## L10 The Chemistry and Biological Activity Studies of *Morinda Elliptica*

**Nordin Hj. Lajis**, Nor Hadiani Ismail, Jasril Karim, Latifah S. Yazan[1], Azimuddin Abdullah[1], A. Manaf Ali[1], Raha A. Rahim[1], Arbakaria Ariff[1], Marziah Mahmood[1], Norio Aimi[2], Hiromitsu Takayama[2], and Mariko Kitajima[2].
Department of Chemistry
[1]Department of Biotechnology
Universiti Putra Malaysia, 43400 Serdang, Selangor, MALAYSIA.
[2]Faculty of Pharmaceutical Sciences
Chiba University, 1-33, Yayoi-cho, Inage-ku, JAPAN

Brine shrimp lethality test has become one of our routine tools in selecting plant materials for further chemical or bioactivity studies in our laboratory. Usually, once a potentially bioactive sample has been identified, it will then be subjected to more elaborate bioassay procedures. Out of more than 200 plant samples tested we found eight samples to be toxic towards brine shrimp larvae.

Table 1. Plant samples showing toxicity towards brine shrimp larvae.

| Plant name | $LC_{50}$ (ppm) |
|---|---|
| *Callophyllum inophyllum* (bark) | 14.5 |
| *Cerbera odolam* (seed) | 31.9 |
| *Cymbopogon nirdus* (aerial part) | 1.1 |
| *Entada* spp. (bark) | 52.1 |
| *Lawsonia inermis* (leaves) | 52.2 |
| *Morinda elliptica* (root) | 116.9 |
| *Morinda citrifolia* (root) | 253.9 |
| *Vitex ovata* (leaves) | 13.2 |

In another study, seventeen Malaysian medicinal plants, including three species of *Morinda*'s were screened for antimicrobial activities against gram negative and gram positive bacteria, fungi, yeast and candida. Methanolic extracts of the plants were prepared and used in the screening. Among the *Morinda* species, *M. elliptica* showed high activity againsts fungi especially *A. niger,*

*Cunninghamella elegans* and *Ca. intermedia.* It also showed moderate antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa.* In view that no reports have been encountered on the plant *Morinda elliptica* and its promising biological activity, the plant has been chosen for further phytochemical work. The species is a member of the family Rubiaceae has been used in the Malay traditional medicinal treatments including, for loss of appetite, headaches, cholera, diarrhoea, fever and haemorrhoids.

The biological activity of the dichloromethane crude extract of the roots of *Morinda elliptica* was evaluated using several established bioassay methods, starting with brine shrimp lethality test followed by antimicrobial assay, antiviral assay and cytotoxic assay. The extract exhibited high toxicity in the brine shrimp lethality test with $LC_{50}$ value of 7.54 ppm. This is indicative of the presence of bioactive compounds in the sample. In the antimicrobial assay, *Morinda elliptica* exhibited high activity against fungi especially *A. niger* and *A. ochraceous*. Moderate activity was also observed against *Ca. intermedia.* Antibacterial activity against *Pseudomonas aeurogenosa* was also significant. Extracts prepared from the leaves and fruits of *Morinda elliptica* was also assayed for antimicrobial activity but were found to be inactive. Two types of virus were used in the antiviral assay, *Vesicular stomatitis* virus (VSV), an RNA virus, and *Herpes simplex* Type I virus (HS I), a DNA virus. The extract was found to be active against *Vesicular stomatitis* virus with minimum inhibition concentration of 1.0 μg/ml. Cytotoxic activity against HeLa was detected with $IC_{50}$ of 3.0 μg/ml.

Following the promising results of the bioassays, the crude extract of roots of *Morinda elliptica* was fractionated using column chromatoghraphy. Combination of fractions collected based on TLC pattern gave six main fractions, A, B, C, D, E, and F. These fractions were also screened for antimicrobial, antiviral and cytotoxic activity. Moderate to strong activity was observed for all fractions with the strongest activity for fraction B. Fraction B inhibited the growth of all tested microbes with inhibition zone diameter between 9 to 26 mm. *Saccharomyces cervisieae* was particularly succeptible to the active component of fraction B. Fraction A also showed good antimicrobial activity and inhibited all microbes with inhibition zone diameter between 11 to 16 mm. Fraction C appeared to be more selective. It inhibited the growth of *Pseudomonas aeurogenosa*, *Aspergillus ochraceous*, *Saccharomyces cervisieae*, *S. lipolytica* and *Candida lipolytica* rather strongly but failed to inhibit the growth of *Bacillus cereus* and *Aspergillus niger*. The results of the quantitative antimicrobial assay using the tube dilution method correlated well

with results using the disc diffusion method. Again, antimicrobial activity was observed for all fractions with strongest activity seen for fraction B. Fraction B gave MIC value of 31 μg/ml or less for all tested microbes.

