PHARMACOGNOSTIC SPECIFICATION OF *KAEMPFERIA GALANGA* RHIZOME IN THAILAND

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ABSTRACT: Dried rhizomes of *Kaempferia galanga* were collected from 15 Thai traditional drug stores of 13 provinces in four regions of Thailand for pharmacognostic specification study**.** Crude drug evaluations were performed by macroscopic and microscopic methods whilst constant numbers due to quality of crude drug were performed by World Health Organization (WHO) guideline standard methods. Anatomical and histological characters showed secretory sac containing volatile oil, parenchyma containing oleoresin and a numerous of starch grains. The mean contents of foreign matter, total ash, acid insoluble ash, ethanol–soluble extractive, water–soluble extractive, loss on drying, moisture and volatile oil were 0.06, 7.03, 3.75, 2.39, 16.05, 10.39, 9.62 and 0.72 % of dry weight respectively. Ethylcinnamate and *p*-methoxy derivative were found as major components of the volatile oil. TLC fingerprint of methanolic extracts and GC fingerprint of volatile oil of the rhizomes were demonstrated.

Key words: Pharmacognostic specification, *Kaempferia galanga*, dried rhizome

INTRODUCTION: *Kaempferia galanga* Linn. (Zingi beraceae Family), common name as “Proh Hom”, is now cultivated quite widely in Southeast Asian countries. It is an important traditional medicine for stomachic, carminative, stimulant and also used in odontaiglia[1-3]. The previous studies of biological activities of its volatile oil showed antimicrobial activities (*S. aureus, B. subtilis, E. coli*), antioxidant activity, rheumatic treatment[4] and some toxicity to brine shrimp[5]. Moreover, it would probably be a good natural sunscreen with antibacterial activity[6]. Toxicity studies of crude rhizome extract of *K. galanga* were found that the hippocratic screening test of ethanolic extract indicated CNS depression for example a decrease in motor activity and respiratory rate and loss of grip and analgesia[7]. The acute and subacute toxicity tests in rats were no significant differences between controls and treated animals of both sexes. Moreover, no sign of irritation was observed during the dermal irritation test of the hexane extraction[7]. This study aimed to examine pharmacognostic specification of *K. galanga* dried rhizome in Thailand for standardization of Thai medicinal crude drug.

MATERIALS: *K. galanga* dried rhizomes were purchased from 15 Thai traditional drug stores located at 4 regions of Thailand as follow, Bangkok (3 stores), Chon Buri, Nakorn Pathom, Kamphaeng Phet, Pichit, Nakorn Sawan, Chiang Mai, Nakorn Ratchasima, Ubon Ratchathani, Roi Et, Songkhla, Trang and Nakorn Sri Thammarat. All crude drugs were identified by one of us (N.R.). Voucher specimens and numbers were deposited at Institute of Health Research, Chulalongkorn University.

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METHODS: Macroscopic, microscopic and constant numbers due to quality of crude drug were examined by World Health Organization (WHO) guideline standard methods[8].

Macroscopic and Microscopic Examination

Size, colour and other visual inspections of crude drugs were examined. Anatomical and histological characters were determined. Transverse sections and powdered samples (ground and sifted through a 250 micron sieve) were inspected respectively under microscope (Olympus BX41) with a magnification of 4x, 10x and 40x and compared the scale with the 0.01 mm micrometer.

Determination of Foreign Matter

Fifty grams of crude drugs were spread in a thin layer and sorted the foreign matter by visual inspection. The remainders of the samples were sifted through a 250 micron sieve. The portions of the sorted foreign matter were then weighed and calculated the content in grams per 100 grams of crude drug.

Determination of Total Ash

Three grams of ground crude drug were accurately weighed and placed in a previously ignited and tared crucible. The samples were spread in an even layer and ignited by gradually increasing the heat to 500-600°C until white then cooled in a desiccator and weighed without delay.

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. Then rinsed the watch-glass with 5 ml of hot water and added this liquid to the crucible. The insoluble matters were collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matters was transferred to the original crucible, dried on a hot plate and ignited again. The crucible was then cooled in a desiccator and weighed without delay.

Determination of Ethanol-Soluble Extractive

Five grams of powdered crude drug were macerated with 100.0 ml of absolute ethanol in a closed conical flask for 6 hours in shaking bath and allowed to stand for 18 hours. They were filtered rapidly to avoid loss of ethanol. Twenty milliliter of the filtrate were evaporated to dryness in a tared small beaker and dried with heat to constantly weight.

