PHARMACOGNOSTIC SPECIFICATION OF *ACORUS CALAMUS* RHIZOMES IN THAILAND

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ข้อกำหนดทางเภสัชเวทของเหง้าว่านน้ำในประเทศไทย

นางสาวอัชฌา สมนึก

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ว่านน้ำ มีชื่อทางวิทยาศาสตร์ว่า *Acorus calamus* Linn. ว่านน้ำเป็นเครื่องยาสมุนไพรที่ใช้ในตำรับยาไทย เช่น ประสะไพล ประสะกานพลู และวิสัมพญาใหญ่ การศึกษานี้มีจุดประสงค์เพื่อจัดทำข้อกำหนดทางเภสัชเวทของเหง้าว่านน้ำในประเทศไทย รวมทั้งวิเคราะห์หาปริมาณสารสำคัญในน้ำมันระเหยของว่านน้ำ โดยวิธีแกสโครมาโทกราฟีแมสสเปกโทรเมทรี โดยศึกษาเหง้าว่านน้ำจาก 15 แหล่งทั่วประเทศไทย วาดภาพลายเส้นแสดงลักษณะทางพฤษศาสตร์ของว่านน้ำ เตรียมเครื่องยาโดยล้างให้สะอาด ตัดเป็นท่อน และอบแห้ง ลักษณะทางมหภาคของเครื่องยามีรูปร่างเป็นแท่งยาวทรงกระบอก หลายขนาด สีน้ำตาลอ่อน มีรากฝอยเป็นเส้นเล็กติดอยู่ตามข้อปล้อง เนื้อภายในสีเนื้อขาว มีกลิ่นหอม ลักษณะเด่นทางจุลภาคของเหง้าว่านน้ำคือ เม็ดแป้ง ต่อมน้ำมัน ผลึกแคลเซียมออกซาเลต การศึกษาเอกลักษณ์ทางเคมี-ฟิสิกส์ของเหง้าว่านน้ำ พบว่า มีปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด น้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณสารสกัดด้วยเอทานอล ปริมาณสารสกัดด้วยน้ำ ปริมาณความชื้น และปริมาณน้ำมันหอมระเหย ร้อยละ 4.49 ± 0.15, 0.83 ± 0.07, 12.23 ± 0.34, 7.32 ± 0.29, 9.53 ± 0.45, 13.15 ± 0.46 และ 1.37 ± 0.11 โดยน้ำหนัก ตามลำดับ การศึกษาสารสกัดจากเอทานอลของเหง้าว่านน้ำด้วยเทคนิคทางทินเลเยอร์โครมาโทกราฟี โดยใช้ตัวทำละลายโทลูอีนต่อเอทิลอะซิเทต (9:1) เป็นเฟสเคลื่อนที่ และตรวจวัดภายใต้แสงอัลตาไวโอเลต ความยาวคลื่น 254 นาโนเมตร และ 365 นาโนเมตร พบว่ามีค่า R*f* เท่ากับ 0.58 น้ำมันว่านน้ำ ประกอบด้วยสารเบตาอาซาโรน (67.5 %) และ แอลฟาอาซาโรน (22.4%) เป็นองค์ประกอบหลัก วิเคราะห์สารเบตาอาซาโรนมีช่วงความเป็นเส้นตรงระหว่าง 0-0.5 มิลลิกรัม/มิลลิลิตร และมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.997 วิธีวิเคราะห์สารแอลฟาอาซาโรนมีช่วงความเป็นเส้นตรงระหว่าง 0-0.1 มิลลิกรัม/มิลลิลิตร และมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.998 ขีดจำกัดของการตรวจพบและขีดจำกัดของการหาปริมาณของสารเบตาอาซาโรน มีค่า 0.0280 และ 0.0850 มิลลิกรัม/ไมโครลิตรของตัวอย่าง และสารแอลฟาอาซาโรน มีค่า 0.0126 และ 0.0382 มิลลิกรัม/ไมโครลิตรของตัวอย่าง ระดับความเที่ยงของวิธีวิเคราะห์สารเบตาอาซาโรน และ แอลฟาอาซาโรน ประเมินจากค่าสัมประสิทธิ์ของการกระจาย มีค่าน้อยกว่าร้อยละ 15 ค่าเฉลี่ยการคืนกลับของสารเบตาอาซาโรนในว่านน้ำ คือ ร้อยละ 100.11-100.40 และ ค่าเฉลี่ยการคืนกลับของสารแอลฟาอาซาโรนในว่านน้ำ คือ ร้อยละ 100.17-100.80 ปริมาณสารเบตาอาซาโรน และ แอลฟาอาซาโรนในน้ำมันว่านน้ำมีค่า 0.259 และ 0.120 มิลลิกรัม/ไมโครลิตรของน้ำมัน ตามลำดับ ผลการศึกษาครั้งนี้สามารถจัดทำเป็นข้อกำหนดมาตรฐานของสมุนไพรว่านน้ำ ในประเทศไทย ซึ่งจะเป็นประโยชน์ต่อการควบคุมคุณภาพและความปลอดภัยในการใช้เครื่องยานี้

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต .....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KEYWORDS : PHARMACOGNOSTIC SPECIFICATION / *ACORUS CALAMUS* / CALAMUS OIL / ASARONE / GAS CHROMATOGRAPHY-MASS SPECTROMERY

ATCHA SOMNUK : PHARMACOGNOSTIC SPECIFICATION OF *ACORUS CALAMUS* RHIZOMES IN THAILAND. ADVISOR : CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 81 pp.

Acorus calamus Linn. dried rhizomes has been traditionally used as crude drug in Thai remedies such as Pra-Sa-Plai, Pra-Sa-Karn-Plu and Wi-Sum-Pa-Ya-Yai. This study aimed to report the current information on the pharmacognostic properties of *A. calamus* dried rhizomes and analysed the chemical constituents of calamus oil by gas chromatography–mass spectrometry (GC/MS). The rhizomes were collected from 15 habitats located at various regions throughout Thailand. The whole plant of *A. calamus* was illustrated in detail. The crude drug was traditionally prepared by cleaning, cutting and drying in a hot air oven. The macroscopic characters were cylindrical pieces, variable in size, pale brown with longitudinal furrows and circular, with pitted scars of rootlets, internally whitish and spongy and odor aromatic. The anatomical and histological characterizations were starch granules, secretory sac containing volatile oil and prismatic crystal of calcium oxalate. The total ash, acid insoluble ash, loss on drying, ethanol–soluble extractive, water–soluble extractive water content and volatile oil content of 4.49 ± 0.15, 0.83 ± 0.07, 12.23 ± 0.34, 7.32 ± 0.29, 9.53 ± 0.45, 13.15 ± 0.46 and 1.37 ± 0.11 % dry weight respectively. Thin-layer chromatographic fingerprints of ethanolic extracts of *A. calamus* dried rhizomes were studied using toluene and ethyl acetate (9:1) as mobile phase. Detection under ultraviolet light (254 nm and 365 nm) as well as spraying with anisaldehyde-sulfuric acid reagent showed the dominant band of α-asarone and β-asarone at R*f* = 0.58. The calamus oil consisted of β-asarone (67.5 %) and α-asarone (22.4%) as main components. Linearity range of β-asarone was 0-0.5 mg/ml with correlation coefficient (r^2) of 0.997 and α –asarone was 0-0.1 mg/ml with correlation coefficient (r^2) 0.998.. LOD and LOQ for β-asarone were 0.0280 and 0.0850 mg/µl of injected sample, 0.0126 and 0.0382 mg/µl of injected sample for α-asarone respectively. The precision was evaluated by the % RSD of repeatability and intermediate precision. β-Asarone was between 1.45-4.67 % RSD and 0.95-2.21 % RSD and α-ssarone showed 2.48-6.58 % and 1.29-3.00 % RSD respectively. The average recoveries were 100.11-100.40 % in β-asarone and 100.17-100.80 % in α-asarone. The quantitative results showed that β-asarone was the highest content in calamus oil (0.259 ± 0.035 mg/µl of oil) whereas α-asarone was found at 0.120 ± 0.020 mg/µl of oil. This study provides scientific information for the quality control of *A. calamus* dried rhizomes including calamus oil composition in Thailand that leads to safe use of this crude drug.

Field of Study : Public Health Sciences ..... Student's Signature .....

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Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

°C	=	Degree Celsius
cm	=	Centimeter
δ	=	Delta
EI	=	Electron Ionization
GC	=	Gas chromatography
GC-MS	=	Gas chromatography-mass spectrometry
HPLC	=	High performance liquid chromatography
hr	=	Hour
LD_{50}	=	Lethal dose 50%
LOD	=	Limit of detection
LOQ	=	Limit of quantitation
m/z	=	Mass-to-charge ratio
µl	=	Microliter
mg	=	Gram
min	=	Minute
ml	=	Milliliter
MS	=	Mass spectrometry
NIST	=	National Institute of Standard and Technology
nm	=	Nanometre
r^2	=	Correlation coefficients
RSD	=	Relative standard deviation
τ	=	Tau
vs	=	Versus

UV	=	Ultraviolet
α	=	Alpha
β	=	Beta

CHAPTER I

INTRODUCTION

Background and significance of the study

For centuries, medicinal plants and their products have been used in worldwide for treating various illnesses and continue to expand. Many crude drugs are expensive, deficient and adulterated part use of medicinal plants. These are problems to develop the medicinal plants and may display undesirable effect. The quality evaluation of medicinal plants is important to develop the traditional medicine and provide valuable information for identification.

Acorus calamus Linn., commonly known as sweet flag or calamus, is a reed like semi-aquatic perennial plant with a stout aromatic rhizome having medicinal properties from the Araceae family, in the genus *Acorus*. *A. calamus* grows worldwide wildly in Europe, North America and East Asia, along swamps, rivers and lakes [1-2]. In Thailand, this plant is widely distributed in marsh areas, mostly in the northern part of Thailand. It is called 'Wan-nam' which has been frequently used in Thai remedies such as Pra-Sa-Plai, Pra-Sa-Karn-Plu and Wi-Sum-Pa-Ya-Yai [3].

The previous studies of biological activities of this plant showed the beneficial effects and the toxicity. The rhizome contains active ingredients possessing antifungal, anti-helminthic, anti-bacterial and insecticide properties [4-7]. In the Ayurvedic system of medicine, the rhizomes are considered to possess anti-spasmodic, carminative, anti-depressant, central nervous system inhibitory and sedative activities [8-10]. This plant contains several active components that are dependent on locations, cytotypes and processing method. However, the chemical investigations of this plant have shown that the most characteristic component of calamus oil has two geometrical isomers of alkenylbenzene compound, asarone which are β-asarone or *cis*-1,2,4-trimethoxy-5-(1-propenyl)benzene and α-asarone or *trans*-1,2,4-trimethoxy-5-(1-propenyl)benzene [6, 8]. Beta–asarone has been demonstrated to be responsible for carcinogenic effects in animal studies, but there are no human data to confirm the carcinogenic potential of this constituent of calamus oil [9].

This study investigated the quality parameters of *A. calamus* rhizome in Thailand by pharmacognostic evaluation and analysed the chemical constituents of calamus oil by gas chromatography–mass spectrometry (GC-MS). The current information can be useful for standardization of *A. calamus* dried rhizomes including calamus oil in Thailand

Objectives of the study

1. To develop the pharmacognostic specification of *A. calamus* dried rhizome in Thailand.
2. To investigate the β-asarone and α-asarone contents of calamus oil in Thailand**.**

CHAPTER II

REVIEW OF LITERATURE

Taxonomy

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Acorales

Family: Araceae

Genus: *Acorus*

Species: *Acorus calamus*

Botanical characteristics

Acorus calamus Linn. (Figure 3) is an aromatic perennial plant with creeping, and cylindrical rhizomes about 1-2.5 cm in diameter, light brown and white internally. The sword-like leaves are between 0.7 and 1.7 cm wide, with average of 1 cm. The sympodial leaf of *A. calamus* is somewhat shorter than the vegetative leaves. The margin is curly-edged or undulate. Plants are very rarely flower or set fruit, the spadix, at the time of expansion, can reach a length between 4.9 and 8.9 cm. The flowers are small and berry-like, long about 3-4 mm. *A. calamus* is infertile and shows an abortive ovary with a shriveled appearance. It inhabits perpetually wet areas like the edges of streams and around ponds and lakes [1-2, 10-12].

Asarone

Formula: $C_{12}H_{16}O_3$

Molecular weight: 208.25

Asarone occurs in nature as a mixture of two geometrical isomers of alkenylbenzene compound, *β*-asarone being the (*Z*)- or *cis*-1,2,4-trimethoxy-5-(1-

propenyl)benzene (Figure 2) and *α*-asarone being the (*E*)- or *trans*-1,2,4-trimethoxy-5-(1-propenyl)benzene (Figure 3) [4, 8].

Figure 1 *Acorus calamus*

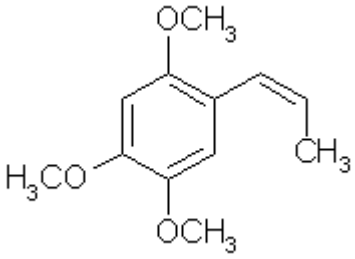

Figure 2 *β*-Asarone

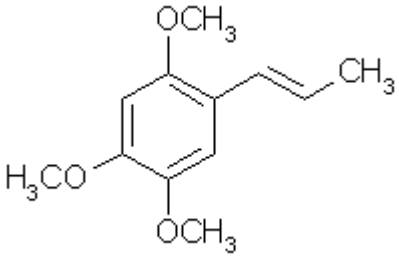

Figure 3 *α*-Asarone

Medicinal uses

A. calamus is a traditional Ayuravedic herbal medicine in India which is considered to possess depression, insomnia, anxiety, psychosis and epilepsy [13]. In Thailand, the roots and rhizomes of *A. calamus* or Wan-nam has been traditionally used for treatment of flatulence, cough and cold, sore throat, headache, asthma and malarial fever. The leaves have been used as poultice for treatment of muscle and joint pain [14]. *A. calamus* dried rhizome is a crude drug ingredient in Thai traditional medicine remedies such as Pra-Sa-Plai (for treatment of dysmenorrhea), Pra-Sa-Karn-Plu (for treatment of abdominal pain due to flatulence) and Wi-Sum-Pa-Ya-Yai (treatment of colic and flatulence) which published in National list of essential medicines - the List of Herbal Medicinal Products A.D.2011 [3, 15].

Chemical constituents

In 1981, Amarendra Patra and Alox K. Mitra reported *cis*-asarone, *trans*-asarone and as components in the oil of *A. calamus* [16].

Calamus oil in Tomsk and Altai regions and Kazakhstas were studies by gas chromatography–mass spectrometry (GC-MS). Samples of these oils were found to contain more than 140 components. The main components were *epi*-shyobunone, shyobunone, preisocalamediol, isocalamendiol, *epi*-acorone and acorone. Moreover, samples of these oils contained a small amount of asarone–type derivatives: γ-asarone and *β*-asarone as well as acorafuran [17].

The fresh rhizome and leaf oils of *A. calamus* from the lower region of the Himalayas were analyzed by gas chromatography-mass spectrometry (GC-MS). *β*-Asarone (83.2%) and *α*-asarone (9.7%) were the major constituents in rhizome oil, while *β*-asarone (85.6%) and linalool (4.7%) were the major constituents in the leaf oil [6].

A. calamus oil in Lithuania were studied by GC-MS presented a higher percentage of *β*–asarone (27.4 - 45.5%) in dried leaves while acorenone (20.86%) was dominant in rhizome followed by isocalamendiol [18]. J. Radusiene *et al.* demonstrated that *β*-asarone (15.7–25.5%), *α*-asarone (1.1–7.7%) (Z)-Methyl

isoeugenol (2.0-4.9%) and γ-asarone (0.6-1.3%) were the main components of essential oil obtained from leaves in wild of Lithuania [19].

Essential oil from dried *A. calamus* rhizomes in Konya (Turkey) was analyzed by GC-MS. The results show that preisocalamendiol (17.3%) represents the major compounds followed by isoshyobunone (13.0%), 1,4-(trans)-1,7(trans)-acorenon(10.5%), camphor (5.9%), 2,6-diepishyobunone (2.6 %) and *β*-gurjunene (2.5%) [20].

A. calamus rhizome powder from Eastern Nepal was determined by gas chromatography–mass spectrometry (GC-MS) found *β*-asarone [21]. Marongin *et al*, (2005) reported that the main components of the supercritical fluid CO_2 extract oil of *A. calamus* were acorenone, iso-acorone, (*z*)-sesquilavandulol, dehydroxy isocalamediol and *β*-asarone *via* GC-MS analysis [22].

