, 32 and 16 μg/ml. The standard solutions (4 ml) were mixed with 20% potassium hydroxide (KOH) solution (1 ml), allowed to stand for 15 minutes and then the absorbance of each solution was determined with Spectro UV-Vis RS at 468 nm, using methanol as a blank. The

standard curve was obtained by plotting the absorbance versus the concentrations of rhinacanthin-C, and calculated the linear regressions and coefficients coefficient of determination ($R^2$).

#### 2.4.8.2 Study on the extraction time

The powdered drug of *Rhinacanthus nasutus* leaves (300 mg) was extracted under reflux condition with methanol (50.0 ml). The extraction time was varied as 30, 60 and 120 minutes. The extract was filtered through a filter paper and the filtrate was evaporated to dryness. The residues were then dissolved in methanol and adjusted to 25.0 ml to afford the stock solution of the sample. The stock solutions of the samples were diluted 10 times with methanol. The obtained solutions (4 ml) were mixed with 20% KOH solution (1 ml), allowed to stand for 15 minutes and then determined the absorbance at 468 nm, using methanol as a blank. The total rhinacanthins of each sample was calculated as rhinacanthin-C using the standard curve of the authentic rhinacanthin-C. The analyses in all experiments were in triplicate.

#### 2.4.8.3 Determination of the total rhinacanthins in *Rhinacanthus nasutus* powdered drugs

Total rhinacanthins from different sources 15 samples (leaves) and 13 samples (roots) were determined. The dried powder of *Rhinacanthus nasutus* (300 mg) was refluxed with methanol (50 ml) for 30 minutes. The extract was filtered through a filter paper and the filtrate was evaporated to dryness. The residues were then dissolved in methanol and adjusted to 25.0 ml to afford the stock solution of the sample. The stock solutions of the samples were diluted 10 times with methanol. The obtained solutions (4 ml) were mixed with 20% KOH solution (1.0 ml), allowed to stand for 15 minutes and then determined the absorbance at 468 nm, using methanol as a blank. The total rhinacanthins of each sample was calculated as rhinacanthin-C using the calibration curve of the authentic rhinacanthin-C. The analyses in all experiments were in triplicate.

### 2.4.9 Distribution of rhinacanthins an the effect of harvesting period on rhinacanthin content in *Rhinacanthus nasutus*

The leaves, stems and roots of *R. nasutus* were separately harvested in April 2003, July 2003, October 2003 and January 2004 from botanical garden, Faculty of Pharmaceutical Sciences, PSU. The plant material were then dried and determined the total rhinacanthin content by the method as described in the section 2.4.8.3.

## 2.5 Data analysis

The data analysis was calculated as the mean ± S.D. for the study in 2.4.4 - 2.4.8. The data in 2.4.9 were analyzed by one way analysis of variance (ANOVA; complete factorial design) (Miller and Miller, 1994). The level of statistical significance was taken at $P < 0.05$.

# CHAPTER 3

# RESULTS AND DISCUSSION

## 3.1 Macroscopic characteristic

### 3.1.1 Plant description

The authentic of *Rhinacanthus nasutus* was collected from the botanical garden, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

The characteristic of *R. nasutus* was described from the authentic plant. *R. nasutus* is a small shrub with 70 - 200 cm height. The stems are erect and branched. The leaves are simple, opposite. The shape of the leaves is lanceolate with 2.5 - 5 cm wide and 6 - 12 cm long. The base of leaves is oblique. The leaves are glabrous yellowish green. Flowers are bisexual, zymgomophic petal and white colour in short axillary clusters. The bract is small. The calyx is devided in to 5 deeply acute parted, light green, 5 - 6 mm long. The corolla tube is bilabiate, upper lip erect, bifid, lower lip 3 lobed. The corolla has brownish purple spots at the throat of the tube. There are 4 stamens with didynamous. The ovary is superior with 2-loculed and ovule free placentation. (Figure 3.1). Unfortunately, the authentic plants in this study have no fruit. However, it has been reported that the fruit of *R. nasutus* is a capsule (Farnsworth and Bunyapraphatsara, 1992).

### 3.1.2 Crude drug description

The characteristic of the dried leaves are greenish-brown with fragment of leaf 2 - 5 cm wide and 3 - 5 cm long (Figure 3.2; A). The taste and odour are characteristic. The dried roots are fragment with 0.2 - 0.4 cm in diameter and 2 - 4 cm long. Odour and taste are characteristic (Figure 3.2; B).

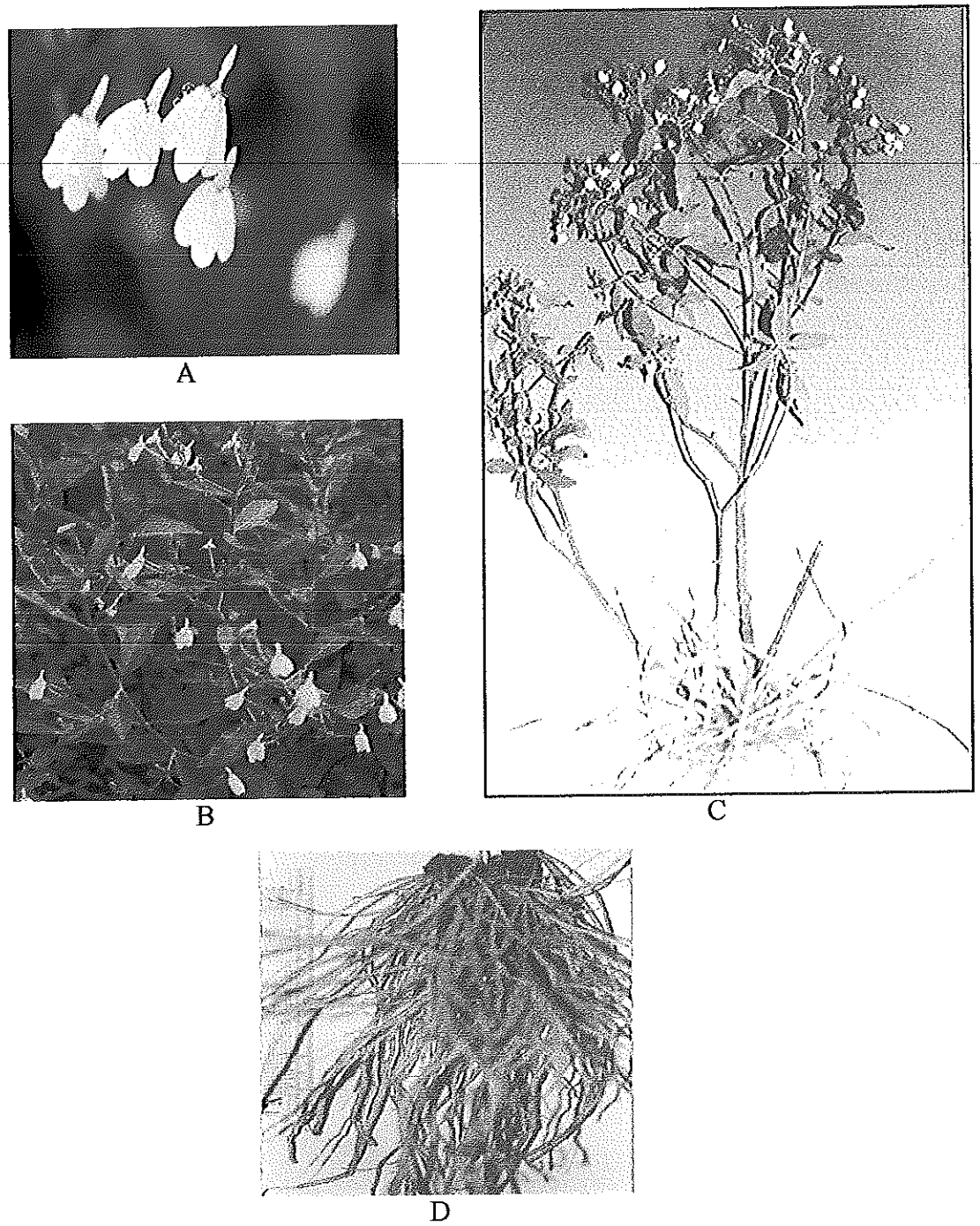



**Figure 3.1** *Rhinacanthus nasutus* (Linn.) Kurz: flowers (A), small shrub of plant (B) whole plant (C), roots (D)

A

B

**Figure 3.2** Crude drugs of *Rhinacathus nasutus*:

A. dried leaves

B. dried roots

## 3.2 Microscopic characteristic

### 3.2.1 Morphology and histology of the leaves (Figure 3.3, Figure 3.4)

The histological characteristic of the leaf in transverse section are described as follows;

1. **Upper epidermis**, which the upper layer of surface view are composing of irregularly shape cells with slightly wavy walls in surface view and rectangular cells in section view. The stoma is absent or very infrequent. There are some large lithocyst cells and glandular trichomes (Figure 3.3; 1 and Figure 3.4; 1).
2. **The mesophyll** consists of a single layer of palisade cells containing chloroplast and globule of pale greenish yellow contents (Figure 3.3; A and Figure 3.4; 2).
3. **Vascular bundle** compose of phloem and xylem surrounded by thin-walled parenchyma cell. Vessel is spiral (Figure 3.4; 4).
4. **Lower epidermis** are similar to those of upper epidermis but they are smaller and more wavy-walled (Figure 3.3; C). There are numerous diacytic stomata (Figure 3.3; C), lithocyst cell containing calcium carbonate and glandular trichomes (Figure3.3; B).

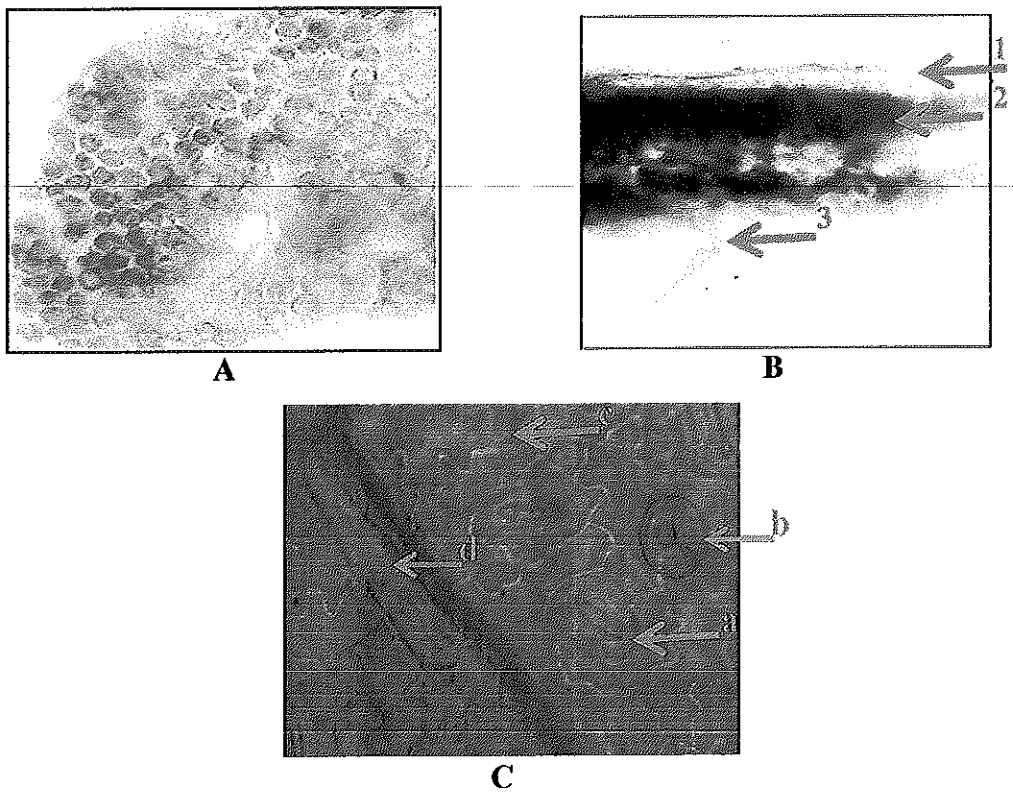



**Figure 3.3** Morphology of *Rhinacanthus nasutus* leaves: **A**. palisade cells with chloroplast, **B**. transverse-section of leaves; upper epidermis (1), palisade cells (2), uniseriate multicellular trichomes (3), **C**. lower epidermis with diacytic stomata (a), glandular trichome (b) irregular shape and wavy wall cell (c), lithocyst cell (d)

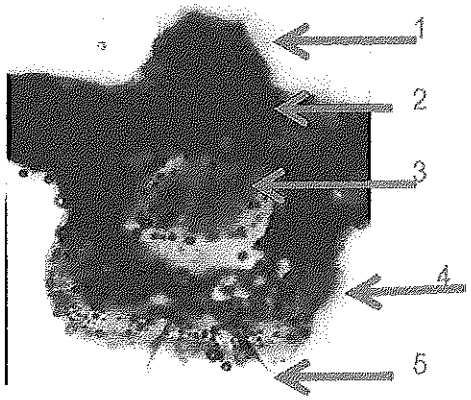



**Figure 3.4** Transverse section of the midrib of *Rhinacanthus nasutus* leaf

1. upper epidermis
2. mesophyll
3. vascular bundle
4. lower epidermis
5. uniseriate trichome

### 3.2.2 Morphology and histology of the roots (Figure 3.5)

The histological characteristic of the roots in transverse section are described as follows;

1. **Epidermis** consists of a single layer of thin-walled cell. The surface view composed of rectangular shape cells. There are some large lithocyst cells.
2. **Collenchyma** is an inner region of the cortex (2). A few of chlorophyll and oil globule are spread surrounded the layer.
3. **Endodermis** consists of a single layer of thin-walled cell and vascular bundles without fiber (3).
4. **Vascular bundle** composes of phloem and xylem (5) surrounded by crystal fiber and lithocyst cells (4).