Determination of Water-Soluble Extractive

Five grams of ground crude drug were macerated with 100.0 ml of distilled water in shaking bath and allowed to stand for 18 hours. They were filtered and 20.0 ml of the filtrate were evaporated to dryness in a tared small beaker and dried at 105°C to constant weight.

Determination of Loss on Drying

Five grams of ground crude drug were weighed in a tared small beaker and dried with heat at 105°C to constantly weight.

Determination of Moisture

Fifty grams of ground crude drug were 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 cooled to room temperature. When water and toluene layers were separated, read off the volume of water.

Determination of Volatile Oil Content

Hundred grams of ground crude drug were added with 600.0 ml of water and distilled by Clevenger apparatus. When volatile oil was completely distilled, the inside of the condenser tube was rinsed with water. Allowed the receiving tube cooled to room temperature. When volatile oil and water layers were separated, read off the volume of volatile oil.

Chemical Identification of Volatile oil

Volatile oil was investigated by capillary column gas chromatography with mass spectrometer as detector (GC/MS). GC/MS qualitative and quantitative analyses

were carried out using a Finnigan Trace GC ultra with Finnigan PolarisQ ion trap detector and BPX5 fused silica column (30 m x 0.25 mm, 0.25 µm film thickness). The injector temperature was 180°C. Sample, 1 µl of the oil, was injected by splitter (1:100) into capillary column. The oven temperature was 60°C for 1 min., then ramp to 240°C with the rate of 3.3°C/ min. Helium was used as carrier gas (flow rate 1 ml/ min). MS was performed by EI positive mode at 70 eV ionization voltages. The constituents of the oil were identified by matching their mass spectra and retention indices with NIST02 MS library and Percentage composition was computed from GC peak areas.

Thin-Layer Chromatographic Identification

One gram of powdered crude drug was refluxed with 20 ml of methanol. The solvent extract was filtered and evaporated to dryness. Dissolved the residue in 0.5 ml of methanol, applied 10 µl to the thin-layer plastic plate coated with silica gel GF254 (Polygram® SIL G/UV254, 0.25 mm thickness, 20 cm x 20 cm), developed the chromatogram in the chamber with the specified solvent. Removed the plate, allowed it to dry in air and observed the produced spots in daylight, under short-wave and long-wave ultraviolet light, sprayed the spots with the specified reagent.

RESULTS: The whole plant of *K. galanga* was shown in Figure 1. The dried rhizomes crude drugs were ovate, oblong or pear-shaped. The colour was light yellow with brown bark. The smell was pleasant odor. The transverse sliced crude drugs were shown in Figure 2. Anatomical characterization of *K. galanga* dried rhizome was showed in figure 3. Secretory sac containing volatile oil, oleoresin, and starch grain were found. Histological characteristics composed of parenchyma, periderm, starch grain, parenchyma containing starch grain, reticulate vessel, annular vessel, reticulate vessel, and spiral vessel (Figure 4). The constant numbers due to quality of *K. galanga* dried rhizomes were shown in Table 1. Volatile oil GC chromatogram and its chemical composition were shown in Figure 5 and Table 2. TLC fingerprints of methanolic extracts of *K galanga* dried rhizomes were shown in Figure 6.

DISCUSSIONS: The study of pharmacognostic specifications of dried *K. galanga* rhizome showed a lot of starch grain in reserved parenchyma and some secretary sacs containing volatile oil. Ethyl cinnamate and derivative, ethyl *p*-methoxycinnamate, were found as major components of volatile oil with specified the contents not less than 38.2 and 8.2% respectively. The previous study of essential oil of *K. galanga* fresh rhizome showed ethyl cinnamate (44.60%), 1,8-cineole (17.40%) and δ-3–carene (11.19%) as major components[9]. There were several toxicity and bioactivity studies of *K. galanga* rhizome extractions and the recent study showed good wound healing in Wistar rat[10]. *K. galanga* could be a potential crude drug for the development of ethnomedical use in further studies.

TLC and GC/MS studies showed characteristic fingerprint profiles which might be used as markers for quality evaluation and standardization of the crude drug. In addition, due to quality of crude drug the constant numbers of foreign matter, total ash, acid insoluble ash, loss on drying and moisture should be not more than 0.06, 7.03, 3.75, 10.39 and 9.62 % of dry weight respectively whilst ethanol–soluble extractive, water–soluble extractive and volatile oil content not less than 2.39, 16.05 and 0.72 % of dry weight respectively. Phytochemical and biological activities should be further studied for the evaluation of crude drug potencies.

