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ANTIVIRAL, CYOTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF ANTHRAQUINONES ISOLATED FROM THE ROOTS OF *MORINDA ELLIPTICA*

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ABSTRACT

2-Formyl-1-hydroxyanthraquinone, along with ten other known anthraquinones (1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, rubiadin-1-methyl ether, soranjidiol, morindone, morindone-5-methyl ether and alizarin-1-methyl ether), isolated from the roots of Morinda elliptica, *were assayed for anti-HIV, cytotoxic and antimicrobial activites. Only damnacanthal showed moderate activity against HIV. It was cytotoxic towards the MCF-7 (breast carcinoma) and CEM-SS (T-lymphoblastic leukaemia) cell line. Nordamnacanthal was very cytotoxic against the CEM-SS cell lines. Other anthraquinones that showed strong cytotoxicity towards the cell lines tested were lucidin-ω-methyl ether (CEM-SS and MCF-7) and rubiadin (CEM-SS). Three anthraquinones viz., nordamnacanthal, damnacanthal and morindone, were found to have strong antimicrobial activity.*

INTRODUCTION

Morinda elliptica L. (Rubiaceae), locally known as "mengkudu kecil", is one of the three species (*M. citrifolia*, *M. elliptica*, *M. corneri*) found in Peninsular Malaysia (Wong, 1984). Different parts of the plant are used for the treatment of several health problems and ailments such as loss of appetite, headache, cholera, diarrhoea, fever, enlarged spleen and wounds (Burkill, 1966). Many anthraquinones have been isolated from various species of *Morinda*. Recently, 2-formyl-1-hydroxyanthraquinone, along with ten other known anthraquinones (1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, rubiadin-1-methyl ether, soranjidiol, morindone, morindone-5-methyl ether and alizarin-1-methyl ether) were isolated from the roots of *M. elliptica* (Ismail et al., 1997). In this paper, we report the anti-HIV, cytotoxic and antimicrobial activites of these compounds.

Keywords: Anthraquinones, antiviral, antimicrobial, cytotoxic, *Morinda elliptica*.

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MATERIALS AND METHODS

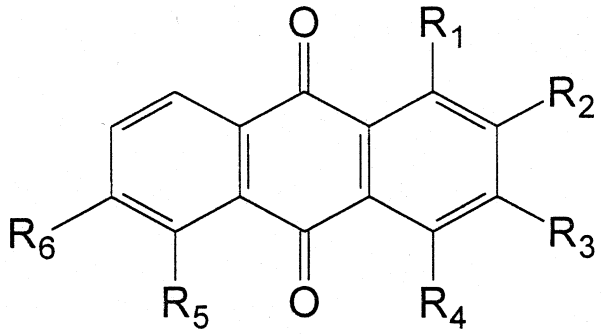
Anthraquinones

A new anthraquinone, 2-formyl-1-hydroxyanthraquinone, and ten known anthraquinones (1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, rubiadin-1-methyl ether, sorajidiol, morindone, morindone-5-methyl ether and alizarin-1-methyl ether) (Fig. 1) were isolated from the roots of *M. elliptica* as described previously (Ismail et al., 1997).

Culture of Cells and Cytotoxicity Assay

The CEM-SS (T- lymphoblastic leukaemia), HeLa (cervical carcinoma) and MCF-7 (breast carcinoma) cell lines were obtained from the National Cancer Institute, Frederick, Maryland, USA. The cells were cultured and maintained as described by Ali et al. (1998).

