PHYTOCHEMICAL AND SELECTED PHARMACOLOGICAL STUDIES OF STANDARDIZED FRUIT EXTRACTS OF MORINDA CITRIFOLIA LINN.

BEH HOOI KHENG

UNIVERSITI SAINS MALAYSIA
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PHYTOCHEMICAL AND SELECTED PHARMACOLOGICAL STUDIES OF STANDARDIZED FRUIT EXTRACTS OF MORINDA CITRIFOLIA LINN.

By

BEH HOOI KHENG

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

February, 2012
Dedicated to my parents, siblings, nephews and niece
ACKNOWLEDGEMENTS

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<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
</tr>
<tr>
<td>BHK</td>
<td>Baby hamster kidney</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BuOH</td>
<td>Butanol</td>
</tr>
<tr>
<td>CAM</td>
<td>Chick Chorioallantoic Membrane</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<tr>
<td>DPPH</td>
<td>1,1-Diphenyl-2-picrylhydrazyl</td>
</tr>
<tr>
<td>EA</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FCR</td>
<td>Folin-Ciocalteu’s reagent</td>
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<tr>
<td>FTC</td>
<td>Ferric thiocyanate</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic acid equivalent</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography - mass spectrometry</td>
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<td>g</td>
<td>Gram</td>
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HCl  Hydrochloric acid
HCT116  Human colon cancer
HDL  High-density lipoprotein
HEp2  Human laryngeal epithiloma
HFD  High fat diet
HL-60  Human promyelocytic leukemia cell lines
HPLC  High-performance liquid chromatography
i.d  Internal diameter
kg  Kilograms
L  Liter
LC$_{50}$  Median lethal concentrations
LDL  Low-density lipoprotein
Na$_2$CO$_3$  Sodium carbonate
m  Meter
MCF-7  Human breast cancer cell lines
mg  Milligram
min  minute
mL  Milliliter
mm  Millimeter
MTT  3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaOH  Sodium hydroxide
nm  Nanometer
<table>
<thead>
<tr>
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<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>ppm</td>
<td>Part per million</td>
</tr>
<tr>
<td>QE</td>
<td>Quercetin equivalent</td>
</tr>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt;</td>
<td>Retardation factor</td>
</tr>
<tr>
<td>S.D</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
</tr>
<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TWEEN</td>
<td>Polysorbate</td>
</tr>
<tr>
<td>U</td>
<td>Unit</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>Vero</td>
<td>African green monkey kidney</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight over weight</td>
</tr>
<tr>
<td>Y79</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µL</td>
<td>Microliter</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometer</td>
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<td>°C</td>
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<td>Total flavonoids content</td>
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<td>Appendix O</td>
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<td>DPPH scavenging activity</td>
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ABSTRAK


Kesan anti-angiogenik dari ekstrak buah telah dinilai dengan menggunakan kaedah membran korioalantoik embrio ayam (CAM). Ekstrak metanol dan fraksi kloroform memberikan kesan anti-angiogenik dengan nilai skor 0.94 dan 1.22 masing-masing. Pengasingan bioaktiviti-bimbingan telah dilakukan dan scopoletin telah dikenalpastikan sebagai salah satu sebatian aktif dalam aktiviti anti-angiogenik (nilai skor 1.39).

Kesan sitotoksik ekstrak buah telah diperhati pada sel kanser payudara (MCF-7) dan sel leukemia manusia (HL-60) dengan menggunakan assai MTT. Ekstrak metanol telah merencat 37.6% sel MCF-7 dan 25.62% sel HL-60 pada kepekatan
30 ug/mL. Fraksi-fraksi yang lain dari buah tala menunjukkan aktiviti sitotoksik yang lebih lemah berbanding dengan ekstrak metanol.


Kajian antioksidan ekstrak buah telah dinilai dalam kajian ini. Fraksi etil asetat mengandungi kandungan fenolik (167.71 ± 5.30 ug/mL GAE) dan kandungan flavonoid (22.30 ± 1.22 ug/mL QE) yang tertinggi. Fraksi tersebut juga mempunyai aktiviti antioksidan yang tertinggi dalam penangkapan radikal DPPH dengan nilai EC50 164.09 ug/mL and mempunyai 83.46% aktiviti antioksidan dalam ujian β-karoten-linoleat pada kepekatan 500 ug/mL.