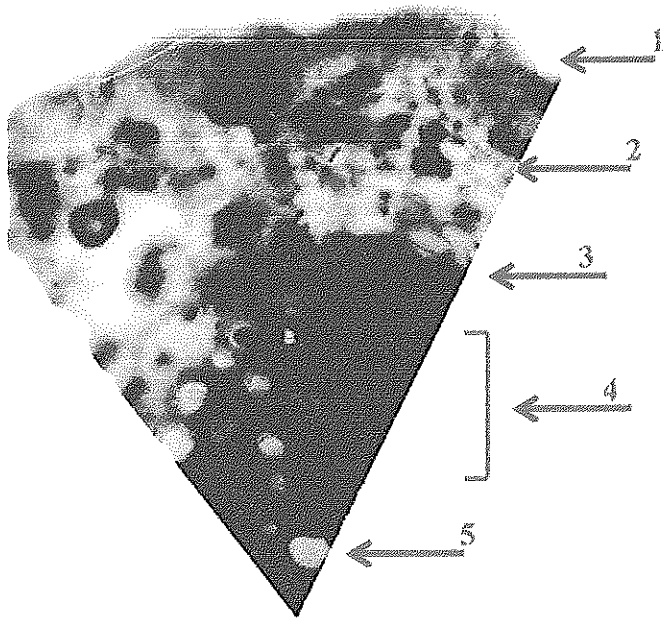


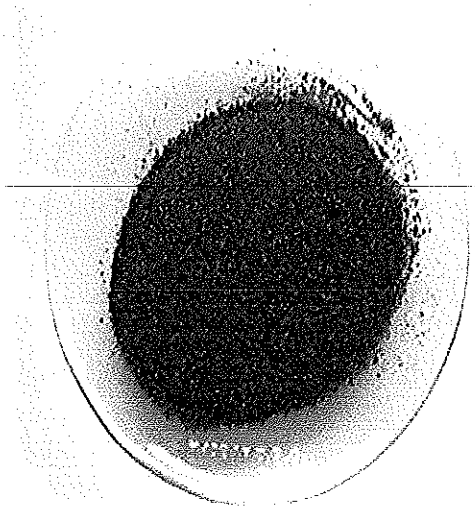

**Figure 3.5** Transverse section of *Rhinacanthus nasutus* root.

1. epidermis
2. cortex with collencyma
3. endodermis
4. vascular bundle
5. xylem

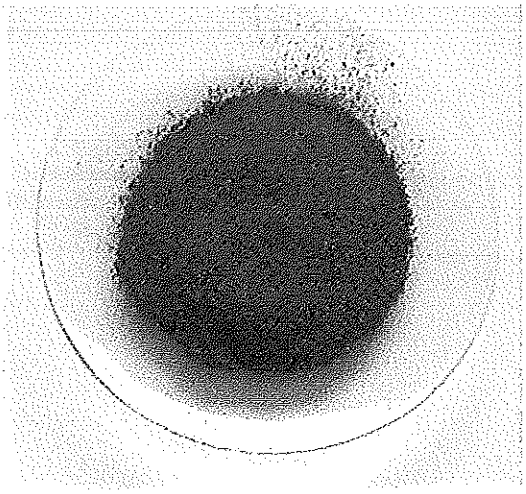
### 3.2.3 Microscopic characteristic of *Rhinacanthus nasutus* powdered drug

**3.2.3.1 Leaf powder**: The powder is yellowish-green with a characteristic odour (Figure 3.6). The characteriatics of the leaf powder (Figure 3.7) are as follows;

1. The epidermis are composed of wavy-walled cells with numerous diacytic stomata (1), glandular trichome (2), uniseriate multicellular trichome and collapse trichome (6).
2. The abundant of mesophyll parenchyma containing chloroplast (3).
3. The lithocyst cell (4) and fragments of the lamina (5) are found scattered.
4. The fragment of covering trichome, multicellular and collapse trichome (6) are found scattered and attached to the fragment of the epidermis. They are uniseriate trichome.
5. The abundant of fragment of epidermis and lithocyst cell (8).
6. The occasional fragments of the fibers with parenchyma containing reddish brown mass (7), fiber with spiral vessel (9) and fragment of fibers (10).



A



B

**Figure 3.6** Powdered drugs of *Rhinacanthus nasutus*

A. leaf powder

B. root powder

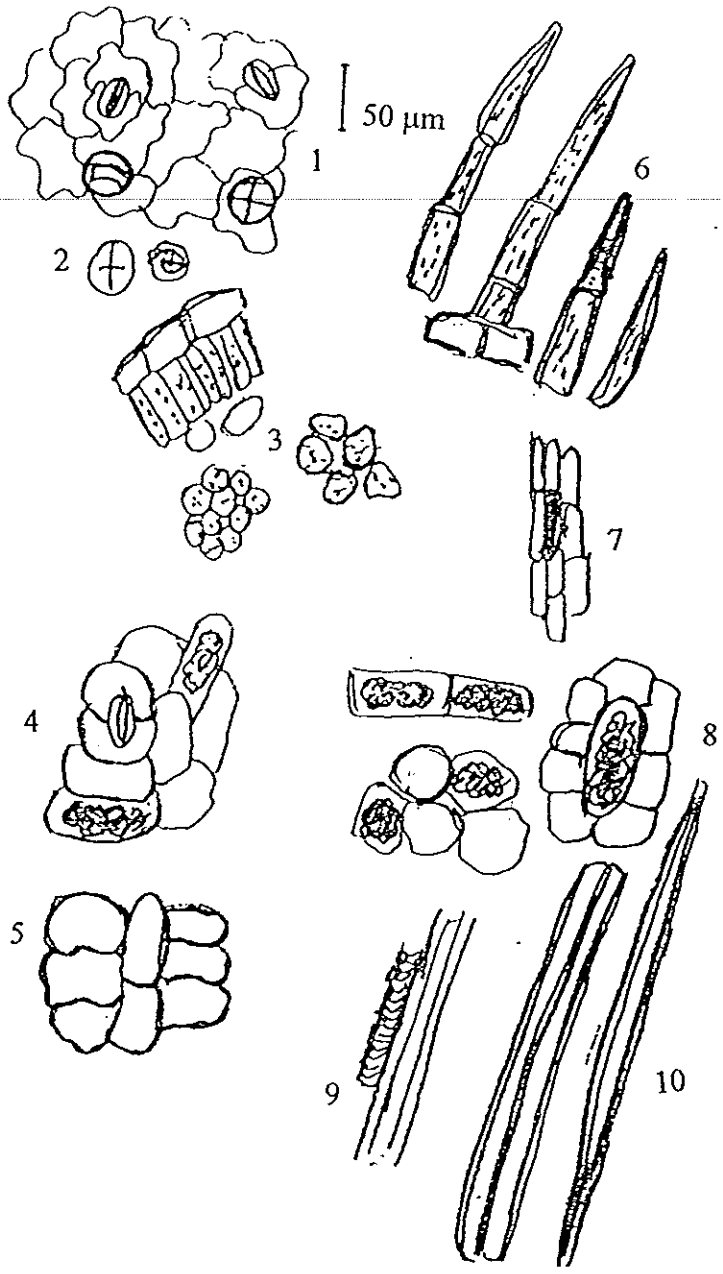



**Figure 3.7** Powdered drugs of *Rhinacanthus nasutus* leaves

1. lower epidermis, diacytic stomata and glandular trichomes
2. glandular trichomes
3. mesophyll parenchyma
4. upper epidermis with lithocyst and diacytic stoma
5. upper epidermis
6. multicellular uniseriate trichomes and collapse trichomes
7. parenchyma with reddish brown mass
8. epidermis and lithocyst cells
9. spiral vessel and fibers
10. fibers

**3.2.3.2 Root powder**: The powder is light brown with characteristic odour. The diagnostic characteristic are as follows (Figure 3.8):

1. The occasional of the small starch granules (1).
2. The cluster of microcrystal of calcium oxalate (2).
3. The fairly abundant of the yellow cork with slightly thick wall cells (3).
4. A few of multicellular uniseriate trichome (4).
5. The fragment of bast fiber (5) and wood fiber (6) with lignified thick walls.
6. The occasional parenchyma with sclereid and stone cells (7), horse shoe shape stone cell (8), parenchyma with oil globules and reddish brown mass (10).
7. Thin wall, non-lignified parenchyma from the cortex, rectangular in shape; parenchyma with lithocyst are larger than leaf powder (9).
8. The fragment of wood parenchyma (11), xylem parenchyma; bordered pit and pitted vessel (12).

The samples of leaves and roots from 10 provinces of Thailand were found to have similar characteristics. The results were shown in Table 3.1 and 3.2. The result of the leaf powder agrees with the previous report (ศิรินทร พิศุทธานันท์ และ นิสิต พิศุทธานันท์, 2543).

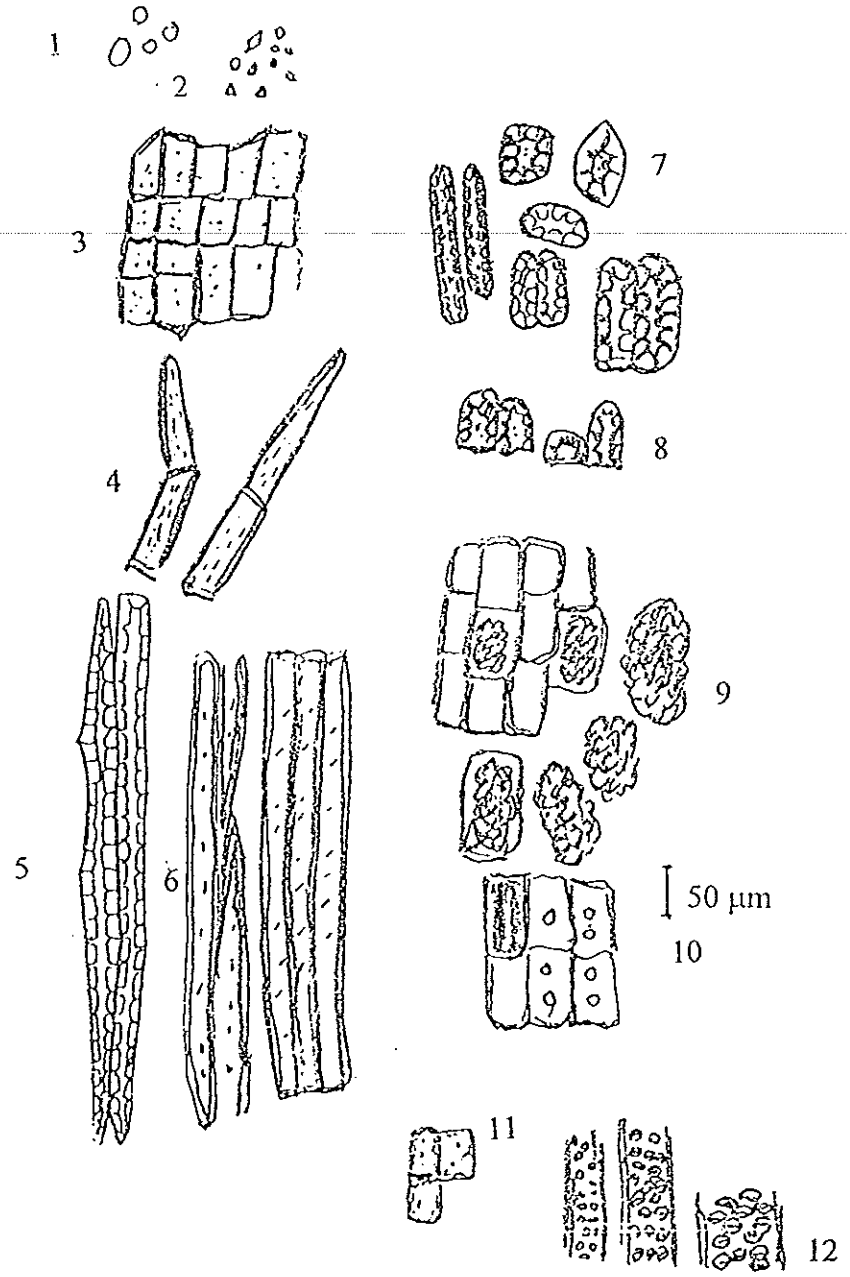



**Figure 3.8** Powdered drugs of *Rhinacanthus nasutus* roots

1. starch grains
2. microcrystal
3. cork cells
4. multicellular uniseriate trichomes
5. bast fibers
6. wood fibers
7. stone cells and sclereids
8. horse shoe shape stone cells
9. parenchyma with lithocyst
10. parenchyma with oil globules and reddish brown mass
11. wood parenchyma
12. pitted and bordered pit vessel

**Table 3. 1** Microscopic data of *Rhinacanthus nasutus* leaf powdered drugs

| Characteristic | Sample No. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Stomata | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | ++ | ++ | ++ |
| Palisade cell | + | + | + | + | + | + | + | + | ++ | ++ | + | ++ | ++ | ++ | ++ |
| Multicellular trichome | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Glandular trichome | + | + | + | + | + | ++ | ++ | ++ | + | + | + | +++ | +++ | ++ | ++ |
| Collapse trichome | + | + | + | + | + | + | + | + | + | + | + | ++ | ++ | ++ | ++ |
| Lithocyst cell | ++ | + | + | + | ++ | + | ++ | ++ | + | + | + | ++ | ++ | ++ | ++ |
| Chlorenchyma | + | + | + | + | ++ | + | + | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ |
| Fiber | ++ | ++ | ++ | ++ | + | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ |
| Reddish brown mass | ++ | ++ | ++ | ++ | + | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |

- absent

+ present

++ moderately

+++ markedly

**Table 3.2** Microscopic data of *Rhinacanthus nasutus* roots powdered drugs

| Characteristic | Sample No. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Cork cell | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ | + | + | + | + | + |
| Trichome | + | + | - | + | + | - | + | + | + | - | + | + | ++ |
| Lithocyst cell | ++ | ++ | ++ | + | ++ | ++ | + | ++ | + | ++ | + | + | + |
| Pitted vessel | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ | + |
| Chlorenchyma | + | + | + | ++ | ++ | + | + | + | + | ++ | ++ | ++ | ++ |
| Wood fiber | + | + | + | + | + | ++ | + | + | + | ++ | ++ | ++ | ++ |
| Bast fiber | + | + | + | + | + | + | + | + | + | + | - | + | + |
| Sclereid | + | + | + | ++ | + | ++ | ++ | + | + | ++ | ++ | ++ | ++ |
| Stone cell | + | ++ | + | ++ | + | + | ++ | + | + | + | + | + | + |
| Starch grain | + | + | + | + | - | + | + | + | + | + | - | + | + |
| Oil globule | + | + | + | + | + | + | + | - | + | + | + | + | + |
| Reddish brown mass | + | ++ | + | ++ | ++ | ++ | ++ | + | + | ++ | ++ | ++ | ++ |

- absent

+ present

++ moderately

+++ markedly

### 3.3 Identification test

#### 3.3.1 Preliminary test

The reaction in this test is on the basis of Borntrager's reaction, which is the specific reaction for quinone compounds, including naphthoquinone and anthraquinone (Trease and Evans, 1983). In an alkaline solution the quinone compounds produce a bathochromic shift and the colour of solution will be change to pink or red. In this study the quinone compounds e.g. rhinacanthins, rhinacanthone, methylanthraquinone, which were accumulated in *Rhinacanthus nasutus* leaves and roots were reacted with 20% potassium hydroxide (KOH) to produce a red colour in ethyl acetate phase (Figure 3.9 and Figure 3.10). The positive result was observed in all tested samples. It implies that all tested samples contain quinone compounds.