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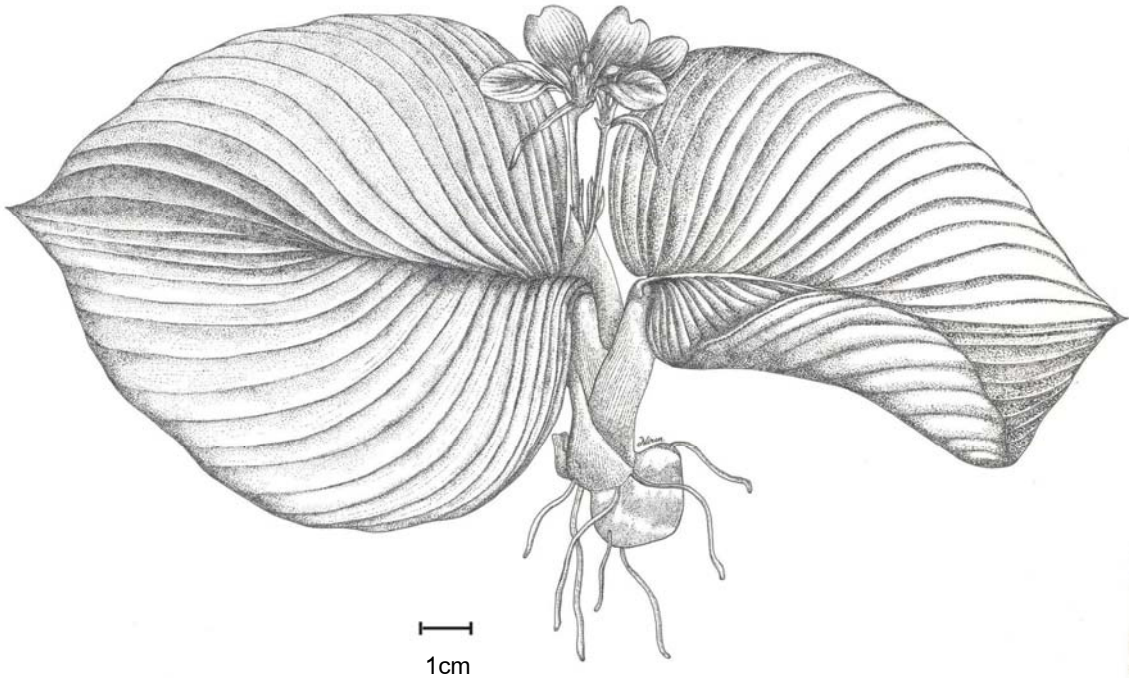


Figure 1 The whole plant of *K. galanga*

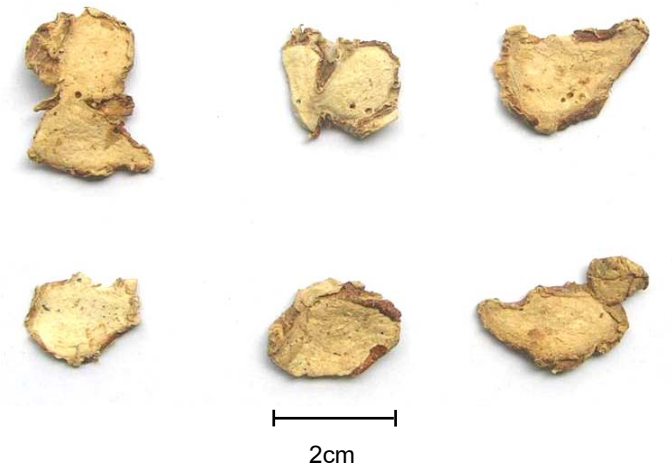


Figure 2 The dried rhizome of *K. galanga* (transverse sliced)

