CHAPTER III

MATERIALS AND METHODS

Materials

1. Eight kinds of Thian, which are Thian Dam, Thian Dang, Thian Khaao, Thian Khaoplueak, Thian Taatakkataen, Thian Yaowapanee, Thian Sattabut, Thian Taakob, and Thian Klethoi, randomly purchased from 5 traditional drugstores in Bangkok metropolitan area, i.e.

1.1 Tai-un-chan, Chakkrawad road, Sampanthawong district, Bangkok. Purchased date 24/5/93, 23/6/93, 27/7/93, 21/9/93, 4/1/94

1.2 Vej-ja-pong, Chakkrawad road, Sampanthawong district, Bangkok. Purchased date 24/5/93, 27/5/93, 23/6/93, 27/7/93, 28/9/93

1.3 Bho-pra-dit, Chakkrawad road, Sampanthawong district, Bangkok. Purchased date 24/5/93, 27/7/93, 28/9/93

1.4 Chao-krom-peur, Chakkrawad road, Sampanthawong district, Bangkok. Purchased date 24/5/93, 27/7/93, 28/9/93

1.5 Ngun-heng-chan, Chakkrawad road, Sampanthawong district, Bangkok. Purchased date 24/5/93, 27/7/93, 28/9/93

2. Two kinds of Thian under the common names anise and caraway, 5 samples each, randomly purchased from 5 supermarkets in Bangkok metropolitan area, i.e.

2.1 Central, Wongsawang road, Bangsue district. Bangkok. Purchased date 18/12/93,17/1/94

2.2 Robinson, Sukhumvit road, Phrakhanong district, Bangkok. Purchased date 20/12/93,14/1/94

2.3 Foodland, Lard prao road, Bangkapi district. Bangkok. Purchased date 22/12/93,25/1/94

2.4 Banglampoo, Ngamwongwan road, Muang district, Nonthaburi. Purchased date 16/12/93,24/1/94

2.5 Jusco, Ratanathibet road, Muang district, Nonthaburi. Purchased date 16/12/93,24/1/94

3. Common planting equipments employed in the planting of Thians such as potting soil, plastic bags, aluminum trays etc.

4. TLC Aluminum sheetsof precoated silica gel 60 F_{254} Merck^{R} 10X10 cm, 0.2 mm thick were employed.

5. Glasswares

erlenmeyer flasks

volumetric flasks

glass funnels

capillary tubes

vials etc.

6. Reagents for TLC

chloroform, AR

ethyl acetate, AR

7. Test reagents

Test reagents for microscopy

The following test reagents were prepared according to the methods described by Hebert and Ellery.[208] (see appendix for preparations and uses.)

chloral hydrate solution

iodine solution

tincture of alkanna

phloroglucin

hydrochloric acid

ruthenium red solution

Test reagents for TLC

The following test reagents were prepared according to the methods described by Stahl.[209] (see appendix)

vanillin-sulphuric acid

Leibermann-Burchard reagent

Kedde reagent

Dragendorff's reagent

8. Equipment for plant identification such as magnifying glass, forceps, blades, needles, etc.

9. Equipment for photography

10. Electro-freezing microtome

11. Microscope attached with photographic equipment and camera lucida

12. Tank for developing TLC plates

13. UV lamp

14. Ultraviolet spectrophotometer were performed on a Jasco UVIDEC 650 double beam spectrophotometer.

Methods

1. Selection of medicinal plants

Five samples of each kind of Thian which will be used in this study were examined. In order to make certain that all 5 samples of each Thian are the same kind of Thian, the following characters were compared:

- morphology of crude drugs
- TLC characters of crude alcoholic extracts
- UV spectra

2. Identification of medicinal plants

2.1 The seeds of each kind of Thian which are healthy and unbroken were sorted out.

2.2 The selected seeds were planted.

2.3 Full grown plants of each kind of Thian were studied in detail and the characters of stems, leaves, flowers, fruits, and seeds were recorded.

2.4 Each kind of Thian was identified by Taxonomic key

2.5 Photographs of various parts of full grown plant of each kind of Thian were taken for the record.

3. Pharmacognostic study

3.1 Morphology of crude drugs

Seeds were studied as of physical characters including occurrences, shapes, sizes, external surfaces, internal appearances as viewed in cross sections, odor, and taste. Photographs of crude drugs were taken for the record.

3.2 Histology

Cross or transverse sections ofeach kind of Thian were prepared on an electro-freezing microtome and the sections were studied under the microscope. Attention should be focused upon the following regions which may show diagnostic characteristics: pericarp, seed coats and appendages (if present), endosperm, and embryo.

Staining test on tissues or cells of the sections using the test reagents for microscopy to study cell contents, cell structure and the compositions of the cells.

Pictures of the sections were taken for the record.

3.3 Powdered drug

The samples of each kind of Thian were ground to powder and studied under the microscope to obtain specific characters

which can be used to identify the powdered drug. The pictures of powdered drug observed under the microscope were drawn by means of the camera lucida.

4. Chromatographic study

4.1 Preparation of crude alcoholic extract

- Five grams of powder of each kind of Thian were macerated in 15 ml of 95% ethanol for 72 hr.
- The whole solution was filtered and the filtrate was evaporate at room temperature until a 5 ml solution was obtained.

4.2 One-dimensional TLC to obtain TLC pattern of each kind of Thian was carried out using the following conditions:

amount : 20 uL
developing solvent : chloroform
distance : 7.5 cm
detection : 1) UV_{254}
2) UV_{365}
3) spray with vanillin-sulphuric acid and activated by heating at 120°C for 5 min
4) spray with Leibermann-Burchard reagent and activated by heating at 100°C for 10 min

5) Kedde reagent

6) Dragendorff's reagent

4.3 Two-dimensional TLC to obtain the characteristic TLC pattern of each kind of Thian was carried out using the following conditions:

amount : 20 uL

developing solvent : 1st dimension : chloroform

2nd dimension : chloroform : ethylacetate(1:1)

distance : 7.5 cm

detection : spray with vanillin-sulphuric acid and activated at 120°C for 5 min

5. Spectrophotometric study

5.1 The crude alcoholic extract of each kind of Thian was diluted (1:100) with 95% ethanol

5.2 Ultraviolet spectra of five samples of each kind of Thian were taken by using quartz cell with path length 1 cm, 95% ethanol was used as reference, scan under 200-500 nm .