

Research Article



The Pharmacognostic Specification of *Acorus calamus* Dried Rhizome with Special Reference to α - and β - Asarone Contents in its Essential Oil

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ABSTRACT

Acorus calamus Linn. dried rhizome is used as crude drug in traditional Thai medicine. The quality specification of this crude drug was investigated. The rhizomes were collected from 15 different areas throughout Thailand. The macroscopic characters were pale brown with longitudinal furrows and circular, with pitted scars of rootlets, internally whitish and spongy and aromatic odor. The microscopic characterization revealed starch granules, secretory sac containing volatile oil and prismatic crystal of calcium oxalate. The total ash, acid insoluble ash, loss on drying, moisture, ethanol-soluble extractive, water-soluble extractive and volatile oil contents were 4.49 ± 0.15 , 0.83 ± 0.07 , 12.23 ± 0.34 , 13.15 ± 0.46 , 7.32 ± 0.29 , 9.53 ± 0.45 and 1.37 ± 0.11 % w/w respectively. TLC fingerprints of ethanolic extracts of *A. calamus* dried rhizomes were demonstrated using silica gel as stationary phase and toluene : ethyl acetate (9:1) as mobile phase. Detection under UV₂₅₄ and spraying with anisaldehyde sulfuric acid reagent showed the dominant band of asarones. Determination of calamus oil by GC/MS using ZB-5 capillary column showed β - and α -asarone at 0.26 ± 0.04 and 0.12 ± 0.02 mg/ μ l respectively.

Keywords: *Acorus calamus*, pharmacognostic specification, calamus oil, asarone isomers.

INTRODUCTION

For centuries, medicinal plants and their products have been used worldwide for treating various illnesses and continue to expand. The quality evaluation of medicinal plant material is important to ensure traditional medicine efficacy. *Acorus calamus* Linn. from the Araceae family, is a reed like semi-aquatic perennial plant with a stout aromatic rhizome. This medicinal plant is commonly known as sweet flag or calamus. This plant is one of traditional Ayurvedic herbal medicines in India used as brain tonic for treatment of mental disorders, for example, depression, insomnia, anxiety, psychosis and epilepsy¹. Crude extracts of calamus rhizomes were demonstrated antimicrobial activities against fungi, yeast and bacteria²⁻⁵. In Thailand, *A. calamus* dried rhizome is a crude drug traditionally used for treatment of flatulence, cough and cold, sore throat, headache, asthma and malarial fever. Methanolic extract of Thai calamus dried rhizome demonstrated high antimicrobial activity against filamentous fungi (*Trichophyton rubrum*, *Microsporium gypseum* and *Penicillium marneffe*); moderate activity against yeasts (*Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*) and low activity against bacteria². β -Asarone is dominant chemical composition of aforementioned calamus rhizome extracts²⁻⁵. The chemical investigations of calamus rhizome and its essential oil showed that major constituent has two geometrical isomers of asarone: α -asarone or *trans*-1,2,4-trimethoxy-5-(1-propenyl)benzene and β -asarone or *cis*-1,2,4-trimethoxy-5-(1-propenyl)benzene. β -Asarone is essentially reported to be rodent carcinogen but

carcinogenic data in human is not concluded⁶. The previous study in 19 years old man who ingested 8 inch of *A. calamus* rhizome with water showed gastrointestinal irritation by vomiting and diaphoresis in several hours without sequelae after supportive care⁷. Recent research on the acute and sub-acute oral toxicity of the hydro-alcoholic extract of *Acorus calamus* rhizomes in mice and rats indicated that the extract was non-toxic, except at high dose of 1,000 mg/kg. Mild histopathological changes were observed in liver tissue without any effect on kidney and cardiac tissues⁸. However, according to β -asarone, *A. calamus* rhizome and calamus oil are generally prohibited from direct addition or use as human food by US-FDA⁹ and is considered to be unfit for human consumption by The Council of Europe Experts on Flavouring Substances. The exception is only for alcoholic beverages traditionally flavoured with calamus which limits at 0.5 mg/kg⁶. The ratio of α and β asarone isomers in *A. calamus* depends on the karyotype or its chromosome number.

