Appendix

Analytical methods

1. Determination of total phenolic content (Julkunen-Titto, 1985)

Chemicals

- Folin and Ciocalteu's Phenol Reagent
- Saturated sodium bicarbonate solution

Method

The extract (0.5 ml) was added with 5 ml of distilled water, followed by 1 ml of Folin and Ciocalteu's Phenol Reagent. After 5 min, 1 ml of saturated sodium bicarbonate solution was added and the color was allowed to develop for 1 h. The absorbance was read at 640 nm by using a UV 1601 spectrophotometer. A standard curve was prepared at the same time by using various concentration of chlorogenic acid ranging from 0 to 100 μg/ml. Total phenolic content was calculated as chlorogenic acid equivalent by using standard curve.

2. Determination of reducing power (Oyaizu, 1986)

Chemicals

- 0.2 M Phosphate buffer, pH 6.6
- 10 % Trichloroacetic acid (TCA)
- 1 % Potassium ferricyanide
- 0.1 % ferric chloride

Method

The extract (0.5 ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %). The mixture was then incubated at 50 ^{o}C for precisely 20 min. An aliquot (2.5 ml) of TCA (10 %) was added to the mixture, which was then centrifuged at 300 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1 %), and the absorbance

was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

3. Determination of scavenging effect on 1,1-diphenyl-2-picryhydrazyl (DPPH) radical (Blois, 1958)

Chemical

- Methanol solution DPPH (at the final concentration of 0.2 mM)

Method

The extract (4.0 ml) was mixed vigorously with 1.0 ml of DPPH and incubated for 30 min. The absorbance of the resulting solution was measured at 517 nm using a 1601 UV spectrophotometer. Values reported are mean from triplicate determinations of the experiment.

4. Determination of scavenging effect on hydroxyl radical (Aruoma, 1994)

Chemicals

- 2.8 mM 2-deoxyribose
- 0.1 M Sodium phosphate
- Stock solution [1% (w/v) TBA in 50 mM sodium hydroxyl plus 2.8% (w/v) TCA]

Method

The reaction mixture (pH 7.4) containing 0.1 mM phosphate buffered saline, 20 μM ferrous ions, 100 μM ascorbate, 1.42 mM H_2O_2, test compound and 2.8 mM deoxyribose (total volume 1.2 ml). The mixture was incubated for 10 min at 37 ^{o}C. One ml stock solution was added to the reaction mixture. It was then heated for 10 min in the boiling water bath, cooled with tap water, and the absorbance of the pink chomogen was read at 532 nm.

5. Determination of thiobarbituric acid substance (TBARS) (Esterbauer and Cheeseman, 1990).

Chemicals

- 0.05 N Sodium hydroxide

- 1 % TBA in 0.05 N Sodium hydroxide

- 25 % Hydrochloric

- 85 % Butanol

Method

Sample (50 mg) was diluted with mixture solution of chloroform and methanol (1:1, V/V). Sample (200 µl) was evaporated. Evaporated sample was mixed with distilled water (1 ml), 1% TBA (0.5 ml) and 25% HCl (0.5 ml), respectively. It was then heated for 15 min in the boiling water bath, cooled with tap water to room temperature. The 85% butanol was added to the sample and shaken vigorously. The butanol layer was pipeted and the absorbance was read at 550 nm. The TBA was calculated in unit of mg malonaldehyde/kg.oil by using malonaldehyde solution as standard curve.

6. Determination of peroxide value (IUPAC, 1979).

Chemicals

- Acetic acid: chloroform (3: 2, V/V)

- Saturated potassium iodide solution

- 0.01 N Sodium thiosulphate solution

- 1% Starch solution

Method

Sample ($\approx$ 1 g) was mixed with a mixture of acetic acid and chloroform, follow by addition of 1.0 ml of potassium iodine. The reaction mixture was left to stand for 5 min in the dark. Distilled water (75.0 ml) was added to the mixture. The mixture was titrated with sodium thiosulphate solution and shaken vigorously until color of mixture was light yellow, then 0.5 ml of starch solution was added. The mixture had blue color. Sodium

thiosulphate was used to titrated until clear solution was obtained. Peroxide value was expressed as meq/kg oil using:

$$\text{Peroxide value (meq/kg oil)} = \frac{(a-b) \times N \times 1000}{W}$$

Where   a = Volume (ml) of sodium thiosulphate of blank

b = Volume (ml) of sodium thiosulphate of sample

N = Concentration of sodium thiosulphate (Normal)

W = weight of sample