Table 1 The constant numbers due to quality of *K. galanga* rhizomes

Specification	Mean ± SD*	Min – Max*	n
Foreign matter	0.06 ± 0.03	0.02 – 0.15	29
Total ash	7.03 ± 1.33	5.32 –10.59	28
Acid-insoluble ash	3.75 ± 8.98	1.99 – 5.82	28
Ethanol-soluble extractive	2.39 ± 0.95	0.70 – 4.30	45
Water-soluble extractive	16.05 ± 2.64	11.00 – 19.20	45
Loss on drying	10.39 ± 0.76	8.35 – 12.23	45
Moisture	9.62 ± 0.92	7.60 – 10.88	30
Volatile oil content	0.72 ± 0.45	0.17 – 2.01	30

*% dry weight

Note: Main compounds of *K. galanga* rhizomes volatile oil were Ethylcinnamate (38.2 ± 4.8 %), Pentadecane (34.6 ± 7.5 %) and ethyl *p*-methoxycinnamate (8.2 ± 4.4 %) respectively.

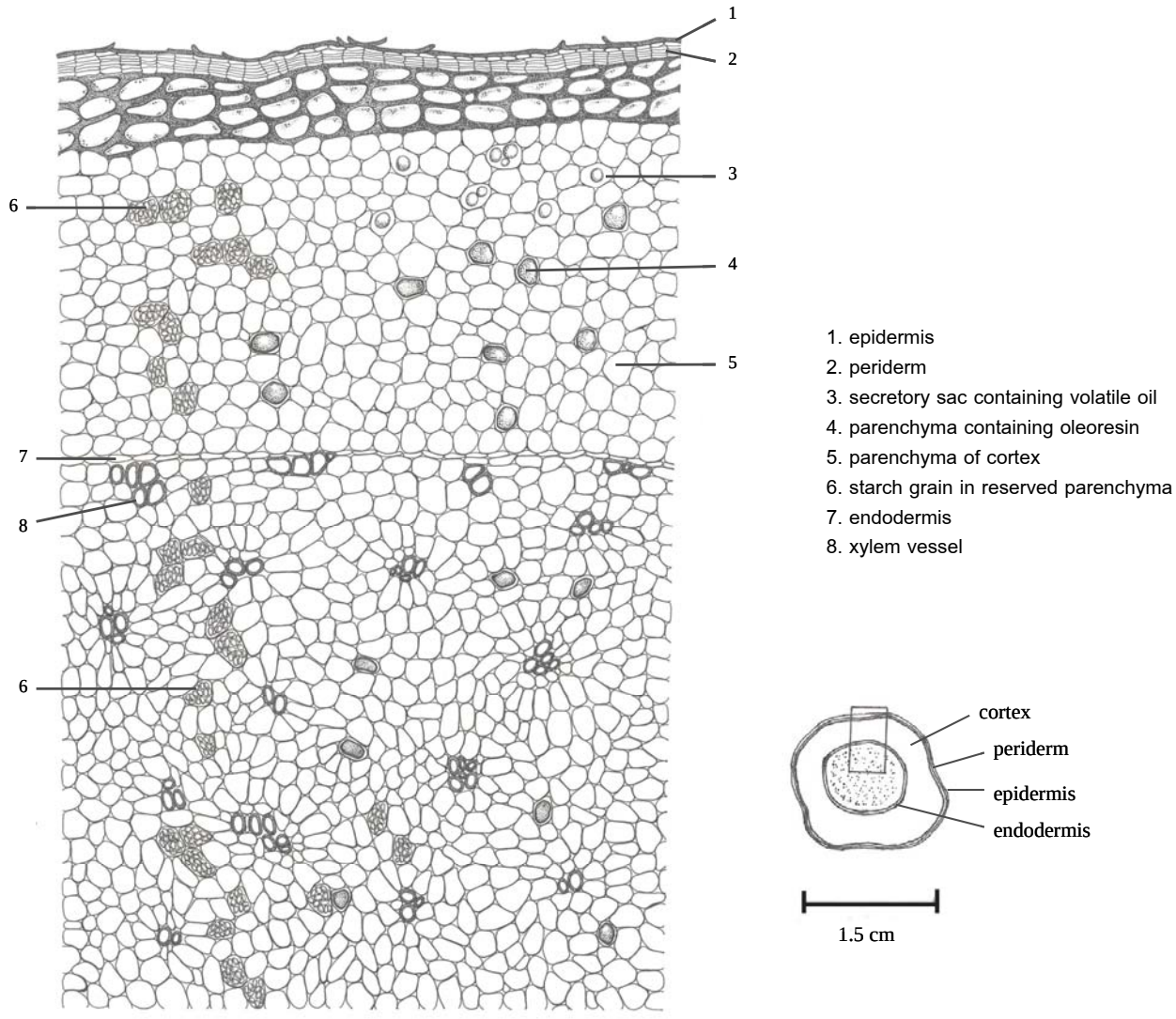
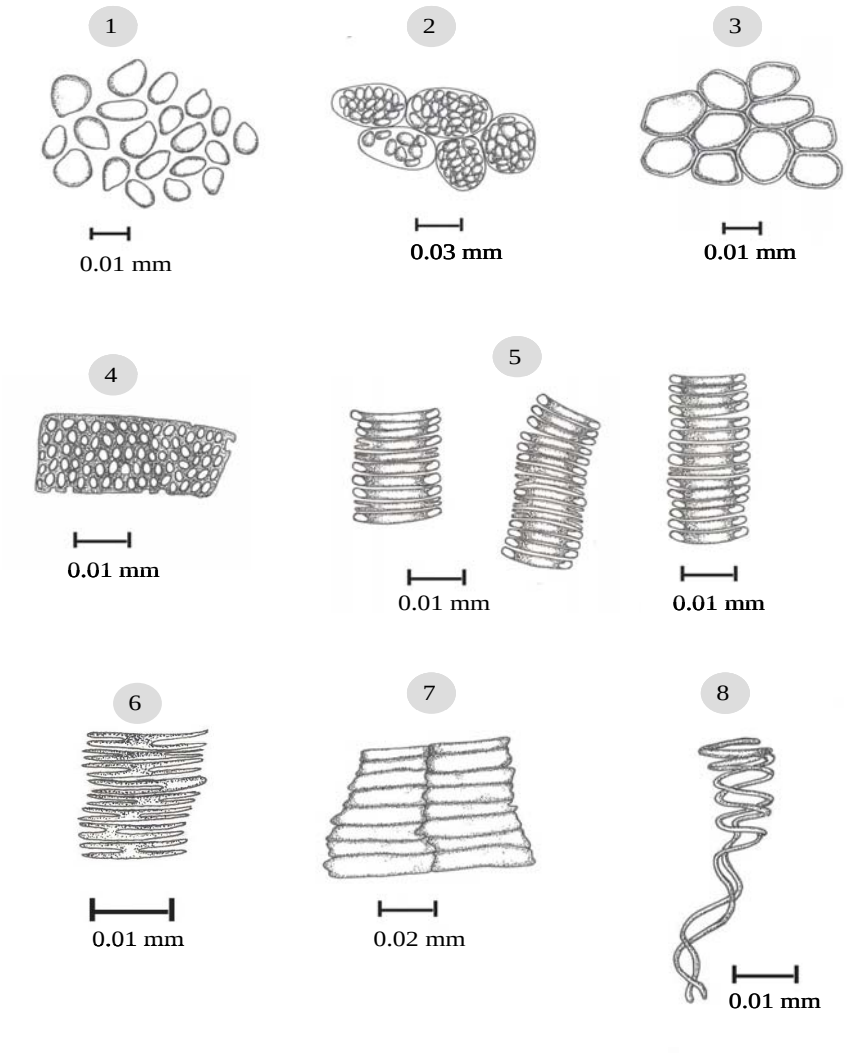
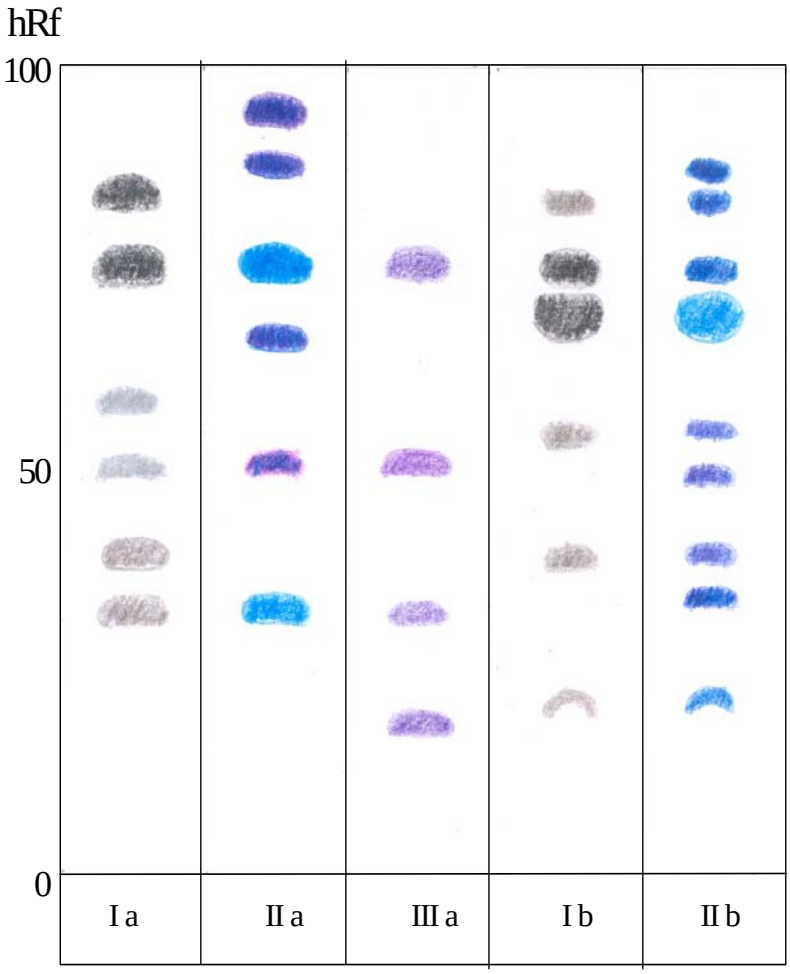