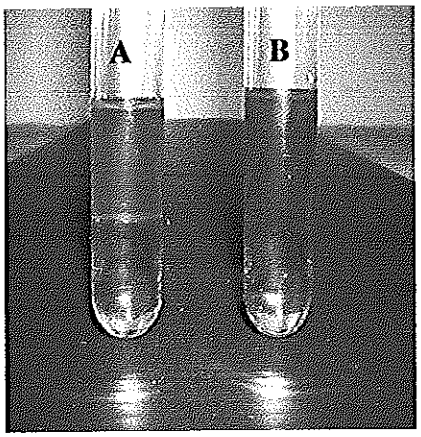



**Figure 3.9** Preliminary test of *Rhinacanthus nasutus* leaf extract
A. ethyl acetate extract + $H_2O$
B. ethyl acetate extract + 20% KOH in $H_2O$

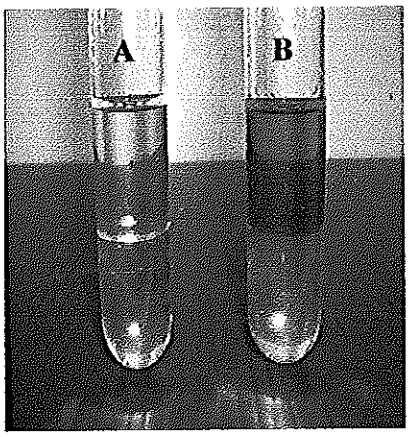



**Figure 3.10** Preliminary test of *Rhinacanthus nasutus* root extract
A. ethyl acetate extract + $H_2O$
B. ethyl acetate extract + 20% KOH in $H_2O$

### 3.3.2 Confirmatory test

A chromatographic technique was used for the confirmatory test of crude drug identification (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1995). An optimum resolution of the rhinacanthin-C and other components in the leaf and root extracts of *Rhinacanthus nasutus* was achieved using silica gel 60 $F_{254}$ as the stationary phase and chloroform : hexane (8 : 2) as the solvent system. The obtained chromatograms were detected under UV 254 nm, UV 366 nm and spraying reagent (20% KOH in methanol). All leaves sample of *R. nasutus* (15 samples) produce a similar pattern of the compounds (Table 3.3). The most chromatogram of the leaf extract shown 10 quenching spots under UV 254 nm. The standard rhinacanthin-C possessed an $hR_f$ value of 54. All leaf samples exhibited the quenching spot at the $hR_f$ value in the ranges of 53 - 56 (Table 3.4). These spots gave a positive result with 20% KOH solution. Thus, they should be the spot of rhinacanthin-C. According to this TLC-developing system, all leaf extract of *R. nasutus* exhibited 3 spots, which gave a positive result to 20% KOH solution (Table 3.4). Thus, this TLC system can detect at least 3 quinone compounds in the leaves.

**Table 3.3** $hR_f$ values of the components in methanolic extract of *Rhinacanthus nasutus* leaves detected by UV 254 nm

| Spot No. | $hR_f$ | Sample No. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1 | 1 - 3 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | 6 - 7 | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + |
| 3 | 12 - 13 | + | + | + | - | - | + | + | - | - | + | + | + | + | + | + |
| 4 | 15 - 17 | + | + | - | + | + | - | + | + | + | + | + | + | - | + | + |
| 5 | 21 - 22 | + | + | + | + | + | + | + | + | - | + | + | - | - | - | + |
| 6 | 32 - 35 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 7 | 48 - 51 | + | + | + | + | + | + | + | + | + | + | + | - | - | + | + |
| 8 | 53 - 56 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 9 | 57 - 60 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 10 | 69 - 70 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| X | 54 | | | | | | | | | | | | | | | |

X = rhinacanthin-C

\+ quenching

\- no quenching

**Table 3.4** $hR_f$ values of the components in methanolic extract of *Rhinacanthus nasutus* leaves

| Spot No. | $hR_f$ | Detection with | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | 20% KOH in methanol |
| 1 | 1 - 3 | quenching | red fluorescence | - |
| 2 | 6 - 7 | quenching | blue fluorescence | pink |
| 3 | 12 - 13 | quenching | red fluorescence | - |
| 4 | 15 - 17 | quenching | red fluorescence | - |
| 5 | 21 - 22 | quenching | red fluorescence | - |
| 6 | 32 - 35 | quenching | red fluorescence | pink |
| 7 | 46 - 48 | - | blue fluorescence | - |
| 8 | 48 - 51 | quenching | - | - |
| 9 | 53 - 56 | quenching | - | pink |
| 10 | 57 - 60 | quenching | red fluorescence | - |
| 11 | 69 - 70 | quenching | red fluorescence | - |
| rhinacanthin-C | 54 | quenching | - | pink |

All root samples of *R. nasutus* (13 samples) also produced a similar pattern of compounds (Table 3.5). All chromatograms of the root extract showed 6 quenching spots under UV 254 nm. All root samples exhibited the quenching spot at the $hR_f$ value in the ranges of 51 - 54 with a positive result to 20% KOH solution (Table 3.6). According to this TLC system, all the root extracts of *R. nasutus* exhibited 5 spots, which gave a positive result to 20% KOH solution (Table 3.6). Thus, TLC system can detect least 5 quinone compounds in root.

**Table 3.5** $hR_f$ values of the components in methanolic extract of *Rhinacanthus nasutus* roots detected by UV 254 nm

| Spot No. | $HR_f$ | Sample No. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1 | 1 - 4 | + | + | + | - | - | + | + | + | + | - | + | - | - |
| 2 | 8 -10 | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | 13 - 15 | + | + | + | - | - | - | + | + | - | + | + | + | + |
| 4 | 29 - 34 | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 5 | 42 - 43 | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 6 | 51 - 54 | + | + | + | + | + | + | + | + | + | + | + | + | + |
| X | 53 | | | | | | | | | | | | | |

X = rhinacanthin-C

\+ quenching

\- no quenching

**Table 3.6** $hR_f$ values of the components in methanolic extract of *Rhinacanthus nasutus* roots

| Spot No. | $hR_f$ | Detection with | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | 20% KOH in methanol |
| 1 | 1 - 4 | quenching | - | pink |
| 2 | 8 - 10 | quenching | - | pink |
| 3 | 13 - 15 | quenching | - | pink |
| 4 | 29 - 34 | quenching | - | - |
| 5 | 42 - 43 | quenching | - | pink |
| 6 | 51 - 54 | quenching | - | pink |
| 7 | 59 - 60 | - | blue fluorescence | - |
| rhinacanthin-C | 53 | quenching | - | pink |

### 3.4 Determination of moisture content

The moisture content of the powdered drugs of *Rhinacanthus nasutus* leaves and roots were determined prior to further investigation. In general, two methods, gravimetric method (loss on drying) and azeotropic method (toluene distillation), are used for the determination of the moisture content of herbal drugs. The gravimetric method is easier to use, but not applicable to drug containing a volatile substance. Generally, the upper limit of the moisture content of an herbal drug is 8 - 14% w/w, with a few exceptions (Dechatiwongse Na Ayudhya *et al.*, 1993). This limitation may extend the duration of an herbal raw material maintenance. The presence of excess water in herbal drug can promote the growth of microbes and the hydrolysis of the constituents leading to deterioration of crude drugs.

**Table 3.7** Moisture content of *Rhinacanthus nasutus* leaves

| No. | Sources | Loss on drying (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 7.1 | 6.1 | 6.2 | 6.5 ± 0.5 |
| 2 | Narathiwat | 5.0 | 4.8 | 4.8 | 4.9 ± 0.1 |
| 3 | Jana, Songkhla | 6.4 | 6.3 | 6.2 | 6.3 ± 0.1 |
| 4 | Phetchabun | 8.7 | 8.4 | 8.7 | 8.6 ± 0.2 |
| 5 | Phang-nga | 5.2 | 5.6 | 5.6 | 5.5 ± 0.2 |
| 6 | Chiang Mai | 5.6 | 5.7 | 5.8 | 5.7 ± 0.1 |
| 7 | Chanthaburi | 6.1 | 5.8 | 5.8 | 5.9 ± 0.2 |
| 8 | Surin | 6.4 | 6.0 | 6.2 | 6.2 ± 0.2 |
| 9 | Pattani | 7.2 | 6.9 | 7.6 | 7.2 ± 0.4 |
| 10 | Phattalung | 5.8 | 5.9 | 5.4 | 5.7 ± 0.3 |
| 11 | Nakhonpathom | 6.5 | 6.5 | 6.4 | 6.5 ± 0.1 |
| 12 | Drug store 1 | 5.3 | 5.4 | 6.1 | 5.6 ± 0.4 |
| 13 | Drug store 2 | 6.1 | 5.8 | 5.5 | 5.8 ± 0.3 |
| 14 | Drug store 3 | 7.0 | 7.0 | 6.7 | 6.9 ± 0.2 |
| 15 | Songkhla, PSU 2 | 6.5 | 6.4 | 6.4 | 6.4 ± 0.2 |

**Table 3.8** Moisture content of *Rhinacanthus nasutus* roots

| No. | Sources | Loss on drying (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 5.5 | 5.6 | 5.6 | 5.7 ± 0.2 |
| 2 | Narathiwat | 5.2 | 4.6 | 4.4 | 4.7 ± 0.4 |
| 3 | Jana, Songkhla | 7.1 | 7.0 | 6.9 | 7.0 ± 0.1 |
| 4 | Phetchabun | 5.6 | 5.8 | 5.9 | 5.8 ± 0.1 |
| 5 | Phang-nga | 5.5 | 5.7 | 5.9 | 5.7 ± 0.2 |
| 6 | Nakhonpathom | 5.8 | 5.4 | 5.6 | 5.6 ± 0.2 |
| 7 | Chanthaburi | 8.4 | 7.8 | 7.7 | 8.0 ± 0.4 |
| 8 | Surin | 7.6 | 7.2 | 6.4 | 7.1 ± 0.6 |
| 9 | Pattani | 7.6 | 7.0 | 6.3 | 7.0 ± 0.6 |
| 10 | Phattalung | 5.4 | 5.6 | 5.4 | 5.5 ± 0.1 |
| 11 | Songkhla, PSU 2 | 4.2 | 4.2 | 4.1 | 4.2 ± 0.1 |
| 12 | Songkhla, PSU 3 | 5.5 | 5.6 | 5.6 | 5.6 ± 0.1 |
| 13 | Songkhla, PSU 4 | 5.7 | 5.4 | 5.5 | 5.5 ± 0.2 |

To evaluate the quality of the crude drugs used in this study, in term of the moisture content, all sample of the leaf and root crude drugs were determined loss on drying using Sartorious Moisture Analyzer. It was found that all the samples use in this study reach the quality of moisture content. The moisture content of *R. nasutus* leaf and root samples were in the ranges 4.9 - 8.6% w/w and 4.2 - 8.0% w/w, respectively (Table 3.7 and 3.8). The moisture content of *R. nasutus* samples purchased from drug stores 4 - 7 (composed of leaves and stems of *R. nasutus*) were in the ranges of 4.9 - 8.6% w/w (Table 3.9). The variation of the moisture content of the *R. nasutus* samples may be due to the sources of raw material, the method of drying and storage.

**Table 3.9** Moisture content of *Rhinacanthus nasutus* crude drugs purchased from drug stores

| No. | Sources | Loss on drying (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Drug store 4 | 6.4 | 6.3 | 6.2 | 6.3 ± 0.1 |
| 2 | Drug store 5 | 5.0 | 4.8 | 4.8 | 4.9 ± 0.1 |
| 3 | Drug store 6 | 8.7 | 8.4 | 8.7 | 8.6 ± 0.2 |
| 4 | Drug store 7 | 7.0 | 7.0 | 6.7 | 6.8 ± 0.2 |

### 3.5 Ash content

The determination of ash content is a method to measure the amount of non-volatile residue when the drug sample is ignited by the method described. Ash may be derived from the plant tissue itself and is usually called the physiological ash. The physiological ash of a plant is a specific property of a plant. Ash also comes from the extraneous substances, such as soil, sand and other foreign matters. The limitation of an ash content is therefore an indicative specification of that herb.

When crude drugs are incinerated, they leave an inorganic ash, which in the case of many crude drugs varies with in fairly wide limits and is therefore of little value for purposes of evaluation (Evans, 1996). The total ash value is of importance and indicates to some extent the amount of care taken in the preparation of the crude drug. In the determination of total ash value, the carbon must be removed at as low temperature (450°C) as possible because alkali chloride, which may be volatile at high temperatures, would otherwise be lost. The total ash usually consists mainly of carbonates, phosphates, silicates and silica. To produce a more consistent ash the EP and BP used a sulfate ash, which involves treatment of the crude drug with dilute sulfuric acid before ignition. In this all oxides and carbonates are converted to sulphates and the ignition is carried out at higher temperature (600°C). If the total ash is treated with dilute hydrochloric acid, percentage of acid insoluble ash may be determined. This usually consists mainly of silica and a high acid insoluble ash in crude drugs and indicates contamination with earthy material (Evans, 1996).

