Natural Product Sciences
8(2) : 48-51 (2002)

Antioxidant Activity of Anthraquinones from *Morinda elliptica*

Nor Hadiani Ismail[1,2], Habsah Mohamad[1], Amran Mohidin[1] and Nordin Hj. Lajis[1,*]

[1]*Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.*
[2]*Current address: Faculty of Applied Science, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*

Abstract – Antioxidative properties of fifteen anthraquinone derivatives, including eleven natural anthraquinones isolated from the roots of *Morinda elliptica* and four from synthetic origin were evaluated using thin layer chromatography (TLC), ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. Five of the compounds, nordamnacanthal, damnacanthal, 2-formyl-1-hydroxyanthraquinone, morindone and alizarin showed higher antioxidative activity than standard natural antioxidant, α-tocopherol, on the FTC assay. Morindone and alizarin showed the strongest antioxidant activity. The results from the bioassay using TBA method correlated well with the results of the FTC method.

Keywords – antioxidant activity, anthraquinone derivatives, *Morinda elliptica*, thin layer chromatography, ferric thiocyanate, thiobarbituric acid.

Introduction

Morinda elliptica is one of the common medicinal plants used in Malaysia. This plant occurs as shrub or small tree commonly found in bushes and lowland forests in the Peninsula Malaysia. Traditionally, different parts of the plants are used in various ways for a number of health problems and ailments. For examples, the leaves may be added to rice for lost of appetite or taken for headache, cholera, diarrhea and fever. They are also applied externally to treat enlarged spleen and wounds. Sometime a lotion is made and used for hemorrhoid and applied upon the body after childbirth (Burkill, 1966). We have previously reported the isolation of eleven anthraquinone derivatives including a new compound, 2-formyl-1-hydroxyanthraquinone, from *Morinda elliptica* (Nor Hadiani *et al.*, 1997). In our efforts to synthesize the new compound, several other anthraquinone compounds were obtained.

In recent years the role of free radicals and reactive oxygen species in human disease processes has become more apparent. Thus the study on antioxidant activity of compounds derived from natural resources has attracted considerable research interest among the scientists (Gordon, 1996). Huang *et al.* (1995) reported the inhibitory effect on lipid peroxidation in rat heart mitochondria of several anthraquinone derivatives containing phenolic groups, which were isolated as active components from root of *Polyganum cuspidatum*. Emodin, alizarin and alizarin complexone were found to significantly inhibit lipid peroxidation. Their potency as inhibitors of lipid peroxidation was higher than that of α-tocopherol.

In this study, the natural anthraquinones isolated from *Morinda elliptica* and those obtained synthetically were evaluated for their antioxidative properties using several methods. Structure-activity relationship for the antioxidant effect of these anthraquinones was established.

Experimental

Anthraquinones – 1-Hydroxy-2-methylanthraquinone, 2-formyl-1-hydroxyanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, soranjidiol, morindone, rubiadin-1-methyl ether, morindone-5-methyl ether and alizarin-1-methyl ether were isolated from roots of *Morinda elliptica* (Nor Hadiani *et al.*, 1997).

Alizarin was purified by column chromatography from commercially available product. Alizarin-2-methyl ether was obtained through methylation of alizarin with dimethyl sulfate in the presence of anhydrous K_2CO_3 in 150 ml acetone. 4-Bromo-1-hydroxyanthraquinone was obtained through Friedel-Craft condensation of phthalic anhydride with 4-bromophenol in the presence of aluminum chloride and sodium chloride as described by Zhang *et al.* (1996). 4-Bromo-1-hydroxyanthraquinone was debrominated to 1-hydroxyanthraquinone using triethylamine, triphenylphosphine,

*Author for correspondence, E-mail: nhlajis@ibs.upm.edu.my

palladium acetate and formic acid as described by Weir *et al.* (1980).

Alizarin-2-methyl ether – Yellow crystals, mp 226-228°C [lit. 230-232°C (EtOH), Burnett (1968)]. UV λ_{max} nm EtOH: 245, 272, 324, 419; UV λ_{max} EtOH/NaOH (nm): 245, 495. IR ν cm^{-1} (KBr): 3449 (OH), 2926, 1664 (C=O unchelated), 1638 (C=O chelated), 1593 (C=C aromatic). MS m/z (rel. int.): 254 (M^+, 100), 225 (54.4), 211 (19.7), 183 (11.7).

