

CHAPTER III

MATERIALS AND METHODS

1. Source of plant materials

The plant materials were collected from various locations in Thailand, as follows:

Sample code	Scientific name	Locality
CIN01	<i>Cinnamomum cf.verum</i>	Chulalongkorn University, Bangkok
CIN02	<i>Cinnamomum cf.iners</i>	Chulalongkorn University, Bangkok
CIN03	<i>Cinnamomum iners</i>	Chulalongkorn University, Bangkok
CIN04	<i>Cinnamomum .tamala</i>	Chulalongkorn University, Bangkok
CIN05	<i>Cinnamomum camphora</i>	Chulalongkorn University, Bangkok
CIN06	<i>Cinnamomum iners</i>	BKF, Bangkok
CIN07	<i>Cinnamomum camphora</i>	BKF, Bangkok
CIN09	<i>Cinnamomum sp.1</i>	Phufoilom, Udon Thani
CIN10	<i>Cinnamomum camphora</i>	Phufoilom, Udon Thani
CIN11	<i>Cinnamomum sp.2</i>	Kudchum Hospital, Yasothon
CIN12	<i>Cinnamomum verum</i>	Kudchum Hospital, Yasothon
CIN13	<i>Cinnamomum cf.iners</i>	Kudchum Hospital, Yasothon
CIN14	<i>Cinnamomum sp.3</i>	Phumakprik, Yasothon
CIN15	<i>Cinnamomum sp.3</i>	Phumakprik, Yasothon
CIN16	<i>Cinnamomum cf.verum</i>	Princess Sirinthorn Garden, Rayong
CIN17	<i>Cinnamomum verum</i>	Princess Sirinthorn Garden, Rayong
CIN18	<i>Cinnamomum cf.iners</i>	Princess Sirinthorn Garden, Rayong
CIN19	<i>Cinnamomum camphora</i>	Princess Sirinthorn Garden, Rayong
CIN20	<i>Cinnamomum cf.subavenium</i>	Phuhinrongkla Nat. Park, Phitsanulok
CIN21	<i>Cinnamomum bejolghota</i>	Doi Phuka Nat. Park, Nan
CIN22	<i>Cinnamomum cf.iners</i>	Sirirukkachat Botanical Garden, Bangkok
CIN23	<i>Cinnamomum camphora</i>	Sirirukkachat Botanical Garden, Bangkok
CIN24	<i>Cinnamomum cf.verum</i>	Sirirukkachat Botanical Garden, Bangkok

Sample code	Scientific name	Locality
CIN25	<i>Cinnamomum porrectum</i>	Khoathaphet, Surat Thani
CIN26	<i>Cinnamomum</i> sp.4	Khoasok Nat. Park, Surat Thani
CIN27	<i>Cinnamomum</i> cf. <i>bejolghota</i>	Khoasok Nat. Park, Surat Thani
CIN28	<i>Cinnamomum pachyphyllum</i>	Khoasok Nat. Park, Surat Thani
CIN29	<i>Cinnamomum pachyphyllum</i>	Khoasok Nat. Park, Surat Thani
CIN30	<i>Cinnamomum</i> sp.5	Khoasok Nat. Park, Surat Thani
CIN33	<i>Cinnamomum subavenium</i>	Khoayai Nat. Park, Nakorn Ratchasima
CIN34	<i>Cinnamomum subavenium</i>	Khoayai Nat. Park, Nakorn Ratchasima
CIN35	<i>Cinnamomum subavenium</i>	Khoayai Nat. Park, Nakorn Ratchasima
CIN36	<i>Cinnamomum subavenium</i>	Khoayai Nat. Park, Nakorn Ratchasima
CIN37	<i>Cinnamomum</i> cf. <i>iners</i>	Khoayai Nat. Park, Nakorn Ratchasima
CIN38	<i>Cinnamomum verum</i>	Kanchanaburi
CIN39	<i>Cinnamomum iners</i>	Phratumnak Suanpathum, Pathum Thani
CIN40	<i>Cinnamomum</i> cf. <i>verum</i>	Phratumnak Suanpathum, Pathum Thani
CIN41	<i>Cinnamomum iners</i>	Phratumnak Suanpathum, Pathum Thani
CIN42	<i>Cinnamomum sintoc</i>	Phratumnak Suanpathum, Pathum Thani
CIN43	<i>Cinnamomum iners</i>	Phratumnak Suanpathum, Pathum Thani
CIN44	<i>Cinnamomum porrectum</i>	Phratumnak Suanpathum, Pathum Thani
CIN45	<i>Cinnamomum verum</i>	Phratumnak Suanpathum, Pathum Thani
CIN46	<i>Cinnamomum iners</i>	Phratumnak Suanpathum, Pathum Thani
CIN47	<i>Cinnamomum camphora</i>	Phratumnak Suanpathum, Pathum Thani
CIN48	<i>Cinnamomum tamala</i>	Phratumnak Suanpathum, Pathum Thani
CIN49	<i>Cinnamomum</i> cf. <i>iners</i>	Phratumnak Suanpathum, Pathum Thani
CIN50	<i>Cinnamomum</i> cf. <i>iners</i>	Phratumnak Suanpathum, Pathum Thani
CIN51	<i>Cinnamomum</i> cf. <i>iners</i>	Phratumnak Suanpathum, Pathum Thani