### 3.5.1 Total ash

The total ash of the powders of leaves (15 samples), roots (13 samples) and the crude drugs purchased from drug stores 4 - 7 (4 samples) were determined the total ash using the method according to THP 1995. The results obtained from these determinations were used to set up the limitation of the total ash of *R. nasutus* leaves and roots, respectively. The results showed that the total ash of *R. nasutus* leaf powders were varied in the ranges of 9.9 - 18.6% w/w (Table 3.10). The leaf sample from Chiang Mai possessed the highest value of the total ash, while the sample from Narathiwat possessed the lowest one. The average value of the total ash from 15 samples of *R. nasutus* leaves was 15.2 ± 2.7% w/w. The limitation of the total ash of *R. nasutus* leaf was set up from the average value plus one S.D. It is therefore set as that not more than 17.9% w/w.

**Table 3.10** Total ash of *Rhinacanthus nasutus* leaves

| No | Sources | Total ash (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 18.0 | 17.0 | 16.4 | 17.2 ± 0.8 |
| 2 | Narathiwat | 9.8 | 9.9 | 9.9 | 9.9 ± 0.1 |
| 3 | Jana, Songkhla | 13.0 | 12.7 | 12.4 | 12.7 ± 0.3 |
| 4 | Phetchabun | 18.0 | 17.9 | 18.0 | 18.0 ± 0.1 |
| 5 | Phang-nga | 15.3 | 15.2 | 15.2 | 15.2 ± 0.1 |
| 6 | Chiang Mai | 18.7 | 18.6 | 18.7 | 18.6 ± 0.1 |
| 7 | Chanthaburi | 13.2 | 13.2 | 13.2 | 13.2 ± 0.0 |
| 8 | Surin | 15.1 | 15.25 | 15.2 | 15.2 ± 0.2 |
| 9 | Pattani | 18.4 | 18.4 | 18.4 | 18.4 ± 0.0 |
| 10 | Phattalung | 15.8 | 15.7 | 15.4 | 15.6 ± 0.2 |
| 11 | Nakhonpathom | 17.8 | 17.4 | 17.6 | 17.6 ± 0.2 |
| 13 | Drug store 2 | 13.2 | 12.3 | 14.1 | 13.2 ± 0.9 |
| 14 | Drug store 3 | 18.2 | 18.1 | 18.2 | 18.2 ± 0.1 |
| 15 | Songkhla, PSU2 | 11.9 | 12.1 | 12.4 | 12.1 ± 0.2 |

The total ash of *R. nasutus* root powders were varied in the ranges of 12.1 - 27.1% w/w (Table 3.11). The root from Phetchabun possessed the highest value of the total ash, while the sample from Songkhla PSU 4 possessed the lowest. The average value of the total ash from 13 samples of *R. nasutus* root was 19.9 ± 4.8% w/w. The limitation of the total ash of *R. nasutus* root was therefore set as that not more than 24.7% w/w. The total ash value of *R. nasutus* is high because there are many lithocyst cells, which contain crystal of calcium carbonate (inorganic matter). The total ash of the roots was higher than that of the leaves, because the roots have more large lithocyst cells. In addition, the roots had more chance of contamination with earthy material than the leaves.

**Table 3.11** Total ash of *Rhinacanthus nasutus* roots

| No. | Sources | Total ash (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 20.8 | 20.0 | 19.5 | 20.4 ± 0.4 |
| 2 | Narathiwat | 18.0 | 18.7 | 17.5 | 18.4 ± 0.7 |
| 3 | Jana, Songkhla | 16.8 | 16.2 | 16.1 | 16.4 ± 0.4 |
| 4 | Phetchabun | 25.5 | 26.2 | 29.5 | 27.1 ± 2.1 |
| 5 | Phang-nga | 24.8 | 25.0 | 28.7 | 26.1 ± 2.3 |
| 6 | Nakhonpathom | 18.6 | 19.0 | 19.0 | 18.8 ± 0.2 |
| 7 | Chanthaburi | 23.4 | 21.8 | 25.5 | 23.5 ± 0.2 |
| 8 | Surin | 12.9 | 13.1 | 12.3 | 12.8 + 0.4 |
| 9 | Pattani | 21.6 | 20.6 | 21.5 | 21.1 ± 0.5 |
| 10 | Phattalung | 20.2 | 20.3 | 20.6 | 20.4 ± 0.2 |
| 11 | Songkhla, PSU 2 | 16.4 | 16.4 | 16.4 | 16.4 ± 0.0 |
| 12 | Songkhla, PSU 3 | 25.4 | 25.7 | 25.6 | 25.5 ± 0.2 |
| 13 | Songkhla, PSU 4 | 11.9 | 12.1 | 12.4 | 12.1 ± 0.3 |

The crude drugs of *R. nasutus* purchased from the drug stores 4 - 7 composed of dried leaves and stems. The total ash of *R. nasutus* crude drugs varied in the ranges of 12.7 - 22.0% w/w (Table 3.12).

Comparison of the total ash of the crude drugs purchased from the drug stores with the limit value of total ash of the leaves exhibited that the crude drugs purchased from the drug stores 5, 6 and 7 were not pass the limitation of leaves. This may be do to the high ratio of stems in the crude drugs.

**Table 3.12** Total ash of *Rhinacanthus nasutus* crude drugs purchased from drug stores

| No. | Sources | Total ash (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Drug store 4 | 13.0 | 12.7 | 12.4 | 12.7 ± 0.3 |
| 2 | Drug store 5 | 19.2 | 19.1 | 19.3 | 19.4 ± 0.4 |
| 3 | Drug store 6 | 22.0 | 22.0 | 22.1 | 22.0 ± 0.1 |
| 4 | Drug store 7 | 18.2 | 18.1 | 18.1 | 18.0 ± 0.2 |

### 3.5.2 Acid insoluble ash

After the total ash determination of *R. nasutus* leaves (15 samples), roots (13 samples) and the crude drugs purchased from drug stores 4 - 7 (4 samples), it was followed by the acid insoluble ash determination. The results obtained from the acid insoluble ash determination of the leaves and roots were used to set up the limitation of the acid insoluble ash of *R. nasutus* leaves and roots, respectively.

The results showed that the acid insoluble ash of *R. nasutus* leaf powders varied in the ranges of 0.1 - 4.9% w/w (Table 3.13). The leaf sample from drug stores 3 possessed the highest value of the acid insoluble ash, while the sample from Nakhonpathom and Songkhla, PSU 2 possessed the lowest one. The average value of the acid insoluble ash from 15 samples of *R. nasutus* leaves was 0.7 ± 0.4 % w/w. The limitation of the acid insoluble ash of *R. nasutus* leaves was set up from the average value plus one S.D. It was therefore set as that not more than 1.1% w/w.

**Table 3.13** Acid insoluble ash of *Rhinacanthus nasutus* leaves

| No. | Sources | Acid insoluble ash (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 0.8 | 1.1 | 0. 9 | 0.9 ± 0.1 |
| 2 | Narathiwat | 0.1 | 0.2 | 0.2 | 0.2 ± 0.1 |
| 3 | Jana, Songkhla | 1.2 | 1.1 | 1.1 | 1.1 ± 0.1 |
| 4 | Phetchabun | 0.7 | 0.8 | 0.7 | 0.7 ± 0.1 |
| 5 | Phang-nga | 0.6 | 0.8 | 0.4 | 0.6 ± 0.2 |
| 6 | Chiang Mai | 0.7 | 0.8 | 0.7 | 0.7 ± 0.1 |
| 7 | Chanthaburi | 0.9 | 0.8 | 0.9 | 0.9 ± 0.1 |
| 8 | Surin | 0.6 | 0.6 | 0.6 | 0.6 ± 0.0 |
| 9 | Pattani | 0.5 | 0.6 | 0.8 | 0.6 ± 0.1 |
| 10 | Phattalung | 0.3 | 0.4 | 0.3 | 0.4 ± 0.1 |
| 11 | Nakhonpathom | 0.1 | 0.1 | 0.0 | 0.1 ± 0.0 |
| 12 | Drug store 1 | 4.0 | 3.1 | 3.4 | 3.5 ± 0.4 |
| 13 | Drug store 2 | 1.7 | 1.8 | 1.8 | 1.7 ± 0.1 |
| 14 | Drug store 3 | 4.9 | 5.1 | 4.6 | 4.9 ± 0.2 |
| 15 | Songkhla, PSU 2 | 0.1 | 0.1 | 0. 0 | 0.1 ± 0.0 |

The acid insoluble ash of *R. nasutus* root powders varied in the ranges of 0.1 - 7.5% w/w (Table 3.14). The sample from Phetchabun possessed the highest value of the acid insoluble ash, while the sample from Nakhonpathom possessed the lowest one. The value of the sample from Phetchabun was unreliable due to the high value of S.D. In addition, the value of the sample from Pattani was too high. Both values were therefore excluded. The average value of the acid insoluble ash was therefore calculated 11 samples of *R. nasutus* roots was 1.0 ± 0.5% w/w. The limitation of the acid insoluble ash of *R. nasutus* roots was therefore set as not more than 1.5% w/w.

**Table 3.14** Acid insoluble ash of *Rhinacanthus nasutus* roots

| No. | Source | Acid insoluble ash (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 1.6 | 1.5 | 1.1 | 1.4 ± 0.2 |
| 2 | Narathiwat | 0.8 | 1.0 | 1.1. | 0.9 ± 0.2 |
| 3 | Jana, Songkhla | 1.2 | 1.1 | 1.1 | 1.1 ± 0.1 |
| 4 | Phetchabun | 4.2 | 11.1 | 9.1 | 7.5 ± 4.5 |
| 5 | Phang-nga | 1.4 | 1.5 | 2.0 | 1.6 ± 0.4 |
| 6 | Nakhonpathom | 0.1 | 0.1 | 0.1 | 0.1 ± 0.0 |
| 7 | Chanthaburi | 0.9 | 0.8 | 0.9 | 0.9 ± 0.1 |
| 8 | Surin | 0.6 | 0.5 | 0.6 | 0.6 ± 0.1 |
| 9 | Pattani | 4.4 | 4.0 | 3.9 | 4.1 ± 0.3 |
| 10 | Phattalung | 1.4 | 1.8 | 2.1 | 1.8 ± 0.3 |
| 11 | Songkhla, PSU 2 | 0.6 | 0.8 | 0.6 | 0.7 ± 0.1 |
| 12 | Songkhla, PSU 3 | 0.6 | 0.7 | 0.7 | 0.7 ± 0.1 |
| 13 | Songkhla, PSU 4 | 0.9 | 1.1 | 1.1 | 1.0 ± 0.2 |

The acid insoluble ash of the crude drugs purchased from the drug stores 4 - 7 were varied in the ranges of 1.8 – 2.4% w/w (Table 3.15). The results showed that all the crude drugs purchased from the drug stores possessed the acid insoluble ash higher than the limitation of the leaves. Thus, these crude drugs are not reach to the quality. This may be due to the mixture of the stems.

**Table 3.15** Acid insoluble ash of *Rhinacanthus nasutus* crude drugs purchased from drug stores

| No. | Sources | Acid insoluble ash (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Drug store 4 | 2.3 | 2.6 | 2.4 | 2.4 ± 0.1 |
| 2 | Drug store 5 | 1.8 | 1.7 | 2.0 | 1.8 ± 0.2 |
| 3 | Drug store 6 | 2.1 | 2.2 | 2.1 | 2.1 ± 0.0 |
| 4 | Drug store 7 | 2.1 | 2.2 | 2.0 | 2.1 ± 0.1 |

### 3.6 Extractive values

The determination of the extractive is a method designed to measure the amount of herbal constituents which are extractable by the solvent under the specific condition. In this study, the water-soluble extractive and ethanol-soluble extractive of *Rhinacanthus nasutus* leaves and roots were examined for setting the limitation.

#### 3.6.1 Water-soluble extractive

The water-soluble extractives of the leaves (15 samples) and roots (13 samples) were determined using the method according THP 1995. The results obtained from this determination were used to set up the limitation of the water-soluble extractives of *R. nasutus* leaves and roots, respectively.

**Table 3.16** Water-soluble extractive of *Rhinacanthus nasutus* leaves

| No. | Sources | Water-soluble extractive value (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S. D. |
| 1 | Songkhla, PSU 1 | 27.6 | 24.6 | 23.6 | 25.3 ± 2.0 |
| 2 | Narathiwat | 34.5 | 38.8 | 32.2 | 35.2 ± 3.5 |
| 3 | Jana, Songkhla | 29.9 | 29.4 | 31.0 | 30.4 ± 0.6 |
| 4 | Phetchabun | 23.7 | 22.2 | 23.4 | 23.1 ± 0.8 |
| 5 | Phang-nga | 25.6 | 25.1 | 23.6 | 24.7 ± 0.9 |
| 6 | Chiang Mai | 22.1 | 22.9 | 22.5 | 22.5 ± 0.3 |
| 7 | Chanthaburi | 27.2 | 27.4 | 27.1 | 27.2 ± 0.2 |
| 8 | Surin | 28.2 | 28.2 | 28.5 | 28.3 ±0.1 |
| 9 | Pattani | 23.2 | 23.6 | 23.2 | 23.3 ± 0.2 |
| 10 | Phattalung | 35.9 | 37.1 | 36.8 | 36.6 ± 0.5 |
| 11 | Nakhonpathom | 15.0 | 15.1 | 14.7 | 14.9 ± 0.2 |
| 12 | Drug store 1 | 22.4 | 22.5 | 22.6 | 22.5 ± 0.1 |
| 13 | Drug store 2 | 19.1 | 19.7 | 19.9 | 19.5 ± 0.4 |
| 14 | Drug store 3 | 19.7 | 19.3 | 19.3 | 19.4 ± 0.2 |
| 15 | Songkhla, PSU 2 | 27.0 | 27.1 | 26.6 | 26.9 ± 0.2 |

It was found that the water-soluble extractives of *R. nasutus* leaf powders varied in the ranges of 14.9 - 36.6% w/w (Table 3.16). The leaf sample from Phattalung possessed the highest value of the water soluble extractive, while the sample from Nakhonpathom possessed the lowest one. The average value of the water-soluble extractive from 15 samples of *R. nasutus* leaves was 24.9 ± 5.2% w/w. The limitation of the water-soluble extractive of *R. nasutus* leaf was set up from the average value minus one S.D. It was therefore set as that not less than 19.7% w/w.

