

CHAPTER IV

RESULTS AND DATA

Part I Identification of Specimens

The stem, flowers, fruits and seeds of bastard cardamom as well as crude drugs of which both entitled “Reo” were collected from different locations in Thailand, those plant materials characters are examined and concluded as follows:

1. *Amomum* sp. A

Two samples of *Amomum* sp. A were collected, one sample is called “Reo” (เร่ว) or “Mak nang” (หมากหนั่ง) from Leang nok tha district, Yasothon province, the other is called “Reo” (เร่ว) or “Reo krawan” (เร่วกระวาน) from Soi dow district, Chantaburi province. The botanical characteristics of the plant is described below and also Figure 4-7.

Pseudostem elongate, 1-2.5 m tall, long and much branched rhizome. Leaves oblong glabrous, 4-7 cm x 35-50 cm, with caudate apex, to 5 cm, without petiole; ligule slightly emarginate, apex broad, 0.3-0.7cm. Inflorescence globose, 3-5 cm; peduncle 5-15 cm long; bracts pinkish to light brown, oblanceolate, 1.5-3 cm long; bracteoles light brown, 1-2 cm long, tubular at the base, apex 2-lobed. Calyx white with pinkish, 1.5-2.5 cm, tubular at the base, apex 3-toothed. Corolla tube as long as calyx, white with pinkish, apex 3-toothed, 1-3 cm long. Labellum obovate and strongly concave, 1.5 cm x 2.5 cm, white with a median yellow band and a dark crimson spots at the base, having a dark crimson stripe on each side of a median yellow band and a dark crimson spots outside the long stripes at the base. Stamen at the base of labellum; staminodes white, 4 mm long, sometime absent; filament white with pinkish at the base, 4 mm long; anther 6 mm long below the crest; crest of connective 3-lobed having spreading side lobes. Fruit obovate, 2-2.5 cm long and 1-1.5 diameter, covered by slender and soft spines, green when young and red when mature. Capsule 3-lobed contains 18-50 seeds; seeds white and red-brown, brown or dark brown when dried, white membranous aril.

Amomum sp. A was intensively checked for their identity such as comparing their characters with descriptions in the Flora of British India (1954), Prosea: Plant resources of South East Asia (1999), Malee Panunumpa’s study (2536), Vallisuta and Vongratanastit’s report (1976) and

with the type herbarium specimens preserved at the Forest herbarium (BKF), National park, Wildlife and plant conservation department, Bangkok. This could lead to the conclusion that *Amomum* sp. A is *Amomum uliginosum* K. D. Koenig.

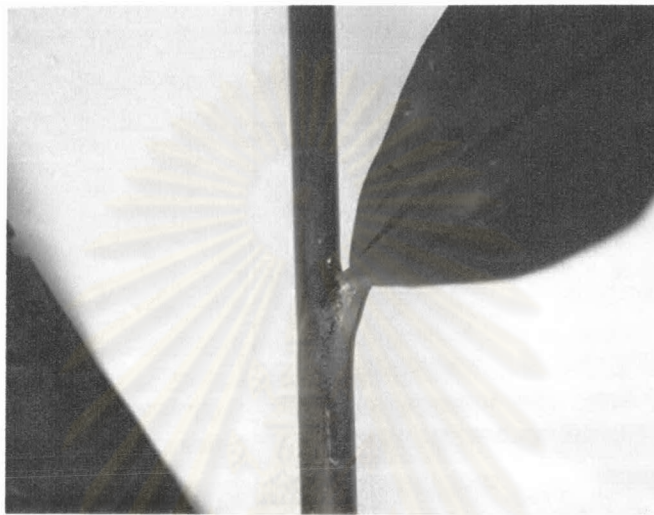


Figure 4 The pseudostem and ligule of *Amomum* sp. A

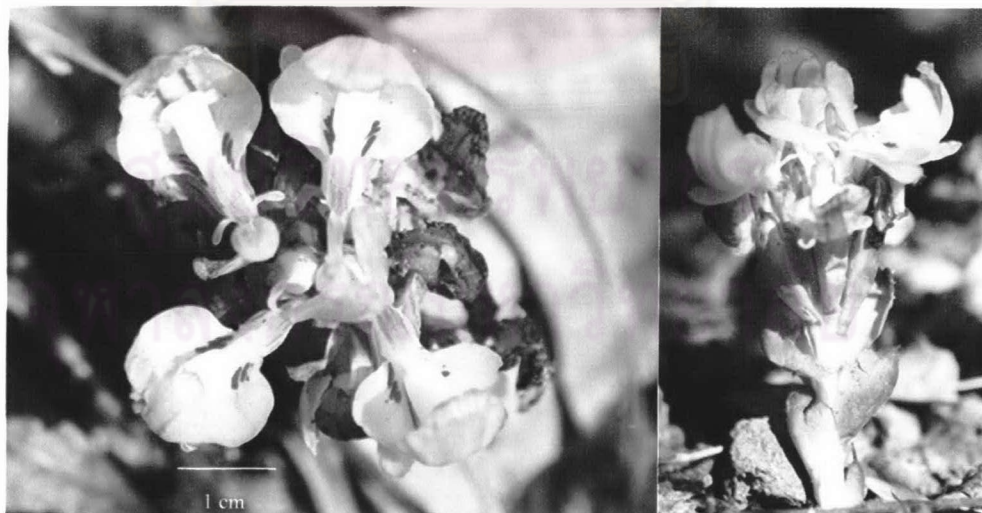


Figure 5 The inflorescence of *Amomum* sp. A



Figure 6 The fruits of *Amomum* sp. A

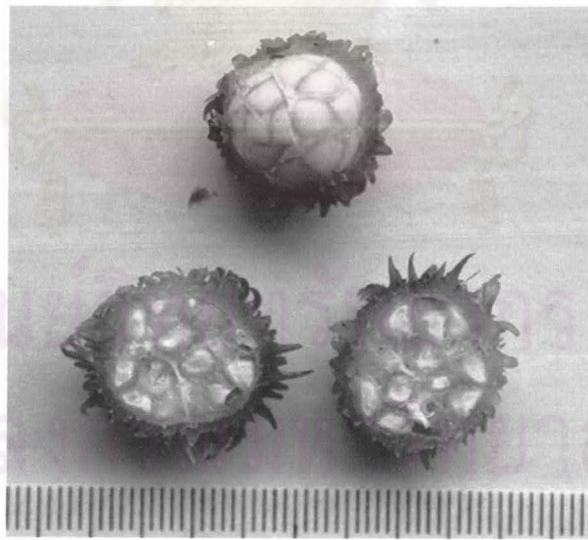


Figure 7 Cross section of fresh fruit of *Amomum* sp. A

2. *Amomum* sp. B

One sample of *Amomum* sp. B was collected, This species is called “Mak nang yai” (หมากหนั่งใหญ่) (Yasothon) or Mak huk (หมากหุก) (Mukdahan) from Dong luong district, Mukdahan province. The botanical characteristics of the plant is described below and also Figure 8-10.

Pseudostem elongate, 0.5-2.8 m tall, reticulate venation on stem. Leaves oblong-lanceolate glabrous, 3.5-10 cm x 35-50 cm, with caudate apex, to 3 cm; petiole 0.5-1 cm long; ligule dark red, coriaceous, 0.5-0.7cm. Inflorescence globose, 5-10 cm; peduncle 6-25 cm long, cover by sheath, 0.9-1.5 cm x 1.5-3.0 cm; bracts 0.9-1.1 cm x 2.2-2.5 cm, oblong-ob lanceolate, red-brown to dark brown; bracteoles light brown, 2.1-2.3 cm long, tubular at the base, apex 2-lobed. Fruit ovate or rounded, 1.5-2.8 cm long and 1.5-2.5 diameter, covered by short and hispid spines, red when mature, apex with persistent calyx. Capsule 3-lobed contains 18-39 seeds; seeds brown to black brown, white membranous aril and black brown to black when dried.