Reference compounds (colchicine – Kyowa Hakko Kogyo, Japan; doxorubicin – Tuobin Pharmaceutical, China; tamoxifen – Torrent Pharmaceuticals Ltd., India) were prepared at a concentration of 10 mg/ml in



R_1	R_2	R_3	R_4	R_5	R_6	Compound name
OH	CH_3	H	H	H	H	1-Hydroxy-2-methylanthraquinone
OH	CHO	H	H	H	H	2-Formyl-1-hydroxyanthraquinone
OH	CHO	OH	H	H	H	Nordamnacanthal
OCH_3	CHO	OH	H	H	H	Damnacanthal
OH	CH_2OCH_3	OH	H	H	H	Lucidin-ω-methyl ether
OH	CH_3	OH	H	H	H	Rubiadin
OH	CH_3	H	H	H	OH	Soranjidiol
OH	CH_3	H	H	OH	OH	Morindone
OCH_3	CH_3	OH	H	H	H	Rubiadin-1-methyl ether
OH	CH_3	H	H	OCH_3	OH	Morindone-5-methyl ether
OCH_3	OH	H	H	H	H	Alizarin-1-methyl ether

Fig. 1. Anthraquinones isolated from the roots of *Morinda elliptica*.

ethanol and working stocks of 60 μg/ml were freshly prepared in serum-free RPMI-1640. Cytotoxicity was determined using the microtitration MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (Mosmann, 1983) in the presence of different concentrations of anthraquinones or reference compounds: 30, 10, 3, 1, 0.3, 0.1 and 0.03 μg/ml (Ali et al., 1996). The fraction of surviving cells was measured relative to the untreated cell population by measuring the optical density at 550 nm with the reference wavelength at 630 nm using a microplate reader. Cytotoxicity was recorded as the 50% cytotoxic concentration (CC_{50}) with reference to the untreated control cells.

Table 1. Cytotoxic activity (CC_{50} in μg/ml) of anthraquinones from *Morinda elliptica*.

	Cell lines		
	HeLa	CEM-SS	MCF-7
Anthraquiones			
1-Hydroxy-2-methylanthraquinone	30	10	30
2-Formyl-1-hydroxyanthraquinone	30	10	10
Nordamnacanthal	30	1.7	10
Damnacanthal	10	4	3
Lucidin-ω-methyl ether	>30	3	3
Rubiadin	>30	3	10
Soranjidiol	>30	10	10
Morindone	10	10	10
Rubiadin-1-methyl ether	30	10	30
Morindone-5-methyl ether	>30	10	30
Alizarin-1-methyl ether	>30	30	10
Reference compounds			
Doxorubicin	11	0.1	29
Tamoxifen	7	36	7
Colchicine	21	0.02	21

Cell lines: HeLa – cervical carcinoma, CEM-SS – T-lymphoblastic leukemia, MCF-7 – breast carcinoma.

Table 2. Antimicrobial activity (MID in μg/disc) of anthraquinones from *Morinda elliptica*.

	P. aeruginosa	*A. ochraceus*	*A. niger*	*C. lipolytica*
Anthraquiones				
1-Hydroxy-2-methylanthraquinone	–	–	>80	–
2-Formyl-1-hydroxyanthraquinone	80	>80	>80	>80
Nordamnacanthal	–	10	20	20
Damnacanthal	10	20	20	20
Lucidin-ω-methyl ether	–	–	–	–
Rubiadin	–	>80	–	–
Soranjidiol	–	–	–	–
Morindone	–	–	–	20
Rubiadin-1-methyl ether	–	–	–	–
Morindone-5-methyl ether	–	–	–	–
Alizarin-1-methyl ether	–	>80	>80	>80

Note: Standard antibiotic; gentamycin (10 μg/disk) was used against *P. aeruginosa* (24 mm diameter inhibition zone); nystatin (100 unit/disk) was used against fungi (25 mm diameter inhibition zone); – = no activity.

XTT Assay for Cytoprotection (Anti-HIV Assay)

The assay was carried out by measuring the ability of the anthraquinones to protect CEM-SS cells after infection with HIV as described previously (Weislow et al., 1989). The cell density and viability of CEM-SS cells at the logarithmic growth phase were determined, and distributed into 96-well tissue culture plates to which serially diluted test compound solutions and media were added. The virus was added to appropriate wells and the plates were incubated for 6 days at 37 °C in a CO_2 incubator. XTT/PMS solution was added into each well and the plates were incubated for four hours at 37 °C. Cell viability was quantified by measuring the absorbance at 450 nm, and 630 nm as the reference wavelength. Cell death was confirmed by microscopic observation. Dextran sulfate was used as a control drug.

