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Potential Mosquito Larvicides from *Morinda citrifolia* Root Extract

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ABSTRACT In our recent investigation on *Morinda citrifolia* as a potential larvicide against the larvae of *Aedes aegypti*, we found 1-hydroxy-2-methyl anthraquinone **(1)** to have good larvicidal activity. Extracts of the roots of *Morinda citrifolia* were also found to be larvicidal.

ABSTRAK Dalam kajian semasa ke atas *Morinda citrifolia* sebagai larvisid yang berpotensi terhadap larva *Aedes aegypti*, didapati bahawa 1-hidroksi-2-metil anthrakuinone **(1)** mempunyai aktiviti larvisida yang baik. Ekstrak-ekstrak daripada akar *Morinda citrifolia* juga didiapati mempunyai sifat tersebut.

(*Morinda citrifolia*, 1-hydroxy-2-methyl anthraquinone, larvicidal activity, *Aedes aegypti*)

INTRODUCTION

Morinda citrifolia is a perennial herb from the Rubiaceae family. Also used in folk remedies for 2000 years, all parts of the plant (including the fruits, leaves, bark and roots) have been investigated and found to have active compounds that have high medicinal values [1]. Anthraquinones which are the major constituents of this plant are well recognized for their anticancer, antibacterial, antiviral and antioxidative activities [2]. Previous studies have reported several anthraquinones from the roots and bark such as damnacanthal, nordamnacanthal, morindone, alizarin, lucidin, rubiadin and other constituents (glycosides, iridoids and coumarin) from various parts of this plant [3]- [5]. These compounds were found to have biological activities such as anticancer, antioxidant, anti-HIV and hypotensive properties [6].

The use of insecticides has been the obvious method for the control of mosquito-borne diseases. However, this has caused much harm to non-target organisms. In Malaysia, the mosquito-borne disease dengue fever has been of great concern to the Health authorities. The use of natural products from plants and essential oils has been suggested to be an alternative to the conventional chemical control. It was reported that anthraquinones such as emodin [7] and tectoquinone [8] possess larvicidal activities against mosquito.

This paper describes the isolation of the anthraquinone-1-hydroxy-2-methyl anthraquinone **(1)** from *Morinda citrifolia* and its larvicidal activity. This is a first report on the larvicidal activity of the compound and plant extracts against *Aedes aegypti*.

MATERIALS AND METHODS

Plant Material - The roots of *Morinda citrifolia* was collected from Wakaf Bharu, Kelantan, Malaysia.

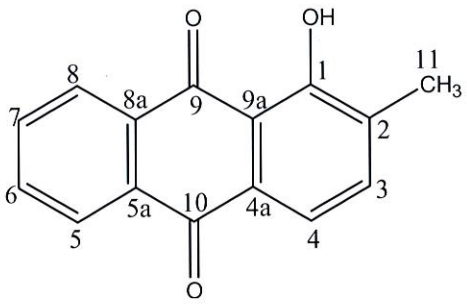
General –Melting points were determined using the Digital Precision Melting Point Apparatus. Infrared spectra were measured in NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shidmazu GCMS-QP5050A spectrometer. ^{1}H, ^{13}C and 2D NMR were obtained using JEOL 400 MHz FTNMR spectrometer with TMS (tetramethylsilane) as internal standard. Ultraviolet spectra were recorded in $CHCl_3$ on a Shidmazu UV-160A, UV-Visible Recording Spectrophotometer.

Extraction and Isolation – Dried roots of *M. citrifolia* (5 kg) were finely ground into powder form. The sample was extracted using solvents with increasing polarity to give the n-hexane (22.9 g), petroleum ether (1.7g), chloroform (28.0g), methanol (70.4g) and acetone (10.1g) extracts. The chloroform extract (9.25g) was chromatographed on a silica gel column chromatography (Kieselgel 60 PF_{254}) with a gradient of hexane-chloroform, chloroform – ethyl acetate, ethyl acetate – methanol and methanol to give 32 fractions. Fraction 2 was repeatedly subjected to further column chromatography purifications to furnish 1-hydroxy-2-methyl anthraquinone **(1)**, (30.7 mg) (hexane – chloroform, 7: 3).