Authentication was achieved through comparison with herbarium specimens in the Forest Herbarium (BKF), National Park, Wildlife and Plant Conservation Department, Thailand.

2. Essential oil composition analysis

2.1 Headspace gas chromatography-mass spectroscopy

Essential oil composition was analyzed using a Varian gas chromatograph (GC) STAR 3400 CX, equipped with a Varian mass spectrometer (MS), SATURN 4D, and a Varian GENESIS Headspace auto-sampler was used. For each sample, a quantity of 0.2 g dry leaves was put in a 20 ml glass crimp top vial and incubated at 110 °C for 30 min. Then it was pressurized for 0.25 min and extracted with carrier gas, kept in 250 µl loop at 150 °C for 0.25 min and finally transferred to the GC with a transfer line at 250 °C. A StabilWax column, 30 m x 0.25 mm i.d. x 0.25 µm film thickness, was used, with a septum-equipped programmable injector (SPI) set at 250 °C, splitless. Carrier gas was helium, with head pressure of 12 psi. The temperature program for analyses was described below.

Temperature (°C)	Rate (°C/min)	Time (min)
60	0	3.0
60 - 200	3	46.7
200	0	10

Mass spectra were taken at 70 eV, with 1 scan per second from 35 until 350 m/z. The relative amount of each compound was calculated as a percentage of total oil using area under the peak (peak area).

2.2 Identification of the components

Identification of the components was based on spectral data computer matching with the NIST mass spectral search program version 1.19 and terpene library, comparison of the retention times and fragment pattern with those reported in the

literatures. Moreover, standard solutions of seven components in the list below were injected as reference solution.

1. (1S)-(-)- α -Pinene	SIGMA Chemical Co
2. (\pm)-Linalool	SIGMA Chemical Co
3. (-)-trans-Caryophyllene	SIGMA Chemical Co
4. Eucalyptol (1,8-Cineol)	CHEM SERVICE
5. Safrole	CHEM SERVICE
6. (+)-3-Carene	Fluka Chemika
7. Eugenol	SIGMA Chemical Co.

3. Chemometric analysis

Hierarchical cluster analysis of the chemical data was performed using the statistical package SPSS version 11.5 for Windows. Between group linkage method with Pearson correlation distance measure was used in clustering. An alternative method of chemometrics, principal component analysis, was performed using MINITAB 13 for Windows.

For the present study, the peak area of 128 volatile compounds were used as dependent variables. Approximately 80% of the compounds have been identified, but as stressed in Dunlop *et. al.* (2004) that complete identification is not necessary for the mathematical analysis.

To reduce the effect of natural variation within species, the peak area from each chromatogram was normalized to 0.00-1.00 range and scored before analysis. The major components of which their normalized quantities were in the range of 1.00-0.90 were scored as 3 while other minor compounds with normalized peak area in the range of, 0.89-0.80, 0.79-0.70, 0.69-0.01 and 0.00 were scored as 2, 1.5, 1 and 0, respectively.