Amomum sp. B was intensively checked for their identity such as comparing their characters with descriptions in the Flora of British India (1954) and Malee Panunumpa’s study (2536). This could be lead to the conclusion that *Amomum* sp. B is *A. fulviceps* Thw.

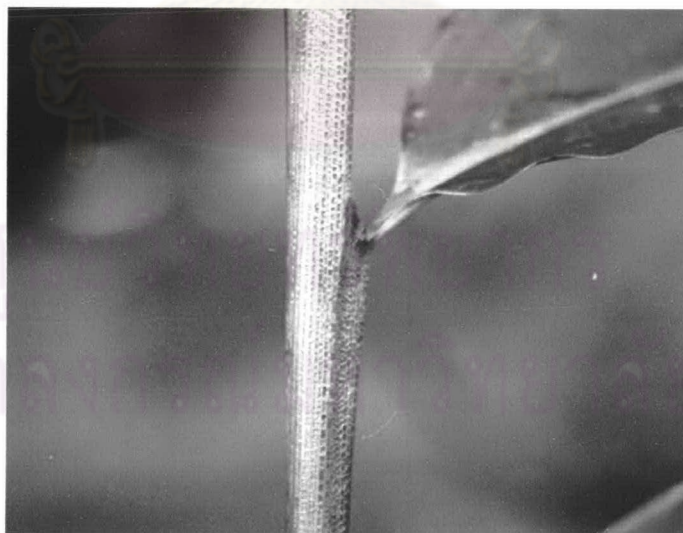


Figure 8 The pseudostem and ligule of *Amomum* sp. B

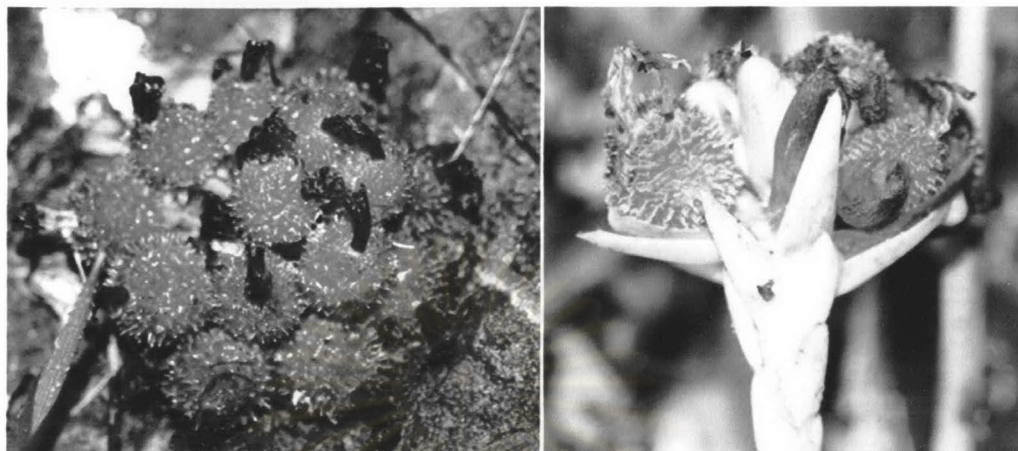


Figure 9 The fruits of *Amomum* sp. B



Figure 10 Cross section of fresh fruit of *Amomum* sp. B

Part II Specification of Reo

The specification of each kind of reo was investigated by pharmacognostic, and gas chromatographic. The results were described in the following section.

Amomum uliginosum K. D. Koenig

The macroscopic and microscopic characters of crude drugs from *A. uliginosum* K. D. Koenig; see below and also Figure 11.

Macroscopic Characters

The aggregated seeds is globular to ovoid, divided in to 3-lobes by thin membranes of septum, and each lobe containing 18-50 seeds which connected longitudinally by aril. Seed red-brown, brown or dark brown, obtusely angular, 3.5 mm in length and 2 mm in diameter. The seed is astringent with a camphor-like odor.



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Figure 11 The dried seeds of *A. uliginosum* K. D. Koenig

A. From Leang nok tha district, Yasothon

B. From Soi dow district, Chantaburi

Microscopic Characters

A. Histology

A cross section of seed of *A. uliginosum* is shown as follows (Figure 12):

- a) A layer of membranous aril (ar), consisting of a very thin-wall cells.
- b) A brown seed-coat consisting of rectangular epidermal layer of thick-walled cells of the testa (ep).
- c) A single layer of rectangular oil cells (oc), usually adhering to the epidermis layer.
- d) A layer of parenchyma cells of the testa (p), composed of a thin-walled cells and vascular bundle (vb).
- e) The sclerenchymatous layer of the testa (sc), composed of a layer of columnar cells which are brownish-yellow in color, each cell contains a nodule of silica.
- f) A large, colourless perisperm (ps), densely filled with starch granules (sta) and some cells contain small prism of calcium oxalate.

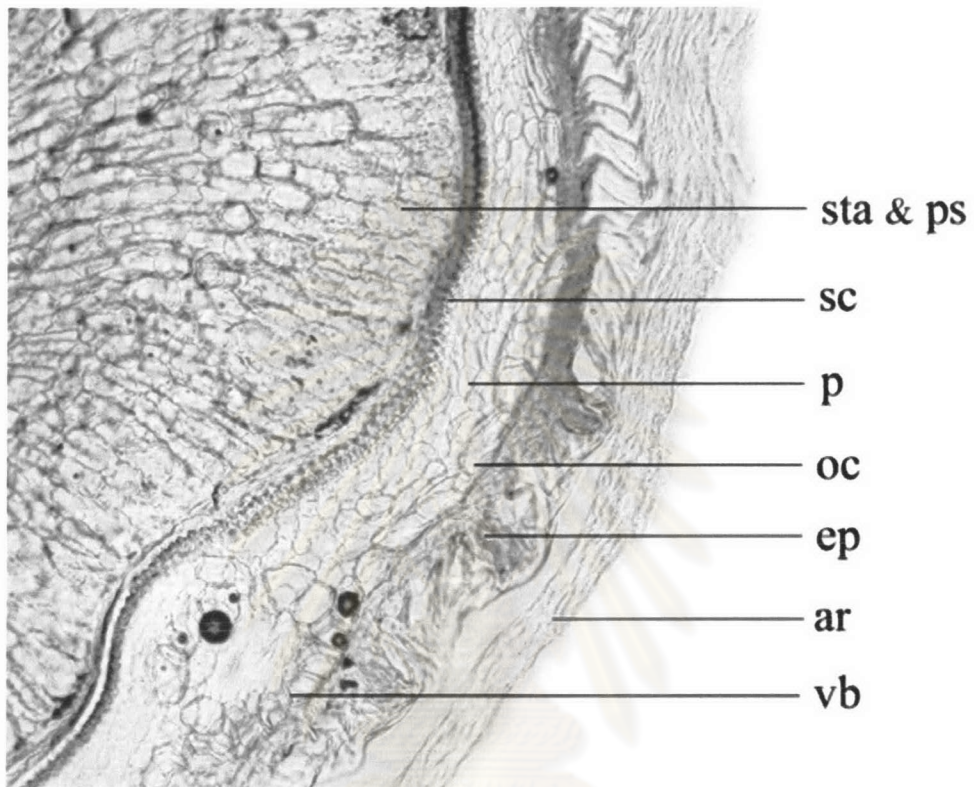


Figure 12 Cross section of dried seed of *A. uliginosum* K. D. Koenig (x 10)

Vascular bundle, (vb); Membranous aril, (ar); epidermis, (ep) of testa; oil cell, (oc); parenchyma, (p) of testa; sclerenchymatous cells, (sc); parenchyma, (p) of perisperm, (ps) containing starch granules, (sta) and calcium oxalate crystals.