PHYTOCHEMICAL AND SELECTED PHARMACOLOGICAL STUDIES
OF STANDARDIZED FRUIT EXTRACTS OF MORINDA CITRIFOLIA
LINN.

ABSTRACT

*Morinda citrifolia* is commonly used in Malaysia by locals as traditional medicine for various diseases. Currently there are eleven registered commercial products of *Morinda citrifolia* fruit available in Malaysia. In this study, preliminary phytochemical screening and physico-chemical studies of the fruit powder were attempted and quality parameter involving chromatographic profiling of the extracts were carried out for standardization.

The fruit extracts were screened for anti-angiogenic effect using *in vivo* chick chorioallantoic membrane assay. Methanolic extract and chloroform fraction showed anti-angiogenic effect with the score value of 0.94 and 1.22, respectively. Bioactivity-guided isolation was performed and scopoletin was identified as one of the active constituents in anti-angiogenic activity (score value 1.39).

Cytotoxic effects of the fruit extracts were observed on breast cancer cell lines (MCF 7) and human leukemia cell lines (HL-60) using MTT cell viability assay. The methanolic extract inhibited 37.6% of MCF 7 cells and 25.62% of HL-60 cells
at concentration of 30 μg/mL. The other fractions of the fruit showed weaker cytotoxic activity than methanolic extract.

Adipogenesis is concomitantly accompanied by new blood vessel growth, and thus suppression of angiogenesis would prevent adipogenesis and obesity. Methanolic extract and chloroform fraction were tested for their anti-obesity and anti-hyperlipidemic effects using high fat diet induced hyperlipidemic rats. The finding showed the fruit extracts influenced triglyceride and cholesterol metabolism in obese rats. The fruit extracts also significantly reduced the percentage of total body weight increased in the rats.

Antioxidant activity of fruit extracts was evaluated in this study. Ethyl acetate fraction contains the highest phenolic content (167.71 ± 5.30 μg/mL GAE) and flavonoid content (22.30 ± 1.22 μg/mL QE). The fraction also possesses the highest antioxidant activity in DPPH scavenging with EC$_{50}$ value 164.09 μg/mL and 83.46% antioxidant activity in β-carotene-linoleate assay at the concentration of 500 μg/mL.

The present studies provided information on the quality and standardization of the fruit extracts. The anti-angiogenic, cytotoxicity, anti-obesity, anti-hyperlipidemic and antioxidant studies of the fruit provided evidence on the traditional use for related disease.
CHAPTER 1
INTRODUCTION

1.1 General
Herbal medicines are the therapeutic experiences of generations of practicing physicians of traditional medicine over hundreds of years and they are known to be oldest health care products that have been used by mankind all over the world to treat various types of ailments (Torey et al., 2010). Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. It is documented that 80% of the world’s population has faith in traditional medicine, particularly plant drugs for their primary healthcare (Dubey et al., 2004).

Modern technological medicine is nowadays much criticized for waiting for diseases to occur and then trying to cure it rather than seeking to prevent it from occurring in the first place (Laurence & Black, 1978). Herbal products have played an important role today not only to heal the diseases but also to prevent the diseases from occurring. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body and lesser side effects (Kamboj, 2000).

There are estimated 350,000 flowering plant species identified so far, about 35,000 species are used worldwide for medicinal purposes (Kong et al., 2003). However,
the number could be much higher as knowledge on the indigenous uses of plants was mostly passed on orally from one generation to another and has largely remained undocumented (Jantan, 2004). Tropical rainforests cover about 12 % of the land area of the earth, Kong et al. (2003) reported that tropical rainforests are a vital source of medicines, there are not more than 1 % of the world's tropical forest plants have been tested for pharmaceutical properties, yet at least 25 % of all modern drugs originally came from rainforests.