Figure 3 Anatomical character (Transverse section) of *K. galanga* rhizome



1. starch grain
2. parenchyma containing starch grain
3. parenchyma, transverse view
4. reticulate vessel
5. annular vessel
6. reticulate vessel
7. periderm
8. spiral vessel

Figure 4 Histological character (powdered) of *K. galanga* rhizome



Solvent systems:

a = Hexane: Ethyl acetate 70:30 b = Chloroform

Detections:

I = detection under UV light (254 nm)

II = detection with vanillin–sulfuric acid

III = detection with Ehrlich's reagent

Figure 6 TLC of methanolic extract of *Kaempferia galanga* rhizome

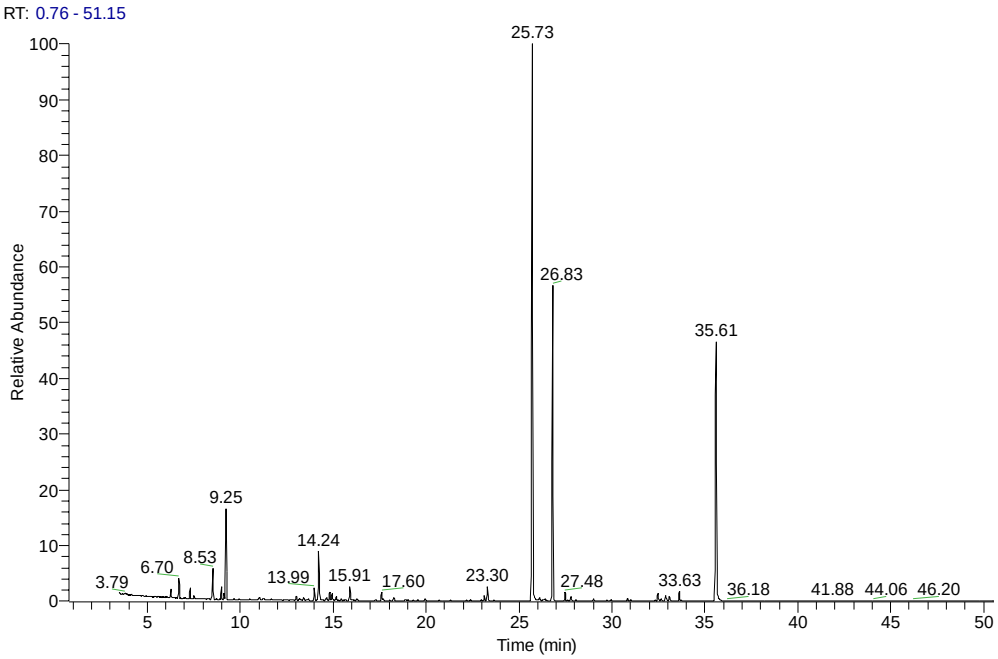


Figure 5 GC fingerprint of *K. galanga* rhizome volatile oil

Table 2 Chemical constituents of *K. galanga* rhizome volatile oil

RT[#]	Chemical composition	KI[##]	Area%	RT[#]	Chemical composition	KI[##]	Area%
6.29	*alpha*-Pinene	939	0.43	15.91	Eucarvone	1199	0.90
6.70	Camphene	953	0.95	17.60	Verbenone	1204	0.51
7.31	*o*-Cymene	1022	0.48	23.14	Tetradecane	1399	0.31
8.53	*alpha*-Terpinene	1018	1.77	23.30	*alpha*-Gurjunene	1409	0.90
8.98	*m*-Cymene	-	0.59	**25.73**	**Ethyl cinnamate**	**1462**	**39.97**
9.12	*d*-Limonene	1031	0.26	**26.83**	**Pentadecane**	**1500**	**20.85**
9.25	1,8-cineol	1033	5.32	27.48	*tau*-Cadinene	-	0.57
13.03	*trans*-2-Caren-4-ol	1136	0.25	27.80	*delta*-Cadinene	1524	0.30
13.99	*p*-Mentha-1,5-dien-8-ol	1166	0.77	32.47	2-Propenoic acid, 3-(4-		
14.24	Borneol	1165	3.22		methoxyphenyl)-, ethyl ester)	-	0.54
14.64	4-Terpineol	1177	0.24	32.90	8-Heptadecene	-	0.41
14.83	*p*-Cymen-8-ol	1183	0.55	33.08	(*Z*)6,(*Z*)9-Pentadecadien-1-ol	-	0.23