European, North American and Kashmir triploid karyotypes contain 3–19% of β -asarone. The variations in tetraploid chemotypes are larger with 10–96% of β -asarone. Indian and south-east Asia tetraploid types contain highest content of β -asarone whereas Japan and East Siberia tetraploid contain about 10–40% of β -asarone^{10,11}. This study aimed to establish the Pharmacognostic specification of *A. calamus* dried rhizome as well as evaluate the contents of α - and β -asarone in calamus oil for standardization of *A. calamus* crude drug as well as its essential oil in Thailand.



MATERIALS AND METHODS

Chemicals

α - and β - Asarone were purchased from Sigma-Aldrich Company Co., St. Louis, MO, USA. Methanol used for GC/MS was HPLC grade. TLC silica gel 60 GF₂₅₄ was purchased from Merck, Germany.

Plant materials

The rhizomes of *A. calamus* were collected from 15 different areas located at four regions in Thailand. All set of crude drugs were authenticated by Ruangrunsi N. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. The rhizomes were cleaned and dried at 45°C and kept in dark cool place.

Pharmacognostic evaluation

The macroscopic character of *A. calamus* was illustrated. The microscopic characterizations were performed on transverse section and powder of *A. calamus* rhizome. The constant numbers of loss on drying, total ash, acid insoluble ash, moisture content, ethanol-soluble extractive, water-soluble extractive and volatile oil content were determined to evaluate the specification of *A. calamus*. Five grams of ground crude drugs were prepared in a pre-weighed small beaker and dried with heat at 105 °C until the constant weight was obtained to determine loss on drying. Total ash was conducted by incinerating of ground crude drugs (3.000 g) at 500 °C for 6 hrs until white and weighing without delay. The remaining ash was added with 25 ml of HCl (70 g/l) and boiled gently for 5 minutes. It was filtered through ashless filter-paper and incinerated at 500 °C for 6 hrs to obtain the acid insoluble ash. Fifty grams of ground crude drugs were distilled with water-saturated toluene by Azeotropic apparatus and the volume of distilled water was recorded as moisture content. Ethanol and water extractive values were performed by macerating ground crude drugs (5.000 g) with 70 ml of solvent in a closed conical flask, shaking for 6 hrs and standing for 18 hrs before filtering. The marc was washed and the filtrate was adjusted to 100 ml. Twenty milliliters of the filtrate were pipetted to pre-weighed beaker and evaporated to dryness. The extract was dried with heat at 105 °C until constant

weight was obtained. Calamus oil content was obtained by weighing 100 grams of ground crude drugs into 600 ml of water and distillation by Clevenger apparatus then reading off the volume of volatile oil. Each experiment was performed in triplicate.

TLC fingerprinting of *A. calamus* dried rhizome

TLC fingerprinting of ethanolic extract of *A. calamus* dried rhizome was determined using silica gel 60 GF₂₅₄ as stationary phase and toluene and ethyl acetate (9:1) as mobile phase. Visualization was performed under UV₂₅₄ and anisaldehyde sulfuric acid reagent.

GC fingerprinting of calamus oil

GC fingerprinting of calamus oil (1:100 in methanol) was determined using ZB-5 capillary column (30 m x 0.25 mm x 0.25 μ m), Finnigan Trace GC ultra and Finnigan Trace DSQ mass spectrometer. The oven temperature was set at 60°C for 1 min then ramped to 240°C with the rate of 3°C/min. The injector temperature was 180°C. Injection volume was 1 μ l with the split ratio of 100:1. Helium was used as carrier gas. The chemical constituents of calamus oil were identified by matching their mass spectra and retention indices with Adams EO Mass Spectral library and NIST05 Mass Spectral library.

Asarone isomers quantitative analysis of calamus oil

The proportion and amount of α -asarone and β -asarone were determined by peak area ratio as well as comparing the area of peak with the calibration curves of standard α - and β - asarone and expressed as mg/ μ l of calamus oil.