4-Bromo-1-hydroxyanthraquinone – Orange crystals, mp 132-136°C, UV λ_{max} EtOH (nm): 264, 326, 405; UV λ_{max} EtOH/NaOH (nm): 270, 311, 494. IR ν cm^{-1} (KBr): 3431 (OH), 2926, 1677 (C=O unchelated), 1641 (C=O chelated), 1591 (C=C aromatic), 710 (C-Br). MS m/z (rel. int.): 302 (M^+, 70.9), 274 (7.2), 246 (13.0), 224 (19.5), 139 (100). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 8.19 (2H, *m*, H-8, H-5), 8.00 (1H, *d*, J=9.15 Hz, H-3), 7.95 (2H, *m*, H-6 and 7), 7.28 (1H, *d*, J=9.15 Hz, H-4).

1-Hydroxyanthraquinone – Yellow-orange crystals, mp 190-192°C [lit. 194.5°C], UV λ_{max} EtOH (nm): 245, 272, 324, 396; UV λ_{max} EtOH/NaOH (nm): 230, 271, 306, 491. IR ν cm^{-1} (KBr): 3443 (OH), 2924, 1674 (C=O unchelated), 1640 (C=O chelated), 1592 (C=C aromatic). ^{1}H-NMR (270 MHz, CD_3Cl) δ ppm: 12.58 (1H, *s*, 1-OH), 8.29 (2H, *m*, H-8, H-5), 7.80 (3H, *m*, H-4, 6 and 7), 7.66 (1H, *t*, J_{ortho}=8.06 Hz, H-3), 7.28 (1H, *dd*, J_{ortho}=8.06 Hz, J_{meta}=2.19 Hz, H-2). ^{13}C-NMR ($CDCl_3$) δ ppm: 188.6 (C=O chelated), 182.3 (C=O unchelated), 162.5 (C-OH), 136.7, 134.6, 134.1, 133.5, 133.4, 133.1, 127.4, 126.9, 124.3, 119.5, 116.1.

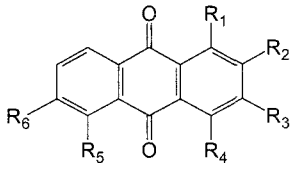
Thin layer chromatography (TLC) Antioxidant Assay – The assay was conducted according to the method described by Chang *et al.* (1983) with slight modification (Masuda *et al.*, 1994). The sample (1 μg, 5 μg and 10 μg) was spotted on silica gel TLC plate with fluorescent indicator (Kielselgel 60 F254). The TLC plate was then sprayed twice with 3% linoleic acid in hexane. The plate was placed under continuous irradiation of UV (254 nm) light using a UV lamp (UVGL-25, UVP Inc., San Gabriel, CA) positioned 2.5 cm above the TLC plate. For 10-15 min, the background of the spots darkened and then a fluorescent spot appeared. The TLC plate was observed every 5 min under continuous irradiation until each fluorescent spot disappeared. The time taken for the disappearance of the fluorescent spot is considered as the induction period for lipid oxidation.

Ferric Thiocyanate (FTC) Antioxidant Assay – The detection of lipid peroxide and the preparation of solutions were carried out according to the method described by Masuda *et al.* (1992). The sample solution was prepared by dissolving the 4 mg test sample (final concentration 0.02% w/v), and linoleic acid (0.10 g) in 8.0 ml of 99.6% ethanol, 8.0 ml of phosphate buffer (0.05 M, pH 7.0) and 3.9 ml of distilled water. The vial containing the solution was then placed in the dark at 40°C. Oxidation of linoleic acid was monitored by the following procedures. To 0.1 ml of sample solution was added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely three minutes after the addition of 0.1 ml of 0.02 M ferrous chloride ($FeCl_2$) in 3.5% hydrochloric acid, the absorbance of the mixture was measured at 500 nm. The measurements were done every 24 hours until one day after the control reached maximum reading.

Thiobarbituric Acid Antioxidant Assay – This test was conducted according to the method of Ottolenghi (1959) and Kikuzaki (1993). The same sample solutions prepared for FTC method were used as sample stock solution. A portion (2 ml) was transferred into a 10 ml graduated screw-capped centrifuge tube. To it was then added 1 ml of 20% aq. trichloroacetic acid and 2 ml of aq. thiobarbituric acid solution. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant liquid was measured at 532 nm. Antioxidant activity was recorded based on absorbance at the final day (8^{th}).

Results and Discussion

In this work, the TLC method described by Chang *et*



R_1	R_2	R_3	R_4	R_5	R_6	
OH	CH_3	H	H	H	H	1-Hydroxy-2-methylanthraquinone (**1**)
OH	CHO	H	H	H	H	2-Formyl-1-hydroxyanthraquinone (**2**)
OH	CHO	OH	H	H	H	Nordamnacanthal (**3**)
OCH_3	CHO	OH	H	H	H	Damnacanthal (**4**)
OH	CH_2OCH_3	OH	H	H	H	Lucidin-ω-methyl ether (**5**)
OH	CH_3	OH	H	H	H	Rubiadin (**6**)
OH	CH_3	H	H	H	OH	Soranjidiol (**7**)
OH	CH_3	H	H	OH	OH	Morindone (**8**)
OCH_3	CH_3	OH	H	H	H	Rubiadin-1-methyl ether (**9**)
OH	CH_3	H	H	OCH_3	OH	Morindone-5-methyl ether (**10**)
OCH_3	OH	H	H	H	H	Alizarin-1-methyl ether (**11**)
OH	OCH_3	H	H	H	H	Alizarin-2-methyl ether (**12**)
OH	H	H	H	H	H	1-Hydroxyanthraquinone (**13**)
OH	H	H	Br	H	H	4-Bromo-1-hydroxyanthraquinone (**14**)
OH	OH	H	H	H	H	Alizarin (**15**)