In Poland dried *A. calamus* whole plant oils were obtained by hydrodistillation found acorenone (14.6%), shyobunone isomer (10.5%) and *β*-asarone (10.4%) [23].

Garneau *et al.* reported that the main components of essential oil from rhizomes of *A. calamus* in Quebec were preisocalamenediol (18 %), acorenone (14.2%), shyobunone (10.8 %), cryptoacorone (7.5 %) and absence of *β*-asarone [24]. Hema Lohani *et al.* studied the essential oil from the dried aerial parts of *A. calamus* and analyzed by GC-MS found that *β*-asarone (74.36 - 92.22%) was the most abundant compound [25].

Wangsittidet T., *et al* (2007) demonstrated that *β*-asarone (82.9%) was the main component in calamus oil from fresh rhizomes in Thailand [26]. The *β*-asarone content in essential oil and crude drug of *A. calamus* rhizome in Thailand were analyzed by gas chromatography and it was shown that *β*-asarone content in oil more than in crude drug [27].

The chemical composition of the essential oil of *A. calamus* depends on the cytotypes according to its chromosome number. Karyological analysis of *Calamus* species indicates that *A. calamus* have four cytotypes (karyotypes): diploid (2x = 24 chromosomes) in North America, triploid (3x = 36 chromosomes) in Europe,

tetraploid (4x = 48 chromosomes) in East Asia, India and Japan, and hexaploid (6x = 72 chromosomes) in Kashmir [16, 17].

The most characteristic component of calamus oil is asarone which formed into 2 isomers, *β*- and *α*- asarone. The concentration of *β*-asarone in *A. calamus* and calamus oil depends on the cytotype of *A. calamus*. The identification of the cytotypes of this species requires the chromosomal analysis rather than determination of anatomical characteristics. *α*-Asarone and *β*-asarone are the major components of triploid and tetraploid *A. calamus* but the diploid *A. calamus* do not contain significant amounts of *β*-asarone which is the carcinogenic compound [18].

Ahlawat *et al.* reported that triploid and tetraploid *A. calamus* was found *β*-asarone content as 6.92–8.0% and 73–88% respectively in essential oil from fresh aerial parts [28]. European and North American triploid presented 3-19% of *β*-asarone in the rhizome oils and 31-44% of *β*-asarone in the leaf top oils [29].

In 2005, alcoholic extracts of both diploid and triploid *A. calamus* was analyzed by gas chromatography–mass spectrometry (GC-MS). Alcoholic extracts of the triploid *A. calamus* presented a higher percentage of *β*-asarone, followed by camphene, E-*β*-ocimene, camphor, calarene, α-selinene and τ-cadinol, when compared to the diploid *A. calamus*. In contrast, alcoholic extracts of diploid *A. calamus* presented higher percentages of iso-shyobunone, *β*-sesquiphellandrene, preiso calamendiol, acorone and completely lacked of *β*-asarone [30]. Methyl isoeugenol (41.5%) and cyclohexanone (21.3%) were identified as the major constituents of the essential oil in Korean *A. calamus* rhizome *via* GC-MS analysis [31].

In tetraploid *A. calamus* showed various amount of *β*-asarone. The tetraploid of Indian type (Indonesian and Taiwan) was reported the *β*-asarone contents in their rhizome oils and leaf top oils up to 96% and 60-70% respectively. The tetraploid of Japan and far-east Russian presence the *β*-asarone contents in their rhizome oils and leaf top oils 10-40% and 20-50% *β*-asarone respectively. On the other hand, tetraploid *A. calamus* from Singapore, Vietnam and Thailand are different from other type by the larger amounts of *β*-asarone in the rhizome oils [6, 17].

In 2007, the average essential oil content was established at the value of 1.50% of dried material in Finland and 1.91% in the Czech Republic [32].

The phytochemical of the *Acorus calamus* rhizome were studied and found the presence of quinone, coumarin, flavone, steroid, phenol, glycosides, terpenoid, tannin, alkaloid and iridoid [33].

Pharmacological activity

In the animal model, it has been reported that Jammu calamus oil exhibited the oral toxicity with LD_{50} of 777 mg/kg in rats [34].

Undiluted calamus oil was not irritated when applied to the back of hairless mice [35] or to intact or abraded rabbit and guinea-pig skin for 24 hr [36].

The previous study in 19 years old man who ingest 8 inch of *A. calamus* rhizome with water showed gastrointestinal irritation by vomiting and diaphoresis in several hours without sequelae after supportive care [37].

An oleoresin from rhizomes of Indian *A. calamus* administered intraperitoneal injection into mice at 0.2 g/kg showed slight sedative activity [38].

Subacute toxicity [39] of *β*-asarone was studies. *β*-asarone has oral 100 mg/kg bw/day in rat and i.p. 184 mg/kg bw/day in mice. Weight loss, reduced thymus weights and liver weights for 5 days after treatment. Subchronic toxicity [39] in rats by feeding the dietary levels tested (0, 100, 250, 500 or 1000 mg/kg bw) for 2 years was reported that given reduce body weight, gross and microscopic changes in the liver and damage to the heart. In a chronic feeding study, *β*-asarone produced an increased incidence in leiomyosarcomas in the small intestines of male rats. *β*-asarone has shown a weak carcinogenic effect in rats at dose levels of 20 mg/kg [39]. No data have been identified in *vivo* genotoxicity of *β*-asarone and on the toxicity of *β*-asarone in humans [9, 39].

The essential oil of Indian *A. calamus* showed anticonvulsant, antiveratrinic and anti-arrhythmic activity [40].

Indian calamus oil administered intraperitoneal injection was found sedative-tranquilizing action in rats, mice, eats, dogs and monkeys, but vomiting was observed in the latter three species [41].

The 50% ethanolic extract of *A. calamus* rhizomes (100 and 200 mg/kg) demonstrated significant hypolipidemic activity. On the other hand, the aqueous extract showed hypolipidemic activity only at a dose of 200 mg/kg [42].

S. Mehrotra., *et al* (2003) demonstrated the antiproliferative and immunosuppressive potential of ethanolic extract of *A. calamus* rhizome *in vitro* [43].

Phongpaichit, S., *et al* (2005) reported that the crude methanol extract of *A. calamus* rhizomes was investigated for its antimicrobial activities on various microorganisms including bacteria, yeasts and filamentous fungi. It exhibited high activity against filamentous fungi: *Trichophyton rubrum*, *Microsporum gypseum*, and *Penicillium marneffei* with IC_{50} values of 0.2, 0.2 and 0.4 mg/ml respectively. However, it showed moderate activity against yeasts: *Candida albicans, Cryptococcus neoformans* and *Saccharomyces cerevisiae* (MIC 0.1-1 mg/ml) and low activity against bacteria (MIC 5->10 mg/ml) [44].

Sundaramahalingam, M., *et al* (2005) found that the ethyl acetate and methanolic extract of *A. calamus* protected most of the changes in the rat brain induced by noise-stress [45].

The previous study reported that *α*-asarone demonstrated the antioxidant activities by against noise-stress induced changes in rat brain [46].

In 2006, the antispasmodic effect of *A. calamus* was studied. The results suggest that the spasmolytic effect of the plant extract is mediated through the presence of Calcium Channel Blockade-like constituents which is concentrated in the *n*-hexane fraction and this study provides a strong mechanistic base for its traditional use in gastrointestinal disorders such as colic pain and diarrhea [47].

In 2009, insulin sensitizing activity and antidiabetic effects of ethyl acetate fraction of *A. calamus* (ACE) *in vitro* and *in vivo* were studied. The result showed that

ACE (12.5 and 25µg/ml) increased glucose consumption mediated by insulin in L6 cells. In diabetic mice, ACE (100 mg/kg) significantly reduced serum glucose, triglyceride, reinforce the decrease of total cholesterol caused by rosiglitazone and markedly reduced free fatty acid (FFA) levels and increased adiponectin levels as rosiglitazone did. Serum insulin was decreased but not significantly. In addition, ACE decreased the intake of food and water, and did not increase body weight gain whereas rosiglitazone did [48].

A. calamus leaf extract inhibits the production of pro-inflammatory cytokines through multiple mechanisms and may be a novel and effective anti-inflammatory agent for the treatment of skin diseases [49].

In 2010, the effects of *β*-asarone on cognitive function and neuronal apoptosis in beta-amyloid hippocampus injection rats and its mechanism of action were studied. The results suggest that *β*-asarone may be a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease [50].

The last data indicate the presence of unique combination of airways relaxant constituents in crude extract of *A. calamus*, provide a pharmacological basis for traditional use of *A. calamus* in disorders of airways [51].

In 2012, the analyze of transformation and excretion of *β*-asarone in rabbits were studied *via* gas chromatography–mass spectrometry (GC-MS). This study reported that *β*-asarone was excreted in urine, feces and bile (62%, 22% and 16%) respectively. Moreover *β*-asarone was excreted in 1 h. and 22% of *β*-asarone was converted into α-asarone [52].

Toxicological activity

β-Asarone is evidentially reported to be rodent carcinogen however carcinogenic data in human is not concluded [53]. Since 1974, the US Food and Drug Administration (FDA) banned the use of *A. calamus* as a flavoring agent in beverages, pharmaceuticals and dental preparations [54]. In the report studied by using the Jammu (Indian) variety of calamus oil which contains 80% *β*-asarone that was more

than the European variety concentrations of *β*-asarone [54]. US-FDA classifies calamus and its derivatives into Subpart C – Substances Generally Prohibited from Direct Addition or Use as Human Food which includes "(a) Calamus is the dried rhizome of *Acorus calamus* L. It has been used as a flavoring compound, especially as the oil or extract. (b) Food containing any added calamus, oil of calamus, or extract of calamus is deemed to be adulterated in violation of the act based upon an order published in the Federal Register of May 9, 1968 (33 FR 6967)." [55].

The Council of Europe Experts on Flavouring Substances classifies *β*-asarone into Category 6 "Plants, animals and other organisms, and parts of these or products thereof, and preparations derived therefrom, which are considered to be unfit for human consumption in any amount." The exception is only for alcoholic beverages traditionally flavoured with calamus which limits at 0.5 mg/kg [53]

Quality control

The quality control of plant material is essential for the utilization of medicinal plants. Their quality control could be efficiently performed only in case their analytical methods and appropriate quality specifications have been established.

In 1986, the Specification of Thai Medicinal Plants vol.1 reported the pharmacognostic specification of *A. calamus* that "Loss on drying: not more than 13%, Total ash: not more than 9%, Acid-insoluble ash: not more than 2%, Alcohol-soluble extractive: not less than 7%, Water-soluble extractive: not less than 7%, Ether-soluble extractive: not less than 0.5%" [56].

In 1993, the pharmacognostic specification of *A. calamus* has been reported that "Foreign matter: not more than 2%, Total ash: not more than 7%, Acid-insoluble ash: not more than 1%, Water content: not more than 12% Ethanol-soluble extractive: not less than 5%, Water-soluble extractive: not less than 11%, Volatile oil content: not less than 1.5%" [27].

The previous reports of *A. calamus* pharmacognostic specification were performed on dried rhizomes of *A. calamus* in Thailand. However, the sample size and sampling location were restricted. This current study investigated the quality

parameters of *A. calamus* dried rhizomes from 15 different locations throughout Thailand accordingly to WHO Quality Control Methods for Herbal Materials.

Parameters standardization [57]

Determination of macroscopic and microscopic description

Medicinal plant materials are categorized according to sensory, macroscopic and microscopic characteristics. Macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface. Microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials. This method using a microscope to identify the structural features, cells, and ergastic substances of herbal samples with application of the knowledge of plant morphology and anatomy so as to authenticate plant species. The drawing will be made using microscope and drawing attachment or camera aided.

Determination of loss on drying

Loss on drying determines both water and volatile matter. For materials, which contain little balance combines the drying, process and weight recording; it is suitable where large numbers of samples are handled and where a continuous record of loss in weight with time is required.

Determination of moisture content

An excess of water in medicinal plant materials will persuade microbial growth which presence of fungi and deterioration by hydrolysis. Limits for water content should be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The azeotropic method (toluene distillation method) gives a direct measurement of the water present in the material being examined. The sample is distilled together with an immiscible solvent, such as toluene or xylene. The water and the solvent are distilled together and separated in the receiving tube on cooling. The volume of water distilled over will be read and the percentage initially present in the sample will be calculated

as a percentage of dried weight. The solvent is saturated with water before use to avoid water absorbed by the solvent during distillation.

Determination of ash content

The ash remaining following incineration of medicinal plant materials is determined by two different methods which measure total ash and acid-insoluble ash.

The total ash method is designed to measure the total amount of material remaining after incineration. This includes both physiological ash which is derived from the plant tissue itself, and non-physiological ash which is the residue of the extraneous matter adhering to the plant surface.

Acid-insoluble ash is the residue obtained after boiling the total ash with around 2 N hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present especially as sand and siliceous earth.

Determination of extractive value

This method determines the amount of active constituents extracted with solvents from an amount of medicinal plant material.

Determination of volatile oil content

Volatile oils are characterized by their odor, oil-like appearance and ability to volatilize at room temperature. Chemically, they are usually composed of mixtures of, for example, sesquiterpenes, monoterpenes, and their oxygenated derivatives. Aromatic compounds predominate in certain volatile oils. The volatile oil is obtained by preparing ground crude drug in water and distillation by Clevenger apparatus**.**

TLC identification

Thin-layer chromatography is particularly valuable for the qualitative determination of small amounts of impurities. Separation by TLC is effected by the application of the mixture or extract as a spot or thin line onto a sorbent that has been applied to a backing plate. Silica gel 60 GF_{254} precoated TLC plates 0.063 - 0.200 mm will be used. The plate is placed into tank with sufficient suitable solvent to just wet

the lower edge of the plate sorbent but not enough to wet the part of the plate where the spots were applied. The solvent front then migrates up the plate through the sorbent by capillary action, a process known as development. Remove the plate, mark the position of the solvent front and allow the solvent to evaporate at room temperature. The spot will be visualized under UV light at 254 nm and 365 nm. Then spray with detecting reagent and heat at 110 °C for 10 min. The information provided by a finished chromatogram includes the migrating behavior of the separated substances. It is given in the form of the retention factor (R_f) value [58, 59].

Gas chromatography–mass spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is the most popular and effective method for separation, identifying and quantifying individual components in a mixture. It has two parts. The gas chromatography (GC) portion is use to separate the chemical mixture and mass spectrometry (MS) portion is use to identify and quantify the chemical [58, 59]. The GC method is suitable for quantitative and qualitative analysis of natural volatile components, and lipophilic compounds with low boiling point and good thermal stability. The components in a mixture can separate base on their volatility. Separation of sample components on a GC column depends on the time, it takes for each individual component to move along and elute from the column, is called the retention time (RT). Although the analysis by HPLC has been mostly for analysis of a wide range of compounds from heat sensitive to nonvolatile compounds, some studies still like to use GC to quantify, because GC provides better resolution due to its longer column than HPLC, and less interference by the mobile phase. MS is the most common detector for the GC to identify unknown chemicals and compounds based on their chemical properties. The MS is an analytical technique that measures the mass-to-charge ratio (*m/z*) [60]. Gas chromatography (GC) separates the components of a mixture and mass spectroscopy (MS) characterizes the individual component. By combining the two techniques, an analysis can be performed both qualitatively and quantitatively. GC-MS is very sensitive and has high resolution [59].

CHAPTER III

MATERIAL AND METHODS

Chemicals and reagents

1. alpha-Asarone, 98% (Sigma-Aldrich Company Co., St. Louis, MO, USA)
2. *cis*-2,4,5-Trimethoxy-1-propenylbenzol, 70% (Sigma-Aldrich Company Co., St. Louis, MO, USA)
3. Methanol, HPLC grade (Labscan, Thailand)
4. Ethanol (Labscan, Thailand)
5. Toluene (Labscan, Thailand)
6. Ethyl acetate, HPLC (J.T. Beaker Chemical Co., USA)
7. Hydrochloric acid (Labscan, Thailand)
8. Anisaldehyde-sulfuric acid reagent
    a. *p*-Anisaldehyde (Sigma-Aldrich Company Co., USA)
    b. Acetic acid, glacial (BDH Chemical Ltd., England)
    c. Methanol (Labscan, Thailand)
    d. Sulfuric acid (Labscan, Thailand)

Materials

1. Zebron ZB 5 capillary column (30 m x 0.25 mm x 0.25µm) (Phenomenex®, CA, USA)
2. TLC Siliga gel 60 GF_{254} (Merck, Germany)
3. Filter paper No.4 Qualitative (Whatman™, UK)
4. Filter paper No.40 Ashless (Whatman™, UK)

Equipments

Gas chromatograph (Trace GC Ultra, Thermo Finigan, USA) equipped with MS detector (DSQ, Thermo Finigan, USA)

Methods

Plant samples collection

Rhizomes of *A. calamus* were collected from 15 habitats that located among several regions throughout Thailand. All set of crude drugs were authenticated by Ruangrungsi N. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. The rhizomes were cleaned, sliced and dried in a hot air oven at 45 °C then kept in well- closed container in a dark place.