RESULTS AND DISCUSSION

Pharmacognostic specification of *A. calamus* dried rhizome

Macroscopic and microscopic specifications were illustrated in Figure 1. The anatomical and histological characterization of the rhizome showed epidermis, collenchyma, cortical fibers, calcium oxalate crystals, oil glands, vascular bundles and starch granules which were agreement with the previous report¹². The constant numbers due to quality of *A. calamus* dried rhizome were shown in Table 1. TLC fingerprint was shown in Figure 2.

Table 1: Physico-chemical specifications of *Acorus calamus* dried rhizome

Content (% by weight)	Mean	SD	Range (Mean \pm 3SD)
Total ash	4.493	0.153	4.035 - 4.950
Acid-insoluble ash	0.832	0.068	0.627 - 1.036
Loss on drying	12.229	0.335	11.224 - 13.235
Moisture	13.149	0.460	11.769 - 14.530
Ethanol-soluble extractive	7.320	0.291	6.449 - 8.192
Water-soluble extractive	9.534	0.453	8.176 - 10.892
Volatile oil	1.369	0.107	1.046 - 1.691



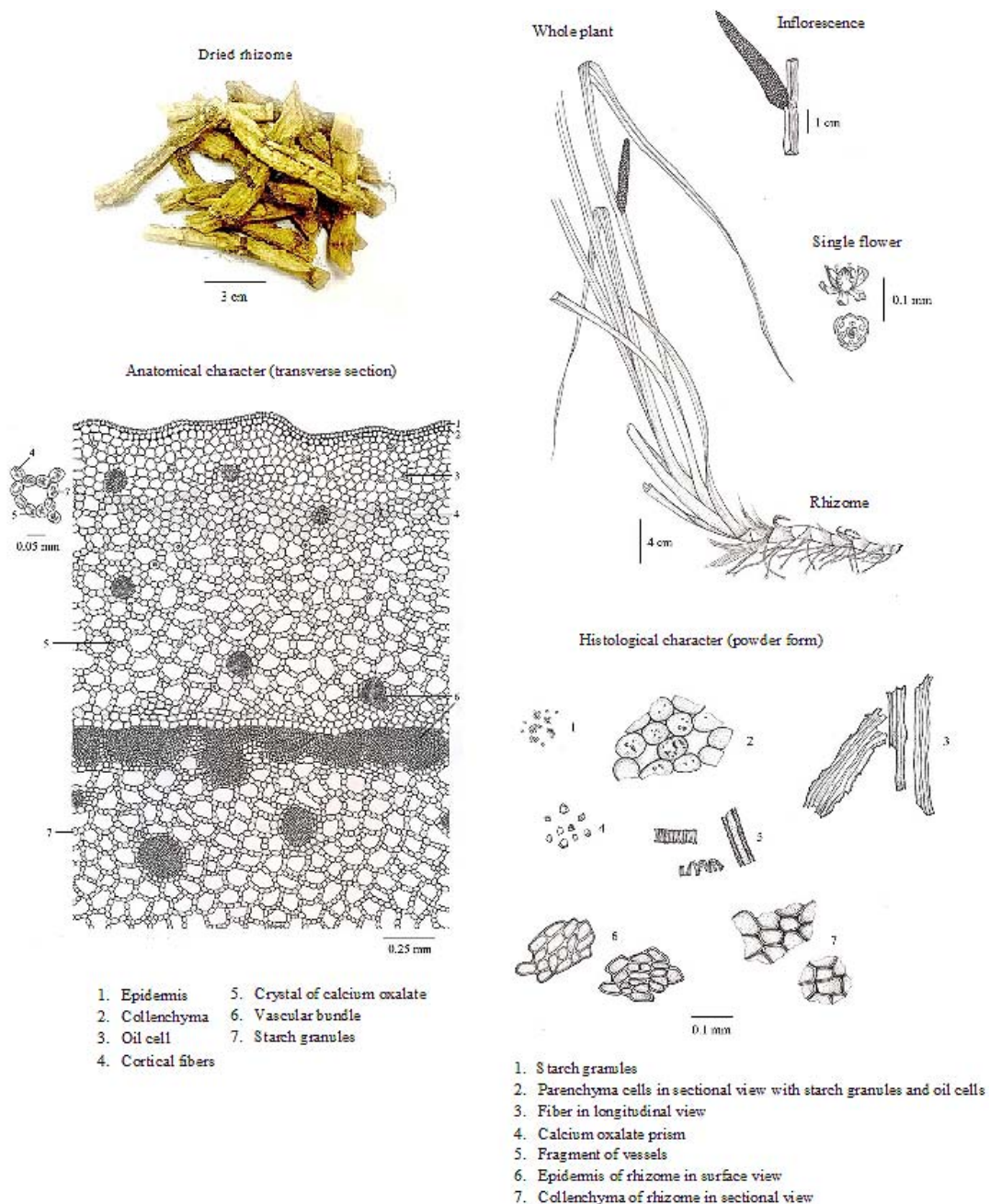


Figure 1: Macroscopic and microscopic characteristics of *Acorus calamus* Linn. dried rhizome

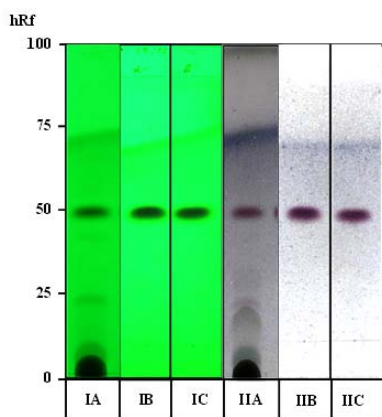
The contents of loss on drying, moisture, ethanol-soluble matter, water-soluble matter and volatile oil were related with the previous study in Thailand¹³. Total ash and acid insoluble ash were less than previously reported which specified the limit at not more than 7-9 % for total ash and not more than 1-2 % for acid insoluble ash¹³. This might be due to the larger sample size and various sample sources in this study. TLC and GC fingerprints were demonstrated and asarones were revealed as main component.