Fig. 1. Anthraquinone Derivatives Evaluated for Antioxidant Activity.

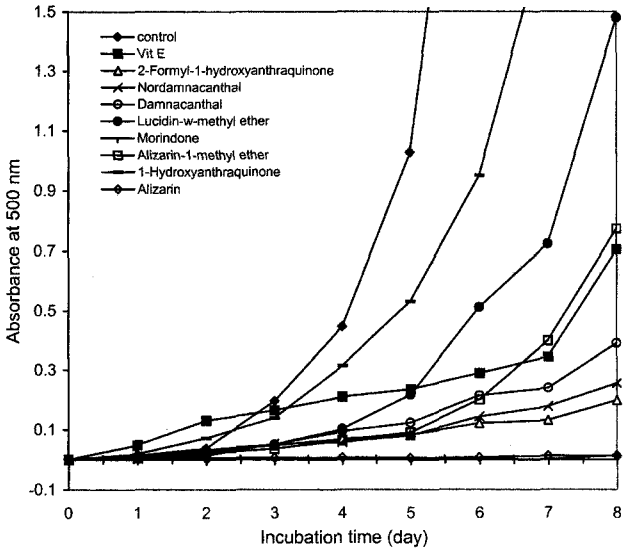


Fig. 2. Graph of Absorbance versus Incubation Time Illustrating Antioxidant Activity of Anthraquinones Measured by FTC Method.

Table 1. Antioxidant Activity of Anthraquinones Measured by TBA Method

Test Compounds	Absorbance at 532 nm (8^{th} day)
Control (no sample)	3.03
BHT (Butylated hydroxy toluene)	0.02
Vit? E (α-Tocopherol)	0.38
1-Hydroxy-2-methylanthraquinone (**1**)	3.62
2-Formyl-1-hydroxyanthraquinone (**2**)	0.15
Nordamnacanthal (**3**)	0.16
Damnacanthal (**4**)	0.23
Lucidin-ω-methyl ether (**5**)	0.95
Rubiadin (**6**)	2.43
Soranjidiol (**7**)	2.43
Morindone (**8**)	0.07
Rubiadin-1-methyl ether (**9**)	2.80
Morindone-5-methyl ether (**10**)	2.34
Alizarin-1-methyl ether (**11**)	1.04
Alizarin-2-methyl ether (**12**)	2.66
1-Hydroxyanthraquinone (**13**)	4.04
4-Bromo-1-hydroxyanthraquinone (**14**)	3.72
Alizarin (**15**)	0.01

al. (1983) was used in the initial evaluation of antioxidant activity of anthraquinones isolated from *Morinda elliptica*. The time taken for the fluorescence spots to disappear when the TLC plate was placed under continuous irradiation of UV (254 nm) light was considered as induction period for lipid oxidation. At 5 μg sample, induction period for lipid peroxidation in the presence of the well known antioxidant, α-tocopherol, was 60 minutes. Nordamnacanthal, damnacanthal, 2-formyl-1-hydroxyanthraquione and morindone seem to show stronger antioxidant property than α-tocopherol with induction period of 105, 85, 65 and 65 minutes, respectively.

The antioxidant activity of the anthraquinones isolated from *M. elliptica* as well as those obtained through synthesis were also measured by ferric thiocyanate (FTC) method and then compared with thiobarbituric acid method at concentration of 0.02% (w/v) of test compounds in aqueous ethanolic solution. The FTC method measures the amount of peroxide in the initial stages of lipid peroxidation. Low absorbance values in the FTC method indicate high level of antioxidant activity. Figure 2 shows the absorbance values of eight anthraquinones that inhibit lipid peroxidation as measured by FTC method. Nordamnacanthal, damnacanthal, 2-formyl-1-hydroxyanthraquinone, morindone and alizarin exhibited higher activity than vitamin E (α-tocopherol). Alizarin and morindone exhibited strongest activity followed by 2-formyl-1-hydroxyanthraquinone, nordamnacanthal and damnacanthal. Antioxidant activity of alizarin-1-methyl ether was comparable to α-tocopherol, while 1-hydroxyanthraquinone exhibited much weaker activity.