In the cytotoxic assay, fractions A, B and D were observed to be cytotoxic towards HeLa and MCF 7 cell lines with strongest activity shown by fraction B, $IC_{50}$=0.3 μg/ml. Fraction C was cytotoxic only towards MCF 7 cell line. All fractions, however showed cytotoxicity toward the non-dividing Vero monolayer cells. Interestingly, antiviral activity against VSV and HSV I of these fractions showed a different profile if compared to that of antimicrobial and cytotoxic assays. Fractions A and B which were significantly active in the antimicrobial and cytotoxic assays were not active in the antiviral assay. Instead, fractions C, E and F were observed to be active against both viruses, although they affect VSV more strongly then does HSV I. Fraction D was active against VSV only.

Table.2 : Antimicrobial Activity of Fractions A to F from Column Chromatography Assayed by Tube Dilution Method. (Minimum Inhibition Concentration, MIC in μg/ml)

| | 14581 | 14591 | 60690 | 398 | 111931 | 20341 | 16617 | 2075 |
|---|---|---|---|---|---|---|---|---|
| Crude | 62 | 62 | 62 | 31 | 31 | 62 | 31 | 31 |
| A | 62 | 62 | 62 | 62 | 62 | 62 | 31 | 31 |
| B | 31 | 31 | 31 | <31 | 31 | <31 | <31 | 31 |
| C | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| D | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 |
| E | 62 | 62 | 62 | 31 | 62 | 62 | 62 | 31 |
| F | 62 | 62 | 62 | 31 | 62 | 62 | 62 | 62 |
| Control | 31 | 31 | 62 | 31 | 31 | 31 | 31 | 31 |

**Control** : Streptomycin
**Microbes** : 14581 *Bacillus megaterium*, 14591 *Bacillus cereus*, 60690 *Pseudomonas aeurogenosa*, 398 *Aspergillus ochraceous*, 111931 *Aspergillus niger*, 20341 *Saccharomyces cervisieae*, 16617 *Saccharomyces lipolytica*, 2075 *Candida lipolytica*.

Table.3 : Cytotoxic Activity of Fractions A to F against HeLa and MCF 7 Cell Lines and Vero Monolayer Cells ($IC_{50}$ in μg/ml)

| | HeLa | MCF 7 | Vero |
|---|---|---|---|
| Crude | 3.0 | 3.0 | 2.0 |
| A | 3.0 | 3.0 | 0.5 |
| B | 0.3 | 0.3 | 0.5 |
| C | - | 3.0 | 5.0 |
| D | 3.0 | 3.0 | 2.0 |
| E | - | - | 5.0 |
| F | - | - | 2.0 |

**Hela** : Cell line derived from cervical carcinoma
**MCF 7** : Cell line derived from breast carcinoma
**Vero** : Monolayer of

Table 4 : Antiviral Activity of Fractions A to F against the VSV and HSV viruses using the Vero Monolayer Cells (Minimum Inhibition Concentration in μg/ml)

| | VSV | HSV I |
|---|---|---|
| Crude | 1.0 | - |
| A | - | - |
| B | - | - |
| C | 0.1 | 5.0 |
| D | 2.0 | - |
| E | 0.3 | 10.0 |
| F | 0.5 | 5.0 |

**VSV** : *Vesicular stomatitis* virus
**HSV I** : *Herpes simplex* Type I virus

Close examination of the root extracts yielded eleven anthraquinones including a new compound, 2-formyl-1-hydroxyanthraquinone. The known compounds are, 1-hydroxy-2-methylanthraquinone, damnacanthal, nordamnacanthal, lucidin-ω-methyl ether, rubiadin, soranjidiol, morindone, rubiadin-1-methyl ether, alizarin-1-methyl ether and morindone-5-methyl ether. Strong activity against several microbes tested were shown by damnacanthal and nordamnacanthal.

Table.5 : Quantitative Antimicrobial Activities of Anthraquinones from *Morinda elliptica*. (MID or Minimum Inhibitory Dose in μg/ml)

| | 14581 | 1447 | 60690 | 398 | 20341 | 16617 | 2075 |
|---|---|---|---|---|---|---|---|
| 1-Hydroxy-2-methylanthraquinone | - | - | - | - | [a]>80 | - | - |
| 2-Formyl-1-hydroxyanthraquinone | >80 | >80 | 80 | >80 | >80 | >80 | >80 |
| Nordamnacanthal | 40 | 20 | - | 10 | 20 | 20 | 20 |
| Damnacanthal | 20 | 10 | 10 | 20 | 20 | 20 | 20 |
| Lucidin-□-methyl ether | - | - | - | - | - | - | - |
| Rubiadin | >80 | - | - | >80 | - | - | - |
| Soranjidiol | - | - | - | - | - | - | - |
| Morindone | - | - | - | - | - | - | 20 |
| Rubiadin-1-methyl ether | - | - | - | - | - | - | - |
| Moridone-5-methyl ether | - | - | - | - | - | - | - |
| Alizarin-1-methyl ether | >80 | - | - | >80 | >80 | >80 | >80 |

[a]>80 mg = +ve in initial qualitative assay but -ve in the quantitative assay.