Calamus oil composition and asarones contents

Calamus oil obtained by hydrodistillation of dried rhizome yields 1.37 ± 0.11% v/w. Figure 3 showed the represented GC chromatogram of calamus oil in Thailand. β-Asarone was major component followed by α-asarone

which confirmed the tetraploid cytotype of *A. calamus* Thailand. The other compositions were methyl isoeugenol, shyobunone, isoelemicin, methyl isoeugenol, β-calacorene, khusinol acetate, shyobunone, guaiol, α-cadinol, elemicin, δ-cadinene, spatulenol, τ-muurolol and rosifoliol sorted by percent peak area respectively.

The contents of asarone isomers in calamus oil determined by peak area ratio method were 67.5 ± 8.1 % for β-asarone (range 52.7 – 79.0%) and 22.4 ± 7.9 % for α-asarone. The contents of asarone isomers in calamus oil quantitated by external standard method were 0.26 ± 0.04 and 0.12 ± 0.02 mg/μl for β- and α-asarone respectively. The β-asarone content in Thai calamus oil was higher than previously reported in China (~0.06 mg/μl)¹⁴.



A. the ethanolic extract, B. α -asarone, C. β -asarone, I. detection under UV light 254 nm,
II. detection with anisaldehyde sulfuric acid reagent

Figure 2: TLC fingerprint of *Acorus calamus* Linn. dried rhizome

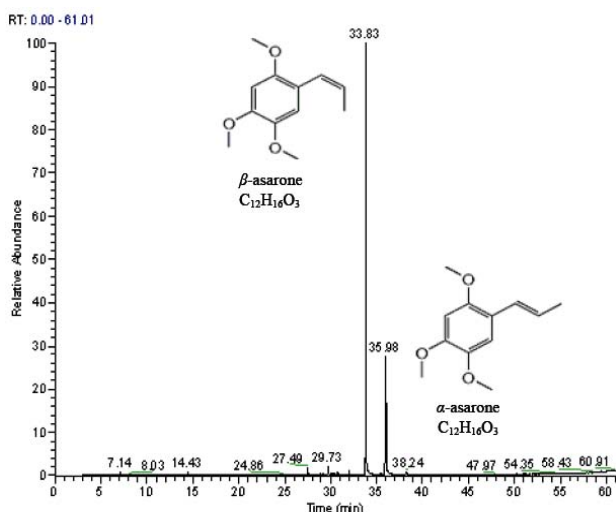


Figure 3: GC chromatogram of the essential oil of *Acorus calamus* Linn. dried rhizome