Pharmacognostic specification [57]

Macroscopic and microscopic description

Identified for size, color and other visual inspection. Performed microscopic histological inspection as described: powdered material, sifted through a 250 micron sieve then inspected under microscope with magnification of 4x, 10x and 40x, compare the scale with the micrometer. Performed microscopic anatomical inspection of transverse section as well. Hand drawing of the whole plant, histological and anatomical characters was exhibited.

Determination of loss on drying

Weighed 5.0 g of ground crude drug in a tared small beaker and dried with heat at 105 °C to constantly weight. Each sample was performed in triplicates.

Determination of ash content

Determination of total ash

Placed 5.0 g of ground crude drug, accurately weighed in a previously ignited and tared crucible. Then ignited it by gradually increasing the heat to 500-600 °C until

white then cooled in a desiccator and weigh without delay. Each sample was performed in triplicates.

Determination of acid-insoluble ash

The crucible containing the total ash was added with 25.0 ml of hydrochloric acid (70 g/l), covered with a watch-glass and boiled gently for 5 minutes. Rinsed the watch-glass with 5.0 ml of hot water and added this liquid to the crucible. Collected the insoluble matters on an ashless filter-paper and washed with hot water until the filtrate was neutral. Transferred the filterpaper containing the insoluble matters to the original crucible, dried on a hot plate and ignite. Cooled in a desiccator and weighed without delay. Each sample was performed in triplicates.

Determination of moisture content

Weighed 50.0 g of ground crude drug, added with 200.0 ml of water-saturated toluene and distilled by Azeotropic distillation. As soon as water was completely distilled, rinsed the inside of the condenser tube with toluene and continued the distillation for 5 more minutes. Allowed the receiving tube to cool room temperature. When water and toluene layers were separated, read off the volume of water. Each sample was performed in triplicates.

Determination of extractive values

Determination of ethanol-soluble extractive value

Macerated 5.0 g of ground crude drug with 100.0 ml of absolute ethanol in a closed conical flask for 6 hours in shaking bath and allowed standing for 18 hours. Filtered rapidly to avoid loss of ethanol. Evaporated 20.0 ml of the filtrate to dryness in a tared small beaker and dried with heat to constantly weight. Each sample was performed in triplicates.

Determination of water-soluble extractive value

Macerated 5.0 g of ground crude drug with 100.0 ml of distilled water in a closed conical flask for 6 hours in shaking bath and allowed standing for 18 hours.

Evaporated 20.0 ml of the filtrate to dryness in a tared small beaker and dried with heat to constantly weight. Each sample was performed in triplicates.

Determination of volatile oil content

Weighed 100.0 grams of ground crude drug, added with 600.0 ml of water and distilled by Clevenger apparatus. When volatile oil was completely distilled, allowed the receiving tube to cool room temperature. When volatile oil and water layers were separated, read off the volume of volatile oil. Each sample was performed in triplicates.

Thin-layer chromatographic identification

The ground crude drug (0.5 g) was macerated with ethanol (10 ml) for 12 hrs. The extract was filtered and evaporated to dryness. The residue was dissolved in methanol (1.0 ml) and 3 µl of this solution was applied on to a thin-layer aluminium plate coated with siliga gel GF_{254}. The TLC plate was then placed in a chamber with toluene and ethyl acetate (9:1) as mobile phase. After development, the plate was removed, and allowed to dry in air and examined under ultraviolet light (254 nm and 365 nm). Then, the plate was sprayed with anisaldehyde-sulfuric acid reagent and heated at 110 °C for 5 min.

Determination of *β*-asarone and *α*-asarone in calamus oil by GC-MS

Calamus volatile oil was extracted from the unpeeled dried rhizome of *A. calamus* by hydro distillation as mentioned above.

Qualitative analyis

Ten microliter of each sample was mixed with 1 ml of HPLC grade methanol. The chemical analysis was performed on a Finnigan Trace GC ultra with Finnigan Trace DSQ mass spectrometer using ZB-5 capillary column (30 m x 0.25 mm x 0.25µm). The oven temperature was 60 ºC for 1 min then ramped to 240 ºC with the rate of 3 ºC/min. The injector temperature was 180 ºC. One microlitre of diluted sample was injected into capillary column with split ratio of 100:1. Helium was used as carrier gas. The *α*- and *β*-asarone were identified by external standard comparison.

The other chemical constituents of calamus oil were identified by matching their mass spectra and retention indices with Adams Essential Oil Mass Spectral library and NIST05 Mass Spectral library and their content ratio was expressed as relative percentage of the total peak areas.

Quantitative analysis

One microliter of each sample was mixed with 1 ml of methanol. The sample solution was analyzed within the same condition GC as use for the sample in qualitative analysis. Analysis of each sample was performed in triplicates. The amount of α-asarone and β-asarone were determined by comparing the area of peak with the calibration curves of standard solutions and were expressed in mean and standard deviation (SD) as mg/µl of oil. Analysis of each sample was performed in triplicates.

Preparation of standard solutions

Stock solution of β-asarone (1.07 mg/ml) was prepared by dissolving 1 µl of β-asarone in 1 ml of methanol. Stock solution of α-asarone (1 mg/ml) was prepared by dissolving 1 mg of α-asarone in 1 ml of methanol. The stock solutions were diluted for standard curves; 0.5, 0.4, 0.3, 0.2, 0.1 mg/ml of β-asarone and 0.25, 0.2, 0.15, 0.1, 0.05 mg/ml of α-asarone. The standards were analyzed under the same condition of GC as used for the samples.

Calibration curve and linearity

The calibration curves were obtained with five concentrations. It was plotted according to the linear regression analysis of the peak areas (y-axis) versus concentrations (x-axis, mg/ml) of β-asarone and α-asarone.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ determination were based on the standard deviation of response and the slope [61]. The LOD and LOQ were calculated as follow:

$$\text{LOD} = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

The slope, S was estimated from the calibration curve of the analyte. The standard deviation of y-intercepts of regression lines was used as the standard deviation, σ.

Precision

The precision of the method was assessed with repeatability and intermediate precision analyses. Three different concentrations of sample which covered the specified range were analyzed in triplicate on 1 day and these were repeated on 3 different days for intermediate precision. The amount of each concentration was determined by comparing the area of peak with the calibration curves of standard solutions. Relative standard deviation (%RSD) was used to measure precision by following formula:

$$\%RSD = (SD \times 100) / mean$$

Where SD = the standard deviation of each measurement

Recovery

Recovery was carried out by spiking method [61] using 3 concentrations of standard solution added to a sample (known amounts of analyte) and the percent recovery was calculated as follow:

$$Recovery\ (\%) = [C/(A+C_0)] \times 100$$

Where C: the amount of β-asarone or α-asarone that found after spiking of the standard solution

A: the amount of those found that before spiking

C_0: the amount of reference standards actually added to the sample

CHAPTER IV

RESULTS

Pharmacognostic specification

The drawing of whole plant of *A. calamus* was illustrated in detail (Figure 4). *A. calamus* crude drug was prepared by cutting the rhizome and dried in a hot air oven at 45°C. The crude drugs were cylindrical or longitudinal pieces, variable in size, pale brown with longitudinal furrows and circular, with pitted scars of rootlets, internally whitish and spongy, odor aromatic (Figure 5).

The anatomical characterization showed epidermis, collenchyma, secretory sac containing volatile oil, crystal of calcium oxalate, cortical fiber, starch granules, and vascular bundle (Figure 6).

The histological characterization was composed of fragments of fiber, calcium oxalate prism, starch granule, epidermis, parenchyma with oil cell and starch granules, collenchymas and fibers (Figure 7).

The constant numbers due to quality parameters of *A. calamus* dried rhizomes were shown in Table 1. The total ash, acid insoluble ash, loss on drying and water content should be not more than 4.49, 0.83, 12.23 and 13.15 % of dry weight respectively whereas ethanol–soluble extractive, water–soluble extractive and volatile oil content should be not less than 7.32, 9.53 and 1.37 % w/w respectively.

Thin layer chromatographic fingerprint of ethanolic extract of *A. calamus* dried rhizome were shown in Figure 8.

Macroscopic characters (Whole plant)

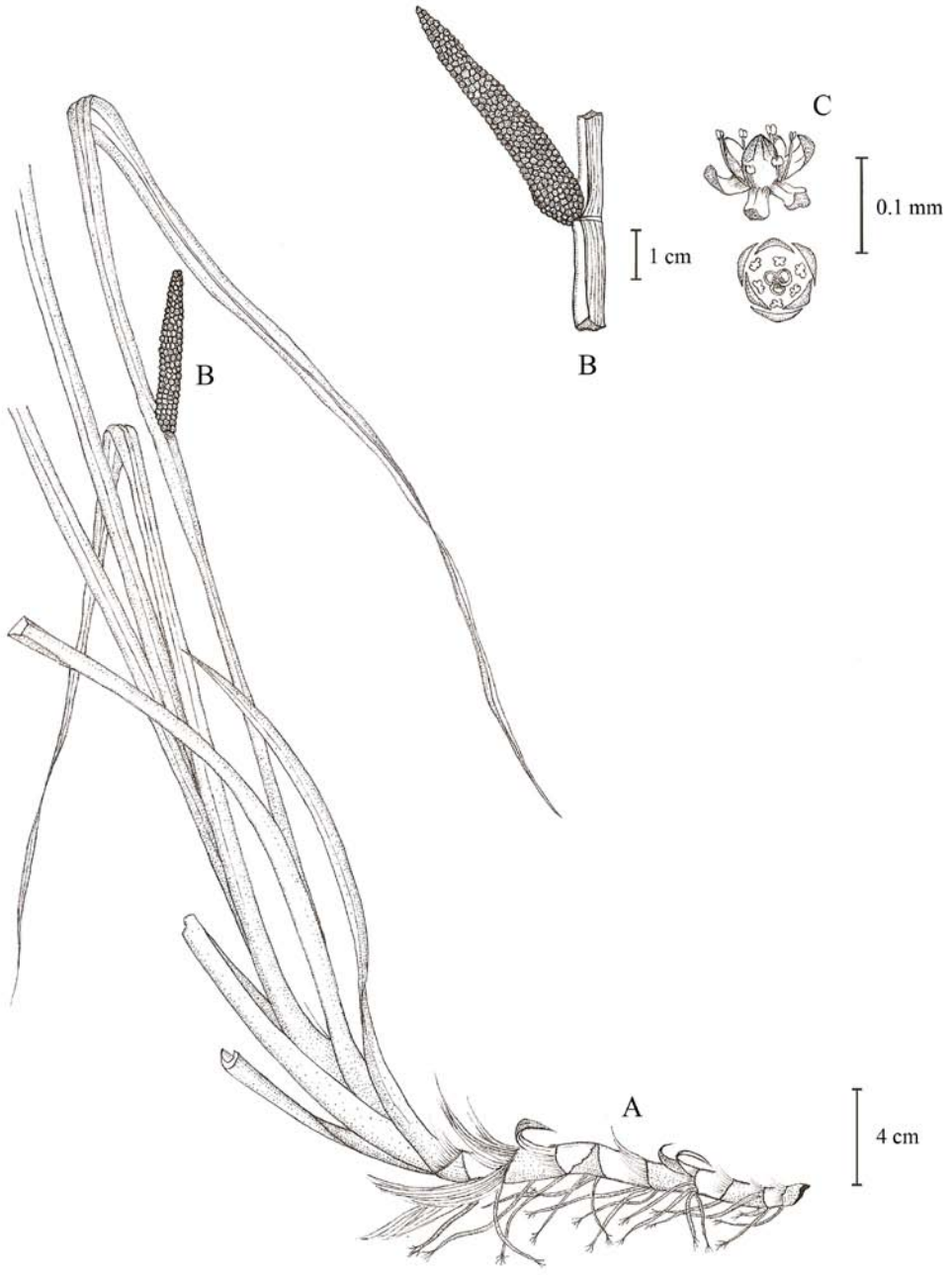

Figure 4 Whole plant of *Acorus calamus* Linn.

A. rhizome

B. inflorescence

C. a single flower, enlarged and diagram of flower

Macroscopic characters (Crude drug)

Figure 5 Crude drug of *Acorus calamus* Linn.

Microscopic characters (Anatomical characters)

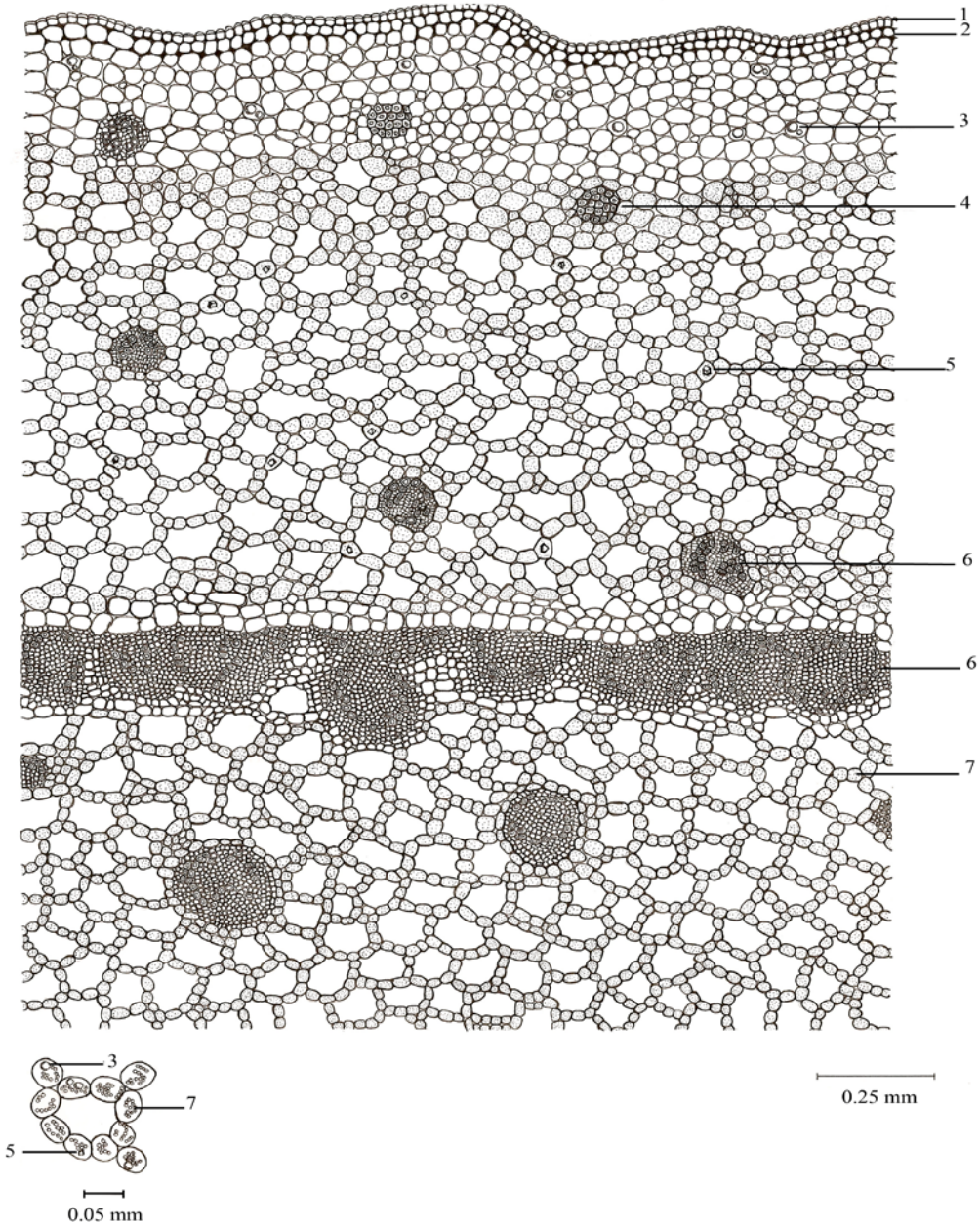

Figure 6 Transverse section of *Acorus calamus* Linn. rhizome:

1. epidermis
2. collenchymas
3. oil cell
4. cortical fiber
5. crystal of calcium oxalate
6. vascular bundle
7. starch granules

Microscopic characters (histological characters)