Strong cytotoxicity against a number of cancer cell-lines were shown by damnacanthal, lucidin-ω-methyl ether as well as rubiadin. In another test for anti-oxidative activity, morindone and 2-formyl-1-hydroxyanthraquinone were found to be the strongest.

Table 6 : Cytotoxic Activities of Anthraquinones from Roots of Morinda elliptica. ($IC_{50}$ in μg/ml)

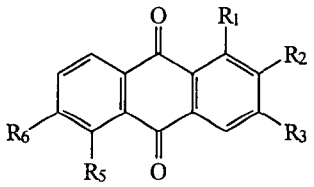
| | Hela ($IC_{50}$) | KU812F ($IC_{50}$) | PN6 ($IC_{50}$) | UACC ($IC_{50}$) | TK10 ($IC_{50}$) | MCF7 ($IC_{50}$) |
|---|---|---|---|---|---|---|
| 1-Hydroxy-2-methylanthraquinone | 30 | 10 | 10 | 30 | 30 | 30 |
| 1-Hydroxy-2-formylanthraquinone | 30 | 3 | 10 | 3 | 10 | 10 |
| Nordamnacanthal | 30 | 3 | 30 | 10 | 10 | 10 |
| Damnacanthal | 10 | 1 | 3 | 3 | 10 | 3 |
| Lucidin-□-methyl ether | nd | 3 | 3 | 10 | 30 | 3 |
| Rubiadin | bd | 3 | 3 | 10 | 10 | 10 |
| Soranjidiol | bd | 10 | 10 | 10 | 10 | 10 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Morindone | 10 | 3 | 10 | 10 | 3 | 10 |
| Rubiadin-1-methyl ether | 30 | 10 | 10 | 30 | 10 | 30 |
| Morindone-5-methyl ether | nd | 10 | 10 | 30 | 10 | 30 |
| Alizarin-1-methyl ether | nd | 30/10 | 30 | 30 | 10 | 10 |

nd - not done; cell lines: HeLa - cervical adenocarcinoma; KU812F - chronic myolegeneous leukemia; PN6 - chronic myolegeneous leukemia; UACC - melanoma TK10 - renal cancer; MCF7 - breast carcinoma

In view of the wide range of biological activities presented by anthraquinones, we embarked into the cell culture of the roots of *M. elliptica.* Preliminary results showed that a high yield (*ca.* 6%) of anthraquinones was produced from dried cell culture suspension of *M. elliptica.* Although the major component isolated from the plant's root extract were also present in the cell cultured suspension, a number of the other anthraquinones, such as damnacanthal, 2-formyl-1-hydroxyanthraquinone, 1-hydroxy-2-methylanthraquinone, morindone-5-methyl ether, and rubiadin-1-methyl ether were not present. On the other hand compounds not previously isolated such as damnacanthol and purpurin-1-methyl ether were detected. Manipulation of parameters used the cell culture has optimized the yields.

Table 7. Anthraquinones Isolated from *M. elliptica*




| Name of compounds | $R_1$ | $R_2$ | $R_3$ | $R_5$ | $R_6$ | Amount |
|---|---|---|---|---|---|---|
| Alizarin-1-methyl ether | $OCH_3$ | OH | H | H | H | Minor |
| Damnacanthal | $OCH_3$ | CHO | OH | H | H | Moderate |
| Damnacanthol | $OCH_3$ | $CH_2OH$ | OH | H | H | Minor* |
| 1-Hydroxy-2-methylanthraquinone | OH | $CH_3$ | H | H | H | Minor |
| 1-Hydroxy-2-formylanthraquinone | OH | CHO | H | H | H | Minor |
| Lucidin-ω-methyl ether | OH | $CH_2OCH_3$ | OH | H | H | Minor |
| Morindone | OH | $CH_3$ | H | OH | OH | Moderate |
| Morindone-5-methyl ether | OH | $CH_3$ | H | $OCH_3$ | OH | Minor |
| Nordamnacanthal | OH | CHO | OH | H | H | Major |
| Purpurin-1-methyl ether | $OCH_3$ | OH | OH | H | H | Minor* |
| Rubiadin | OH | $CH_3$ | OH | H | H | Moderate |
| Rubiadin-1-methyl ether | $OCH_3$ | $CH_3$ | OH | H | H | Major |
| Soranjidiol | OH | $CH_3$ | H | H | OH | Moderate |

* Compounds isolated only from the cell culture suspension.