Introduction

*M. citrifolia* belongs to the Rubiaceae family and comprises 80 species. This plant is found in South East Asia, Caribbean countries, Australia and Central-South America [1,2]. *M. citrifolia* has been used as a medicine for many ailments such as dysentery, heartburn, liver diseases, diabetes, high blood pressure, muscle aches, headaches, heart diseases, cancer, gastric ulcers and arthritis [3,4]. *M. citrifolia* has approximately 200 phytochemical compounds which are distributed throughout the plant [5,6]. Anthraquinones, a major bioactive compound, is present in different parts of the plant [3,5]. Among the compounds found in the fruit of this plant are 2-methoxy-1,3,6-trihydroxyanthraquinone, 5,15-dimethylmorindol, 1,6-dihydroxy-5-methoxy-2-methoxymethylanthraquinones,5,7-trihydroxy-6-methoxy-2-methoxymethylanthraquinones, 1,3-dimethoxyanthraquinone and 1,2-dihydroxyanthraquinone [7-10]. *M. citrifolia* leaves contain 2-methoxy-1,3,6-trihydroxyanthraquinone, 5,15-dimethylmorindol, 1,3-dihydroxy-2-methylol-9,10-anthraquinone, 1,2-dihydroxyanthraquinoneand 1,3-dihydroxy-2-methylanthraquinone [8,11]. The many compounds found in the root of this plant include dammacanthal, nordammacanthal, tectoquinone and others [12,13]. These compounds have antibacterial, antifungal and other biological activities [14].

Materials and Methods

Plant collection

The fresh ripe fruit and leaves of *M. citrifolia* were collected from Sendayan Valley, Seremban, Malaysia in November, 2010. This plant was identified at the herbarium under the registration numbers KLU 22480. All samples were washed under tap water and dried in an oven at 40°C for 3 days. The plant materials were then put through a grinder with a mesh size of 2 mm.

Anthraquinones extracts from *M. citrifolia* fruit and leaves

This method is based on [15]. The dried powder of the fruit and leaves of this plant (50 g) were added to 100 ml of methanol and 150 ml of distilled water and refluxed for 3 hours. Then, the extract was added to 4ml of concentrated HCl with 5% of methanol solution and refluxed for 6 hr. Extraction was conducted with chloroform and filtered. Chloroform was then evaporated at 40°C using a rotary evaporator until the solvent was removed (Heidolph WB2000, Germany). The product yield was 0.47% of the original material.

Anthraquinones extracts from *M. citrifolia* root

The dried powder of the root of this plant was extracted with 200 ml of ethanol in the Soxhlet apparatus for 4 hours. The extract was filtered and ethanol removed at 40°C using a rotary evaporator (Heidolph WR2000, Germany). The SEP-PAK C18 column was used to purify the product. The sample was eluted from the column using 100% ethanol as the mobile phase. The ethanol in the elute was evaporated to dryness under reduced vacuum at 40°C. All anthraquinones extracts of different
parts of this plant were tested using a standard protocol [16]. These extracts were mixed with organic solvent and filtered. The aqueous phase added to NH4OH solution. The pink or violet colour indicated the presence anthraquinones in these extracts.

**Thin Layer Chromatography (TLC) and IR spectrometry**

TLC chromatography based on the method [15]. Anthraquinones fractions of all parts of the bioactive compounds were loaded on TLC plates 60 F254 (Merck, Germany). The mobile phase dichloromethane: methanol (9:1) and spray by using KOH reagent to get the red colour of the bands of anthraquinones fractions. All TLC plates were visualized under UV light at wavelength 245 nm and 356 nm. Then, the IR spectrum of these compounds was recorded by FTIR (Perkin Elmer spectrum 400 FT-IR, UK) at room temperature from 400 to 4000 cm$^{-1}$ for scanning directly.

**Results and Discussion**

TLC results of anthraquinone extract from *M. citrifolia* revealed six red bands for the fruit, five for the leaves and four for the roots under UV light at wavelength 245 nm and 356 nm. The anthraquinones extracts of fruit, leaves and roots of *M. citrifolia* were typically visualised as a red colouration on thin-layer plates sprayed with KOH. This study was in agreement with the work of Stawadhari, Deshpande, Hashmi and Syed [17] which identified some bioactive components such as anthraquinones, saponine, and scopolein in *M. citrifolia* fruit by using the TLC technique.

The results of the IR spectra (Figures 1, 2 and 3) exhibited strong absorption bands at 3408.83, 3275.52 and 3308.88 cm$^{-1}$ for anthraquinone extract from the fruit, leaves and roots of *M. citrifolia* respectively identified by O-H stretching, C-H stretching groups were detected at the bands 2954.74 and 2857.38 cm$^{-1}$ (fruit), 2928.13 cm$^{-1}$ (leaf), 2922.31 cm$^{-1}$ and 2852.95 cm$^{-1}$ (root). The C=O group of *M. citrifolia* fruit was detected at the band 1726.27 cm$^{-1}$, leaves at 1600.06 cm$^{-1}$ and roots at 1630.71 cm$^{-1}$. According to published studies, anthraquinones with these functional main groups have been shown to be present in fruit, leaves and roots of *M. citrifolia* [10-12,18].

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