Bioassay – Tests for larvicidal activity were conducted using standard protocols from WHO standard susceptibility tests in the laboratory (1981) [9]. The eggs of *Aedes aegypti* were hatched in dechlorinated water. 0.1g of sample was dissolved in 10 ml acetone to prepare a 10,000 ppm stock solution. Ten fourth-instar larvae were then introduced into a glass containing dechlorinated water. The extract standard solution was then added to each glass. The stock solution extracts were tested at concentrations of 50 ppm, 100 ppm and 150 ppm for range finding. A control solution was prepared using 750 µl of acetone and dechlorinated water. Tests were then carried out for 7 concentrations. Each treatment was done in two replicates. Mortality of the larvae was recorded after 24-hours. The LC_{50} and LC_{90} that represent the 50% and 90% mortality of the larvae at the particular concentrations were calculated using the Probit Analysis Programme.

1-Hydroxy-2-methyl anthraquinone **(1)**. Yellow solid with melting point 180°C - 181°C (Lit. 183°C-184°C) [8]. UV ($CHCl_3$) λ_{max} nm (log ε): 276 (3.33), 275 (3.44), 273 (3.52), 266 (3.65), 259 (3.80), 249 (3.18). IR ν_{max} cm^{-1} (NaCl): 3438 (OH bonded group), 2926 (C-H stretch), 2856, 1670 (C=O), 1634 (C=C), 1590 1456(C=C), 1430, 1358, 1290, 1264, 1198, 1154. EIMS m/z (rel int): 238 (100), 237 (23), 181 (25), 152 (21), 76 (29). NMR data (See Table 1).

RESULTS AND DISCUSSION



(1)

1-Hydroxy-2-methyl anthraquinone **(1)**, a yellow solid with m.p 180°C – 181°C (Lit. 183°C-184°C) [10], gave a molecular ion peak at m/z 238 in the EI-MS spectrum. This molecular mass agrees with the molecular formula $C_{15}H_{10}O_3$. The FTIR spectrum showed absorptions for the hydroxyl group at 3438 cm^{-1} and carbonyl group at 1670 cm^{-1}. The singlet peaks at δ 12.87 and δ 2.30 (H-11) in the ^1H NMR spectrum indicated the presence of the chelated hydroxyl and the aryl methyl group in the compound. Meanwhile, two doublet signals at δ 7.44 (H-3) and δ 7.65 (H-4), a triplet signal at δ 7.72 (H-6, H-7) and a broad doublet of doublet at δ 8.19 (H-5, H-8), all integrating for two Hs each, correlate to the ^{13}C signals at δ 137.2 (C-3), δ 119.2 (C-4), δ 134.0 (C-6), δ 134.5 (C-7), δ 127.3 (C-5) and δ 126.8 (C-8), respectively. This supports the presence of aromatic protons. The ^{13}C resonances at δ 182.4 and δ 188.9 for C-9 and C-10 suggested two carbonyl groups that attributed to the skeleton for an anthraquinone. The other feature of the ^{13}C NMR spectrum as shown by the peak at δ 16.2 represents the methyl carbon. Confirmation of the proposed structure was carried out using 2D NMR. From the COSY spectrum, the aromatic protons at δ 7.44 (H-3) and δ 7.65 (H-4) were found to be coupled to each other. Also, a triplet at δ 7.72 was assigned to H-6 and H-7. A broad doublet of doublet at δ 8.19 which integrates for two protons was found to be coupled to the triplet at δ 7.72. This doublet of doublet was assigned to H-5 and H-8. The singlet signal for the methyl protons at C-2 gave a ^2J correlation with the carbon signal at δ 135.0 (C-2) and a ^3J correlation with δ 161.0 (C-1) in the HMBC spectrum. Hence the methyl group was assigned to C-2. It was also observed that the aromatic proton at δ 7.44 (H-3) gave ^3J connectivity with the carbon

signal at δ 161.0 (C-1), δ 131.3 (C- 4a), δ 16.2 (C-11) and a ^2J connectivity with δ 119.2 (C-4). Hence the signal at δ 7.44 was assigned to H-3. Similarly, δ 7.65 was assigned to H-4 based on its ^3J correlation with δ 135.0 (C-2), δ 182.4 (C-10) and δ 115.2 (C-9a). Thus, this compound was assigned as 1-Hydroxy-2-methyl anthraquinone, previously isolated from *Rubia cordifolia* [11], [12] and *Asperula laurina* [13]. Our spectral data are in agreement with those reported. Figure 1 shows the HMBC correlations between the protons and the carbons of **(1).**