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B. Powdered drugs

A brownish powder with the following tissue fragments (Figure 13):

- a) The abundant fragments of the epidermis of the testa, underlying the epidermis, elongated cells of the hypodermis are associated along with the epidermal cells (1), usually associate with the pigment cell (2).
- b) The pigment cells of the testa consisting of a layer of orange to reddish brown cell in surface view (3), (4).
- c) The thin-walled parenchyma cells (5).
- d) The sclerenchymatous layer of testa, compose of a single layer of thick-walled cells which are brownish-yellow color and each cell contains a nodule of silica. Occasional fragments of this layer may be found immature cells (6), mature cells (7), sectional view (8) or surface view from below (9).
- e) The spiral vessels usually found associated with thin walled parenchyma (10).
- f) The stone cells (11) which are thick-walled cells with numerous pits.
- g) The dark brown pigment (12), which occur singly or in group.
- h) The prisms of calcium oxalate (13) and the cluster of prisms (14) are found scattered in fragment of septum.
- i) A large perisperm cells containing starch granules and small prism of calcium oxalate (15), (16).
- j) The cluster of starch granules (17) which are dispersed from perisperm cells.

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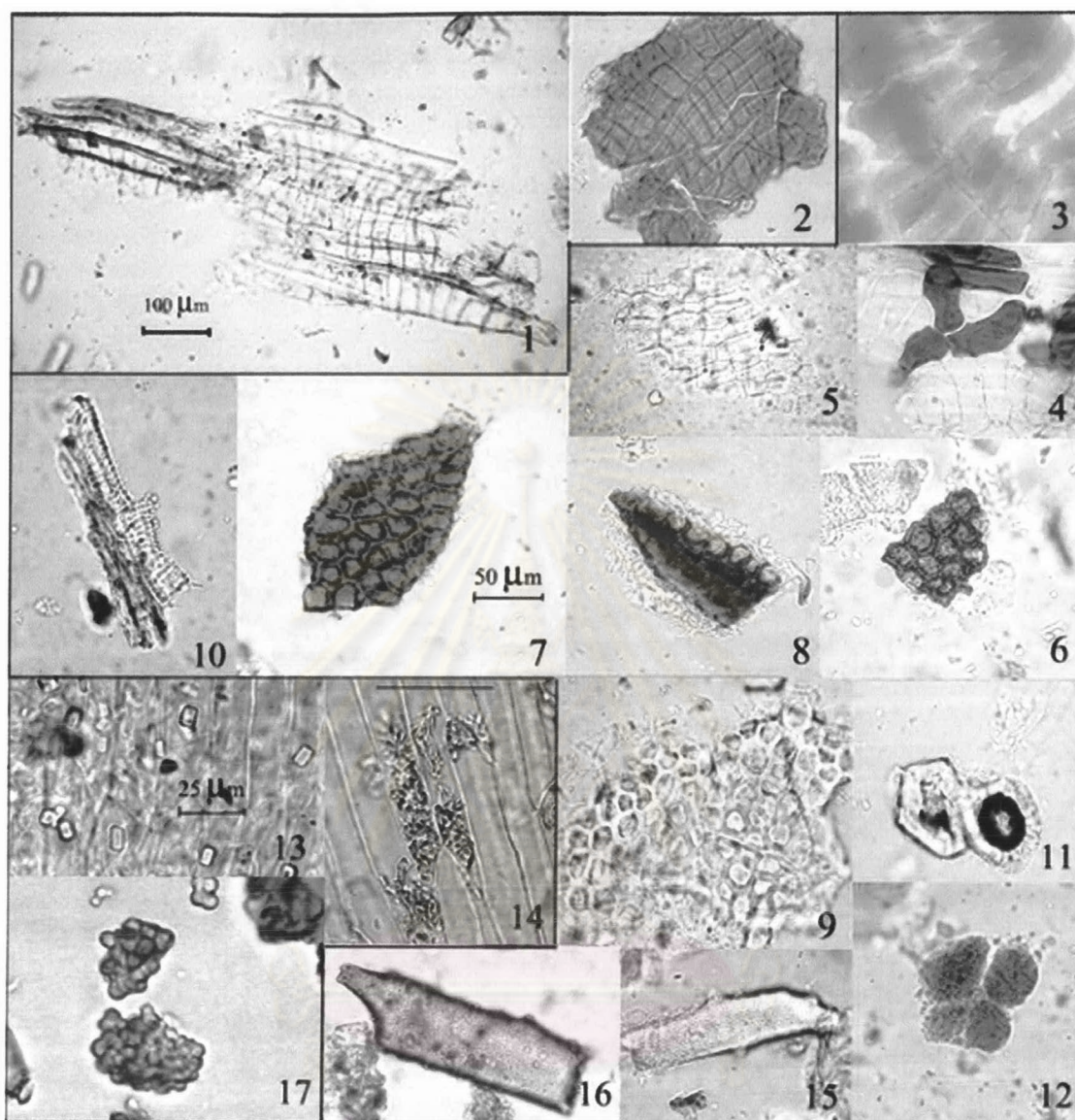


Figure 13 Powdered drug of the seeds of *A. uliginosum* K. D. Koenig

- | | | | |
|-----|---------------------------------|-------|--|
| 1-2 | Epidermis of the testa | 12 | Dark brown pigment |
| 3-4 | Pigment cells of the testa | 13-14 | Prisms of calcium oxalate |
| 5 | Thin-walled parenchyma cells | 15-16 | Perisperm cells containing prisms of calcium oxalate |
| 6-9 | Sclerenchymatous layer of testa | 17 | Cluster of starch granules |
| 10 | Spiral vessels | | |
| 11 | Stone cells | | |

The Volatile Oil Content and Composition

Table 5 Volatile oil content of *Amomum uliginosum* K. D. Koenig

Crude drug sample	Volatile oil content	
	Steam distillation \cong (%w/w)	Supercritical fluid extraction (%w/w)
<i>A. uliginosum</i> (Soi daw)	2.48*	1.64*
<i>A. uliginosum</i> (Leang nok tha)	2.49*	2.30*
Mean	2.485	1.97

\cong Assumed that v/w is nearly equivalent to w/w.

* The value of each sample is mean of two experiments.

In addition to the volatile oil content, composition of the volatile oil obtained from reo at Soi daw and Leang nok tha that extracted by different methods were also compared by using gas chromatography (GC). The resulted gas chromatograms exhibited the similarity of volatile oil contents between the two sources and two methods of extraction as shown in Figure 14.

To identify the compositions from chromatograms, volatile oil samples were injected to gas chromatography-mass spectroscopy (GC-MS) analysis. Peak numbers and name of each composition as well as its chemical group are shown in Table 6 and the content of volatile oil compositions are shown in Table 7.