**Table 3.17** Water-soluble extractive of *Rhinacanthus nasutus* roots

| No. | Sources | Water soluble extractive value (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S. D. |
| 1 | Songkhla, PSU 1 | 32.9 | 31.2 | 30.5 | 31.5 ± 1.2 |
| 2 | Narathiwat | 19.3 | 19.8 | 20.4 | 19.8 ± 0.6 |
| 3 | Jana, Songkhla | 40.2 | 39.7 | 41.1 | 40.3 ± 0.7 |
| 4 | Phetchabun | 44.2 | 36.5 | 40.0 | 40.2 ± 3.8 |
| 5 | Phang-nga | 29.8 | 26.5 | 25.7 | 27.3 ± 2.1 |
| 6 | Nakhonpathom | 18.6 | 19.0 | 19.0 | 18.9 ± 0.2 |
| 7 | Chanthaburi | 21.6 | 22.7 | 22.4 | 22.2 ± 0.6 |
| 8 | Surin | 25.3 | 25.3 | 25.5 | 25.4 ± 0.1 |
| 9 | Pattani | 30.2 | 35.2 | 33.1 | 33.8 ± 2.5 |
| 10 | Phattalung | 31.2 | 27.7 | 27.1 | 28.7 + 2.2 |
| 11 | Songkhla, PSU 2 | 46.2 | 45.7 | 46.0 | 46.0 ± 0.2 |
| 12 | Songkhla, PSU 3 | 39.0 | 39.7 | 41.9 | 40.2 ± 1.4 |
| 13 | Songkhla, PSU 4 | 41.6 | 41.1 | 39.8 | 40.8 ± 0.9 |

The water-soluble extractives of *R. nasutus* root powders varied in the ranges of 18.9 - 46.0% w/w (Table 3.17). The root sample from Songkhla, PSU 2 possessed the highest value of the water soluble extractive, while the sample from Nakhonpathom possessed the lowest one. The average value of the water-soluble extractive from 13 samples of *R. nasutus* roots was 30.4 ± 8.9% w/w. The limitation

of the water-soluble extractive of *R. nasutus* leaf was set up from the average value minus one S.D. It was therefore set as that not less than 21.4% w/w.

**Table 3.18** Water-soluble extractive of *Rhinacanthus nasutus* crude drugs purchased from drug stores

| No. | Sources | Water soluble extractive value (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S. D. |
| 1 | Drug store 4 | 17.6 | 22.2 | 18.6 | 19.5 ± 2.4 |
| 2 | Drug store 5 | 22.5 | 22.5 | 22.6 | 22.5 ± 0.1 |
| 3 | Drug store 6 | 19.1 | 19.7 | 20.0 | 19.6 ± 0.5 |
| 4 | Drug store 7 | 19.7 | 19.3 | 19.3 | 19.4 ± 0.2 |

The results of the water-soluble extractives of the crude drugs purchased from the drug stores 4 - 7 exhibited that the value of the water-soluble extractives were in the ranges of 19.4 - 22.5% w/w (Table 3.18). It was found that all the crude drugs purchased from drug stores pass the water-soluble extractive limitation of the leaves except the sample from drug store 7.

### 3.6.2 Ethanol-soluble extractive

The ranges of ethanol-soluble extractive in the leaves of *R. nasutus* were 1.3 - 7.2% w/w and the average value was 3.8 ± 1.6% w/w. The maximum value was from Songkhla, PSU 2 and the minimum value is from Phetchabun. The limitation of the ethanol-soluble extractive value of *R. nasutus* leaves is therefore set as that not less than 2.2% w/w.

**Table 3.19** Ethanol-soluble extractive of *Rhinacanthus nasutus* leaves

| No. | Sources | Ethanol-soluble extractive value (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S. D. |
| 1 | Songkhla, PSU 1 | 5.3 | 5.4 | 5.2 | 5.3 ± 0.1 |
| 2 | Narathiwat | 4.9 | 4.3 | 4.6 | 4.6 ± 0.3 |
| 3 | Jana, Songkhla | 3.9 | 4.4 | 4.0 | 4.1 ± 0.2 |
| 4 | Phetchabun | 1.0 | 1.5 | 1.5 | 1.3 ± 0.3 |
| 5 | Phang-nga | 3.2 | 3.1 | 3.2 | 3.2 ± 0.1 |
| 6 | Chiang Mai | 4.4 | 4.4 | 4.5 | 4.4 ± 0.1 |
| 7 | Chanthaburi | 4.0 | 3.1 | 4.2 | 3.8 ± 0.6 |
| 8 | Surin | 5.1 | 5.4 | 5.4 | 5.3 ± 0.2 |
| 9 | Pattani | 5.1 | 5.2 | 5.2 | 5.2 ± 0.1 |
| 10 | Phattalung | 3.6 | 4.4 | 3.6 | 3.9 ± 0.5 |
| 11 | Nakhonpathom | 2.2 | 2.3 | 2.4 | 2.3 ± 0.1 |
| 12 | Drug store 1 | 1.8 | 1.7 | 2.0 | 1.8 ± 0.2 |
| 13 | Drug store 2 | 2.9 | 3.0 | 2.9 | 2.9 ± 0.1 |
| 14 | Drug store 3 | 2.9 | 2.9 | 3.0 | 2.9 ± 0.1 |
| 15 | Songkhla, PSU 2 | 7.2 | 7.1 | 7.3 | 7.2 ± 0.1 |

The ranges of ethanol-soluble extractive in the roots of *R. nasutus* were 2.4 – 7.4% w/w and the average value was 5.4 ± 1.5% w/w. The maximum value was from Jana, Songkhla and the minimum value was from Nakhonpathom. The limitation of the ethanol-soluble extractive values of *R. nasutus* roots is therefore set as that not less than 3.9% w/w.

The roots of *R. nasutus* exhibited the ethanol extractive higher than the leaves. It implies that the root of *R. nasutus* contained the chemicals constituents which can dissolve in the ethanol higher than the leaves.

**Table 3.20** Ethanol-soluble extractive of *Rhinacanthus nasutus* roots

| No. | Sources | Ethanol-soluble extractive value (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S. D. |
| 1 | Songkhla, PSU 1 | 7.0 | 6.7 | 7.0 | 6.9 ± 0.1 |
| 2 | Narathiwat | 7.0 | 5.8 | 6.0 | 6.3 ± 0.1 |
| 3 | Jana, Songkhla | 7.4 | 7.4 | 7.3 | 7.4 ± 0.1 |
| 4 | Phetchabun | 3.4 | 2.6 | 2.5 | 2.8 ± 0.6 |
| 5 | Phang-nga | 4.4 | 4.2 | 4.2 | 4.3 ± 0.1 |
| 6 | Nakhonpathom | 2.5 | 2.4 | 2.4 | 2.4 ± 0.1 |
| 7 | Chanthaburi | 4.3 | 4.4 | 4.1 | 4.3 ± 0.1 |
| 8 | Pattani | 5.8 | 5.8 | 6.0 | 5.9 ± 0.1 |
| 9 | Surin | 5.3 | 5.3 | 5.3 | 5.3 ± 0.0 |
| 10 | Phattalung | 5.6 | 5.6 | 5.4 | 5.5 ± 0.1 |
| 11 | Songkhla, PSU 2 | 6.3 | 6.8 | 6.4 | 6.5 ± 0.3 |
| 12 | Songkhla, PSU 3 | 5.8 | 5.9 | 5.8 | 5.8 ± 0.1 |
| 13 | Songkhla, PSU 4 | 6.2 | 6.3 | 6.4 | 6.3 ± 0.1 |

**Table 3.21** Ethanol-soluble extractive of *Rhinacanthus nasutus* crude drugs purchased from drug stores

| No. | Sources | Ethanol-soluble extractive value (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S. D. |
| 1 | Drug store 4 | 2.3 | 2.6 | 2.4 | 2.4 ± 0.1 |
| 2 | Drug store 5 | 4.2 | 3.9 | 3.9 | 4.0 ± 0.1 |
| 3 | Drug store 6 | 2.1 | 2.2 | 2.1 | 2.1 ± 0.1 |
| 4 | Drug store 7 | 2.1 | 2.2 | 2.0 | 2.1 ± 0.1 |

The range of ethanol-soluble extractive of the crude drugs purchased from drug stores 4 - 7 were in the ranges of 2.1 - 4.0% w/w. The crude drugs purchased from the drug stores 6 and 7 do not pass the limitation of ethanol-soluble extractive of leaves, but the crude drugs purchased from drug stores 4 and 5 pass the limitation.

### 3.7 Quantitative determination of total rhinacanthins

In this study, quantitative determination of the total rhinacanthins in *Rhinacanthus nasutus* leaves and roots were achieved using the method that has been previously developed using a UV-vis spectrophotometry with colorimetric techniques (Kongchai and Panichayupakaranant, 2002) The colorimetric technique used in the assay is on the basis of Borntrager's reaction with some modification.

The content of total rhinacanthins in the leaf extract of *R. nasutus* was calculated as rhinacanthin-C, using the standard curve of rhinacanthin-C. The standard curve of the authentic rhinacanthin-C was observed to be linear in the concentration ranges of 16 - 160 µg/ml with $R^2$ value of 0.9999. The equation of $Y = 0.0037X - 0.0218$ was fit to the standard curve (Figure 3.11).

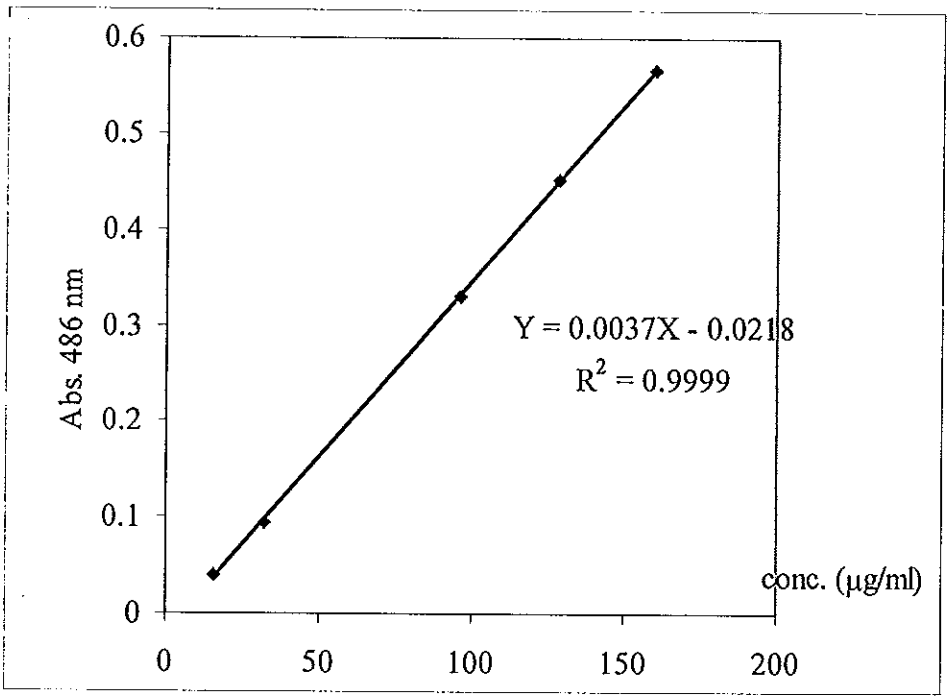



**Figure 3.11** Standard curve of rhinacanthin-C

### 3.7.1 Study on the extraction times

The duration of the extraction is an essential factor for the experiment. In this study, the powdered drugs were extracted with methanol under refluxing. Study on the time course of the extraction found that an increasing of the extraction time from 30 minutes a lower content of total rhinacanthins, calculated as rhinacanthin-C was observed (Figure 3.12). This may be due to the less stability of rhinacanthins at the high temperature. Thus, the suitable time for the extraction of *R. nasutus* powdered drug should be 30 minutes.

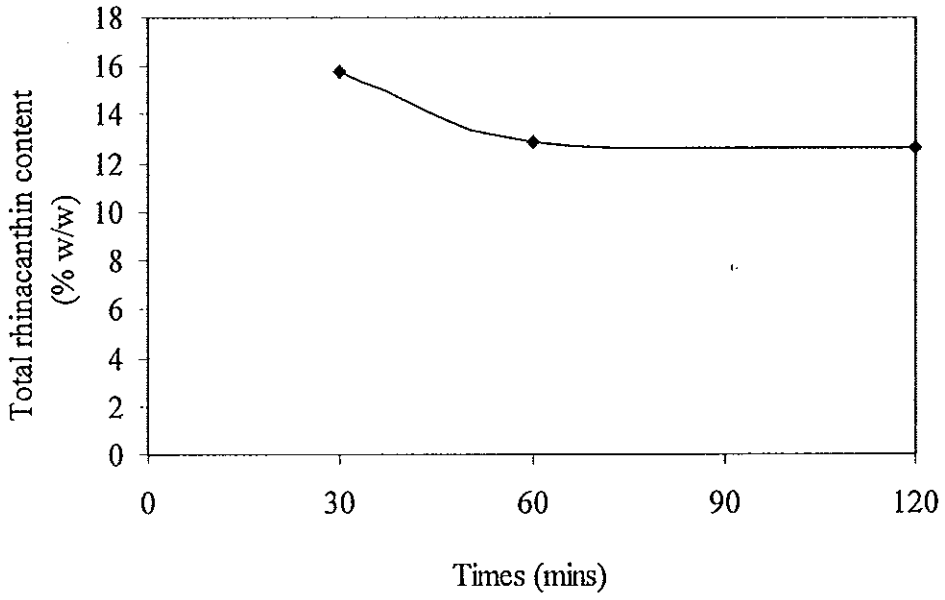



**Figure 3.12** Effect of extraction time on the total rhinacanthin content in *Rhinacanthus nasutus*

The data from quantitative analysis of the total rhinacanthins in the powders of *R. nasutus* leaves (15 samples) and roots (13 samples) were used to set up the lower limitation of the total rhinacanthin content in *R. nasutus* leaves and roots, respectively. The results showed that the total rhinacanthin contents in *R. nasutus* leaf powders were varied in the ranges of 0.9 – 4.5% w/w (Table 3.22). The leaf sample from Narathiwat possessed the highest value of total rhinacanthins, while the sample from the drug store 3 possessed the lowest one. The average value of the total rhinacanthins from 15 samples of *R. nasutus* leaves was 2.2 ± 1.1% w/w. The lower

limitation of the total rhinacanthins in *R. nasutus* leaf was set up from the average value minus one S.D. It was therefore set as that not less than 1.1% w/w.

