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Isolation and Identification of Anthraquinones Extracted From *Morinda Citrifolia* L. (Rubiaceae)

Abdul Mushin M Shami*

Institute of Biological Science, University of Malaya, Malaysia

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***Corresponding author**

Abdul Mushin M Shami, Institute of Biological Science, University of Malaya, Malaysia, Tel: +6012-2447910; Fax: +603-796748; Email: aashbio@yahoo.com

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Abstract

The purpose of the study was to isolate the anthraquinone fractions from the fruit, leaves and roots of *Morinda citrifolia*. Anthraquinones extracts from the fruit, leaves and roots of *M. citrifolia* exhibited 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 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), 2922.31cm^{-1} and 2852.95 cm^{-1} (root). The C=O group of *M. citrifolia* fruit were detected at the band 1726.27 cm-1, leaves at 1600.06 cm^{-1} and roots at 1630.71 cm^{-1}. It could be concluded that the anthraquinone of the plant had a good anthraquinones can be a new source of antimicrobials against pathogenic bacteria and antioxidant source.

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 damnacanthal, nordamnacanthal, 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 and roots 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 methanolic 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 WB2000, 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

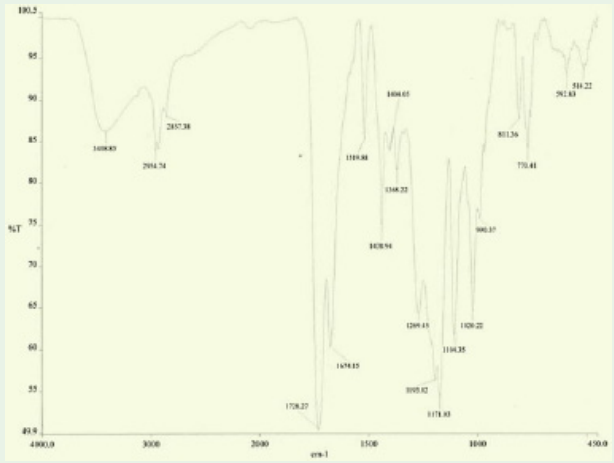

Figure 1: IR spectra of anthraquinones extracted from the fruit of *M. citrifolia.*

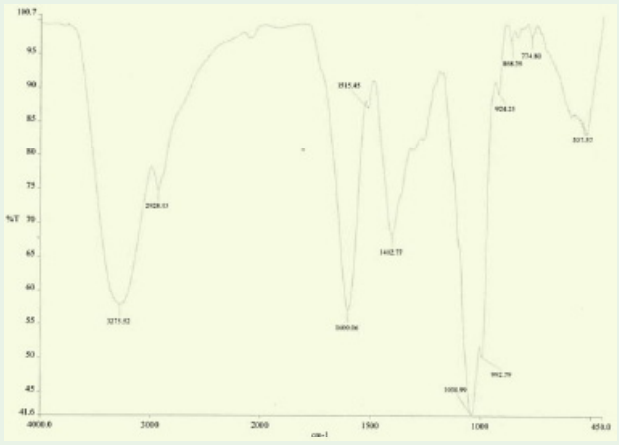

Figure 3: IR spectra of anthraquinones extracted from the roots of *M. citrifolia*.

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 NH_4OH 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 Stawadhar, Deshppande, Hashmi and Syed [17] which identified some bioactive components such as anthraquinones, saponine, and scopoltein in *M. citrifolia* fruit by using the TLC technique.

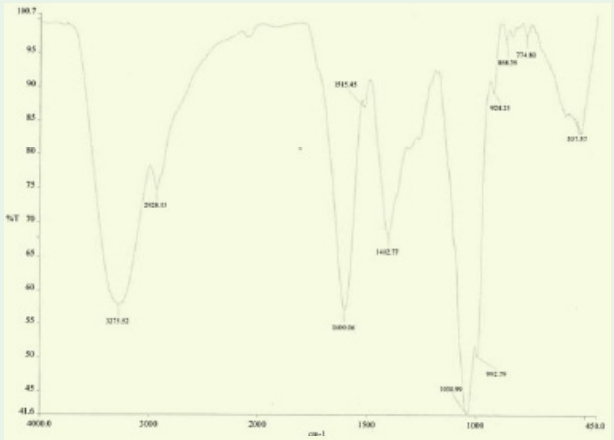

Figure 2: IR spectra of anthraquinones extracted from the leaves of *M. citrifolia.*

The results of the IR spectra (Figures1, 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), 2922.31cm^{-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].

