CHAPTER 3

3 MATERIALS AND METHODS

3.1 Overview

This chapter presents the materials and chemical used for the experimental study. The details methods study for the extraction, proximate analysis (total phenolic content (TPC) and total flavonoids content (TFC)) and ultra-performance liquid chromatography (UPLC) were presented in this chapter.

3.2 Chemicals

Ethanol (99%), HPLC grade acetonitrile, trifluoroacetic acid (99%), sodium hydroxide (98%), sodium nitrite and aluminum hexachloride (99.9%) were purchased from Sigma Aldrich (St. Louis, MO). Propanol was obtained from Fisher Scientific (Pittsburgh, PA). HPLC grade methanol was obtained from Fluka (USA) and Folin-Ciocalteu reagent was obtained from Merck (Darmstadt, Germany). The chemical of ethanol, propanol, methanol, HPLC grade acetonitrile, HPLC grade methanol, trifluoroacetic acid and Folin-Ciocalteu reagent were in liquid phase. Sodium hydroxide, sodium nitrite and aluminum hexachloride were in solid phase.
3.3 Plant Materials

White flowered of Orthosiphon stamineus leaves samples is collected in Gambang, Pahang, Malaysia. Then, the leaves were washed and dried in an oven at 35 °C for a few days. After that, the dried leaves were grounded to powder form and kept in the tight plastic bag at room temperature to avoid the moisture absorption.

![Figure 3.1](image)

**Figure 3.1:** (A) Orthosiphon stamineus (white) flower, (B) Orthosiphon stamineus leaves

3.4 Extraction methods

3.4.1 Microwave-assisted extraction

Misai kucing extracts were prepared by using Microwave Organic Synthesis Reactor (M.A.D Technology, 2012) at 230°C as control temperature and the range of power within 25W to 250W for 0.5min to 6min. Hence, before the extraction part, the powder has been sieves and 125µm were chosen to use and the powder was moisture content by using a moisture analyzer (AND MS-70) at temperature 105°C. The dried plant powder were weighted based on solid to liquid ratio (0.1 to 10%) and mixed with
5mL of various concentration of ethanol (40 to 100%) into vial. The parameter is manipulated by using RSM in order to get better extraction yield. Use the different concentration of ethanol in water for extraction of phenolic compound will be safe and efficient (Li et al., 2012).

Figure 3. 2: (A) Vial with cap, (B) Microwave Organic Synthesis Reactor