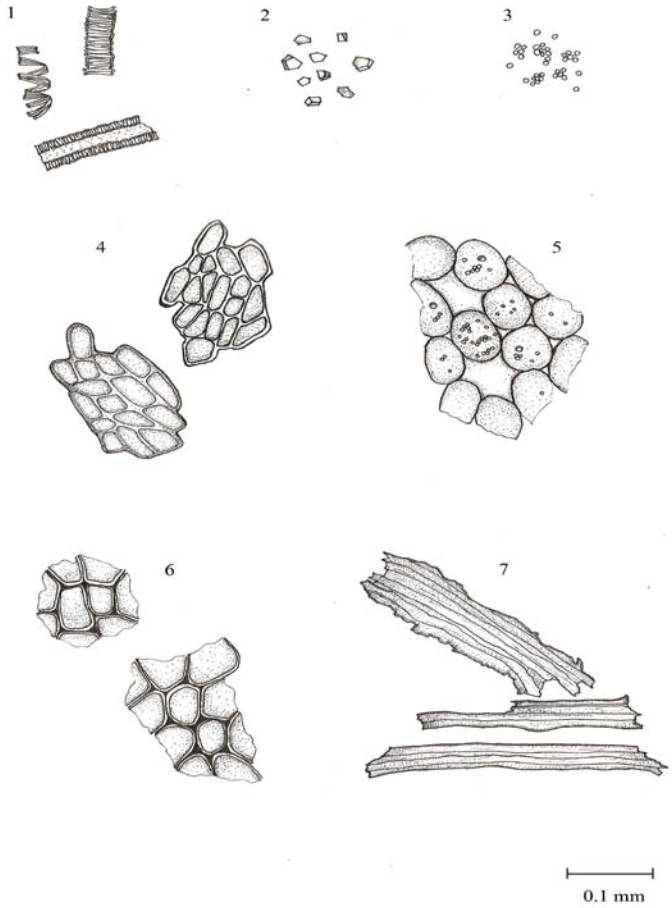

Figure 7 Powder of *Acorus calamus* Linn. rhizome:

1. fragments of fiber
2. calcium oxalate prism
3. starch granule
4. epidermis of rhizome in surface view
5. parenchyma in sectional view with oil cell and starch granules
6. collenchymas of the rhizome in sectional view
7. fibers

Table 1 The constant numbers due to quality parameters of *Acorus calamus* rhizomes

Specification (%by weight)	**Mean ± SD[a]**	**Min – Max**
Loss on drying	12.23 ± 0.34	11.81 - 12.78
Total ash	4.49 ± 0.15	3.88 - 5.32
Acid-soluble ash	0.83 ± 0.07	0.70 - 1.03
Ethanol–soluble extractive	7.32 ± 0.29	7.02 - 8.45
Water–soluble extractive	9.53 ± 0.45	6.74 - 11.58
Water content	13.15 ± 0.46	12.13 - 14.20
Volatile oil content*	1.37 ± 0.11	1.20 - 1.53

[a] The parameters were shown as grand mean ± pooled SD. Samples were from 15 different sources throughout Thailand. Each sample was done in triplicate.

* ml/100 g

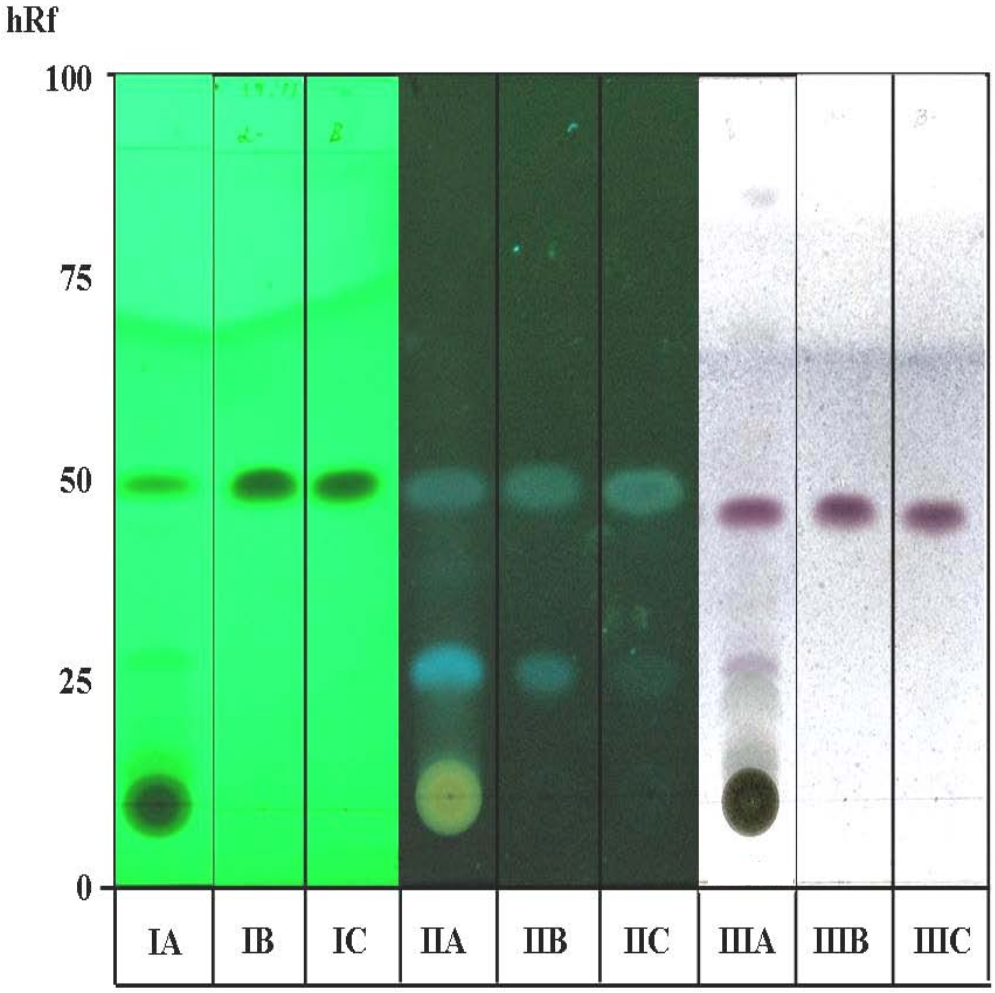

Figure 8 Thin layers chromatographic fingerprint

A: the ethanolic extract of *Acorus calamus* dried rhizome

B: α-asarone

C: *β*-asarone

Detection

I = detection under UV light 254 nm

II = detection under UV light 365 nm

III = detection with anisaldehyde-sulfuric acid reagent

Calamus oil constituents

The major chemical constituent of the calamus oil from 15 different areas in Thailand was revealed as *β*-asarone (67.5 ± 8.1 %) followed by α-asarone (22.4 ± 7.9 %). Figure 9 and Table 2 showed the GC chromatogram and chemical constituents of calamus oil which revealed the composition of asarone in major and other minor essential oil.

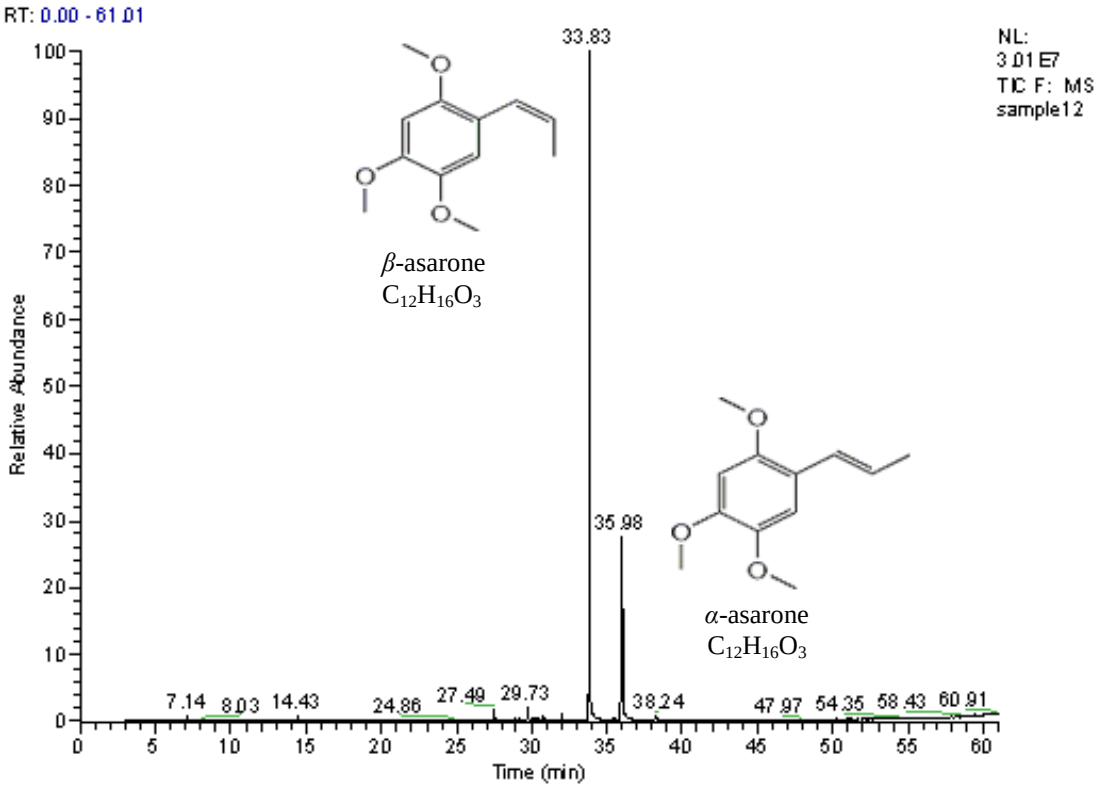

Figure 9 GC chromatogram of calamus oil

Table 2 The chemical constituents of volatile oil of *Acorus calamus* rhizomes (calamus oil)

RT	Name of compound	Area %	KI
28.05	Methyl isoeugenol <Z>	4.25	1453
29.54	Shyobunone	0.29	
29.64	Methyl isoeugenol <E>	0.99	1492
30.35	Shyobunone	1.74	
30.72	Cadinene <delta>	0.11	1523
30.97	unidentified	0.35	
31.48	Calacorene <beta>	0.47	1565
31.92	Elemicin	0.12	1557
32.62	Isoelemicin <Z>	1.60	1570
32.81	Spatulenol	0.11	1578
33.74	Rosifoliol	0.07	1600
34.51	Guaiol	0.16	1600
34.55	Asarone <beta>	71.84	1617
35.25	tau – Muurolol	0.11	1642
35.71	Cadinol <alpha>	0.13	1654
35.96	unidentified	0.11	
36.10	Khusinol acetate	0.47	1823
36.62	Asarone <alpha>	16.36	1676
37.90	unidentified	0.17	
38.88	unidentified	0.55	

The mass spectrum of *β*-asarone (Figure 10) showed a parent peak at *m/z* 208 corresponding to the molecular formula α-asarone $C_{12}H_{16}O_3$. The spectrum contained peaks corresponding to major fragments at *m/z* value of 208, 193, 194, 165, 162, 137, 91, 77 and 69. Similarly, The mass spectrum of *α*-asarone (Figure 11) showed a parent peak at *m/z* 208 corresponding to the molecular formula *α*-asarone $C_{12}H_{16}O_3$. The spectrum contained peaks corresponding to major fragments at *m/z* value of 208, 193, 165, 162, 137, 133, 91, 77 and 69.

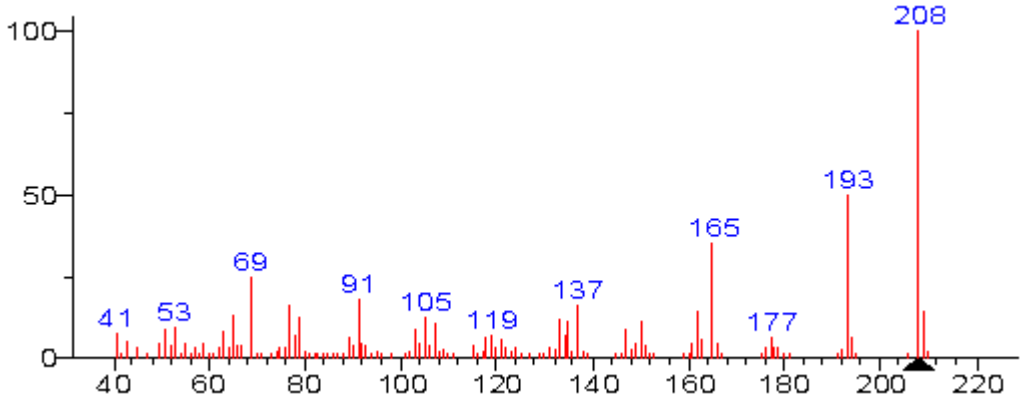

Figure 10 Mass spectrum of *β*-asarone

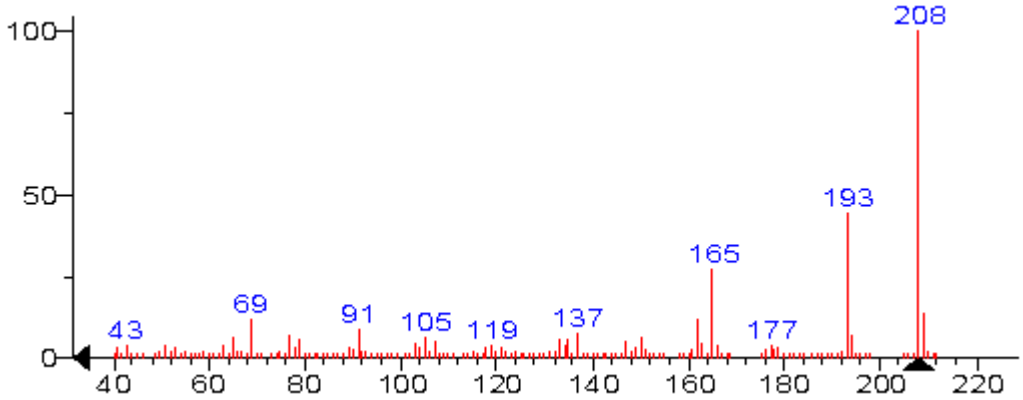

Figure 11 Mass spectrum of *α* –asarone

Calibration curve and linearity

The calibration curves were obtained by plotting the peak area of standards against five concentrations. The regression equations for the linear portion of *β*-asarone and *α*-asarone were y = 62796x – 44008 and y = 56631x – 49650 respectively (y referred to the peak area; x referred to the concentration of the unknown). The correlation coefficients (r^2) of of *β*-asarone and *α*-asarone were 0.997 and 0.998 respectively. Linearity range of *β*-asarone was 0-0.5 mg/ml and of *α*-asarone was 0 - 0.1 mg/ml (Figure 12, 13).

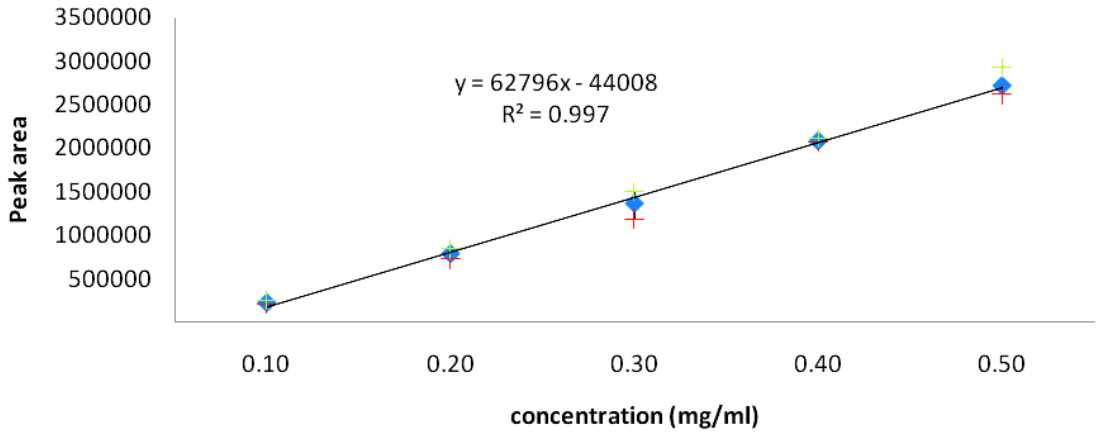

Figure 12 calibration curve of *β*-asarone

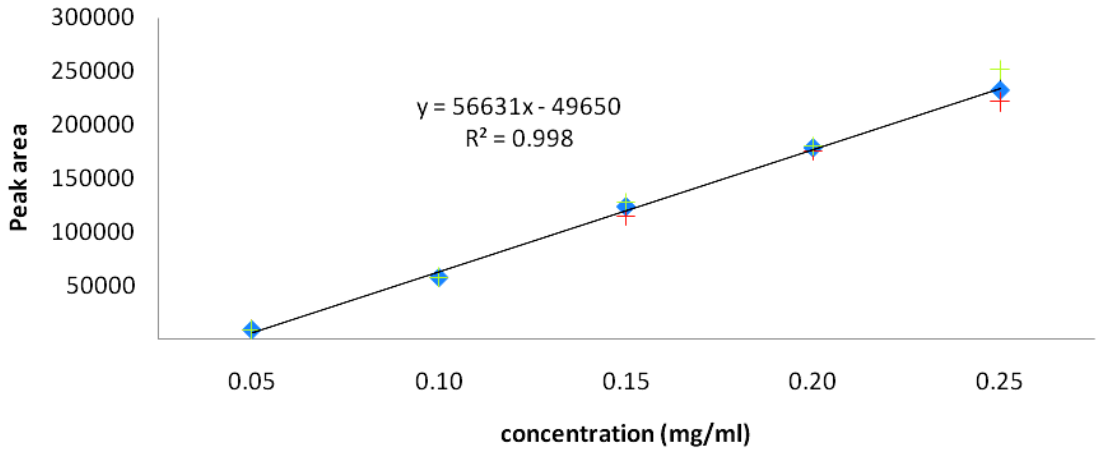

Figure 13 calibration curve of *α*-asarone

LOD and LOQ

In this study, LOD and LOQ determination were based on the standard deviation of response and the slope [61]. LOD and LOQ value for *β*-asarone were 0.0280 and 0.0850 mg/µl of sample, 0.0126 and 0.0382 mg/µl of sample for *α*-asarone respectively.