14.95	*p*-Cymen-8-ol	1183	0.47	33.63	Heptadecane	1700	0.63
15.17	*alpha*-Terpineol	1189	0.23	**35.61**	**Ethyl p-methoxycinnamate**	**-**	**18.35**

[#]Retention time (minute) [##]Kovat's index[11]

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คุณสมบัติทางเภสัชเวทของเหง้าเปราะหอมในประเทศไทย

ธนสร ตันศฤงฆาร[1] ชนิดา พลานุเวช[1] อนุสรณ์ รังสิโยธิน[1] สมชาย อิสระวานิช[1] นิรันดร์ วิพันธุ์เงิน[1] อัญชลี จูฑะพุทธิ[2] นิจศิริ เรืองรังษี[1,3,*]

[1]สถาบันวิจัยวิทยาศาสตร์การแพทย์ จุฬาลงกรณ์มหาวิทยาลัย [2]กรมพัฒนาการแพทย์แผนไทยและ การแพทย์ทางเลือก กระทรวงสาธารณสุข นนทบุรี 11000 [3]ภาควิชาเภสัชเวท คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10330

บทคัดย่อ: ศึกษาคุณสมบัติทางเภสัชเวทของเหง้าเปราะหอมแห้งที่เก็บจากร้านยาแผนโบราณ 15 แห่ง ใน 13 จังหวัด จาก 4 ภาค ของประเทศไทย วิเคราะห์ลักษณะมหทรรศน์ จุลทรรศน์ ของเครื่องยา และวิเคราะห์ค่าคงที่ต่างๆเพื่อบอกถึงคุณภาพของเครื่องยา ตามวิธีการขององค์การอนามัยโลก ลักษณะทางกายวิภาคและเนื้อเยื่อวิทยา พบถุงเก็บน้ำมันระเหย โอลีโอเรซินและเม็ดแป้งในเซลล์พาเรนไคมา ค่าเฉลี่ยของปริมาณสารปนเปื้อน เถ้ารวม เถ้าที่ไม่ละลายในกรด สารสกัดด้วยเอทานอล สารสกัดด้วยน้ำ น้ำหนักที่หายไปเมื่อทำให้แห้ง ความชื้น และน้ำมันระเหย มีค่าร้อยละ 0.06, 7.03, 3.75, 2.39, 16.05, 10.39, 9.62 และ 0.72 โดยน้ำหนักแห้ง ตามลำดับ องค์ประกอบหลักทางเคมีของน้ำมันระเหย คือ เอธิลซินนาเมต และอนุพันธ์พาราเมท็อกซีซินนาเมต การศึกษาทางโครมาโตกราฟีแสดงทีแอลซีฟิงเกอร์ปริ้นท์ของสารสกัดเมทานอล และจีซีฟิงเกอร์ปริ้นท์ของน้ำมันระเหยของเหง้าเปราะหอม

คำสำคัญ: คุณสมบัติทางเภสัชเวท เปราะหอม เหง้าแห้ง

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