**Table 3.22** Total rhinacanthin content of *Rhinacanthus nasutus* leaves

| No. | Sources | Total rhinacanthins (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 4.5 | 4.5 | 4.4 | 4.4 ± 0.1 |
| 2 | Narathiwat | 4.2 | 4.3 | 4.9 | 4.5 ± 0.4 |
| 3 | Jana, Songkhla | 2.8 | 2.6 | 2.8 | 2.7 ± 0.1 |
| 4 | Phetchabun | 1.5 | 1.0 | 1.2 | 1.2 ± 0.2 |
| 5 | Phang-nga | 1.8 | 1.9 | 1.4 | 1.7 ± 0.3 |
| 6 | Chiang Mai | 2.3 | 2.2 | 2.2 | 2.2 ± 0.0 |
| 7 | Chanthaburi | 2.1 | 2.0 | 2.1 | 2.1 ± 0.1 |
| 8 | Surin | 1.0 | 1.0 | 1.0 | 1.0 ± 0.0 |
| 9 | Pattani | 1.1 | 1.2 | 1.2 | 1.2 ± 0.0 |
| 10 | Phattalung | 1.6 | 1.5 | 1.5 | 1.6 ± 0.1 |
| 11 | Nakornpathom | 1.8 | 1.7 | 1.7 | 1.7 ± 0.1 |
| 12 | Drug store 1 | 2.2 | 2.2 | 2.3 | 2.2 ± 0.1 |
| 13 | Drug store 2 | 1.6 | 1.7 | 1.6 | 1.6 ± 0.0 |
| 14 | Drug store 3 | 0.9 | 0.9 | 0.9 | 0.9 ± 0.0 |
| 15 | Songkhla, PSU 2 | 3.3 | 3.3 | 3.3 | 3.3 ± 0.0 |

The total rhinacanthin contents in *R. nasutus* root powders varied in the ranges of 1.6 - 5.7% w/w (Table 3.23). The root sample from Songkhla, PSU 4 possessed the highest value of total rhinacanthins, while the sample from Jana, Songkhla possessed the lowest one. The average value of the total rhinacanthins from 13 samples of *R. nasutus* roots was 3.4 ± 1.3% w/w. The lower limitation of the total rhinacanthins of *R. nasutus* roots was set up from the average value minus one S.D. It was therefore set as that not less than 2.1% w/w.

**Table 3.23** Total rhinacanthin content of *Rhinacanthus nasutus* roots

| No. | Sources | Total rhinacanthins (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Songkhla, PSU 1 | 4.6 | 4.7 | 4.7 | 4.7 ± 0.0 |
| 2 | Narathiwat | 4.8 | 4.7 | 4.8 | 4.8 ± 0.0 |
| 3 | Jana, Songkhla | 1.7 | 1.6 | 1.6 | 1.6 ± 0.0 |
| 4 | Petchabun | 2.3 | 1.8 | 2.1 | 2.1 ± 0.2 |
| 5 | Phang-nga | 2.5 | 2.5 | 2.4 | 2.5 ± 0.0 |
| 6 | Nakhonpathom | 1.8 | 1.7 | 1.6 | 1.6 ± 0.1 |
| 7 | Chanthaburi | 4.2 | 4.0 | 4.2 | 4.1 + 0.1 |
| 8 | Pattani | 3.3 | 3.3 | 3.5 | 3.4 ± 0.1 |
| 9 | Surin | 3.6 | 3.8 | 3.2 | 3.6 ± 0.3 |
| 10 | Phattalung | 2.1 | 2.0 | 2.3 | 2.1 ± 0.2 |
| 11 | Songkhla, PSU 2 | 4.0 | 4.2 | 4.1 | 4.2 ± 0.0 |
| 12 | Songkhla, PSU 3 | 4.6 | 4.4 | 4.0 | 4.3 ± 0.3 |
| 13 | Songkhla, PSU 4 | 5.8 | 5.6 | 5.6 | 5.7 ± 0.1 |

**Table 3.24** Total rhinacanthin content of *Rhinacanthus nasutus* crude drugs purchased from drug stores

| No. | Sources | Total rhinacanthins (% w/w) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Mean ± S.D. |
| 1 | Drug store 4 | 1.2 | 1.0 | 0.9 | 1.0 ± 0.1 |
| 2 | Drug store 5 | 2.2 | 2.1 | 2.2 | 2.2 ± 0.1 |
| 3 | Drug store 6 | 0.9 | 0.9 | 0.8 | 0.9 ± 0.1 |
| 4 | Drug store 7 | 0.8 | 0.8 | 0.7 | 0.8 ± 0.1 |

The results of the total rhinacanthins content of the crude drugs from the drug stores showed that the value were varied in the ranges 0.8 - 2.2% w/w (Table 3.24). It was found that the crude drugs purchased from the drug stores do not pass the lower limitation of total rhinacanthins content, except the crud drug purchased from the drug stores 5. These may be due to the higher ratio of stems in the crude drugs.

### 3.7.2 Distribution of rhinacanthins in *Rhinacanthus nasutus* and the effect of harvesting period

Determination of total rhinacanthin content in the leaves, stems and roots of *R. nasutus*, which were collected at a different period of times, had demonstrated that rhinacanthins markedly accumulated in the roots and leaves, but less accumulated in the stems (Table 3.25). The result is harmonious with the recommend part use of *R. nasutus* in traditional and primary health care systems (Farnsworth and Bunyapraphatsara, 1992). As regards the effect of harvesting period it was found that the leaves and roots harvested in July gave higher amounts of rhinacanthins (Table 3.25). In July, *R. nasutus* begins to blossom. Thus, *R. nasutus* leaves and roots should be harvested when blossom.

**Table 3.25** Total rhinacanthin content in leaves, stems and roots of *Rhinacanthus nasutus* harvested in different times

| Period of harvesting | Total rhinacanthins (% w/w) Mean ± S.D. | | |
|---|---|---|---|
| | Leaves | Stems | Roots |
| April 2003 | 3.6 ± 0.1* | 2.1 ± 0.0* | 4.3 ± 0.3* |
| July 2003 | 5.6 ± 0.0* | 1.0 ± 0.0* | 5.7 ± 0.1* |
| October 2003 | 4.4 ± 0.1* | 0.6 ± 0.0* | 4.7 ± 0.0* |
| January 2004 | 3.3 ± 0.0* | 0.8 ± 0.0* | 4.2 ± 0.0* |

N = 3.

* Statistical significance was taken at $P < 0.05$ when compare the data within the same column.

Although the leaves and roots of *R. nasutus* that harvested in other periods gave a lower content of total rhinacanthins, they still passed the lower limit of the total rhinacanthin contents.

# CHAPTER 4

# CONCLUSIONS

*Rhinacanthus nasutus* (Linn.) Kurz (Thong pan chang) is an herb used in Thai traditional medicines which neither pharmacognostic and chemical specification have been reported. In the present study, the pharmacognostic and chemical specifications, including macroscopic and microscopic characteristics, powdered drugs, identification, loss on drying, total ash, acid insoluble ash, ethanol soluble extractive, water soluble extractive and the total rhinacanthin content of *R. nasutus* leaves and roots were investigated. The standard methods that were used in this study was followed to the Thai Herbal Pharmacopoeia. The plant samples were collected from the fields as well as purchased from the drug stores, which were at least ten sources from different provinces of Thailand. The number of the leaf and root samples used in this study are 15 and 10, respectively. The result from this study lead to an establishment of pharmacognostic and chemical information of *R. nasutus* leaves and roots, which are useful for the further establishment of the monograph in the Thai Herbal Pharmacopoeia. The pharmacognostic and chemical information of *R. nasutus* leaves and roots were draw as shown in the monographs.

The distribution of rhinacanthins in various parts of *Rhinacanthus nasutus* plant as well as the effect of harvesting period on the content of rhinacanthins in the leaves, stems and roots were studied. It was found that rhinacanthins were higher accumulated in the roots and leaves, respectively, while the accumulation in the stems was very low. In addition, harvesting in July or in blossom lead to the highest content of rhinacanthins in both roots and leaves.

# MONOGRAPH

## ใบทองพันชั่ง (THONG-PHAN-CHANG, BAI)

**Rhinacanthus Nasutus Leaf**

**Synonyms** *Rhinacanthus communis* Nees

**Rhinacanthus Nasutus leaf is the dried leaf of *Rhinacanthus nasutus* (Linn.) Kurz (*Rhinacanthus communis* Nees) Family Acanthaceae.**

**Constituents** rhinacanthin-C, rhinacanthin-D, rhinacanthin-I, rhinacanthin-J, rhinacanthin-E, rhinacanthin-F, rhinacanthin-N, rhinacanthone, 2,6-dimethoxy benzoquinone, syringic acid, 2-methoxy-4-propionylphenol, umbelliferone, methylpheophorbide-A, 2-methylanthraquinone, methyl-α-D-galactopyranoside, 4-acetonyl-3,5-dimethoxy-*p*-quinol, sitosterol-β-D-glucopyranoside, stigmasterol-β-D, glucopyranoside, 3,4-dimethylphenol-β-D-glucopyranoside, 3,4,5-trimethylphenol-β-D-glucopyranoside.

**Description of the plant** shrub, erect, branched 1.5 to 2 m height, simple leaves, opposite, entire, lanceolate, base often oblique 2.5 to 5 cm wide and 4 to 6 cm long, glabrous, yellowish green, inflorescence, axillary and terminal, white color, bisexual flower, zymgomophic, bract small, calyx, 5 corolla 2 lipped, stamen 4, didynamous, ovary superior, ovule free central placentation, capsule fruit (Figure 4.1).

**Description** Odour and taste are characteristic

*Macroscopic* *R. nasutus* leaves are broken, brown dried leaves about 1.5 to 2 cm in length and 0.2 to 0.6 cm diameter.

*Microscopic* Transverse section of the leaf shows upper epidermis, which composed of irregularly shaped cells with slightly wavy walls in surface view and rectangular cells in section view. The stomata are absent or very infrequent. There are some large lithocyst cells and glandular trichomes with a unicellular stalk and 4 to 7 cell head and multicellular uniseriate trichomes. The mesophyll consists of a single layer of palisade cells, containing chloroplast in the surface view. Lower epidermis are similar to those of upper epidermis, but they are smaller and more wavy-walled. Numerous diacytic stomata, lithocyst cell and glandular trichomes are also present.

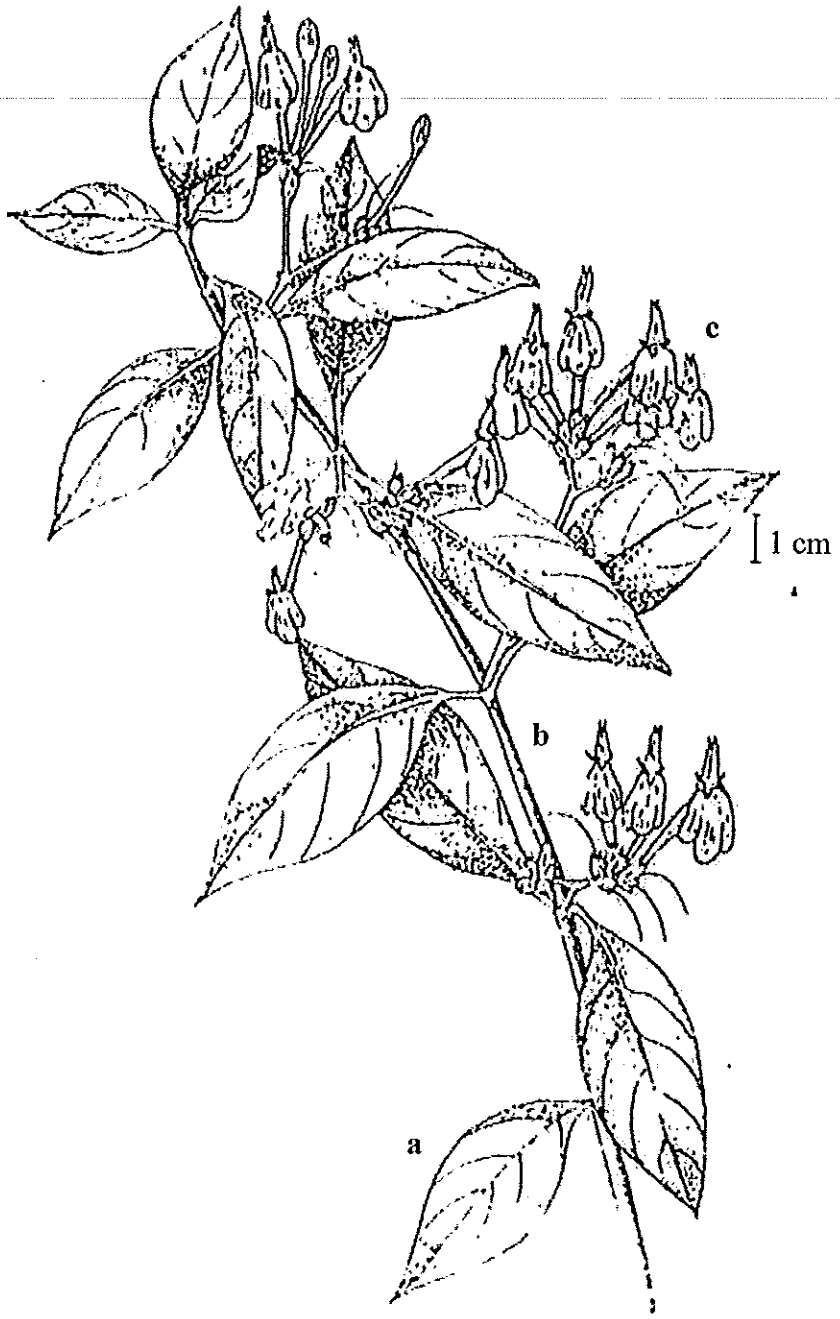



**Figure 4.1** *Rhinacanthus nasutus* (Linn.) Kurz; leaves (a), stem (b) and (c) flowers (Farnsworth and Bunyapraphatsara, 1992)