Precision

Repeatability was analyzed in triplicate of 3 concentrations of sample on 1 day and the intermediate precision was analyzed by comparing on 3 different days. The %RSD of repeatability and intermediate precision of *β*-asarone and *α*-asarone were presented in Table 3.

The %RSD of repeatability of *β*-asarone at 0.10, 0.30 and 0.50 mg/µl oil were 4.67, 2.36 and 1.45 respectively. The %RSD of intermediate precision of *β*-asarone was 2.21, 1.02 and 0.95 respectively. The %RSD of repeatability of *α*-asarone at 0.05, 0.15 and 0.25 mg/µl oil were 6.58, 3.52 and 2.48 respectively. The %RSD of intermediate precision of *α*-asarone was 3.00, 2.24 and 1.29 respectively.

Table 3 Percentage of relative standard deviation of repeatability and intermediate precision analysis

	Concentration (mg/µl oil)	**Repeatability %RSD**	**Intermediate precision %RSD**
β-asarone	0.10	4.67	2.21
	0.30	2.36	1.02
	0.50	1.45	0.95
α-asarone	0.05	6.58	3.00
	0.15	3.52	2.24
	0.25	2.48	1.29

Recovery

The recovery of *β*-asarone and *α*-asarone was performed on samples spiked with three different concentrations of standard (0.1, 0.2, 0.3 mg of *β*-asarone and 0.05, 0.1, 0.2 mg of *α*-asarone). The percentage recoveries of *β*-asarone and *α*-asarone were presented in Table 4-5.

Table 4 Recovery study of *β*-asarone

Amount of *β*-asarone added (mg)	**Amount of *β*-asarone detected (mg/µl)**	**Recovery (%)**
0	0.108	
0.1	0.209	100.40
0.2	0.308	100.15
0.3	0.408	100.11

Table 5 Recovery study of *α*-asarone

Amount of *α*-asarone added (mg)	**Amount of *α*-asarone detected (mg/µl)**	**Recovery (%)**
0	0.080	
0.05	0.130	100.52
0.1	0.181	100.80
0.2	0.280	100.17

The *β*-asarone and *α*–asarone contents in calamus oil

The *β*-asarone and *α*-asarone were the most characteristic components in calamus oil. The study demonstrated that the *β*-asarone content in calamus oil from dried rhizomes in Thailand was more than *α*–asarone content (0.259 ± 0.035 *vs* 0.120 ± 0.020 mg/µl respectively).

CHAPTER V

DISCUSSION AND CONCLUSION

Although many medicinal plants are used in worldwide for therapeutic uses, lack of information and education makes consumer easy victim of market exploitation and herbal myths. Quality evaluation for identification of this medicinal plant is essential [62]. In Thailand and other country, *A. calamus* has been used in medicinal purpose, in perfumes, and in beverages [4-8]. In Thailand, this plant is called 'Wan-nam' which has been frequently used in many Thai remedies [3].

In this study, standardization parameters of *A. calamus* rhizome in Thailand were conducted according to the World Health Organization (WHO) guidelines for herbal standardization [57]. The macroscopic and microscopic observed in this study were agreement in the earlier reported by the faculty of pharmacy, Mahidol University which reported of epidermis, collenchyma, cortical fibers, calcium oxalate crystals, oil glands, vascular bundles and starch granules [56].

The constant numbers of loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content were related with the previous study in Thailand [27, 56]. Total ash and acid insoluble ash were less than previously reported which specified the limit at not more than 7-9 % for total ash and not more than 1-2 % for acid insoluble ash [27, 56]. This might be due to the larger sample size and various sample sources. Thin-layer chromatographic fingerprints of ethanolic extracts of *Acorus calamus* dried rhizomes were performed using toluene and ethyl acetate (9:1) as mobile phase. The band was clearly detected under ultraviolet lights (254 nm and 365 nm) and anisaldehyde-sulfuric acid reagent. The dominant band of α-asarone / β-asarone was shown at R*f* 0.58 (Figure 8). TLC is appropriate fast screening method in order to identify plants according to their chemical constituents [58]. However, this technique cannot separate the alpha and beta isomers of asarone.

Gas chromatography coupled with mass spectrometry (GC/MS) is the most reliable method for detection of essential oil including calamus oil [57-59]. For

asarone isomers determination by GC/MS, the mass spectrum between alpha- and beta- asarone is identical. The external standard method was used to identify each isomer by the retention time matching. The peak at the retention time around 34.55 min corresponded to *β*-asarone which mass spectrum was characterized by the presence of abundant ions at m/z 208 (Figure 10). Similarly, the mass spectrum of *α*-asarone (Figure 11) showed a parent peak at *m/z* 208 at the retention time around 36.62 min. The retention indices of *β*- and *α*- asarone shown as Kovat's Index were reported of 1617 and 1676 [63]. The elution profile of asarones by GC/MS in this study was in the same way as reference. The stationary phase used in this study were Zebron ZB 5 which contained 5% phenyl and 95% dimethylpolysiloxane equivalent to the one used by Kovat. Nevertheless, the retention time could switch over based on each GC condition such as temperature programming. External standard method using standard *β*- and *α*- asarone provided more accuracy of the results.

The major chemical constituent of the calamus oil from dried rhizome crude drugs in Thailand were revealed as 67.5 ± 8.1 % *β*-asarone followed by 22.4 ± 7.9 % *α*-asarone (Figure 9 and Table 2). *β*-Asarone content in calamus oil from dried rhizomes in this study was less than the content in calamus oil from fresh rhizomes previously reported. Wangsittidet T., *et al*. as well as Raina, R. V., *et al*. demonstrated 82.9% and 83.2% of *β*-asarone in calamus oil from fresh rhizomes respectively [6, 26]. The minimum and maximum area percent of *β*-asarone in calamus oil obtained from 15 *A. calamus* rhizome sources were 52.66% and 78.96% respectively. This high content of *β*-asarone confirmed the tetraploid cytotype of *A. calamus* in Thailand [17]. The minor chemical constituents of the calamus oil in Thailand were demonstrated for example methyl isoeugenol, shyobunone, isoelemicin, *β*-calacorene, khusinol acetate, guaiol, *α*-cadinol, elemicin, *δ*-cadinene, spatulenol, *τ*-muurolol and rosifoliol. The chemical compositions of calamus oil among countries also vary depended on external factors such as growing habitat, geographical condition and processing method

In addition to area percent quantitative method, multiple point external standard method was performed. The correlation coefficient (r^2) of linear calibration curves were obtained with good responds (0.997 and 0.998) for *β*-asarone and *α*-

asarone respectively. The detection response of *α*-asarone was about 10% greater than *β*-asarone. This finding was in accordance with the other study [64]. LOD and LOQ were 0.0280 and 0.0850 mg/µl of calamus oil for *β*-asarone and 0.0126 and 0.0382 mg/µl of calamus oil for *α*-asarone respectively. The % RSD of *β*-asarone was between 1.45-4.67 % RSD and 0.95-2.21 % RSD for repeatability and intermediate precision respectively. *α*-Asarone showed 2.48-6.58 % RSD for repeatability and 1.29-3.00 % RSD for intermediate precision. The precision should not exceed 15% RSD [65, 66]. The average recoveries were 100.11-100.40 % in *β*-asarone and 100.17-100.80 % in *α*-asarone. The results demonstrated that the method was suitable for determination of *β*-asarone and *α*-asarone in calamus oil. Therefore, GC/MS method was effective implemented for qualitative and quantitative analysis of calamus oil. The quantitative results were found that *β*-asarone content in calamus oil was 0.259 ± 0.035 mg/µl of oil whereas α-asarone content was 0.120 ± 0.020 mg/µl of oil. The *β*-asarone content in this study was different from previously reported in China (~0.060 mg/µl) [67]. The *β*-asarone content in dried rhizomes based on the yield of calamus oil, that was 1.37 ± 0.11 g / 100 g dried rhizomes, was calculated to be 0.36 ± 0.08 g / 100 g dried rhizomes. Three traditional Thai medicine formularies containing *A. calamus* dried rhizomes included Pra-Sa-Plai (for treatment of dysmenorrhea), Pra-Sa-Karn-Plu (for treatment of abdominal pain due to flatulence) and Wi-Sum-Pa-Ya-Yai (treatment of colic and flatulence). These formularies have been published in National list of essential medicines - the List of Herbal Medicinal Products A.D.2011 [15]. The amounts of *A. calamus* dried rhizomes in 3 ingredients per one dose (1 g) were 0.049, 0.016 and 0.018 g of Pra-Sa-Plai, Pra-Sa-Karn-Plu and Wi-Sum-Pa-Ya-Yai respectively. They were corresponded to *β*-asarone content of 0.174, 0.057 and 0.064 mg per dose of these formularies respectively. The daily dosages were 3 times a day for Pra-Sa-Plai and Pra-Sa-Karn-Plu and every 4 hours for Wi-Sum-Pa-Ya-Yai [15]. Even though these estimated doses intake of *β*-asarone were not exceeded the limits at 0.5 mg/kg excepted by The Council of Europe Experts on Flavouring Substances [53], the carcinogenic possibility of *β*-asarone should be concerned.

The results of these investigations could serve as the pharmacognostic specification and chemical constituents of *A. calamus*. This study revealed the high content of β-asarone in calamus oil which considered as carcinogen, so it should be aware of the calamus oil utilization.

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APPENDICES

APPENDIX A

Data of pharmacognostic characters of *Acorus calamus* rhizome

Table 6 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Bangkok 1 (1)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	3.94		
	2	3.92		
	3	3.88	3.91	0.03
Acid insoluble	1	0.75		
	2	0.69		
	3	0.88	0.77	0.10
Loss on drying	1	11.99		
	2	11.79		
	3	11.70	11.83	0.15
Moisture content	1	13.00		
	2	13.20		
	3	13.00	13.06	0.12
Ethanol-soluble extractive	1	7.28		
	2	8.73		
	3	7.24	7.75	0.85
Water-soluble extractive	1	10.79		
	2	10.56		
	3	11.20	10.85	0.32
Volatile oil content	1	1.40		
	2	1.20		
	3	1.40	1.33	0.12

Table 7 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Bangkok 2 (2)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	3.88		
	2	3.90		
	3	3.91	3.90	0.016
Acid insoluble	1	0.75		
	2	0.75		
	3	0.78	0.76	0.014
Loss on drying	1	13.47		
	2	11.65		
	3	11.77	12.30	1.017
Moisture content	1	13.00		
	2	13.00		
	3	13.00	13.00	0.00
Ethanol-soluble extractive	1	7.02		
	2	7.06		
	3	7.08	7.05	0.03
Water-soluble extractive	1	10.14		
	2	9.85		
	3	10.30	10.10	0.23
Volatile oil content	1	1.20		
	2	1.40		
	3	1.20	1.27	0.12

Table 8 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Samut Prakan (3)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	4.70		
	2	4.70		
	3	4.26	4.552	0.252134
Acid insoluble	1	0.91		
	2	0.74		
	3	0.89	0.847	0.096374
Loss on drying	1	12.59		
	2	12.56		
	3	12.53	12.561	0.0333
Moisture content	1	13.00		
	2	12.91		
	3	13.50	13.14	0.32
Ethanol-soluble extractive	1	8.62		
	2	8.51		
	3	8.22	8.45	0.21
Water-soluble extractive	1	10.70		
	2	12.81		
	3	11.23	11.58	1.09
Volatile oil content	1	1.40		
	2	1.60		
	3	1.60	1.53	0.12

Table 9 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Phra Nakhon Si Ayutthaya (4)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	5.26		
	2	5.21		
	3	5.24	5.24	0.02
Acid insoluble	1	0.89		
	2	0.79		
	3	0.92	0.87	0.07
Loss on drying	1	11.93		
	2	11.70		
	3	11.94	11.86	0.14
Moisture content	1	12.80		
	2	13.00		
	3	12.80	12.87	0.12
Ethanol-soluble extractive	1	7.10		
	2	7.01		
	3	7.07	7.06	0.05
Water-soluble extractive	1	8.91		
	2	8.94		
	3	9.09	8.98	0.10
Volatile oil content	1	1.40		
	2	1.20		
	3	1.40	1.33	0.12

Table 10 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Chanthaburi (5)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	3.92		
	2	3.91		
	3	4.00	3.94	0.05
Acid insoluble	1	0.68		
	2	0.67		
	3	0.78	0.71	0.06
Loss on drying	1	11.74		
	2	11.95		
	3	11.73	11.81	0.13
Moisture content	1	13.00		
	2	13.00		
	3	12.60	12.87	0.23
Ethanol-soluble extractive	1	7.14		
	2	7.55		
	3	7.03	7.24	0.27
Water-soluble extractive	1	7.73		
	2	8.75		
	3	7.98	8.16	0.53
Volatile oil content	1	1.20		
	2	1.40		
	3	1.40	1.33	0.12

Table 11 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Phrae (6)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	3.90		
	2	3.98		
	3	3.92	3.93	0.04
Acid insoluble	1	0.68		
	2	0.67		
	3	0.78	0.71	0.06
Loss on drying	1	11.94		
	2	11.89		
	3	12.08	11.97	0.10
Moisture content	1	13.00		
	2	13.50		
	3	13.00	13.17	0.29
Ethanol-soluble extractive	1	7.19		
	2	7.12		
	3	7.24	7.18	0.06
Water-soluble extractive	1	10.54		
	2	10.28		
	3	9.93	10.25	0.31
Volatile oil content	1	1.40		
	2	1.60		
	3	1.40	1.47	0.12

Table 12 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Uttaradit (7)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	4.61		
	2	4.68		
	3	4.59	4.63	0.05
Acid insoluble	1	0.86		
	2	0.77		
	3	0.69	0.77	0.08
Loss on drying	1	12.78		
	2	12.83		
	3	12.72	12.78	0.05
Moisture content	1	12.00		
	2	12.20		
	3	12.20	12.13	0.12
Ethanol-soluble extractive	1	7.75		
	2	7.88		
	3	8.32	7.98	0.30
Water-soluble extractive	1	10.01		
	2	10.26		
	3	10.07	10.11	0.13
Volatile oil content	1	1.40		
	2	1.20		
	3	1.40	1.33	0.12

Table 13 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Lampang (8)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	4.67		
	2	4.07		
	3	4.30	4.35	0.31
Acid insoluble	1	0.77		
	2	0.67		
	3	0.65	0.70	0.06
Loss on drying	1	12.79		
	2	12.63		
	3	12.36	12.59	0.21
Moisture content	1	13.20		
	2	15.00		
	3	13.40	13.87	0.99
Ethanol-soluble extractive	1	7.17		
	2	6.97		
	3	6.92	7.02	0.13
Water-soluble extractive	1	8.64		
	2	8.60		
	3	8.44	8.56	0.11
Volatile oil content	1	1.20		
	2	1.40		
	3	1.40	1.33	0.12