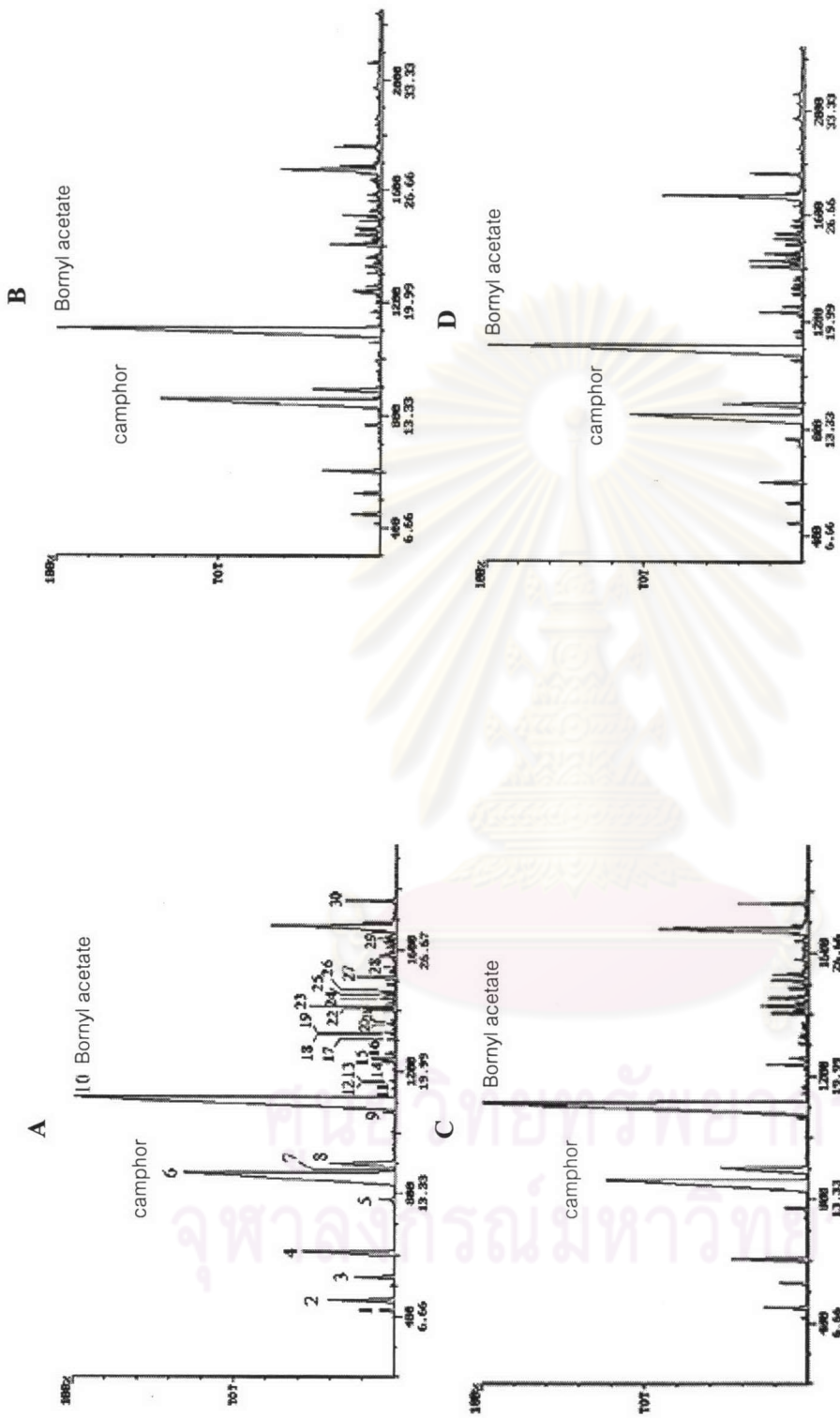


Figure 14 Gas chromatogram of volatile oil from dried seeds of *A. uliginosum* K. D. Koenig

A. Soi dow: Steam distillation

B. Soi dow: Supercritical fluid extraction

C. Leang nok tha: Steam distillation

D. Leang nok tha: Supercritical fluid extraction

Table 6 Volatile oil compositions from dried seeds of *A. uliginosum* K. D. Koenig

Peak No.	Compound	Category group
1	α -Pinene	Monoterpene hydrocarbons
2	Camphene	
3	β -Myrcene	
4	Limonene	
5	Fenchyl alcohol	Oxygenated monoterpene
6	Camphor	
7	Camphene hydrate	
8	Borneol	
9	Fenchone	
10	Bornyl acetate	
11	<i>cis</i> -Geraniol	Sesquiterpene hydrocarbons
12	Elixene	
13	δ -Elemene	
14	Ylangene	
15	Copaene	
16	β -Elemene	
17	Caryophyllene	
18	Germacrene B	
19	α -Bergamotene	
20	β -Farnesene	
21	Aromadendrene	
22	γ -Muurolene	
23	δ -Selinene	
24	α -Elemene	
25	β -Bisabolene	Oxygenated sesquiterpene
26	δ -Cadinene	
27	Nerolidol	
28	δ -Cadinol	
29	α -Bisabolol	
30	Farnesyl acetate	

Table 7 The content of volatile oil compositions from dried seeds of *A. uliginosum* K. D.

Koenig

Compound	Content (% relative*)	
	Steam distillation	Supercritical fluid extraction
α -Pinene	0.31	0.08
Camphene	1.60	0.66
β -Myrcene	0.94	0.63
Limonene	3.20	1.72
Fenchyl alcohol	0.20	0.34
Camphor	26.96	25.61
Camphene hydrate	0.03	0.04
Borneol	4.14	4.67
Fenchone	0.29	0.35
Bornyl acetate	45.79	45.35
<i>cis</i> -Geraniol	0.08	0.09
Elixene	0.04	0.07
δ -Elemene	0.07	0.16
Ylangene	0.03	0.10
Copaene	0.66	1.13
β -Elemene	0.25	0.36
Caryophyllene	0.20	0.31
Germacrene B	0.08	0.14
α -Bergamotene	0.14	0.28
β -Farnesene	0.14	0.21
Aromadendrene	0.06	0.19
γ -Murolene	0.75	1.56
δ -Selinene	0.33	0.24
α -Elemene	0.53	0.95
β -Bisabolene	0.29	0.48
δ -Cadinene	0.42	0.65
Nerolidol	0.02	0.07
δ -Cadinol	0.04	0.03
α -Bisabolol	0.19	0.18
Farnesyl acetate	0.93	1.06

* % relative = % integrated area

Each value represented the median value of two extracted of *A. uliginosum* K. D. Koenig from Soi dow and Leang nok tha.

Amomum fulviceps Thw.

The macroscopic and microscopic characters of crude drugs from *A. fulviceps* Thw.; see below and also Figure 15.

Macroscopic Characters

The mostly aggregated seeds is ovoid, divided in to 3-lobes by thin membranes of septum, and each lobe containing 18-39 seeds of which connected longitudinally by aril. Seed brown to black brown, obovoid, 3-5 mm in length and 2-3 mm in diameter. The seed is slightly odor.



Figure 15 The dried seeds of *Amomum fulviceps* Thw.

A. The dried aggregated seeds of *A. fulviceps* Thw.

B. Separated lobe of fruits cover with septum

Microscopic Characters

A. Histology

A cross section of the seed of *Amomum fulviceps* Thw. is shown as follows (Figure 16):

- a) A layer of membranous aril (ar).
- b) A brown seed-coat consisting of rectangular epidermal layer of thick-walled cells of the testa (ep).
- c) A layer of parenchyma cells of the testa consisting of brownish material (p).
- d) Sclerenchymatous layer of testa containing silica (sc).
- e) A large, colourless perisperm (ps), densely filled with starch granules (sta) and some cells contain rosette aggregates of calcium oxalate.



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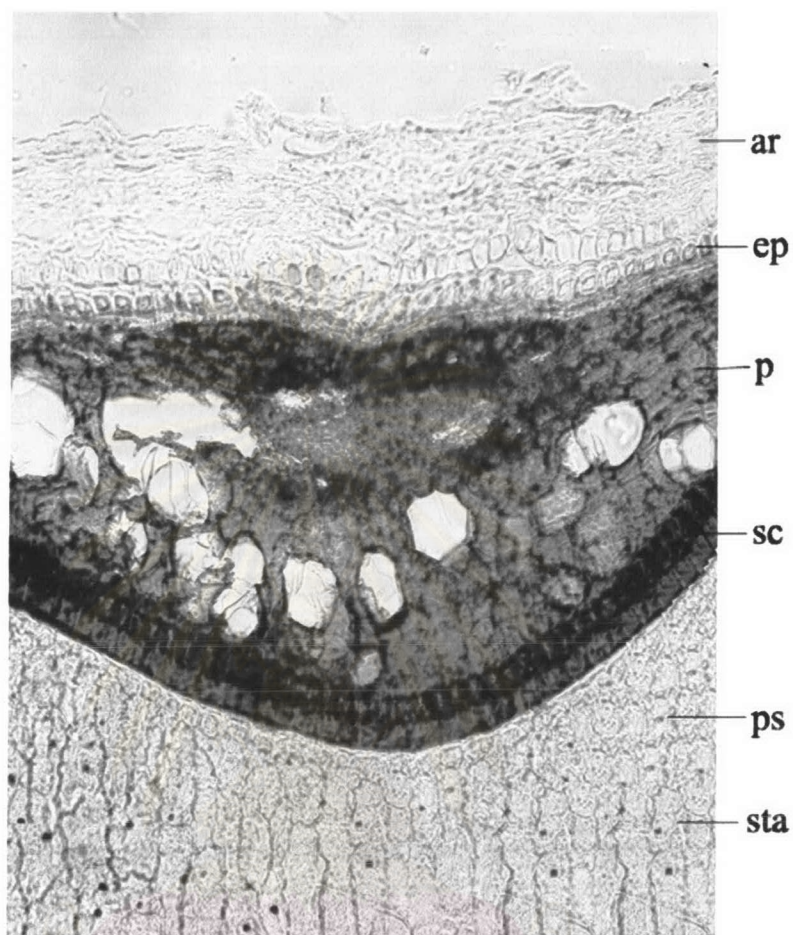


Figure 16 Cross section of dried seed of *A. fulviceps* Thw. (x 40)

Membranous aril, (ar); epidermis, (ep) of testa; parenchyma, (p) of testa; sclerenchymatous cells, (sc); parenchyma, (p) of perisperm, (ps) containing starch granules, (sta) and calcium oxalate crystals.