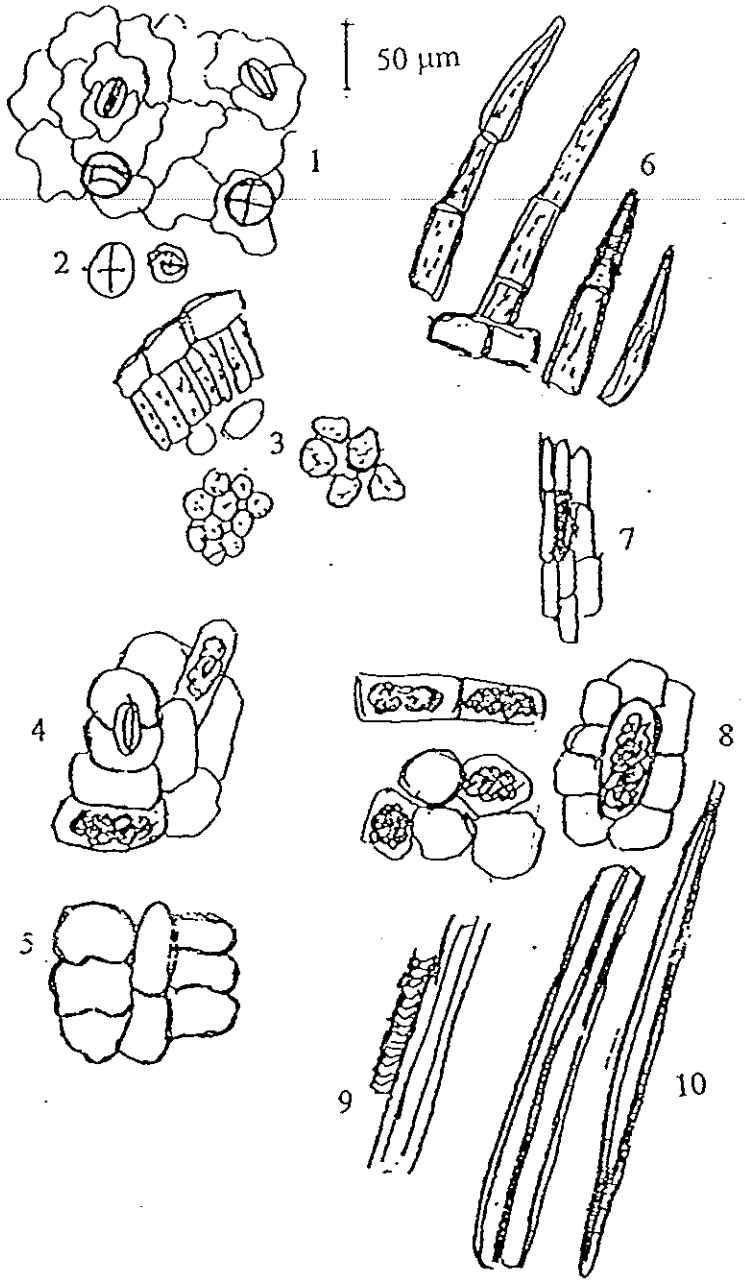



**Figure 4.2** Powdered drugs of *Rhinacanthus nasutus* leaves:

1. lower epidermis, diacytic stomata and glandular trichomes
2. glandular trichomes
3. mesophyll parenchyma
4. upper epidermis with lithocyst and diacytic stoma
5. upper epidermis
6. multicellular uniseriate trichomes and collapse trichomes
7. parenchyma with reddish brown mass
8. epidermis and lithocyst cell
9. spiral vessel and fibers
10. fibers

*Rhinacanthus nasutus* in powder possesses the diagnostic microscopical characters of the leaf with addition characters. The powder is yellowish-green with a characteristic odor. The diagnostic characters of transverse section of the leaf are the abundant fragments of the lamina in surface view. The upper epidermis is composed of slightly wavy walled cells. The lower epidermis is composed of wavy-walled cells with numerous diacytic stomata. Covering trichome, glandular trichome and lithocyst cells are found on both epidermis. The fragment of covering trichomes and collapse are found scattered and attached to the fragment of the epidermis, they are uniseriate. The occasional fragments of the fibers and xylem are found scatter (Figure 4.2).

**Packaging and storage** *R. nasutus* should be kept in dry place and well closed container.

**Identification**

**A. Preliminary Test**

Accurately weigh 100 mg of sample, sonicate with ethyl acetate for 30 minutes, filter through filter paper. Mix 3 ml of the solution with 3 ml of 20 per cent w/w potassium hydroxide in water. The ethyl acetate layer become orange to red.

**B. Confirmatory test**

1. Test solution: weigh 500 mg of the powder sample is reflux with 10 ml of methanol for 10 minutes, allow to cool and filter through filter paper and concentrate the filtrate to 5 ml.

2. Apply to the plate, 10 µl of the test solution. Carry out the test describe in the "Thin Layer Chromatography". Using silica gel as the coating substance and chloroform : hexane (8 : 2) as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. After removal of the plate, allow it to dry in the air and examine under ultraviolet light (254 nm, 366 nm). Spray the plate with 20 per cent w/w potassium hydroxide in methanol. Several 10 quenching spots under UV 254 nm and 3 spots, which give positive result to 20% potassium hydroxide solution are observed (Table 4.1 and Figure 4.3).

**Loss on drying** Not more than 10 per cent w/w

**Total ash** Not more than 17.9 per cent w/w

**Acid insoluble ash** Not more than 1.1 per cent w/w

**Ethanol soluble-extractive** Not less than 2.2 per cent w/w

**Water soluble-extractive** Not less than 19.5 per cent w/w

**Total rhinacanthins** Not less than 1.1 per cent w/w

**Table 4.1** $hR_f$ values of the components in methanolic extract of *Rhinacanthus nasutus* leaves

| Spot No. | $hR_f$ | Detection with | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | 20% KOH in methanol |
| 1 | 1 - 3 | quenching | red fluorescence | - |
| 2 | 6 - 7 | quenching | blue fluorescence | pink |
| 3 | 12 - 13 | quenching | red fluorescence | - |
| 4 | 15 - 17 | quenching | red fluorescence | - |
| 5 | 21 - 22 | quenching | red fluorescence | - |
| 6 | 32 - 35 | quenching | red fluorescene | pink |
| 7 | 46 - 48 | - | blue fluorescence | - |
| 8 | 48 - 51 | quenching | - | - |
| 9* | 53 - 56 | quenching | - | pink |
| 10 | 57 - 60 | quenching | red fluorescence | - |
| 11 | 69 - 70 | quenching | red fluorescence | - |

* rhinacanthin-C

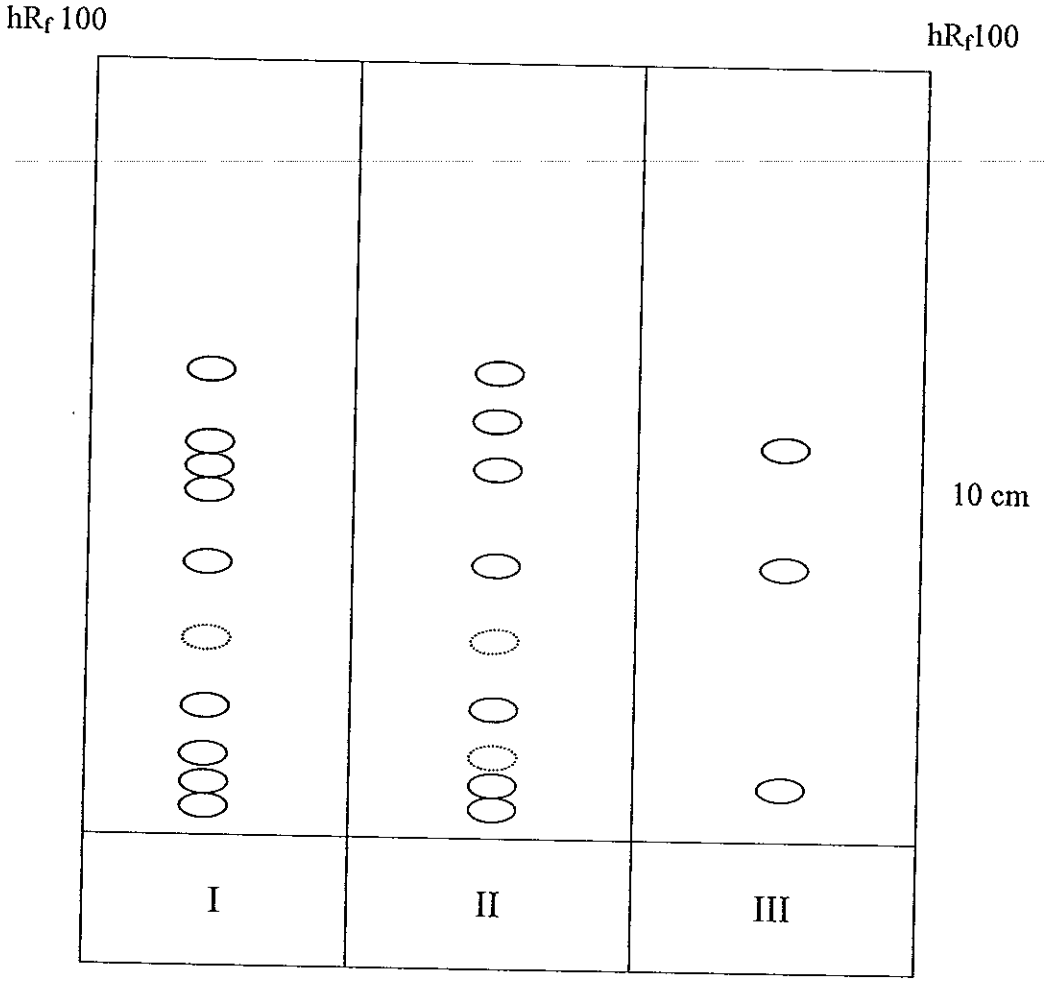



**Figure 4.3** TLC chromatogram of the methanolic extract of *Rhinacanthus nasutus* leaves

I = detection under UV light (254 nm)

II = detection under UV light (366 nm)

III = detection with 20 per cent potassium hydroxide in methanol

⬭ = spots appear in some batches

**รากทองพันชั่ง (THONG-PHAN-CHANG, RAAG)**

**Rhinacanthus Nasutus root**

**Category** Antifungal

**Rhinacanthus Nasutus root is the dried roots of *Rhinacanthus nasutus* (Linn.) Kurz (*Rhinacanthus communis* Nees) Family Acanthaceae.**

Constituents rhinacanthin-A, rhinacanthin-B, rhinacanthin-C, rhinacanthin-D, rhinacanthin-G, rhinacanthin-H, rhinacanthin-I, rhinacanthin-J, rhinacanthin-K, rhinacanthin-L, rhinacanthin-M, rhinacanthin-N, rhinacanthin-O, rhinacanthin-P, rhinacanthin-Q, dehydro-(-lapachone,p-hydroxy-benzaldehyde, allantoin, methyl-vanillate, syringaldehyde, 2-methoxy-4-propionylphenol, methylpheophorbide-A, wogonin, oroxylin, amyrin, glutinol, lupeol, (+)-praeruptorin, (β-sitosterol and stigmasterol.

**Description of the plant** Shrub, erect, branched 1.5 to 2 m height, simple leaves, opposite, entire, lanceolate, base often oblique 2.5 to 5 cm wide and 4 to 6 cm long, glabrous, yellowish green, inflorescence, axillary and terminal, white colour, bisexual flower, zymgomophic, bract small, calyx, 5 corolla 2 lipped, stamen 4, didynamous, ovary superior, ovule free central placentation, capsule fruit (Figure 4.1).

**Description** Odour and taste are characteristics.

*Macroscopic* The root occour as a mixture of short form of root and broken of bark root with brown to dark brown colour about 2 to 4 cm in length and 0.2 to 0.4 cm diameter.

*Microscopic.* Transverse section of the roots composing of irregularly shape cells with slightly wavy walls in surface view and rectangular cells in section view.

The root in powdered drugs possesses the diagnostic microscopical characters of the unground drug (Figure 4.4).