Table 14 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Khon Kaen (9)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	5.33		
	2	5.29		
	3	5.32	5.31	0.02
Acid insoluble	1	1.03		
	2	1.03		
	3	1.03	1.03	0.00
Loss on drying	1	11.82		
	2	11.73		
	3	12.12	11.89	0.20
Moisture content	1	12.00		
	2	13.00		
	3	13.25	12.75	0.66
Ethanol-soluble extractive	1	7.01		
	2	7.43		
	3	7.32	7.25	0.22
Water-soluble extractive	1	10.84		
	2	10.65		
	3	10.79	10.76	0.10
Volatile oil content	1	1.40		
	2	1.60		
	3	1.60	1.53	0.12

Table 15 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Roi Et (10)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	3.91		
	2	3.89		
	3	3.90	3.90	0.01
Acid insoluble	1	0.87		
	2	0.72		
	3	0.78	0.79	0.07
Loss on drying	1	11.95		
	2	11.87		
	3	12.43	12.08	0.30
Moisture content	1	13.00		
	2	12.60		
	3	13.00	12.87	0.23
Ethanol-soluble extractive	1	7.14		
	2	7.04		
	3	6.94	7.04	0.10
Water-soluble extractive	1	9.13		
	2	7.89		
	3	8.95	8.66	0.67
Volatile oil content	1	1.20		
	2	1.20		
	3	1.20	1.20	0.00

Table 16 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Sakon Nakhon (11)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	5.28		
	2	5.69		
	3	4.90	5.29	0.39
Acid insoluble	1	1.07		
	2	1.00		
	3	0.88	0.98	0.10
Loss on drying	1	12.21		
	2	12.12		
	3	11.72	12.02	0.26
Moisture content	1	13.00		
	2	14.60		
	3	15.00	14.20	1.06
Ethanol-soluble extractive	1	7.22		
	2	7.21		
	3	7.04	7.16	0.10
Water-soluble extractive	1	7.89		
	2	8.26		
	3	8.98	8.38	0.56
Volatile oil content	1	1.20		
	2	1.20		
	3	1.20	1.20	0.00

Table 17 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Kanchanaburi (12)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	4.72		
	2	4.64		
	3	4.61	4.66	0.06
Acid insoluble	1	0.87		
	2	0.93		
	3	0.88	0.89	0.03
Loss on drying	1	12.53		
	2	12.46		
	3	12.73	12.57	0.14
Moisture content	1	13.50		
	2	14.00		
	3	14.50	14.00	0.50
Ethanol-soluble extractive	1	7.08		
	2	7.00		
	3	7.35	7.14	0.18
Water-soluble extractive	1	6.54		
	2	7.05		
	3	6.62	6.74	0.27
Volatile oil content	1	1.40		
	2	1.60		
	3	1.60	1.53	0.12

Table 18 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Phetchaburi (13)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	3.88		
	2	3.84		
	3	3.91	3.88	0.04
Acid insoluble	1	0.67		
	2	0.73		
	3	0.80	0.73	0.07
Loss on drying	1	11.97		
	2	12.92		
	3	12.01	12.30	0.54
Moisture content	1	13.00		
	2	12.60		
	3	12.80	12.80	0.20
Ethanol-soluble extractive	1	7.40		
	2	6.92		
	3	7.24	7.19	0.25
Water-soluble extractive	1	10.04		
	2	9.04		
	3	9.20	9.42	0.54
Volatile oil content	1	1.20		
	2	1.20		
	3	1.40	1.27	0.12

Table 19 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Krabi (14)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	5.32		
	2	5.32		
	3	5.31	3.91	0.03
Acid insoluble	1	0.92		
	2	0.97		
	3	0.95	0.77	0.10
Loss on drying	1	12.04		
	2	12.03		
	3	12.27	11.83	0.15
Moisture content	1	13.20		
	2	13.20		
	3	13.20	13.20	0.00
Ethanol-soluble extractive	1	6.98		
	2	7.01		
	3	7.57	7.19	0.33
Water-soluble extractive	1	10.64		
	2	10.28		
	3	10.37	10.43	0.19
Volatile oil content	1	1.20		
	2	1.40		
	3	1.40	1.33	0.12

Table 20 Total ash, acid insoluble ash, loss on drying, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content (% by weight) of *Acorus calamus* rhizome from Songkhla (15)

Parameter	Crude drug sample	Amount (% by weight)	Mean	SD
Total ash	1	4.43		
	2	4.66		
	3	4.72	3.90	0.02
Acid insoluble	1	0.89		
	2	0.94		
	3	0.81	0.76	0.01
Loss on drying	1	12.73		
	2	12.76		
	3	12.84	12.30	1.02
Moisture content	1	13.25		
	2	13.50		
	3	13.25	13.33	0.15
Ethanol-soluble extractive	1	6.96		
	2	7.01		
	3	7.34	7.10	0.20
Water-soluble extractive	1	9.72		
	2	9.94		
	3	10.44	10.03	0.37
Volatile oil content	1	1.40		
	2	1.60		
	3	1.60	1.53	0.12

Anisaldehyde-sulfuric acid reagent

Procedure: Mix 0.5 ml of p-anisaldehyde, 10 ml of glacial acetic acid, 85 ml of methanol and 5 ml of sulfuric acid (~1760 g/l).

APPENDIX B

Data of β-asarone and α-asarone determination

Formulas

- LOD = $\frac{3.3\sigma}{S}$

- LOQ = $\frac{10\sigma}{S}$

  Where σ = the standard deviation of the response

  S = the slope of the calibration curve

- %RSD = (SD x 100) / mean

- Recovery (%) = [C/(A+C_0)] x 100

  Where C: the amount of β-asarone or α –asarone that found after spiking of the standard solution

  A: the amount of those found that before spiking

  C_0: the amount of reference standards actually added to the sample

Table 21 Percent areas of *β*-asarone and *α*-asarone in caamus oil

No. of sample	**Area %**	
	***β*-asarone**	***α*-asarone**
1	71.84	16.36
2	55.84	30.61
3	63.43	24.59
4	73.61	19.63
5	74.07	18.41
6	64.11	26.34
7	76.27	13.25
8	52.66	35.92
9	63.66	26.53
10	66.82	21.46
11	72.82	18.35
12	74.07	18.41
13	67.33	22.53
14	55.59	36.91
15	72.82	18.35
Mean	67.50	22.40
SD	8.10	7.90

Table 22 *β*-asarone and *α*-asarone contents (mg/μl of oil) in calamus oil (n=3)

No. of sample	*β*-asarone	*α*-asarone
1	0.239±0.039	0.086±0.013
2	0.164±0.022	0.098±0.029
3	0.164±0.022	0.141±0.044
4	0.276±0.002	0.107±0.006
5	0.314±0.064	0.157±0.008
6	0.233±0.037	0.105±0.015
7	0.396±0.029	0.172±0.020
8	0.156±0.017	0.107±0.028
9	0.212±0.006	0.080±0.010
10	0.237±0.007	0.080±0.010
11	0.263±0.025	0.163±0.002
12	0.289±0.086	0.153±0.014
13	0.237±0.038	0.106±0.019
14	0.161±0.019	0.112±0.032
15	0.484±0.000	0.138±0.000
Grand mean	**0.259±0.035**	**0.120±0.020**

APPENDIX C

GC chromatogram of calamus oil

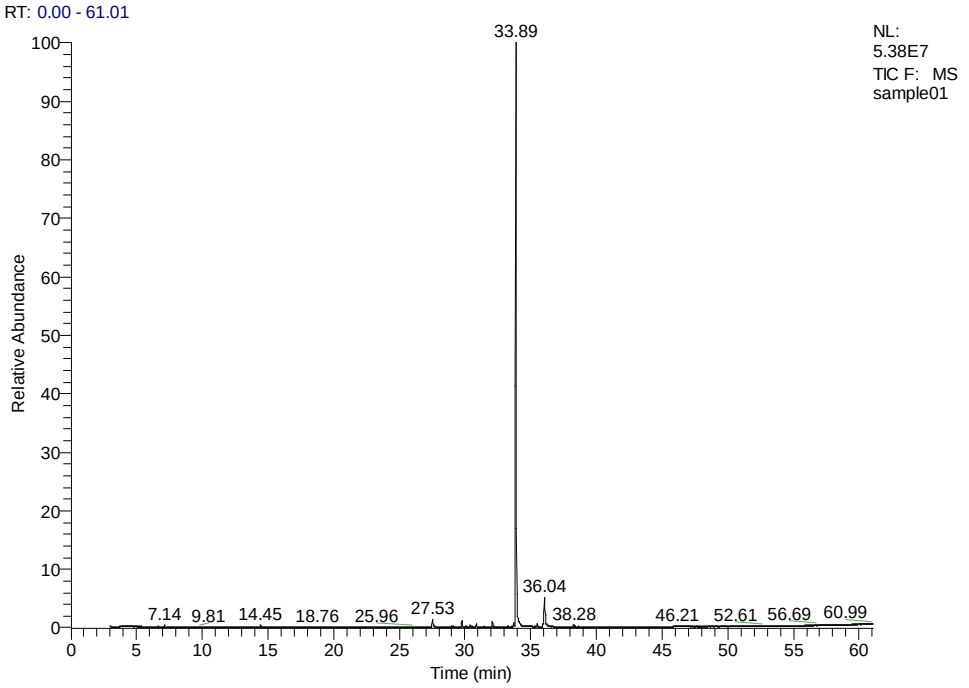

Figure 14 GC chromatogram of calamus oil (1)

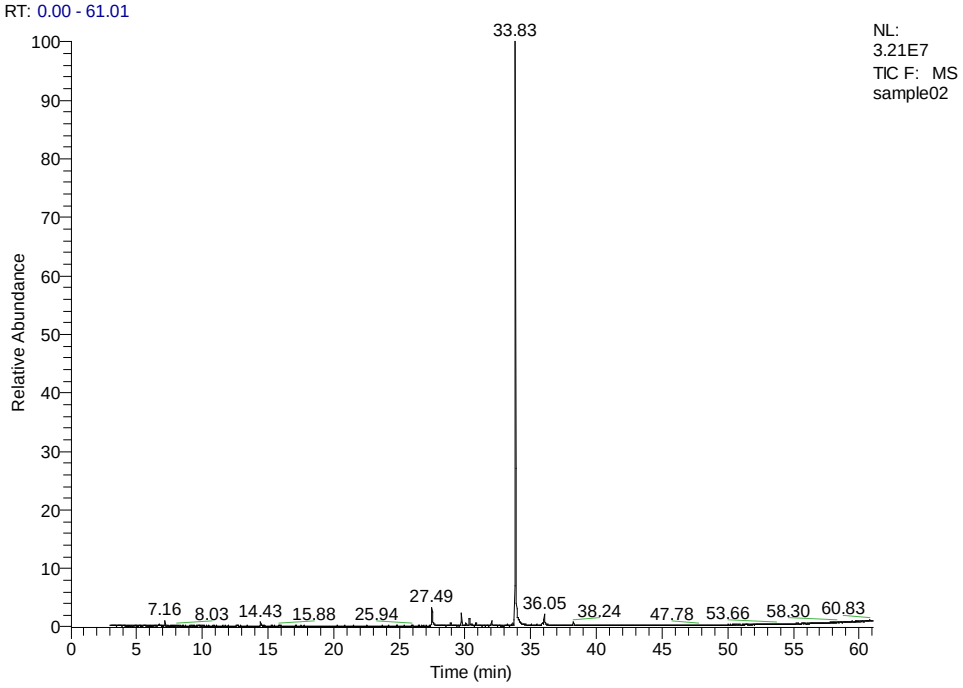

Figure 15 GC chromatogram of calamus oil (2)

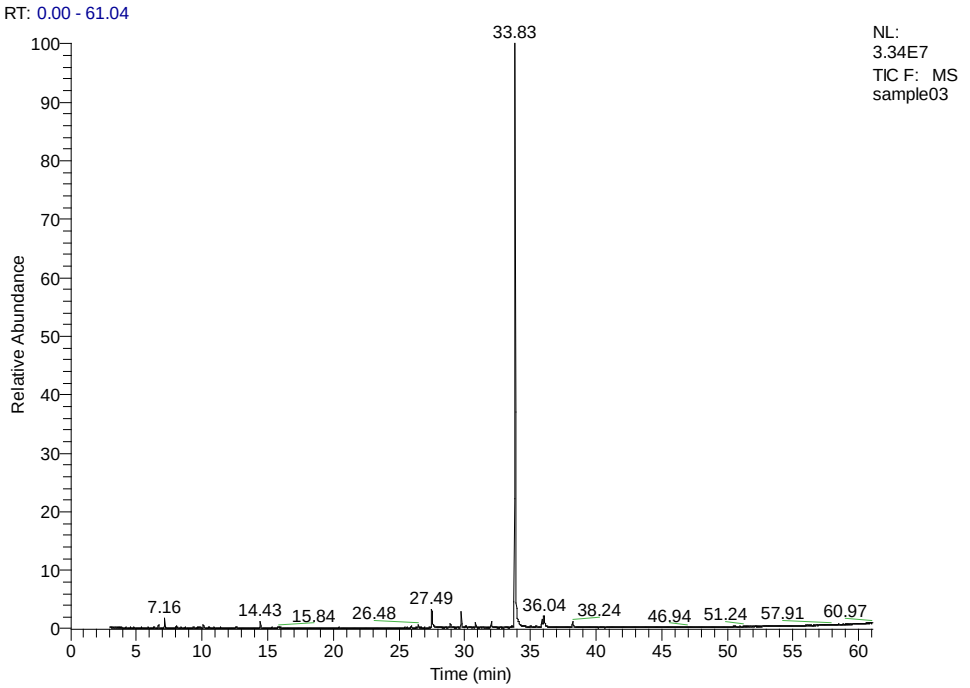

Figure 16 GC chromatogram of calamus oil (3)

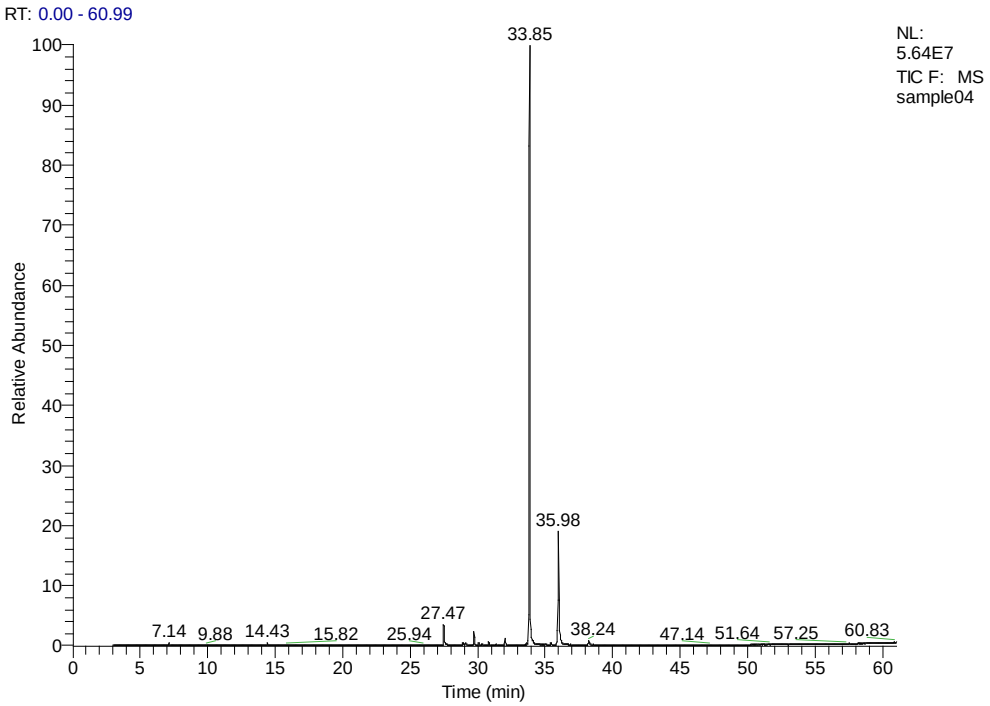

Figure 17 GC chromatogram of calamus oil (4)