Antimicrobial Assay

Four microorganisms were used, i.e., *Pseudomonas aeruginosa*, *Saccharomyces Aspergillus ochraceus*, *A. niger* and *Candida lypolitica*. The source of microorganism, maintenance of cultures and determination of antimicrobial activity have been described previously (Ali et al., 1995). Standard antibiotics, gentamycin and nystatin (BBL®, Beckon Dickinson Microbiology Systems, USA) were used as control.

RESULTS AND DISCUSSION

The anthraquinones tested in the XTT cytoprotection assay were 2-formyl-1-hydroxyanthraquinone, nordamnacanthal, damnacanthal, morindone, rubiadin-1-methyl ether and alizarin-1-methyl ether. Only damnacanthal showed moderate activity with 72.4% cytoprotection against HIV. The 50% effective concentration (EC_{50}) value was 3.43. The control compound, dextran sulfate, which gave 100% cytoprotective effect with EC_{50} of 0.53 μg/ml.

Table 1 shows the CC_{50} values of the isolated anthraquinones against various tumor cell lines. Three cytotoxic reference compounds were used in the cytotoxic assay; doxorubicin, a natural product compound which intercalates between DNA base pairs; tamoxifen, an estrogen antagonist which binds to estrogen receptor, and colchicine, a natural product mitotic inhibitor which disrupts microtubullar protein. The CEM-SS cell line was very sensitive towards colchicine and doxorubicin (CC_{50} = 0.02-0.1 μg/ml) whereas the HeLa and MCF-7 cell lines were sensitive towards tamoxifen (CC_{50} = 7 μg/ml). Among the three cell lines tested, CEM-SS was the most sensitive towards nordamnacanthal (CC_{50} = 1.7 μg/ml), damnacanthal (CC = 4 μg/ml), lucidin-ω-methyl ether (CC_{50} = 3 μg/ml) and rubiadin (CC_{50} = 3 μg/ml). Other anthraquinones showed moderate activity (CC_{50} = 10 μg/ml) towards the CEM-SS cell line, except alizarin-1-methyl ether, which gave a CC_{50} value of 30 μg/ml. The MCF-7 cell line was very sensitive towards damnacanathal and lucidin-ω-methyl ether (CC_{50} = 3 μg/ml). Most of the anthraquinones were weakly cytotoxic towards the HeLa cell line except for damnacanthal and morindone, both of which showed moderate cytotoxic activity. Structurally, all the four anthraquinones that showed strong cytotoxicity (nordamnacanthal, damnacanthal, lucidin-ω-methyl ether and rubiadin) were hydroxylated at carbon number three and possessed a hydroxyl at carbon number one and/or a *ortho*-formyl group at carbon number two.

Three anthraquinones, i.e., nordamnacanthal, damnacanthal and alizarin-1-methyl ether, showed

strong antimicrobial activity when tested against one bacterium and three fungi (Table 2). Only 2-formyl-1-hydroxyanthraquinone and damnacanthal were active against *P. aeruginosa* with MID values of 80 μg/disk and 10 μg/disk, respectively. For antifungal activity, damnacanthal and nordamnacanthal showed very strong activity against all the test fungi with MID values of 10 to 20 μg/disk. Morindone, however, showed very strong antifungal activity only against *C. lipolytica* (MID = 20 μg/disk). The antimicrobial activity of the three anthraquinones was most probably due to the presence of a formyl group at carbon-2 and a hydroxyl (nordamnacanthal and damnacanthal) and 1,2-dihydroxyl groups (morindone).

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