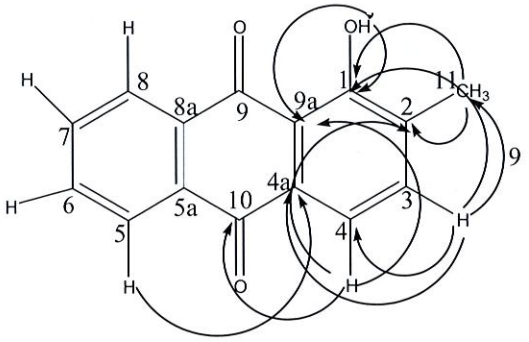


Figure 1: HMBC correlations of 1-hydroxy-2-methyl anthraquinone **(1)**

Table 1. ^{1}H-NMR and ^{13}C NMR Spectral Data of 1-hydroxy-2-methyl anthraquinone (1) in CDC

Position	δ_H (ppm)	δ_C (ppm)	HMBC correlations
1-OH	12.87 (1H, s)	161.0	C-1 (161.0) (^2J), C-9a (115.2) (^3J)
2	-	135.0	
3	7.44 (1H, d, *J*=7.32 Hz)	137.2	C-1 (161.0) (^3J), C-4 (119.2) (^2J), C-4a (131.3) (^3J), C-11 (16.2) (^3J)
4	7.65 (1H, d, *J*=7.32 Hz)	119.2	C-2 (135.0) (^3J), C-9a (115.2) (^3J) , C-10 (182.4) (^3J)
4a	-	131.3	
5	8.19 (1H, br dd, *J*=8.28Hz)	127.3	C-4a (131.3) (^4J)
5a	-	133.8	
6	7.72 (1H, t, *J*=4.14Hz)	134.0	
7	7.72 (1H, t, *J*=4.14Hz)	134.5	
8	8.19 (1H, br dd, *J*=8.28Hz)	126.8	
8a	-	133.3	
9	-	188.9	
9a	-	115.2	
10	-	182.4	
11	2.30 (3H, s)	16.2	C-1 (161.0) (^3J), C-2 (135.0) (^2J)

Table 2. LC_{50} and LC_{90} values for the plant extracts and pure compound from *M. citrifolia* against *A. aegypti* larvae

Extracts/compound	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Slope +/- S.E
Petroleum ether extract	14.6	19.1	11.06 +/- 1.78
Chloroform extract	6.0	8.7	8.07 +/- 1.54
Acetone extract	33.2	64.3	4.47 +/- 0.84
[a]1-Hydroxy-2-methyl anthraquinone	1.8	2.9	6.41 +/- 1.11
[b] *Asimina Triloba*	20.0	-	-

[a] For the test on the pure compound, a 5000 ppm stock solution was prepared and range finding was carried out at concentrations of 5 ppm, 10 ppm and 15 ppm. The control was prepared using 150 µl of acetone and dechlorinated water. [b] Positive control (Mikolajczak *et al.,* 1988) [14]

Table 2 displays the LC_{50} and LC_{90} of the extracts and pure compound against the larvae of *A. aegypti*. Of the five extracts (n-hexane, petroleum ether, chloroform, methanol, acetone) tested in the screening, three showed promising bioactivity, which had LC_{50} values of less than 50 µg/ml against fourth-instar *A. aegypti* larvae. These are the petroleum ether, chloroform and acetone extracts.

When comparing the LC_{50} values, it was observed that the chloroform extract gave the highest mortality, with an LC_{50} value of 6.0 µg/ml. Therefore, it is considered to be the most bioactive extract against the larvae and followed by the petroleum ether extract (LC_{50} = 14.6 µg/ml). Even though the acetone extract is also toxic against the larvae, it exhibited the least activity compared to the other two extracts with an LC_{50} value of 33.2 µg/ml.

Meanwhile, 1-hydroxy-2-methyl anthraquinone is also highly toxic with an LC_{50} value of 1.8µg/ml. Based on a study by Morimoto *et al.* [15], it was reported that a methyl group at the C-2 position of the anthraquinone is important for antifeedant activity against the common cutworms. A study by Cheng *et al.* [8] on larvae of *A. aegypti* using six available anthraquinones (anthraquinone, alizarin, 1-hydroxyanthraquinone, anthraquinone-2-carboxylic acid, 2-hydroxymethylanthraquinone and emodin) and tectoquinone, an anthraquinone that has a methyl group substituted at C-2 position indicate that an anthraquinone with a methyl group has the most potent larvicidal activity. This explains the significant result in the bioassay of 1-hydroxy-2-methyl anthraquinone which carries a methyl group in C-2 against the larvae of *A. aegypti*.

The chloroform extract of *M. citrifolia* and 1-hydroxy-2-methyl anthraquinone can be considered to be potential larvicides against the larvae of *A. aegypti*.

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