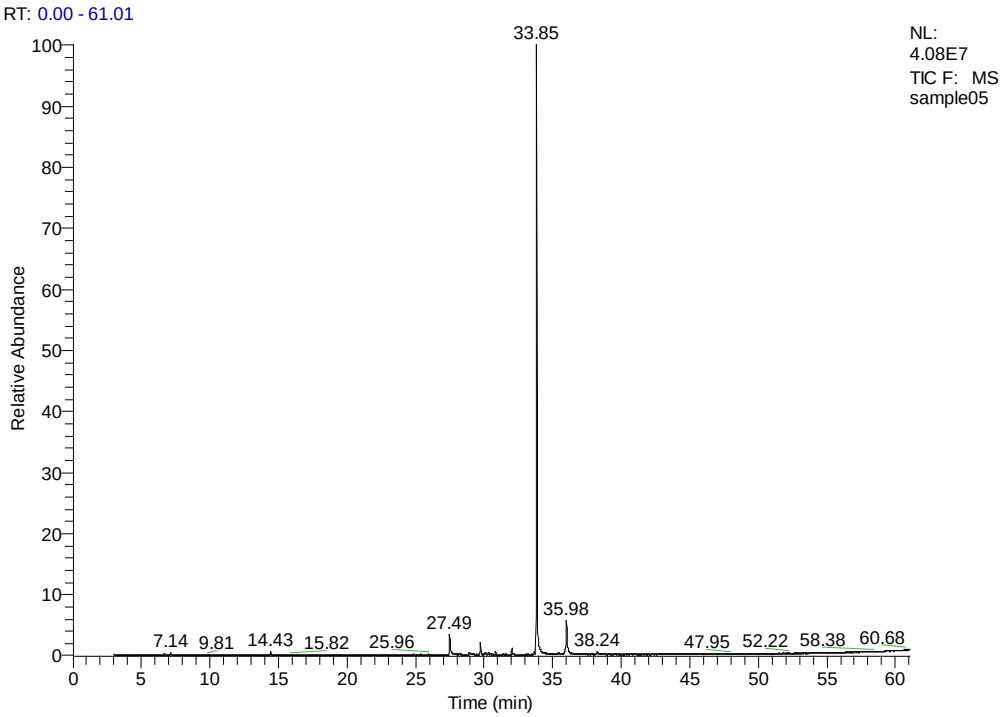

Figure 18 GC chromatogram of calamus oil (5)

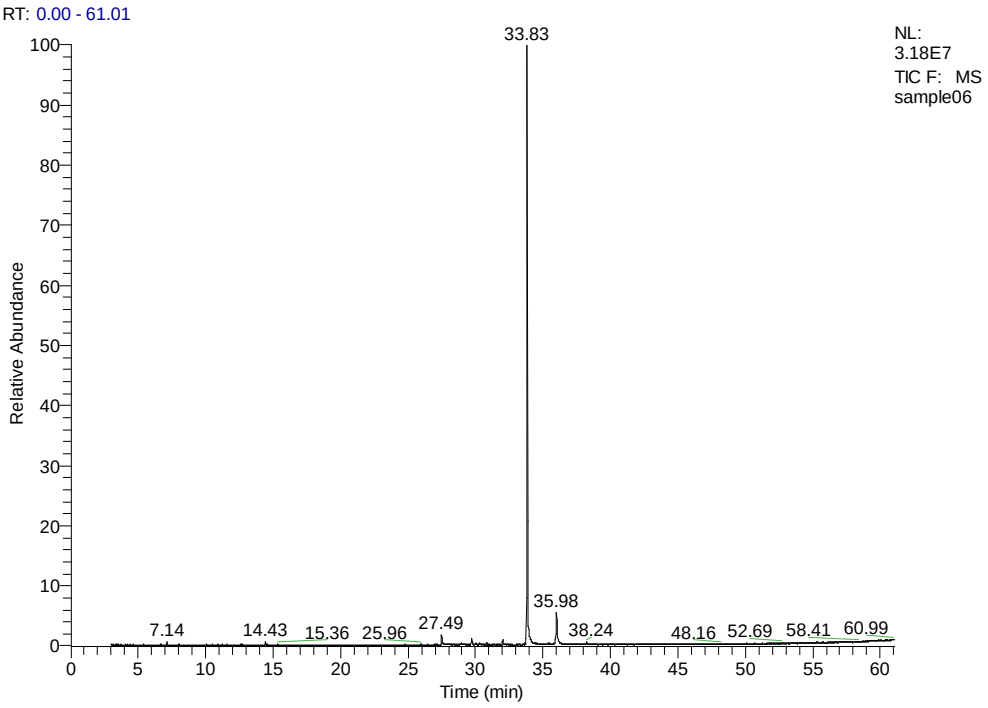

Figure 19 GC chromatogram of calamus oil (6)

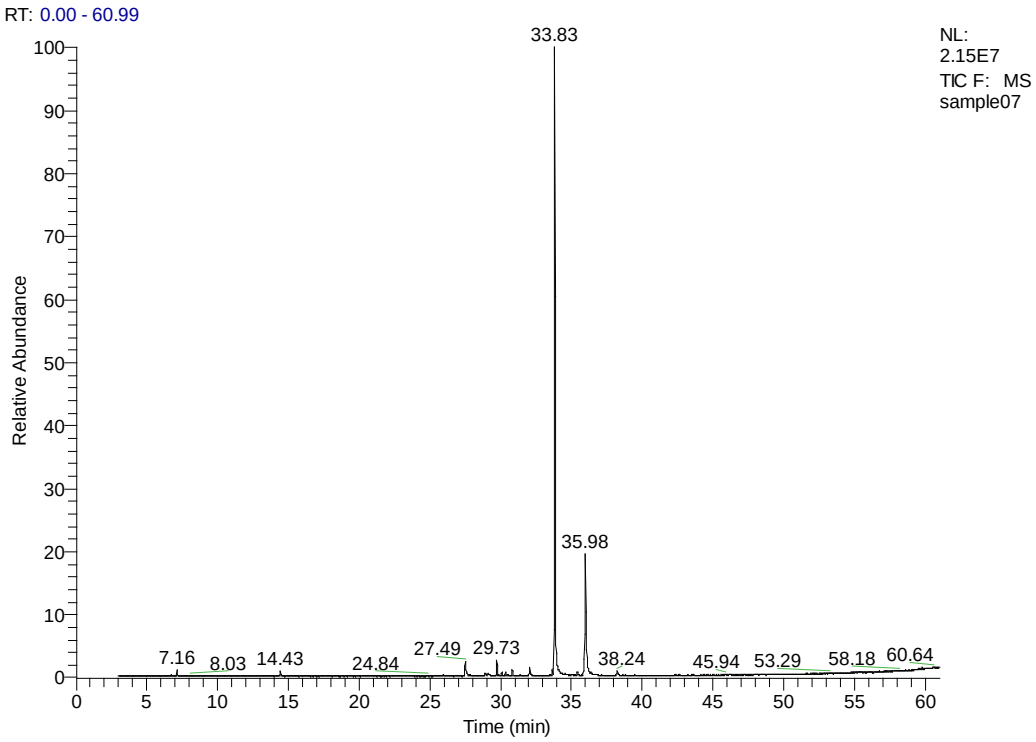

Figure 20 GC chromatogram of calamus oil (7)

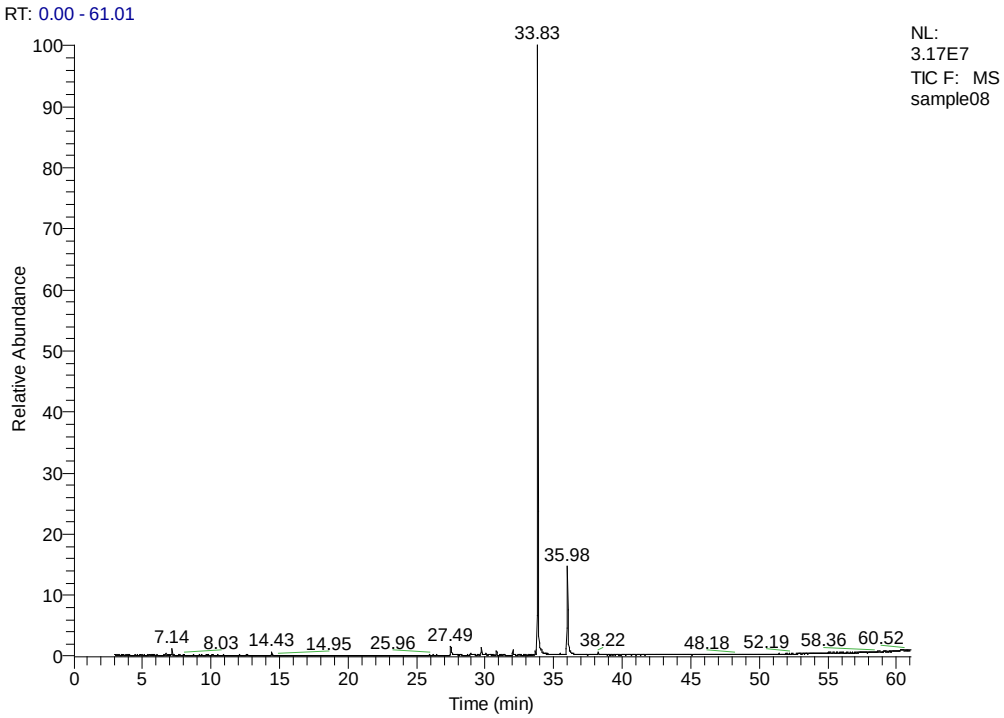

Figure 21 GC chromatogram of calamus oil (8)

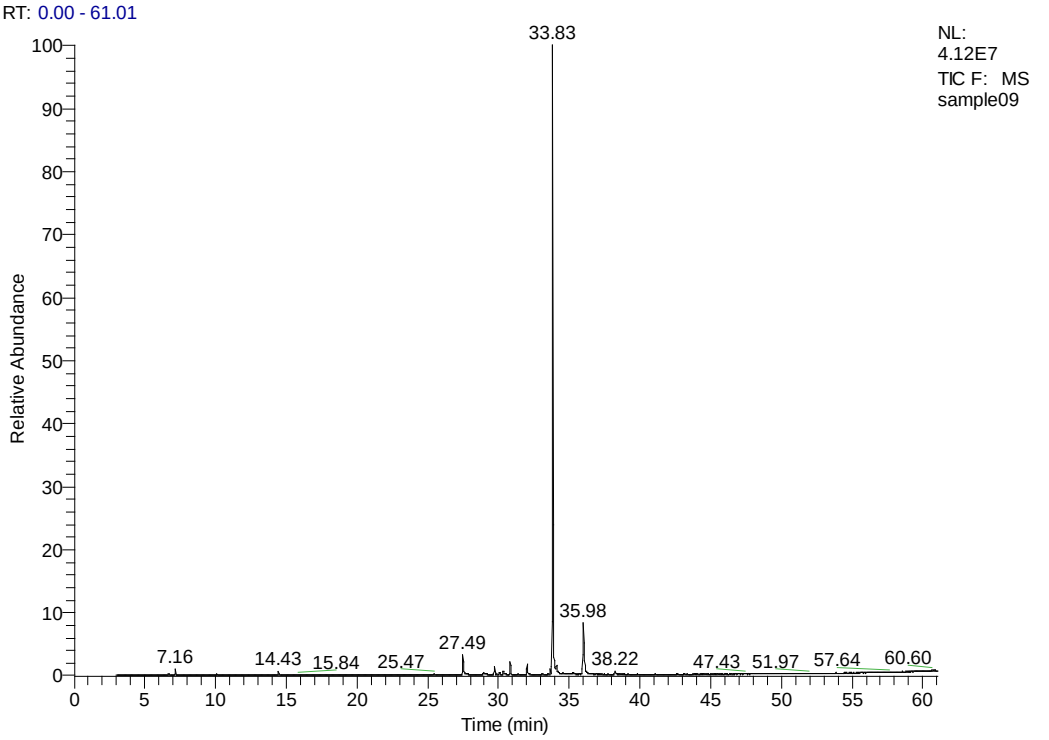

Figure 22 GC chromatogram of calamus oil (9)

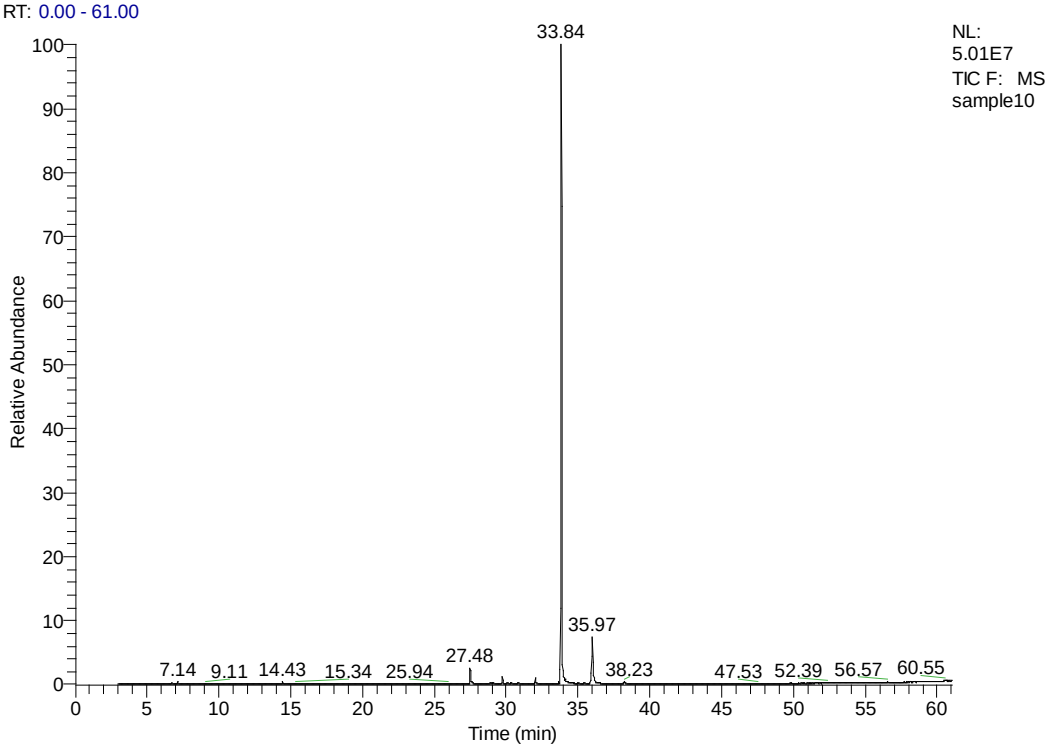

Figure 23 GC chromatogram of calamus oil (10)

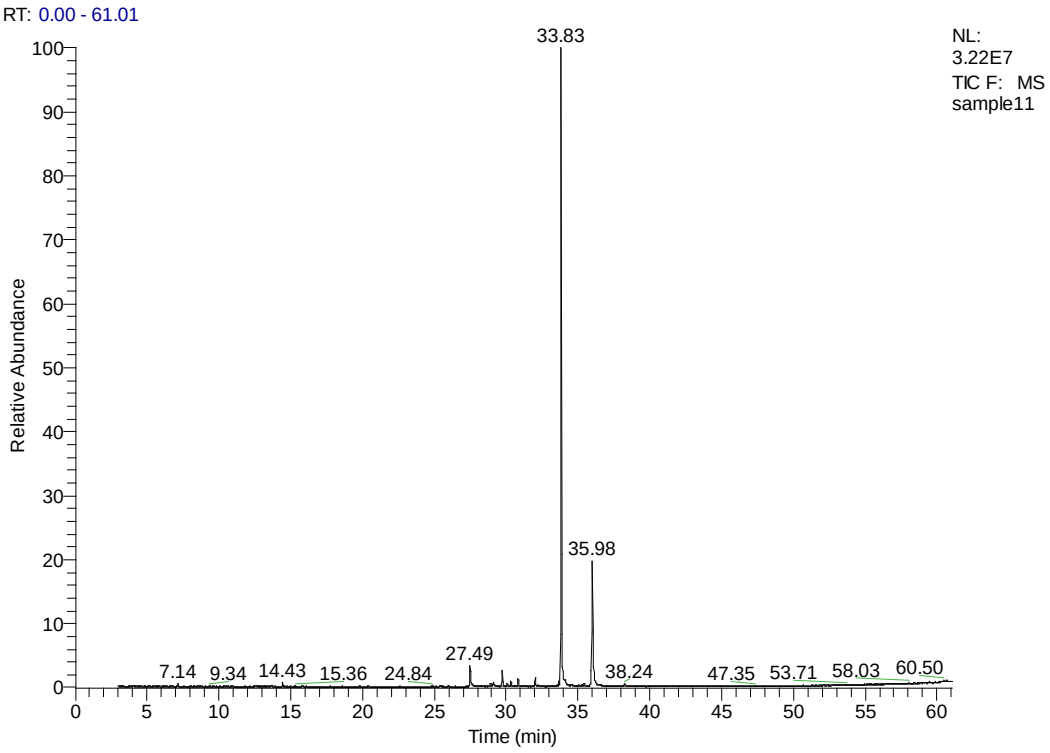

Figure 24 GC chromatogram of calamus oil (11)