*Morinda citrifolia* is commonly used in Malaysia by local people as folk remedy to cure or to prevent diseases. There are eleven registered commercial products of *M. citrifolia* fruit available in the market. In Malaysia, *Morinda citrifolia* L. (mengkudu besar) and *Morinda elliptica* Ridl. (mengkudu hutan) are the two common *Morinda* species. Both of the species have their therapeutic effect. *M. citrifolia* is widely used to treat diabetes and *M. elliptica* is widely used to treat diarrhoea (Ong & Nordiana, 1999).

1.2 Phytochemical and biological activities of genus *Morinda*

The genus *Morinda* (Rubiaceae) is made up of around 80 species (Chan-Blanco *et al.*, 2006). In the Indo-Pacific region, species diversity is highest in Near Oceania with attenuation into Remote Oceania. The genus *Morinda* includes trees, shrubs, and vines (McClatchey, 2003). *Morinda* is a genus of the family Rubiaceae and has long been known to contain substantial amount of anthraquinones. About 90%
of these compounds occur as derivatives of 9,10-anthracenedione with several hydroxy and other functional groups, such as methyl, hydroxymethyl and carboxyl (Jasril et al., 2003). Hydroxyanthraquinones are the active principles of many phyto-therapeutic drugs (Wolfle et al., 1990).

1.2.1 *Morinda elliptica*

*Morinda elliptica* Ridl. is a small plant known as “mengkudu kecil”. It is a shrub or small tree, growing wild in newly developed areas or in bushes. It is a native plant of Asia and Polynesia used in traditional folk medicine such as cholera, diarrhea, piles, headache and to increase appetite (Ismail et al., 1997; Ishak et al., 2010).

Ismail et al. (1997) reported a new anthraquinone and 10 known anthraquinones. The anthraquinone, 2-formyl-1-hydroxyanthraquinone and 10 known anthraquinones, 1-hydroxy-2-methylantraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, soranjidiol, morindone, rubiadin-1-methyl ether, alizarin-1-methyl ether and morindone-5-methyl ether were isolated from roots of *M. elliptica*. The structures of the isolated compounds were shown in Figure 1.1.

Jasril et al. (2003) tested the antitumor and antioxidant activities of six anthraquinones (nordamnacanthal, alizarin-1-methyl ether, rubiadin, soranjidiol,
lucidin-ω-methyl ether and morindone) of *M. elliptica*. All compounds exhibited stronger antitumor activity than the reference compounds genistein and quercetin. In antioxidant assay using ferric thiocyanate (FTC) method, nordamnacanthal and morindone showed stronger antioxidant activity than α-tocopherol. However when the compounds were assayed for scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, only morindone was considered to be active as free radical scavenger. This observation suggested that radical scavenging is less prominent in nordamnacanthal as compared to morindone. The differences between the two compounds are that the formyl at C-2 and hydroxyl at C-3 in nordamnacanthal are replaced by a methyl and proton groups, respectively in morindone.
Figure 1.1 Isolated compounds from the roots of *Morinda elliptica*
Figure 1.1 (continued) Isolated compounds from the roots of *Morinda elliptica*
1.2.2  *Morinda morindoides*

*Morinda morindoides* Milne-Redh, is one of the most popular medicinal plants currently used in villages and towns in Democratic Republic of Congo in traditional medicine. An aqueous decoction of fresh leaves, which is the typical traditional remedy used for the treatment of various illnesses among which are diarrhoea and constipation associated with intestinal worms (Cimanga *et al*., 2010).

Marie-Genevieve *et al*. (2010) reported that the *in vitro* effect of the toluene, methyl tert-butyl ether (MtBE), ethyl acetate (EtOAc), n-butanol (n-BuOH) and water extracts from *M. morindoides* leaves on their cytotoxicity effect against leukemic cell lines. They found that both toluene and MtBE extracts exhibited a significant cytotoxic effect on the cell lines. The highest cytotoxicity was obtained with toluene extract. By contrast, EtOAc, n-BuOH and water extracts did not affect cell viability of the three cell lines tested.