B. Powdered drugs

A brownish powder with the following (Figure17):

- a) The abundant fragments of the epidermis of the testa, underlying the epidermis, elongated cells of the hypodermis are lying axis to the epidermis cells (1), usually thickened wall (2).
- b) The parenchyma cells that different shape and size of walls(3-5).
- c) The sclerenchymatous layer of testa, compose of a single layer of thick-walled cells which are brownish-yellow color and each cell contains a nodule of silica. Occasional fragments of this layer may be found immature cells (6) or mature cells (7).
- d) The spiral vessels usually found associated with thin walled parenchyma (8).
- e) The large dark brown pigment (9), which occur singly or in group.
- f) The calcium oxalate crystals are found abundant in fragment of septum and perisperm cells, they occurs as rosette aggregates (10), under the polarized microscope (11), and also as prisms (12).
- g) A large perisperm cells containing starch granules and rosette aggregate of calcium oxalate crystals(13).
- h) The starch granules (14) which are fragment from perisperm cells.

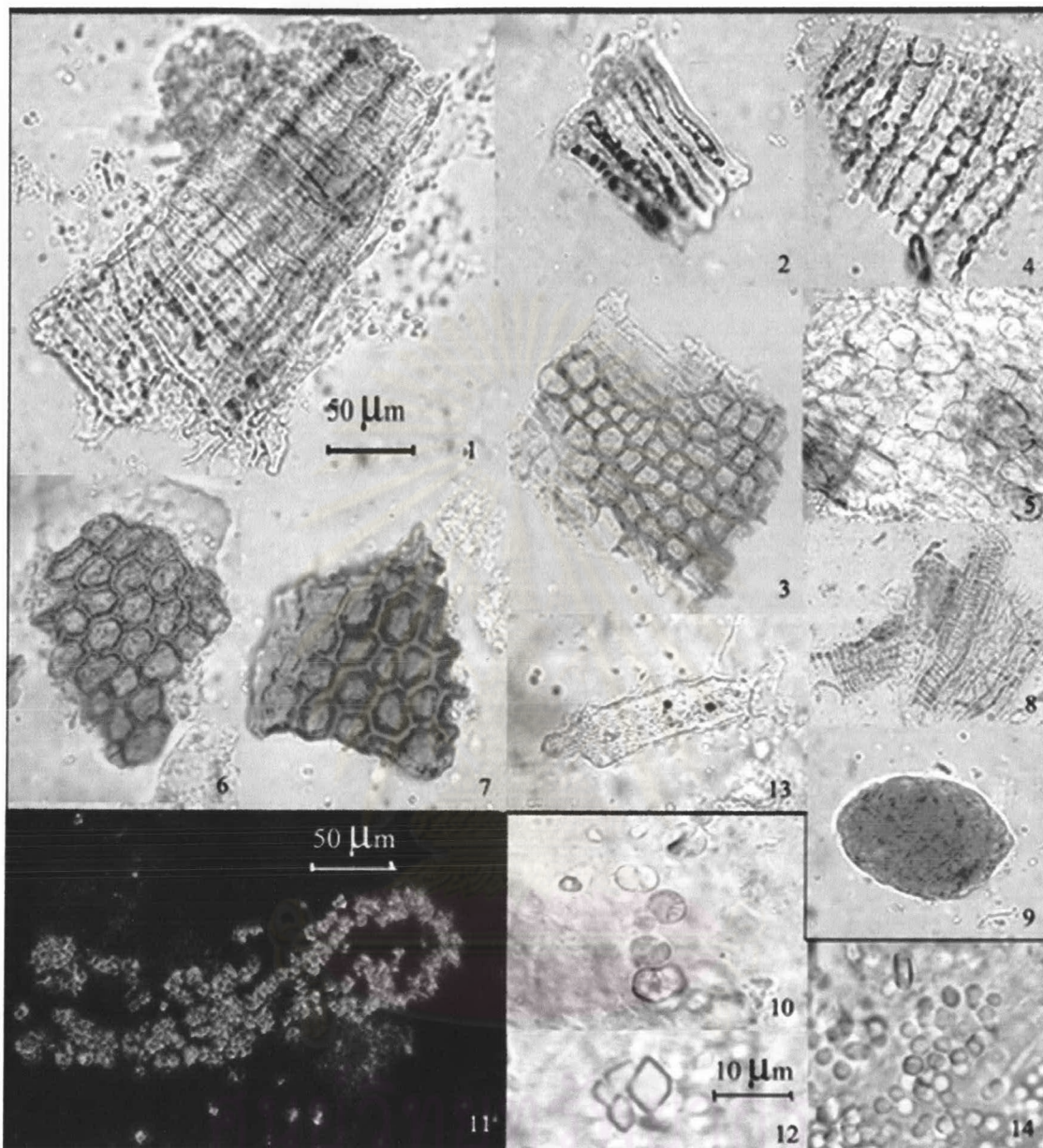


Figure 17 Powdered drug of the seeds of *A. fulviceps* Thw.

- | | | | |
|-----|---------------------------------|-------|--|
| 1-2 | Epidermis of the testa | 10-11 | rosette aggregates of calcium oxalate crystal |
| 3-5 | Parenchyma cells | 12 | Prisms of calcium oxalate |
| 6-7 | Sclerenchymatous layer of testa | 13 | Perisperm cells containing calcium oxalate crystal |
| 8 | Spiral vessels | 14 | Starch granules |
| 9 | Dark brown pigment | | |

The Volatile Oil Content and Composition

Volatile oil of the seeds of *A. fulviceps* Thw. was extracted by steam distillation and supercritical fluid extraction, but lesser content was obtained from steam distillation, thus the extract from supercritical fluid method (0.85% w/w) was selected to run gas chromatography.

Composition of the volatile oil is shown in Figure 18, where as numbers and name of each component as well as its chemical group are summarized and the content of volatile oil compositions are shown in Table 8.