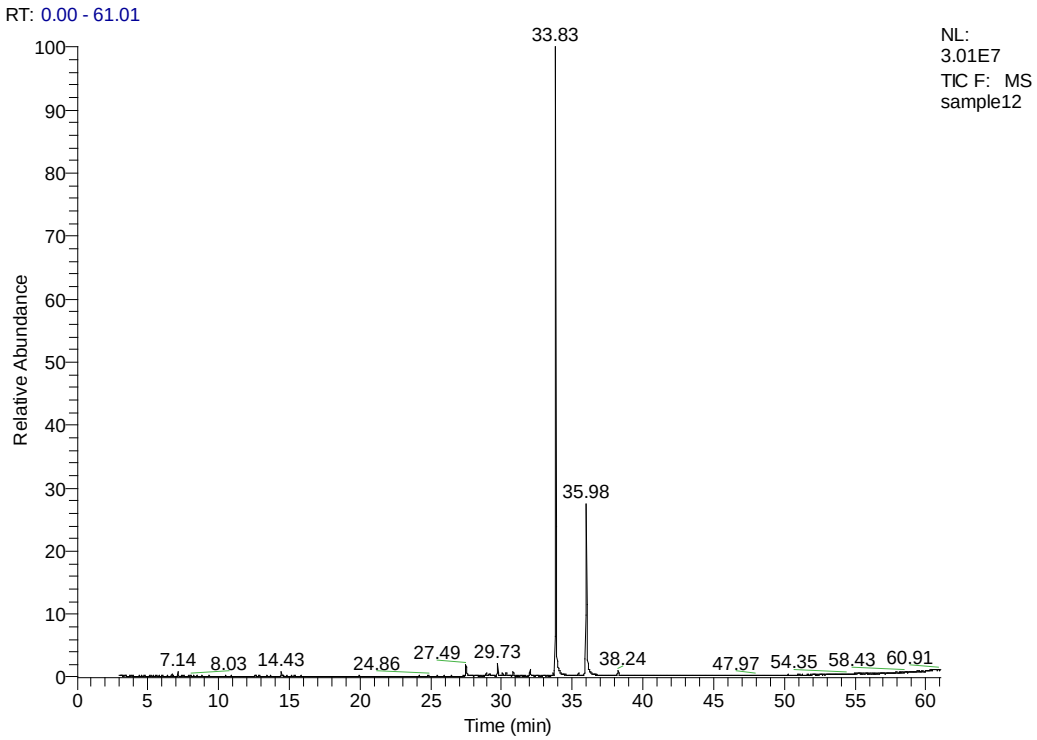

Figure 25 GC chromatogram of calamus oil (12)

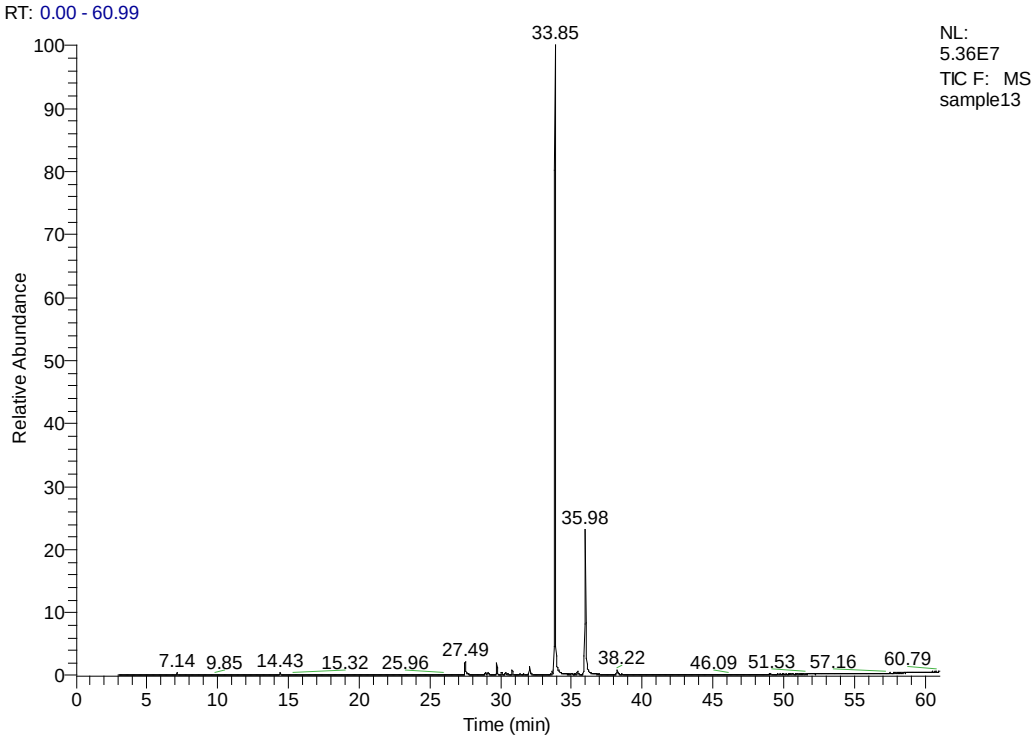

Figure 26 GC chromatogram of calamus oil (13)

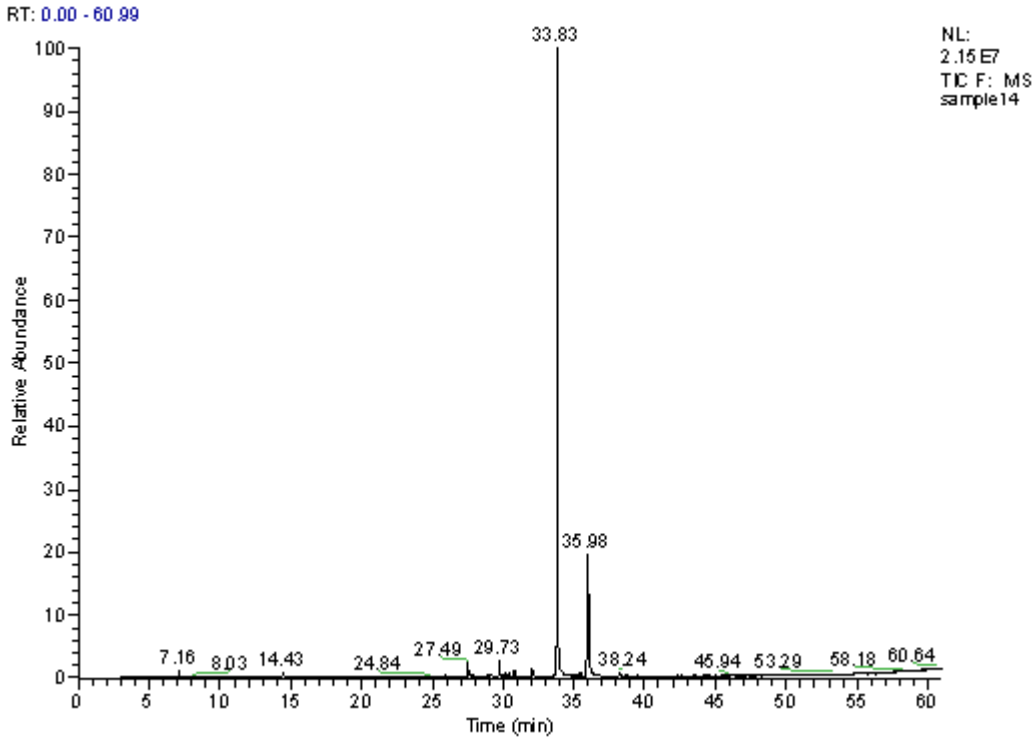

Figure 27 GC chromatogram of calamus oil (14)

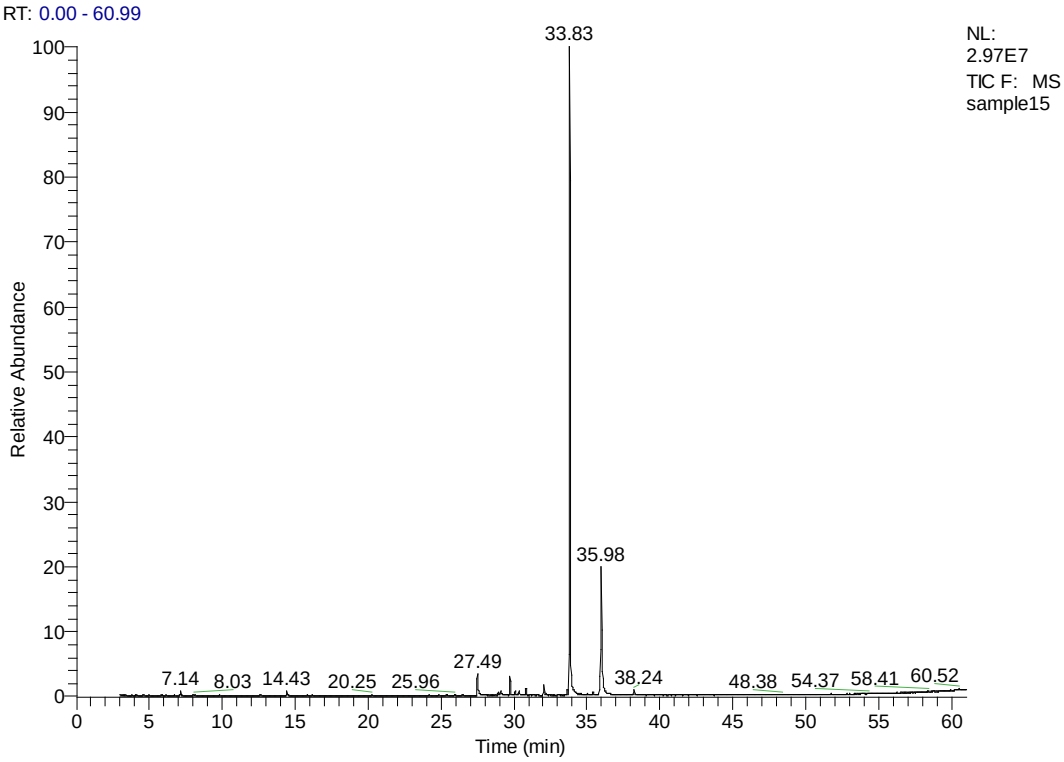

Figure 28 GC chromatogram of calamus oil (15)

APPENDIX D

Thai remedies

Pra-Sa-Plai (ประสะไพล)

สูตรตำรับ ในผงยา 162 กรัม ประกอบด้วย เหง้าไพล หนัก 81 กรัม ผิวมะกรูด เหง้าว่านน้ำ หัวกระเทียม หัวหอม พริกไทยล่อน ดอกดีปลี เหง้าขิง เหง้าขมิ้นอ้อย เทียนดำ เกลือสินเธาว์ หนักสิ่งละ 8 กรัม การบูร หนัก 1 กรัม

ข้อบ่งใช้ 1. ระดูมาไม่สม่ำเสมอหรือมาน้อยกว่าปกติ

2. บรรเทาอาการปวดประจำเดือน

3. ขับน้ำคาวปลาในหญิงหลังคลอดบุตร

ขนาดและวิธีใช้

กรณีระดูมาไม่สม่ำเสมอหรือมาน้อยกว่าปกติ

ชนิดผง รับประทานครั้งละ 1 กรัม ละลายน้ำสุก วันละ 3 ครั้ง ก่อนอาหาร เป็นเวลา 3-5 วัน เมื่อระมาให้หยุดรับประทาน

ชนิดแคปซูล ชนิดเม็ด ชนิดลูกกลอน รับประทานครั้งละ 1 กรัม วันละ 3 ครั้ง ก่อนอาหาร เป็นเวลา 3-5 วัน เมื่อระดูมาให้หยุดรับประทาน

กรณีปวดประจำเดือน

ในกรณีที่มีอาการปวดประจำเดือน เป็นประจำ ให้รับประทายาก่อนมีประจำเดือน 2-3 วัน ไปจนถึงวันแรกและวันที่สองที่มีประจำเดือน

ชนิดผง รับประทานครั้งละ 1 กรัม ละลายน้ำสุก วันละ 3 ครั้ง ก่อนอาหาร

ชนิดแคปซูล ชนิดเม็ด ชนิดลูกกลอน รับประทานครั้งละ 1 กรัม วันละ 3 ครั้ง ก่อนอาหาร

กรณีขับน้ำคาวปลาในหญิงหลังคลอดบุตร

ชนิดผง รับประทานครั้งละ 1 กรัม ละลายน้ำสุก วันละ 3 ครั้ง ก่อนอาหาร ให้รับประทานจนกว่าน้ำคาวปลาจะหมด แต่ไม่เกิน 15 วัน

ชนิดแคปซูล ชนิดเม็ด ชนิดลูกกลอน รับประทานครั้งละ 1 กรัม วันละ 3 ครั้ง ก่อนอาหาร ให้รับประทานจนกว่าน้ำคาวปลาจะหมด แต่ไม่เกิน 15 วัน

Pra-Sa-Karn-Plu (ประสะกานพลู)

สูตรตำรับ ในผงยา 250 กรัม ประกอบด้วย ดอกกานพลู หนัก 125 กรัม เปลือกซิก หนัก 10 กรัม เหง้าขมิ้นชัน หนัก 8 กรัม เปลือกเพกา เปลือกขี้อ้าย หนักสิ่งละ 4 กรัม เหง้าขิงแห้ง ดอกดีปลี หนักสิ่งละ 3 กรัม เหง้าไพล รากเจตมูลเพลิงแดง เถาสะค้าน รากช้าพลู หนักสิ่งละ 2 กรัม พริกไทยล่อน หนัก 1 กรัม เหง้าว่านน้ำ หัวกระชายการบูร หนักสิ่งละ 4 กรัม รากแฝกหอม หัวเปราะหอม รากกรุงเขมา ใบกระวาน ลูกกระวาน ลูกผักชีลา หนักสิ่งละ 4 กรัม เนื้อไม้ ลูกจันทน์ หนักสิ่งละ 8 กรัม เทียนดำ เ ทียนขาว โกฐสอ โกฐกระดูก หนักสิ่งละ 4 กรัม

ข้อบ่งใช้ บรรเทาอาการปวดท้อง จุกเสียด แน่นเฟ้อจากอาหารไม่ย่อย เนื่องจากธาตุไม่ปกติ

ขนาดและวิธีใช้

ชนิดผง รับประทานครั้งละ 1 กรัม ละลายน้ำกระสายยา วันละ 3 ครั้ง หลังอาหาร เมื่อมีอาการ

น้ำกระสายยาที่ใช้ ใช้ไพลเผาไฟพอสุกฝนกับน้ำปูนใสเป็นน้ำกระสาย ถ้าหาน้ำกระสายยาไม่ได้ให้ใช้น้ำสุกแทน

ชนิดเม็ด และชนิดแคปซูล รับประทานครั้งละ 1 กรัม ละลายน้ำกระสายยา วันละ 3 ครั้ง หลังอาหาร เมื่อมีอาการ

Wi-Sum-Pa-Ya-Yai (วิสัมพญาใหญ่)

สูตรตำรับ ในผงยา 108 กรัม ประกอบด้วย ดอกดีปลี หนัก 54 กรัม ลูกผักชีลา ลูกจันทน์ ดอกจันทน์ หนักสิ่งละ 8 กรัม ลูกกระวาน ดอกกานพลู โกฐสอ โกฐเขมา โกฐหัวบัว โกฐเชียง โกฐจุฬาลัมพา เปลือกอบเชย เปลือกสมุลแว้ง เนื้อลูกสมอเทศ เนื้อลูกสมอไทย เหง้าว่านน้ำ เถาบอระเพ็ด เหง้าขิงแห้ง รากพญารากขาว หนักสิ่งละ 2 กรัม

ข้อบ่งใช้ บรรเทาอาการท้องอืด ท้องเฟ้อ จุกเสียด

ขนาดและวิธีใช้ รับประทานครั้งละ 1 กรัม ละลายน้ำสุก หรือผสมน้ำผึ้งปั้นเป็นลูกกลอนทุก 4 ชั่วโมง

VITA

Miss Atcha Somnuk was born on September 6, 1987 in Phrae, Thailand. She receives her Bachelor's degree of Applied Thai Traditional Medicine with second class honor from School of Health Sciences, Mae Fah Luang University, Thailand in 2009.

Publications

Atcha, S., Palanuvej, C., and Ruangrungsi, N. Pharmacognostic Specification and α - / β - Asarone Contents of *Acorus calamus* Rhizome in Thailand. Proceedings of the 7th Indochina Conference on Pharmaceutical Sciences, pp. 296 - 299. Bangkok, 2011