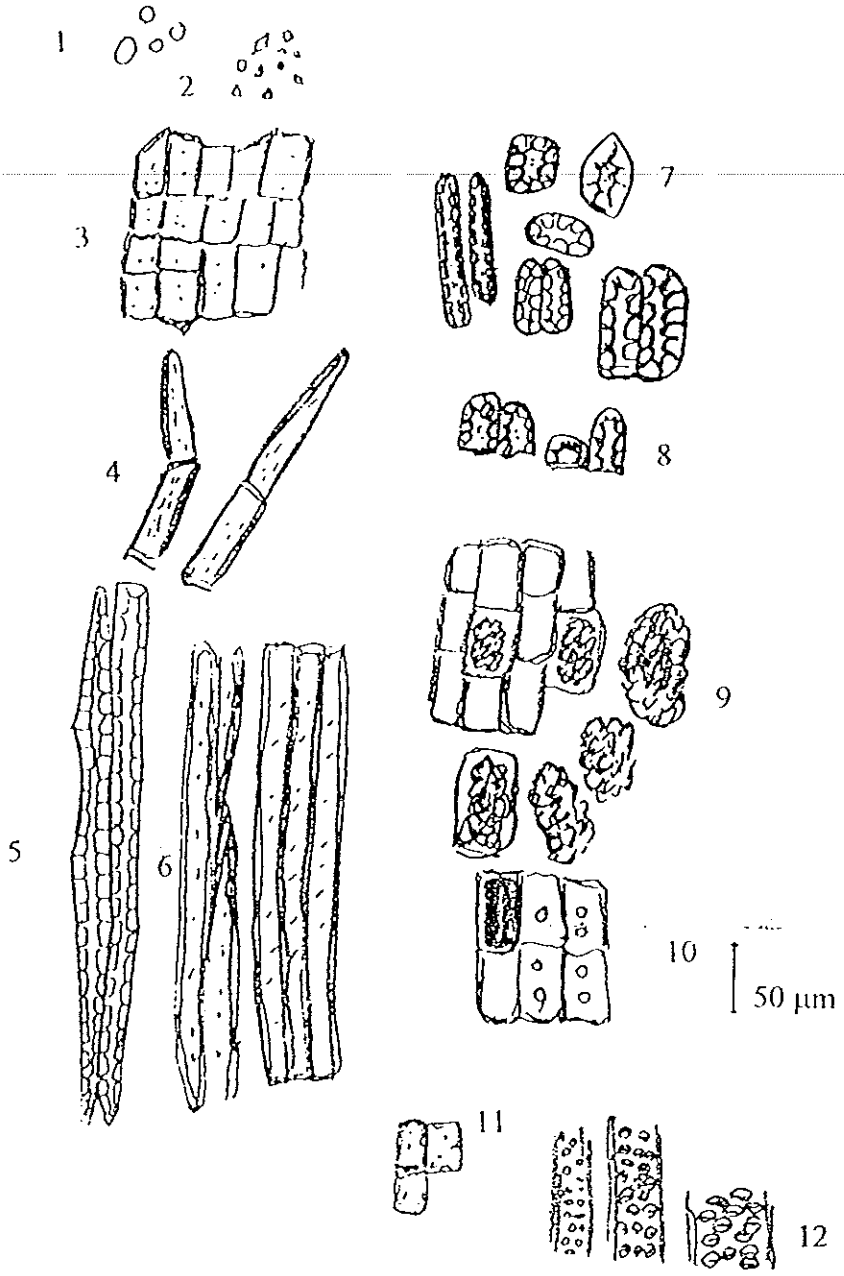



**Figure 4.4** Powdered drugs of *Rhinacanthus nasutus* roots

1. starch grains
2. microcrystal
3. cork cells
4. multicellular uniseriate trichomes
5. bast fibers
6. wood fibers
7. stone cells and sclereids
8. horse shoe shape stone cells
9. parenchyma with lithocyst
10. parenchyma with oil globules and reddish brown mass
11. wood parenchyma
12. pitted and bordered pit vessel

The bast fiber and multicellular uniseriate trichomes are absent or infrequency and large lithocyst cells (Figure 4.4).

The powder is yellowish-brown. Odour and taste are characteristic.

**Packaging and storage** *R. nasutus* should be store in dry place and well closed container.

**Identification**

**A. Preliminary Test**

Accurately weigh 100 mg of sample, sonicat with the ethyl acetate for 30 minutes, filter through filter paper. Mix 3 ml of solution with 3 ml of 20 per cent potassium hydroxide in water. The ethyl acetate layer become pink to red.

**B. Confirmatory test**

1. Test solution: weigh 500 mg of the powder sample is reflux with 10 ml of methanol for 10 minutes, allow to cool and filter throughout the filter paper and concentrate the filtrate to 5 ml.

2. Apply to the plate, 10 µl of the test solution. Carry out the test describe in the "Thin-Layer Chromatography". Using silica gel as the coating substance and chloroform : hexane (8 : 2) as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. After removal of the plate, allow it to dry in the air, and examine under ultraviolet light (254 nm, 366 nm). Spray the plate with 20% potassium hydroxide in methanol. Several 6 quenching spots under UV 254 nm and 5 spots, which give positive result to 20% potassium hydroxide in methanol are observed (Table 4.2 and Figure 4.5).

**Loss on drying** Not more than 10 per cent w/w

**Total ash** Not more than 24.7 per cent w/w

**Acid insoluble ash** Not more than 1.5 per cent w/w

**Ethanol soluble extractive** Not less than 3.9 per cent w/w

**Water soluble extractive** Not less than 22.9 per cent w/w

**Total rhinacanthins** Not less than 2.1 per cent w/w

**Table 4.2** $hR_f$ values of the components in methanolic extract of *Rhinacanthus nasutus* roots

| Spot No. | $hR_f$ | Detection with | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | 20% KOH in methanol |
| 1 | 1 - 4 | quenching | - | pink |
| 2 | 8 - 10 | quenching | - | pink |
| 3 | 13 - 15 | quenching | - | pink |
| 4 | 29 - 34 | quenching | - | - |
| 5 | 42 - 43 | quenching | - | pink |
| 6* | 51 - 54 | quenching | - | pink |
| 7 | 59 - 60 | - | blue fluorescence | - |

* rhinacanthin-C

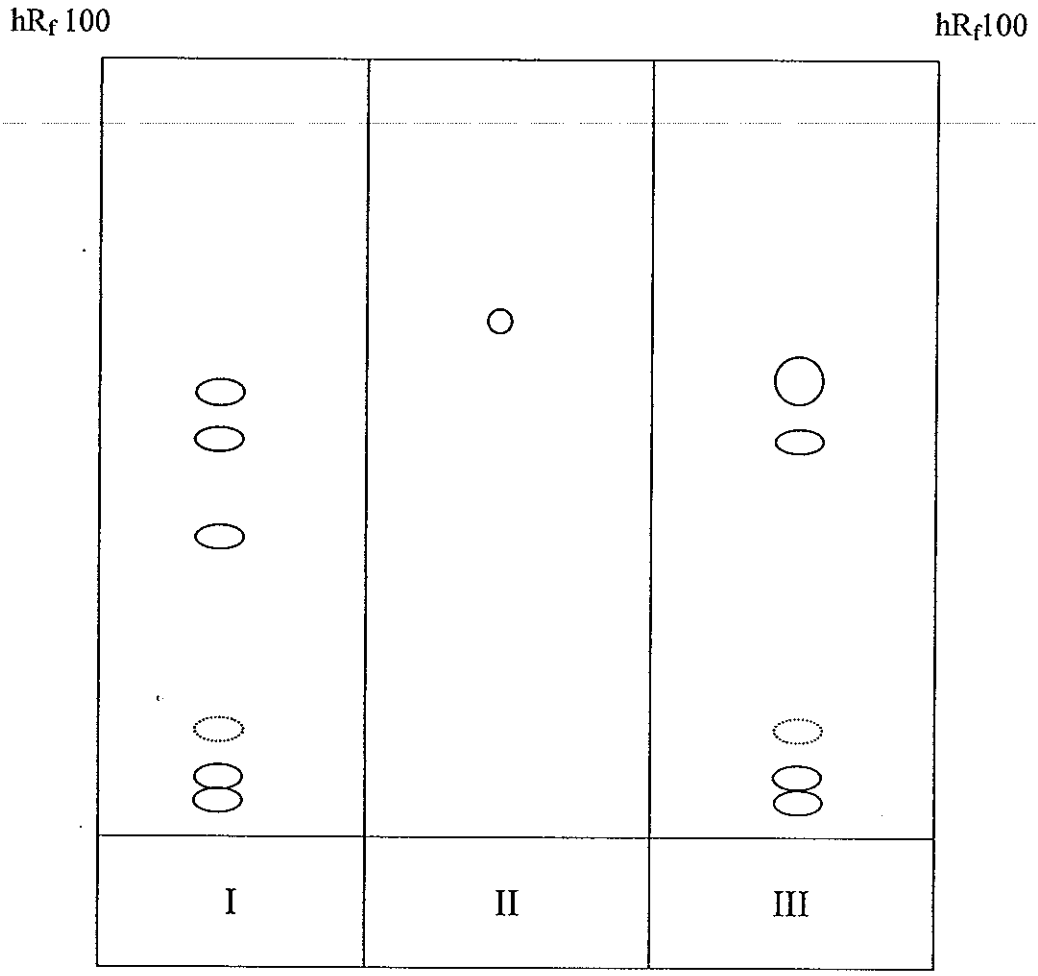



**Figure 4.5** TLC chromatogram of the methanolic extract of *Rhinacanthus nasutus* roots

I = detection under UV light (254 nm)

II = detection under UV light (366 nm)

III = detection with 20 per cent potassium hydroxide in methanol

◌ = spots appear in some batches

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# APPENDIX

## 1. Reagents

1.1 Chloroform water

Shake 2.5 ml of chloroform with 900 ml of water until dissolved and dilute with water to 1000 ml.

1.2 Hydrochloric Acid, 2 M

HCl = 36.46

Dilute 170 ml of hydrochloric acid with sufficient water to produced 1000 ml.

1.3 Potassium hydroxide 20 % in methanol

Dissolve 20 g of potassium hydroxide with 90 ml of methanol until dissolved and dilute with methanol to 100 ml.

1.4 Potassium hydroxide 20 % in water

Dissolve 20 g of potassium hydroxide with 90 ml of water until dissolved and dilute with water to 100 ml.

## 2. Determination the technique for analytical thin layer chromatography (TLC)

(Kirchner, 1967)

**Material**

Leaves and roots of *Rhinacanthus nasutus* were collected and dried in the oven 50°C for 2 hrs. The technique for analytical thin layer chromatography (TLC) was used. The conditions for the analytical TLC used in this study are as follows:

| | | |
|---|---|---|
| Adsorbent | : | Silica gel 60 $F_{254}$ precoated plate |
| Solvent system | : | Chloroform : Hexane (8 : 2) |
| Layer thickness | : | 0.2 mm |
| Distance | : | 10.0 cm |
| Temperature | : | Laboratory temperature (30 - 37° C) |

**Method of extraction**

500 mg of the sample in dried powder, add 10 ml methanol, refluxed on water bath for 10 minutes, allowed to cool. After filtered through a plug of cotton

wool, the filtrates were evaporated to nearly dryness. The crude extract obtain were then kept in well close containers.

**Method of chamber saturation**

Care should be taken to saturate the chromatographic chamber with solvent vapour as completely as possible before use since it is strongly influences $R_f$ values.

In order to accomplish uniform saturation, the solvent system (100 ml of chloroform : hexane (8 : 2) ) was introduce into the chamber, of which the walls were lined as completely as possible with filter paper, 60 minutes before the introduction of the chromatographic plate. The vessel was then swirled round so that the filter paper was soaked with solvent.

**Method of application**

Take standard and test solutions with capillary tube 10 µl. Spot on TLC plate in the same plan, 2 cm far from bottom and dry. Take it in to tank, which already prepaired the mobile phase of solvent mixture, allowed in room temperature until the solvent front to the top 10 cm and dry to test.

**Method of detection**

1. Ultraviolet light

The chromatogram were examined under 254 nm and 366 nm ultraviolet light.

2. 20 % potassium hydroxide in methanol

Procedure: The chromatogram was sprayed with the reagent and allowed to dry in the air for 15 minutes.

**3. Sources of *Rhinacanthus nasutus***

In this study. *R. nasutus* raw materials were from different sources as showed in Table A.

**Table A** Sources of *Rhinacanthus nasutus*

| Samples | Plant part | Sources | Harvesting period | Purchased time |
|---|---|---|---|---|
| Songkhla, PSU 1 | Leaves and roots | Faculty of Pharmaceutical Sciences, Prince of Songkla University | October 2003 | - |
| Songkhla, PSU 2 | Leaves and roots | Faculty of Pharmaceutical Sciences, Prince of Songkla University | April 2003 | - |
| Songkhla, PSU 3 | Roots | Faculty of Pharmaceutical Sciences, Prince of Songkla University | July 2003 | - |
| Songkhla, PSU 4 | Roots | Faculty of Pharmaceutical Sciences, Prince of Songkla University | January 2003 | - |
| Narathiwat | Leaves and roots | Botanical garden, Amphur Muang, Narathiwat | October 2003 | - |
| Jana, Songkhla | Leaves and roots | Amphur Jana, Songkhla | October 2003 | - |
| Petchabun | Leaves and roots | Amphur Nhongphai, Phetchabun | - | February 2003 |
| Phang-nga | Leaves and roots | Amphur Tahaupa, Phang-nga | - | January 2003 |
| Chiang Mai | Leaves | Amphur Muang, Chiang Mai | - | February 2003 |
| Chantaburi | Leaves and roots | Amphur Khlung, Chantaburi | - | October 2003 |

**Table A** (continued)

| Samples | Plant part | Sources | Harvesting period | Purchased time |
|---|---|---|---|---|
| Surin | Leaves and roots | Amphur Muang, Surin | September 2003 | - |
| Pattani | Leaves and roots | Amphur Mae Lan, Pattani | January 2004 | - |
| Phattalung | Leaves and roots | Amphur Khaochaison, Phattalung | December 2003 | - |
| Nakhonpathom | Leaves and roots | Amphur Muang, Nakhonpathom | - | March 2004 |
| Drug store 1 | Leaves | Drug store, Amphur Hatyai, Songkhla | - | March 2003 |
| Drug store 2 | Leaves | Drug store Amphur Jana, Songkhla | - | October 2003 |
| Drug store 3 | Leaves | Drug store, Amphur Hatyai, Songkhla | - | October 2003 |
| Drug store 4 | Aerial part | Drug store, Amphur Muang, Nakhonpathom | - | May 2003 |
| Drug store 5 | Aerial part | Drug store, Amphur Hatyai, Songkhla | - | March 2003 |
| Drug store 6 | Aerial part | Drug store, Amphur Jana, Songkhla | - | October 2003 |
| Drug store 7 | Aerial part | Drug store, Amphur Hatyai, Songkhla | - | October 2003 |