Cimanga and co-workers reported *M. morindoides* leaf extracts have been shown to possess antiprotozoal activity particularly against *Entamoeba histolytica* (Cimanga *et al*., 2006). Extracts, fractions and some isolated compounds from *M. morindoides* leaves were tested for their potential *in vitro* antiamoebic activity. Results indicated that the aqueous decoction (dried extract) and 80% methanolic extract displayed an appreciable antiamoebic activity. The CHCl$_3$, EtOAc and n-BuOH soluble fractions from the partition of 80% methanolic extract exhibited an
average antiamoebic activity. The residual water-soluble fraction showed a weak effect against *Entamoeba histolytica*.

A number of isolated compounds from the leaf of *M. morindoides* were reported (Cimanga *et al.*, 2006). The structures of the compounds are shown in Figure 1.2.

![Chemical structures of isolated compounds](image)

**Figure 1.2** Isolated compounds from the leaf of *Morinda morindoides*
Figure 1.2 (continued) Isolated compounds from the leaf of *Morinda morindoides*
Figure 1.2 (continued) Isolated compounds from the leaf of *Morinda morindoides*
1.2.3 *Morinda officinalis*

*Morinda officinalis* How, is one of the traditional Chinese plants grows in humid areas of southeast China. It has been reported to possess the ability to reinforce kidney function, strengthen the tendons and bones and relieve rheumatic condition. This plant is also claimed for its anti-diabetic effects and may have an antidepressant-like action (Soon & Tan, 2002; Zhang *et al.*, 2002).

Zhang and co-workers (2009) reported the polysaccharides from the roots of *M. officinalis* were found to have significant anti-fatigue activity by using mice weight-loaded swimming model. The activity may be related to the anti-stress and enhancing immunity effects of *M. officinalis*. The anti-fatigue activity of the polysaccharides may partially explained the tonic property in traditional medicine, which provided scientific evidence for traditional medicine and further development of medicinal products for prevention and treatment of diseases related to chronic fatigue syndromes.

Five anthraquinones including alizarin-1-methylether, 1,2-dimethoxy-3 hydroxyanthraquinone, 1-hydroxy-3-hydroxymethylantraquinone, rubiadin-1-methylether and anthragallol-2-methylether were isolated from the dried roots of *M. officinalis* How (Zhu *et al.*, 2009). The structures of the isolated compounds are shown in Figure 1.3.
22. alizarin-1-methylether  
23. 1,2-dimethoxy-3 hydroxyanthraquinone

24. 1- hydroxy-3-hydroxymethylantraquinone  
25. rubiadin-1-methylether

26. anthragallol-2-methylether

**Figure 1.3** Compounds isolated from the dried roots of *M. officinalis* How
1.2.4 *Morinda lucida*

*Morinda lucida* Benth., is a tropical West Africa rainforest tree also called Brimstone tree. Different parts of the plant are attributed with diverse therapeutic benefits. For example, in Southern Cameroon, cold decoction of the plant leaves is used for the treatment of fever. However, in most parts of West Africa, the bitter water decoction of the plant bark, root and leaf are used as bitter tonic and as astringent for dysentery, abdominal colic and intestinal worm infestation (Adeneye & Agbaje, 2008).

Raji *et al.* (2005) investigated the effect of *M. lucida* on the reproductive activity of male albino rats. *M. lucida* leaf extract did not cause any changes in body and somatic organ weights, but significantly increased the testis weight (*P* < 0.05). The sperm motility and viability, and the epididymal sperm counts of rats treated for 13 weeks were significantly reduced (*P* < 0.05). Sperm morphological abnormalities and serum testosterone levels were significantly increased (*P* < 0.05). There were various degrees of damage to the seminiferous tubules. The extract reduced the fertility of the treated rats by reducing the litter size.

The antibacterial activity of *M. lucida* was investigated against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* using the Kirby–Bauer agar diffusion method. The bark methanolic extract of *M. lucida* inhibited the growth of the above mentioned
bacteria with the MIC values below 20 mg/mL. Phytochemical screening was performed on the plant extract and the tests suggested the presence of saponins, flavonoids, alkaloids, terpenoids, tannins and anthraquinones in the plant (Gbedema et al., 2010).