Thiobarbituric acid (TBA) method measured the degradation of peroxide to lower molecular weight compounds during oxidation. Table 1 shows the absorbance values of all tested anthraquinones at 532 nm measured on the eighth day (one day after the control reached maximum) using this method. The absorbance values for damnacanthal, nordamnacanthal, 2-formyl-1-hydroxyanthraquinone, morindone and alizarin (0.25, 0.16, 0.15, 0.07 and 0.01, respectively) were lower than that of α-tocopherol (0.38), indicating stronger antioxidative property. The absorbance showed by alizarin was in fact comparable to butylated hydroxytoluene (BHT), a commercially used synthetic antioxidant.

Structurally, alizarin and morindone possessed two hydroxyl groups arranged in *ortho* position. Both compounds exhibited strong antioxidant activity. In morindone-5-methyl ether, the 5-hydroxyl group of morindone had been methylated thus destroying the *ortho* arrangement of hydroxyl groups. Morindone-5-methyl ether did not show antioxidant activity, so were alizarin-1-methyl ether and alizarin-2-methyl ether, confirming the necessity of having two *ortho* arranged hydroxyl groups.

In 2-formyl-1-hydroxyanthraquinone, a hydroxyl group and a formyl group are arranged in *ortho* position. This compound also exhibited antioxidant activity, although it was weaker than alizarin and morindone but significantly stronger than α-tocopherol. Nordamnacanthal and damnacanthal which possess the same feature also exhibited good antioxidant activity. From these observations, it can be concluded that the formyl group next to a hydroxyl in anthraquinone derivatives also plays an important role in antioxidant activity.

Rubiadin, an anthraquinone which possesses 1,3-dihydroxy structure did not show antioxidative activity. Lucidin-ω-methyl ether, another 1,3-dihydroxyanthraquinone, on the other hand showed only weak antioxidative activity This finding showed that hydroxyl groups arranged in *meta* positions are less crucial for antioxidative property of anthraquinones. The monohydroxyanthraquinones did not show antioxidant activity as expected.

Acknowlegdements

The authors would like to thank Prof. Nakatani and Dr. Kikuzaki for their advice on the assay methods, and acknowledged the support from Universiti Teknologi MARA for the study leave granted to NHI and the Ministry of Science for the financial project under IRPA (09-02-04-0067) programme.

References

Burkill, I.H. (1966) *A Dictionary of Economics Products of the Malay Peninsular.* 2 vols. London: Crown Agents to the Colonies (Reprint: Kuala Lumpur, Ministry of Agriculture and Cooperatives).

Chang, W.H., H.X. Luu and A.C. Cheng. A TLC-fluorescent method for detecting and evaluating individual antioxidative components. *J. Food Sci.* **48**, 658-659 (1983).

Huang, S.S., S.F. Yeh and C.Y. Hong. Effect of anthraquinone derivatives on lipid peroxidation in rat heart mitochondria: structure-activity relationship. *Journal Natural Products.* **58**(9), 1365-1368 (1995).

Gordon, M.H. Dietary Antioxidants in Disease prevention. *Natural Products Report*, 13(**4**), 265-273 (1996).

Kikuzaki, H. and N. Nakatani. Antioxidant effects of some ginger constituents. *J. Food Sci.* **58**(6), 1407-1410 (1993).

Masuda, T. and A. Jitoe. antioxidative antiimflamatory compounds from tropical gingers: Isolation, structure determination, and activities of cassumunins A, B, and C, new complex curcuminoids from *Zingiber cassumunar. J. of Agricultural and Food Chem.* **42**, 1850-1856 (1994).

Masuda, T., J. Isobe, A. Jitoe and N. Nakatani. Antioxidative curcuminoids from rhizome of *Curcuma xanthorrhiza. Phytochemistry.* **31**, 3645-3647 (1992).

Nor Hadiani, I., A.M., Ali, N. Aimi, M. Kitajima, H. Takayama and N.H. Lajis. Anthraquinones from *Morinda elliptica. Phytochemistry.* **45**(8), 1723-1725 (1997).

Ottolenghi, A. Interaction of Ascorbic Acid and Mitochondrial Llipids. *Arch. Biochem. Biophys.* **79**, 355 (1959).

Weir, J.R., B.A. Patel and R.F. Heck, Palladiumcatalyzed Triethylammonium Formate Reductions. 4. Reduction of Acetylenes to Cis Monoenes and Hydrogenolysis of Tertiary Allylic Amines. *J. Org. Chem.* **45**, 4926-4931 (1980).

Zhang, X., B.W. Fox and J.A. Hadfield (1996) Preparation of Naturally Occurring Anthraquinones. *Synthetic Commun.* **26**(1), 49-62.

(Accepted March 30, 2002)