CHAPTER III

MATERIALS AND METHODS

3.1 Plant materials

Fresh roots of *Morinda citrifolia* are harvested, washed, and ground in liquid nitrogen to an average size of 0.2 mm in diameter. The ground samples ware oven dried in at 45 ° C for 3 days, and then kept in a dry place until use.

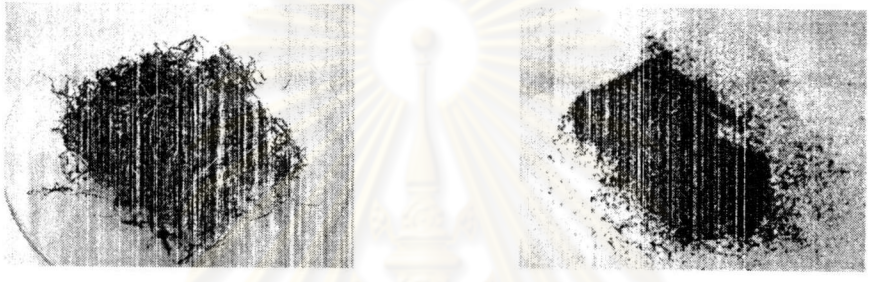

Figure 3.1 Dried roots and ground roots of *Morinda citrifolia*

3.2 Conventional extraction

3.2.1 Solvent extraction

The roots of *Morinda citrifolia* (0.1g) was extracted in 10 ml of organic solvent in a 125 ml flask extraction. The extract was then filtered with a filter paper (Whatman, no.1 125 dia.). The concentration of anthraquinones was measured by a spectrophotometer, Genesys20 (Thermo spectronic, USA). After extraction, the anthraquinones remained in the sample residue determined by extracting the residue repeatedly in three 10-ml volumes of solvent or until the extract was clear. The residue extracts were combined and the concentration was measure spectrophotometrically.

3.2.2 Soxhlet extraction

A classical soxhlet apparatus were employed in order to compare with the other results. 0.1 g of sample was placed into the cartridge with 200 ml of solvent (ethanol) in flask (500 ml). Extraction was carried out for 2-3 hours. The anthraquinones concentration was measured by spectrophotometer.

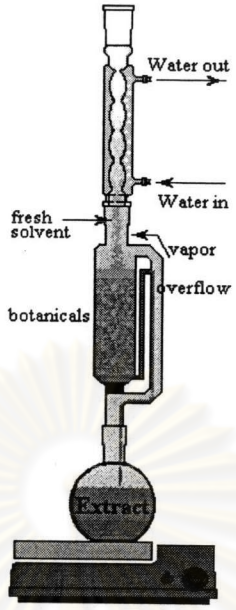

Figure 3.2 .2 Soxhlet apparatus

3.2.3 Ultrasonic extraction

For the ultrasonic extraction experiments, an ultrasonic bath was used as an ultrasound source. The bath, 275DAE (Crest Ultrasonics, USA), was basically a rectangular container (23.5 cm × 13.3 cm × 10.2 cm), to which two 38.5 kHz transducers were annealed at the bottom, and the bath power rating was 270 W on the scale of 0 to 9. The extraction of anthraquinones was performed by adding 0.1 g of ground dried roots into 10 ml of solvent in a 28 ml tube. The tube was then partially immersed into the ultrasonic bath, which contains 2.2 L of water. The bottom of the flask was approximately 5 cm above the bottom of the bath. The solvent surface in the flask was kept at the level of the water in the ultrasonic bath. Water in the ultrasonic bath was circulated and regulated at constant desired temperatures to avoid the water temperature rise, caused by ultrasonic exposure. After extraction, the anthraquinones remained in the sample residue determined by extracting the residue repeatedly in three 10-ml volumes of solvent or until the extract was clear. The residue extracts were combined and the concentration was measure spectrophotometrically.

3.2.4 Microwave assisted extraction

For the microwave extraction experiment, the microwave, MARS5 (thai unique, USA) was used in experiment. The extraction of anthraquinones was performed by adding 0.1 g of ground dried roots into 10 ml of solvent in the vessel. The vessels were placed symmetrically in the microwave field. The extraction was carried out by controlling the condition selected: power applied and irradiation time, composition of solvent (ethanol:water), type of solvent, temperature.

After irradiation, the solution was filtered thorough filter paper (Whatman, no.1 125 dia.). The anthraquinones concentration was calculated by spectrophotometer. Operating conditions tested for ultrasonic extraction and microwave extraction are summarized in Table 3.1.

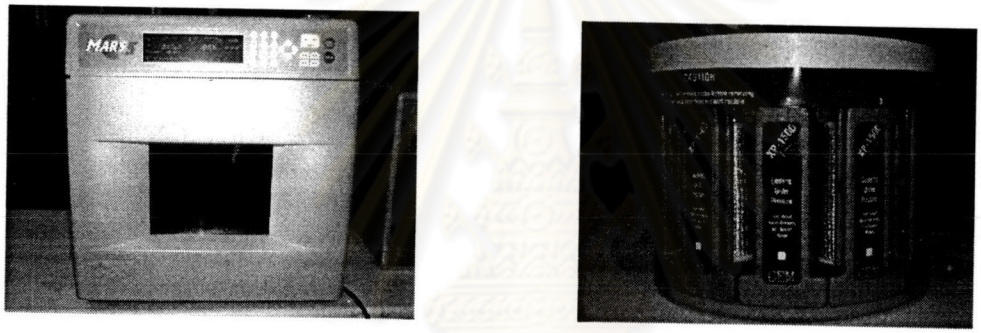

Figure 3.2.4 Microwave apparatus (MARS 5)

Table 3.1: Parameter condition in experiment

Parameters	Conditions	
	Ultrasonic	Microwave
Temperature	25, 45, 60 ° C	60, 80,100, 120 ° C
Power	(power setting 3, 6, 9) 90, 180, 270 W	60% (1200 W)
Solvent composition (ethanol:water)	20:80, 50:50, 80:20	
Time radiation	15, 30, 45, 60 min	5, 10, 15, 20 min
Type of solvent	acetone, methanol, ethanol, acetonitrile	
Approx. Materials size	0.2 mm	

3.3 Measurement of anthraquinones concentration

After each extraction, the solid plant sample was filtered out and the concentration of the anthraquinones extract was analyzed by measuring the absorbance at 435 nm, following the spectroscopic method modified from *Zenk et al., 1975*. A calibration curve of Alizarin (or 1,2- dihydroxyanthraquinone) solutions was used as a reference.

Figure 3.3 Spectrophotometer

3.4 Antioxidant activity measurement

Antioxidant activity of antraquinones extracts obtained using ultrasound assisted extraction, microwave assisted extraction and classical extraction methods was be tested and compared, using DPPH method modified from that described in previous research (*Ollanketo et al., 2002*). For the purposes of comparing the antioxidant activity in various extracts, concentration of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index. To find this value, the extract is diluted in series with ethanol and 2 ml of each diluted extract was added to 2 ml of 110 μM DDPH solution. The solutions were mixed using a vortex and the mixture was then incubated for 4 hours in darkness at room temperature, after which the absorbance was measured at the wavelength of 517 nm using ethanol as a reference.

IC_{50} can found from the plot of percent inhibition (PI) versus the corresponding concentration of anthraquinones. The values of PI can be calculated using the following equation:

$$PI\ (\%) = [1-(A_t / A_r)] \times 100 \qquad (3.1)$$

A_t and A_r are absorbance of test sample and absorbance of the DPPH reference, respectively.