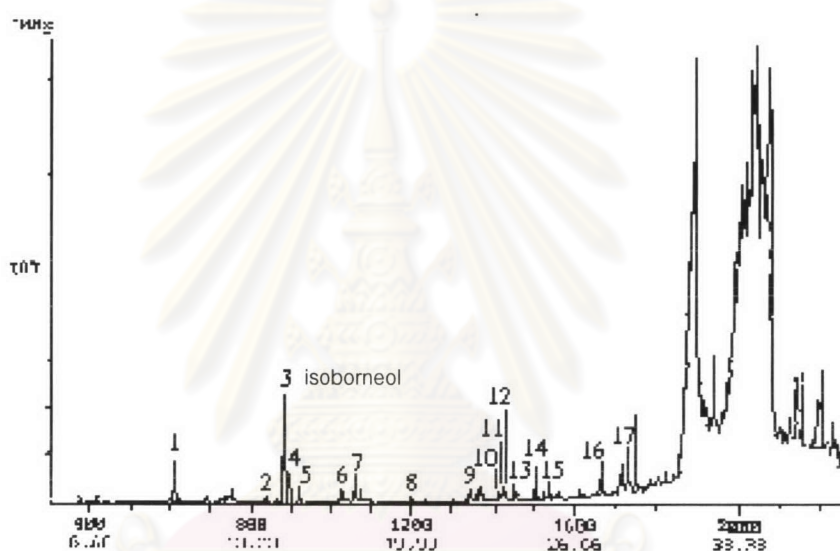


Figure 18 Gas chromatogram of volatile oil from dried seeds of *A. fulviceps* Thw. extracted by supercritical fluid extraction

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Table 8 The contents of volatile oil and other compositions from dried seeds of *A. fulviceps* Thw., extracted by supercritical fluid extraction

Peak no.	Compound	Category group	Content (%relative*)
1	Eucalyptol	Monoterpene hydrocarbons	0.26
2	Camphor		0.03
3	Isoborneol		0.68
4	4-Terpeneol		0.06
5	α -Terpineol		Oxygenated monoterpene
6	<i>cis</i> -Geraniol		0.07
7	Bornyl acetate		0.04
8	Isoeugenol		0.02
9	α -Caryophyllene		Sesquiterpene hydrocarbons
10	α -Guaiene	0.04	
11	γ -Selinene	0.04	
12	Caryophyllene	0.04	
13	Elixene	Oxygenated sesquiterpene	0.06
14	Nerolidol		0.13
15	<i>trans</i> -Farnesol		0.20
16	Palmitic acid	Wax	0.21
17	Oleic acid		0.13

* %relative = % integrated are

Crude Drugs of Purchased Reo

The macroscopic characters of reo purchased from the traditional drugstores throughout Thailand (according to Table 3) are shown in Figure 19- 35 and Table 9

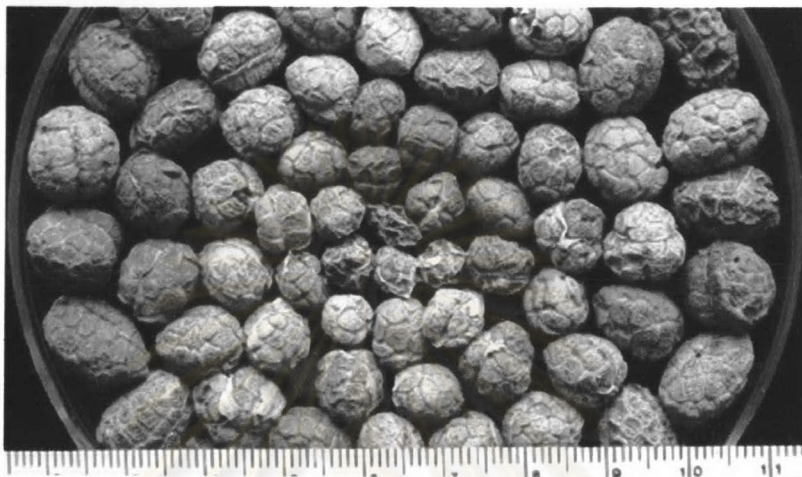


Figure 19 Crude drug from Chiang Mai 1

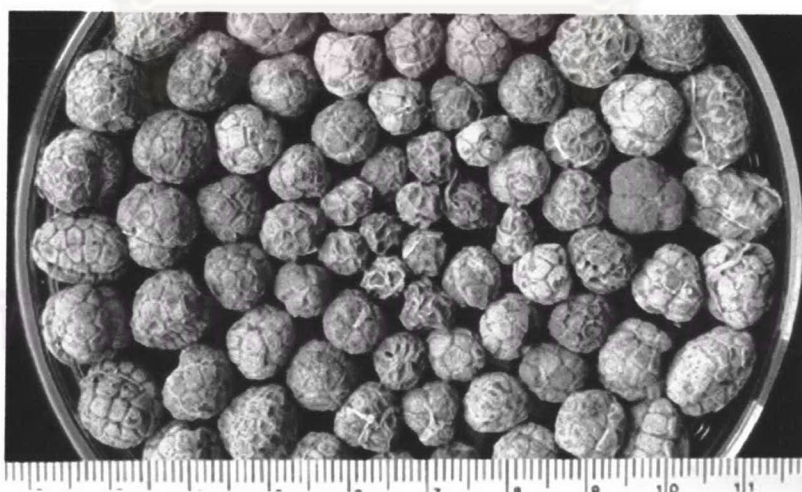


Figure 20 Crude drug from Chiang Mai 2



Figure 21 Crude drug from Sukhothai



Figure 22 Crude drug from Bangkok 1



Figure 23 Crude drug from Bangkok 2



Figure 24 Crude drug from Bangkok 3



Figure 25 Crude drug from Bangkok 4-a



Figure 26 Crude drug from Nakhonpathom



Figure 27 Crude drug from Chon Buri



Figure 28 Crude drug from Chanthaburi



Figure 29 Crude drug from Khon Kaen



Figure 30 Crude drug from Roi Et



Figure 31 Crude drug from Ubon Ratchathani



Figure 32 Crude drug from Surat Thani



Figure 33 Crude drug from Songkhla-a



Figure 34 Crude drug from Bangkok 4-b



Figure 35 Crude drug from Songkhla-b (adulterant)

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Table 9 Macroscopic characters of purchased reo

Source	Seed character	Shape	Size	Color	Odor and taste
Chiang Mai 1	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	6 to 15 mm long and 6 to 12 mm wide	Pale brown, brown to dark brown	Astringent with aromatic odor and taste
Chiang Mai 2	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 15 mm long and 5 to 10 mm wide	Red-brown to purple	Astringent with aromatic odor and taste
Sukothai	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 16 mm long and 5 to 13 mm wide	Pale brown, red-brown to purple	Astringent with aromatic odor and taste.
Bangkok 1	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 13 mm long and 5 to 11 mm wide	Grayish-brown, brown to dark brown	Astringent with aromatic odor and taste
Bangkok 2	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 13.5 mm long and 5 to 10 mm wide	Grayish-brown, yellow-brown to dark brown	Astringent with aromatic odor and taste
Bangkok 3	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular-ovoid	Nearly sized, 5 to 13 mm long and 5 to 11 mm wide	Mostly red-brown to purple and some brown to dark brown	Astringent with aromatic odor and taste

Table 9 Macroscopic characters of purchased reo (continued)

Source	Seed character	Shape	Size	Color	Odor and taste
Bangkok 4-a	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 15 mm long and 5 to 11 mm wide	Yellow-brown to dark brown	Astringent with aromatic odor and taste
Bangkok 4-b	The compact masses of the seed, without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid, difficult to break into 3 fragments	11 to 17.5 mm long and 11 to 15 mm wide	Yellow-brown, red-brown to brown	Mild aromatic odor and taste.
Nakhonpathom	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 14 mm long and 5 to 10 mm wide	Brown, red-brown to purple	Astringent with aromatic odor and taste
Chonburi	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Nearly shaped, mostly ovoid	5 to 15 mm long and 5 to 11 mm wide	Yellow-brown to brown.	Astringent with aromatic odor and taste
Chanthaburi	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Nearly shaped, mostly globular	6 to 17 mm long and 6 to 12 mm wide	The color tone of crude drugs are yellow and red brown	Astringent with aromatic odor and taste
Khon Kaen	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 12 mm long and 5 to 10 mm wide	Different color such as pale brown, yellow-brown, brown, and dark brown	Astringent with aromatic odor and taste

Table 9 Macroscopic characters of purchased reo (continued)

Source	Seed character	Shape	Size	Color	Odor and taste
Roi Ed	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	7 to 16 mm long and 7 to 13 mm wide	Pale brown to brown	Astringent with aromatic odor and taste
Ubon Ratchathani	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid and oblong-ovoid for large size	7 to 18 mm long and 7 to 13 mm wide	Brown to purple	Astringent with aromatic odor and taste
Suradthani	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 14 mm long and 5 to 11 mm wide	Grayish-brown to brown	Astringent with aromatic odor and taste
Songkhla-a	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Globular to ovoid	5 to 14 mm long and 5 to 12 mm wide	Pale brown to brown.	Astringent with aromatic odor and taste
Songkhla-b	The seeds aggregated without pericarp, divides in to 3-lobed by thin-septum	Mostly is ovoid and some globular	8 to 19 mm long and 8 to 15 mm wide	Dark brown	Mild aromatic odor and taste.