CONCLUSION

This study provides scientific information for the quality control of *A. calamus* dried rhizomes in Thailand including calamus oil composition with special reference to asarone isomer contents. The uses of *A. calamus* crude drug as well as calamus oil in traditional Thai medicine should be concerned for the possibility of β -asarone toxicity.

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REFERENCES

- Dandiya PC, Sharma JD, Studies on *Acorus calamus* Part V. Pharmacological actions of asarone and β -asarone on central nervous system, Indian Journal of Medical Research, 50, 1962, 46-60.
- Phongpaichit S, Pujenjob N, Rukachaisirikul V, Ongsakul M, Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn., Songklanakarin Journal of Science and Technology, 27(Suppl. 2), 2005, 517-523.
- Kumar SS, Akram AS, Fareed Ahmed TS, Mohamed Jaibir MS, Phytochemical analysis and antimicrobial activity of the ethanolic extract of *Acorus calamus* rhizome, Oriental Journal of Chemistry, 26(1), 2010, 223-227.
- Devi SA, Ganjewala D, Antimicrobial activity of *Acorus calamus* (L.) rhizome and leaf extract, Acta Biologica Szegediensis, 53(1), 2009, 45-49.
- Joshi N, Prakash O, Pant AK, Essential oil composition and *in vitro* antibacterial activity of rhizome essential oil and β -asarone from *Acorus calamus* L. collected from lower Himalayan region of Utarakhand, Journal of essential oil-bearing plants, 15(1), 2012, 32-37.
- Council of Europe, Committee of Experts on Flavouring Substances, Active principles (constituents of toxicological concern) contained in natural sources of flavourings, Health Protection of the Consumer Series, Strasbourg: Council of Europe Press, 2005, 41-47. Available from: http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/flavouring_substances/Active%20principles.pdf.
- Vargus CP, Wolf LR, Gamm SR, Koontz K, Getting to the root (*Acorus calamus*) of the problem, Journal of Toxicology, 36, 1998, 259-260.
- Muthuraman A, Singh N, Acute and sub-acute oral toxicity profile of *Acorus calamus* (Sweet flag) in rodents. Asian Pacific Journal of Tropical Biomedicine, Supplement 2, 2012, S1017-S1023.
- U.S. Food and Drug Administration, Code of Federal Regulations – Title 21, Volume 3, Part 189, Sec. 189.110 Calamus and its derivatives [Revised as of April 1, 2013], Available from: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/cfrsearch.cfm?fr=189.110.
- Raina VK, Srivastava SK, Syamasunder KV, Essential oil composition of *Acorus calamus* L. from the lower region of the Himalayas, Flavour and Fragrance Journal, 18, 2003, 18-20.
- Ahlatwari A, Katoch M, Ram G, Ahuja A, Genetic diversity in *Acorus calamus* L. as revealed by RAPD markers and its relationship with β -asarone content and ploidy level, Scientia Horticulturae, 124(2), 2010, 294-297.
- Neha B, Honey J, Ranjan B, Mukesh B, Pharmacognostical and preliminary phytochemical investigation of *Acorus calamus* Linn., Asian Journal of Pharmaceutical Research, 2(1), 2012, 39-42.
- Department of Medical Sciences, Chemical specification of Thai herbal drugs. Volume I. Bangkok: Ministry of Public Health, 1993.
- Wagner H, Bauer R, Melchart D, Xiao PG, Staudinger A, Chromatographic fingerprint analysis of herbal medicine. Volume II. New York: Springer-Verlag, 2011, 777-789.

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