CHAPTER III

MATERIALS AND METHODS

1. Source of plant material

The plant materials were collected from various localities in Thailand and different periods of time as follows:

Number	Name	Place
1	*Aegle marmelos* Corr.	Faculty of Pharmaceutical Science Chulalongkorn University, Bangkok, January 1996
2	*Atalantia monophylla* Correa	Rayong, June 1996
3	*Citrus aurantifolia* Swing.	Khamsakaesang Nakornratchasima, March 1996
4	*Citrus hystrix* DC.	Khamsakaesang Nakornratchasima, March 1996
5	*Citrus maxima* Merr.	Faculty of Pharmaceutical Science Chulalongkorn University, Bangkok, February 1996.
6	*Citrus medica* Linn.	Rangsit , Prathumthani, March 1996.
7	*Citrus reticulata* Blanco	Rangsit , Prathumthani, March 1996.
8	*Clausena anisata* Hook.	Kanchanaburi, January 1996
9	*Clausena excavata* Burm.	Faculty of Pharmaceutical Science Chulalongkorn University, Bangkok, January 1996.

Number	Name	Place
10	*Feronia limonia* Swing.	Faculty of Pharmaceutical Science Chulalongkorn University, Bangkok, January 1996.
11	*Glycosmis pentaphylla* Corr	Saraburi , February 1996.
12	*Hesperethusa crenulata* Roem.	Rayong , June 1996
13	*Micromelum minutum* Wight& Arn.	Sakae-Raj Environmental Research Station, Pak Thong Chai Nakorn Ratchasima , in April 1996
14	*Murraya paniculata* Jack.	Sakae-Raj Environmental Research Station, Pak Thong Chai Nakorn Ratchasima , in April 1996
15	*Paramignya scandens* Craib.	Sakae-Raj Environmental Research Station, Pak Thong Chai Nakorn Ratchasima , in April 1996
16	*Toddalia asiatica* Lamk.	Sakae-Raj Environmental Research Station, Pak Thong Chai Nakorn Ratchasima , in April 1996
17	*Triphasia trifolia* P. Wils.	Faculty of Pharmaceutical Science Chulalongkorn University, Bangkok, January 1996.
18	*Zanthoxylum limonella* Alston.	Sakae-Raj Environmental Research station, Pak Thong Chai , Nakorn Ratchasima , in April 1996

Authentication was achieved through comparison with herbarium specimen in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Coorperative, Thailand.

2. Volatile oil content and composition.

2.1 Volatile oil content determination.

Volatile oil was determined by the method described in the association of official analytical chemists (method 962.17,AoAc,1990). One hundred and fifty grams of each sample were put into a 500 ml round bottom flask. The tridistilled water were added into the flask to about half full and a few pieces of boiling chips. The flask was conneted to the apparatus for the determination of volatile oil (Fig. 1). The content of the flask was distilled until two consecutive reading taken at one hour interval showed no change in oil content (about four hours). After cooling, the oil volume was measured, calculated and expressed as millilitre of the oil per one hundred grams of sample. The volatile oil obtained was then collected and stored at 4 °C until being analysed for its chemical composition by GC-MS.

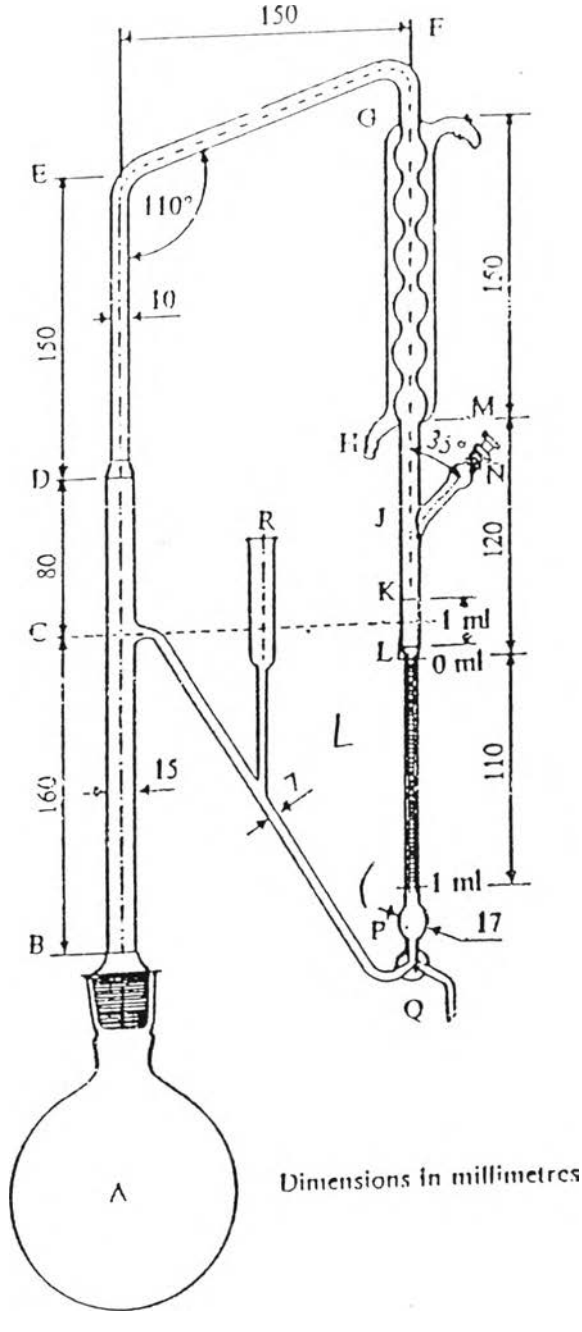

Figure 1 Apparatus for volatile oil content determination.

2.2 Gas chromatography - mass spectrometry

For identification of the composition of essential oil, a gas chromatography-mass spectrometry (GC-MS) was used. The essential oil was diluted to 1:100 in methanol before being injected into GC-MS system. The condition of GC-MS was described below. The spectra were recorded and compared with the terpene library.

GC-MS Condition

Instrument model

Column	fused silica capillary column (30 mm. x 0.25 mm. i.d.) coated with DB-5 (J&W) film thickness 0.25 μm
Column programming	60-180 °C rate 3 °C/min
Injector temperature	220 °C
Helium carrier gas	1 ml/min
Split ratio	100 : 1
Accelerating voltage	1700 volts
Sample size	1 μl
Solvent	methanol were HPLC grade

Identification of the component

Identification of the components was based on GC retention times computer matching with of terpene library, comparison of the fragment pattern with those reported in the literature.