The Volatile Oil Content and Composition

The content of volatile oil and composition of commercial reo were compared between steam distillation and supercritical fluid extraction method. The volatile oil content are shown in Table 10 and volatile composition are shown in Table 11 and Figure 36. Each value represents the mean of fifteen separate preparations of volatile oil from reo which were purchased from traditional drugstores throughout Thailand.

Table 10 Volatile oil content of reo which were purchased from traditional drugstores

Crude drug sample	Volatile oil content	
	Steam distillation \cong (%w/w)	Supercritical fluid extraction (%w/w)
Chiang Mai 1	2.50	1.57
Chiang Mai 2	2.56	2.47
Sukothai	2.46	1.69
Bangkok 1	2.27	2.12
Bangkok 2	2.24	1.53
Bangkok 3	2.05	1.70
Bangkok 4-a	2.86	1.50
Nakornpathom	2.28	1.77
Chonburi	2.25	2.87
Chantaburi	2.77	1.92
Khon Kaen	3.17	3.25
Roi Ed	2.86	2.29
Ubon Ratchathani	2.23	2.39
Surat Thani	2.37	2.09
Songkhla-a	2.37	1.32
Mean	2.48	1.95
SD	0.31	0.49

\cong Assumed that v/w is nearly equivalent to w/w

Table 11 The components and the content of volatile oil from purchased reo

Compound	Content (%relative*)	
	Steam distillation	Supercritical fluid extraction
α -Pinene	0.37 \pm 0.23	0.07 \pm 0.13
Camphene	2.72 \pm 2.87	0.79 \pm 0.45
β -Myrcene	1.22 \pm 0.64	0.61 \pm 0.30
Limonene	3.59 \pm 1.30	1.58 \pm 0.67
Fenchyl alcohol	0.27 \pm 0.28	0.30 \pm 0.20
Camphor	27.41 \pm 5.45	25.55 \pm 8.04
Camphene hydrate	0.07 \pm 0.07	0.36 \pm 1.24
Borneol	7.21 \pm 1.69	7.93 \pm 4.96
Fenchone	0.32 \pm 0.41	0.22 \pm 0.09
Bornyl acetate	31.35 \pm 8.02	29.42 \pm 9.89
<i>cis</i> -Geraniol	0.04 \pm 0.06	0.05 \pm 0.06
Elixene	tr**	0.01 \pm 0.03
δ -Elemene	0.05 \pm 0.05	0.10 \pm 0.07
Ylangene	0.04 \pm 0.03	0.12 \pm 0.17
Copaene	0.29 \pm 0.26	0.38 \pm 0.33
β -Elemene	0.29 \pm 0.15	0.38 \pm 0.19
Caryophyllene	1.03 \pm 1.87	0.70 \pm 1.21
Germacrene B	0.14 \pm 0.38	0.24 \pm 0.42
α -Bergamotene	0.11 \pm 0.06	0.15 \pm 0.10
β -Farnesene	0.18 \pm 0.11	0.27 \pm 0.16
Aromadendrene	0.10 \pm 0.14	0.11 \pm 0.09
γ -Muurolene	0.53 \pm 0.23	0.96 \pm 0.55
δ -Selinene	0.29 \pm 0.21	0.28 \pm 0.35
α -Elemene	0.41 \pm 0.36	0.45 \pm 0.40
β -Bisabolene	0.40 \pm 0.34	0.43 \pm 0.26
δ -Cadinene	0.33 \pm 0.37	0.31 \pm 0.18
Nerolidol	0.12 \pm 0.30	0.15 \pm 0.17
δ -Cadinol	0.10 \pm 0.07	0.10 \pm 0.07
α -Bisabolol	0.14 \pm 0.06	0.16 \pm 0.07
Farnesyl acetate	0.63 \pm 0.56	1.12 \pm 0.89

* %relative = %integrated area

tr** = trace

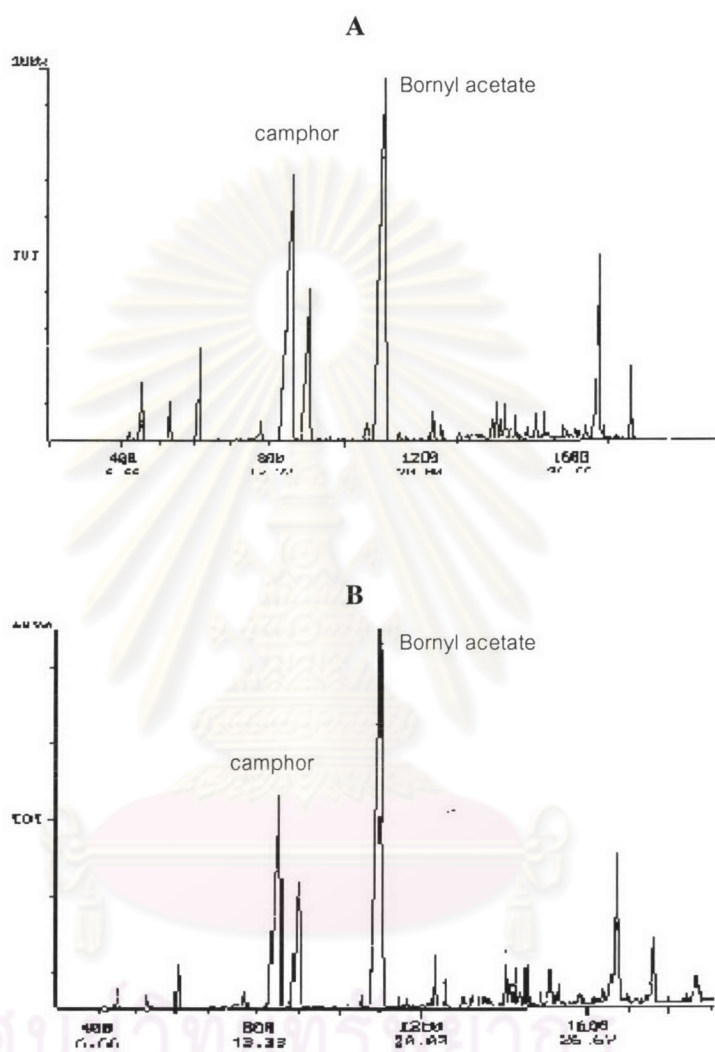


Figure 36 Gas chromatogram of volatile oil from purchased reo

A. Steam distillation

B. Supercritical fluid extraction

Volatile oil content of the other species claimed as reo and found as adulteration (Songkhla-b) and the less popular consumed species found in only one herb store (Bangkok 4-b) are shown in the Table 12 below.

Table 12 Volatile oil content of the other species claimed as reo

Crude drug sample	Volatile oil content	
	Steam distillation \cong (%w/w)	Supercritical fluid extraction (%w/w)
Songkhla-b	*	0.13
Bangkok 4-b	0.95	0.70

\cong Assumed that v/w is nearly equivalent to w/w

* Volatile oil less than distilled

In conclusion, as same as self collected plants specimens previously identified as *Amomum uliginosum* K. D. Koenig, composition of the volatile oil obtained from each specie that extracted by different methods were also compared by using gas chromatography (GC). The resulted GC-chromatograms and volatile oil constituents are similar to all fifteen samples in two methods, so sample's agent only of each methods are shown (Figure 36). Separately, the two different samples of which less popular available (Songkhla-b and Bangkok 4-b), the resulted GC-chromatograms and volatile oil constituents are shown in Figure 37 and Table 13-14, respectively.