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 Stawadhar, Deshppande, Hashmi and Syed [17] which identified some bioactive components such as anthraquinones, saponine, and scopoltein in *M. citrifolia* fruit by using the TLC technique. IR spectrometry revealed anthraquinones with functional main groups O-H, C=O and C-H in fruit, leaves and roots of *M. citrifolia*. 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]. In conclusion, this is the first report that studied isolation and identification of anthraquinones extracts from *M. citrifolia*. Anthraquinones extracted from *M. citrifolia* identified important compounds which may be used to develop biopharmaceuticals against infectious diseases with antioxidants source in future.

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References

1. Nelson C. *Morinda citrifolia* (noni). Species profiles for Pacific Island forestry. 2006; 4: 1-13.

2. Morton J. The ocean-going noni, or Indian Mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colorful" relatives. Economic Botany. 1992; 46: 241-256.

3. Chan-Blanco Y, Vaillant F, Mercedes Perez A, Reynes M, Brillouet JM, Brat P. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. Journal of Food Composition and Analysis. 2006; 19: 645-654.

4. Müller JC, Botelho GG, Bufalo AC, Boareto AC, Rattmann YD. *Morinda citrifolia* Linn (Noni): *in vivo* and *in vitro* reproductive toxicology. J Ethnopharmacol. 2009; 121: 229-233.

5. Singh DR. *Morinda citrifolia* L. (Noni): A review of the scientific validation for its nutritional and therapeutic properties. Journal of Diabetes and Endocrinology. 2012; 3: 77-91.

6. Dussossoy E, Brat P, Bony E, Boudard F, Poucheret P. Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice (*Morinda citrifolia* L.). J Ethnopharmacol. 2011; 133: 108-115.

7. Pawlus AD, Su BN, Keller WJ, Kinghorn AD. An anthraquinone with potent quinone reductase-inducing activity and other constituents of the fruits of *Morinda citrifolia* (noni). J Nat Prod. 2005; 68: 1720-1722.

8. Deng S, West BJ, Jensen CJ, Basar S, Westendorf J. Development and validation of an RP-HPLC method for the analysis of anthraquinones in noni fruits and leaves. Food Chemistry. 2009; 116: 505-508.

9. Lin CF, Ni CL, Huang YL, Sheu SJ, Chen CC. Lignans and anthraquinones from the fruits of *Morinda citrifolia*. Nat Prod Res. 2007; 21: 1199-1204.

10. Siddiqui BS, Sattar FA, Ahmad F, Begum S. Isolation and structural elucidation of chemical constituents from the fruits of *Morinda citrifolia* Linn. Arch Pharm Res. 2007; 30: 919-923.

11. Takashima J, Ikeda Y, Komiyama K, Hayashi M, Kishida A. New constituents from the leaves of *Morinda citrifolia*. Chem Pharm Bull (Tokyo). 2007; 55: 343-345.

12. Lv L, Chen H, Ho CT, Sang S. Chemical components of the roots of Noni (*Morinda citrifolia*) and their cytotoxic effects. Fitoterapia. 2011; 82: 704-708.

13. Sang S, Ho CT. Chemical components of noni (*Morinda citrifolia* L.) root. In: Herbs: challenges in chemistry and biology, ACS (American Chemical Society) Symposium Series. 2006; 925.

14. Zahin M, Aqil F, Khan M, Ahmad I. Ethnomedicinal plants derived antibacterials and their prospects. Ethnomedicine: A Source of Complementary Therapeutics. Research Signpost, India. 2010:149-178.

15. Smita N, Sushma M. Preliminary physicochemical and phytochemical evaluation of *Morinda citrifolia* fruit extractives. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2: 150-154.

16. Sarker S, Nahar L. Chemistry for pharmacy students: general, organic and natural product chemistry. John Wiley & Sons; 2007.

17. Stawadhar PN, Deshppande HW, Hashmi S, Syed KA. Nutritional composition and identification of some of bioactive components in *Morinda citrifolia* juice. International Journal of Pharmcy and Pharmaceutical Science. 2011; 3: 58-60.

18. Ee GC, Wen YP, Sukari MA, Go R, Lee HL. A new anthraquinone from *Morinda citrifolia* roots. Nat Prod Res. 2009; 23: 1322-1329.