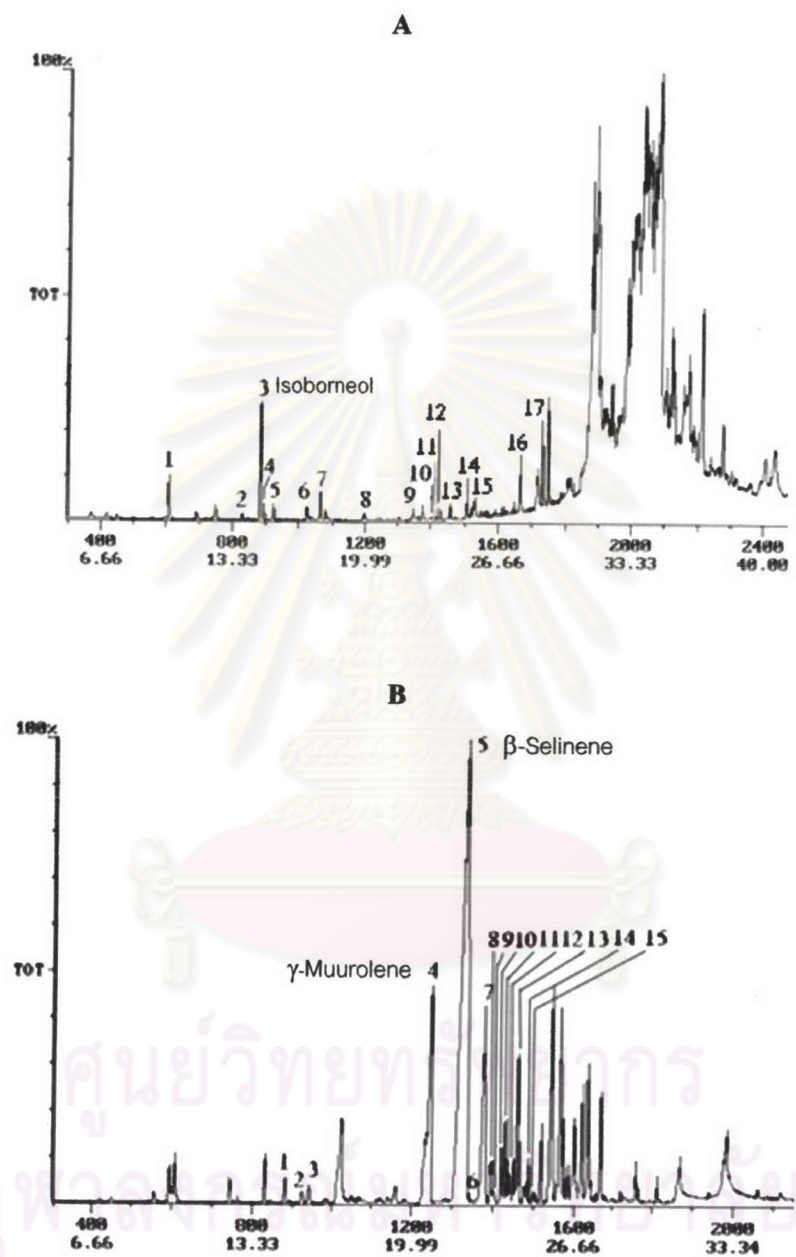


Figure 37 Gas chromatogram of volatile oil of purchased reo from

A. Songkhla-b

B. Bangkok 4-b

Table 13 The components and the content of compound from Songkhla-b sample by supercritical fluid extraction method

Peak No.	Compound	Content (%relative*)
1	Eucalyptol	0.22
2	Camphor	0.02
3	Isoborneol	0.71
4	4-Terpeneol	0.06
5	α -Terpineol	0.05
6	<i>cis</i> -Geraniol	0.04
7	Bornyl acetate	0.03
8	Isoeugenol	0.02
9	α -Caryophyllene	0.05
10	α -Guaiene	0.04
11	γ -Selinene	0.05
12	Caryophyllene	0.04
13	Elixene	0.08
14	Nerolidol	0.17
15	<i>trans</i> -Farnesol	0.22
16	Palmitic acid	0.23
17	Oleic acid	0.10

* % relative = % integrated area

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Table ..14 The components and the content of compound from Bangkok 4-a sample by supercritical fluid extraction method

Peak No.	Compound	Content (%relative*)
1	Borneol	0.50
2	α -Terpineol	0.28
3	<i>cis</i> -Sabinol	0.46
4	γ -Muurolene	9.14
5	β -Selinene	39.03
6	α -Amorphene	0.06
7	α -Caryophyllene	6.63
8	β -Cubebene	1.21
9	α -Muurolene	0.22
10	δ -Selinene	1.38
11	γ -Selinene	0.53
12	Germacrene D	0.56
13	δ -Cadinene	2.85
14	α -Gurjunene	0.14
15	Caryophyllene oxide	0.17

* % relative = % integrated area

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Part III Qualitative determination of commercial crude drugs

The crude drugs which were purchased from traditional drugstores throughout Thailand can be distinguishable into 3 species according to the results of macroscopical and gas chromatography. The majority of crude drugs are *Amomum uliginosum* K.D.Koenig, found fifteen samples from sixteen times of bought. *A. fulviceps* Thw. had the half adulterant of *A. uliginosum* K.D.Koenig which was purchased from Songkhla and the one sample is *Alpinia* sp. which was purchased from Bangkok. According to the above results commercial drugs could be identified and carried out further qualitative analysis as follow (Table 15-17).

Table 15 Foreign matter of reo which were purchased from traditional drugstores

Crude drug sample	Foreign matter (%)
Chiang Mai 1	4.16
Chiang Mai 2	1.31
Sukothai	0.63
Bangkok 1	0.59
Bangkok 2	2.17
Bangkok 3	0.67
Bangkok 4-a	5.46
Nakornpathom	1.65
Chonburi	0.54
Chantaburi	0.44
Khon Kaen	0.27
Roi Ed	0.77
Ubon Ratchathani	0.98
Surat Thani	0.73
Songkhla-a	29.46
Mean	1.45
SD	1.53

All of crude drugs were free from moulds, insects and other animal contamination. The crude drugs were adulterated by either part of pericarp or organs of other plants, but crude drugs that purchased from Songkhla was half adulterated by another sample (Songkhla-b), so this sample was not use to calculated the mean and SD.

Table 16 Moisture content, total ash and acid insoluble ash of reo

Crude drug sample	Moisture content (%)	Ash content (%)	
		Total ash	Acid insoluble ash
<i>Amomum uliginosum</i> (Soi daw)	1.76	7.54	2.59
<i>A. uliginosum</i> (Leang nok tha)	1.81	8.04	3.01
Chiang Mai 1	1.88	7.59	3.43
Chiang Mai 2	1.78	8.64	3.42
Sukhothai	1.80	8.11	2.97
Bangkok 1	1.64	9.18	3.09
Bangkok 2	1.59	8.26	3.26
Bangkok 3	1.49	8.26	2.08
Bangkok 4-a	1.83	8.23	2.61
Nakorn Pathom	1.91	8.85	3.10
Chon Buri	1.76	8.24	3.29
Chantaburi	1.69	7.78	2.83
Khon Kaen	1.85	7.99	3.05
Roi Et	1.78	8.61	3.02
Ubon Ratchathani	2.00	8.34	3.71
Surat Thani	1.73	7.18	2.95
Songkhla-a	1.88	9.28	3.14
Mean	1.78	8.24	3.03
SD	0.12	0.56	0.37

Table 17 Moisture content, total ash and acid insoluble ash of the other species

Crude drug sample	Moisture content (%)	Ash content (%)	
		Total ash	Acid insoluble ash
<i>A. fulviceps</i> Thw.	2.67	5.13	1.98
Songkhla-b	2.84	5.94	2.28
Bangkok 4-a	1.91	4.48	1.51

Each crude drug sampling was tripled for ash content, but was seconded for moisture content.



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