The chemical constituents isolated from *M. lucida* were reported by Adesogan (1973). The structures of these chemical constituents are shown in Figure 1.4.
Figure 1.4 Compounds isolated from *Morinda lucida* Benth.
33. rubiadin-1-methylether

34. 2-methyl-anthraquinone

35. 1-methoxy-2-methyl-anthraquinone

36. hexacosanoic acid

Figure 1.4 (continued) Compounds isolated from *Morinda lucida* Benth.
1.2.5 *Morinda angustifolia*

*Morinda angustifolia* Roxb, is a resourceful perennial undershrub, and widely distributed in the southwestern mountainous areas of China, and nearby countries, such as Burma, Laos, Thailand and India. The major usage of the plant is to make yellow fabric dye stuff (Xiang *et al*., 2008).

Bhuyan and Saikia (2005) reported that the isolated morindone from the root benzene extract of *M. angustifolia* was the colour component responsible for dyeing. Dyes derived from natural sources have emerged as important alternatives to synthetic dyes, which have been reported to have carcinogenic effects. Recently with the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has once again gained interest.

Xiang and co-workers (2008) studied the antimicrobial activity of *M. angustifolia*. Six isolated compounds (1,8-dihydroxy-2-methyl-3,7- dimethoxyanthraquinone, lucidin 3-O-β-primeveroside, 1,3-dihydroxy-2-methylantraquinone, lucidin-ω-ethyl ether, lucidin-ω-butyl ether and damnacanthol) were tested for their antimicrobial potential against *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Sarcina lutea*, *Staphylococcus aureus, Aspergillus niger, Candida albicans* and *Saccharomyces sake*. Among the compounds, 1,8-dihydroxy-2-methyl-3,7-dimethoxy anthraquinone demonstrated the most significant antimicrobial activity against *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Sarcina lutea,*
Candida albicans and Saccharomyces sake. The structures of the isolated compounds are shown in Figure 1.5.

Figure 1.5 Structures of the isolated compounds from the roots of M. angustifolia
1.3 Plant *Morinda citrifolia* Linn.

1.3.1 Plant taxonomy

Kingdom: Plantae

Sub kingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliopsida

Class: Magnoliopsidae

Subclass: Asteridae

Order: Rubiales

Family: Rubiaceae

Genus: *Morinda*

Species: *Morinda citrifolia*

Common name: Noni (Hawaii or island of Polinesia) or mengkudu (Malaysia)

Figure 1.6 The fruit of *Morinda citrifolia* (with flowers)

Figure 1.7 Young fruit of *Morina citrifolia*
1.3.2 Plant morphology

*Morinda citrifolia* is a bush or small tree, 3–10 m tall, with abundant wide elliptical leaves (5–17 cm length, 10–40 cm width). The small tubular white flowers are grouped together and inserted on the peduncle. The petioles leave ring-like marks on the stalks and the corolla is greenish white (Chan-Blanco et al., 2006).

*M. citrifolia* fruit (Figure 1.6 and Figure 1.7) is oval in shape and will turn from a greenish to a yellowish-white color when it ripens. It has a bitter taste and exhales a strong butyric acid-like pungent smell. The seeds have a distinct air chamber, and can retain viability even after floating in water for months (Nelson, 2003). The roots and inner bark may have little coloration or may range from bright yellow to red.

1.3.3 Plant habitat and distribution

The plant habitat ranges from tropical rainforest to dry lowland plains, from the coast to elevated inland sites. It can tolerate exposed sites as well as the relatively thin and infertile soils. The plant is present worldwide predominantly in tropical countries. *M. citrifolia* occurs in Africa, Australia, Barbados, Cambodia, Caribbean, Cayman Islands, Cuba, Dominican Republic, El Salvador, Fiji, Florida, French West Indies, Guadeloupe, Guam, Haiti, Hawaii, India, Jamaica, Java, Laos, Malaysia, Marquesas Islands, Philippines, Polynesia, Puerto Rico, Raratonga,
Samoa, Seychelles, Solomon Islands, Southeast Asia, St. Croix, Surinam, Tahiti, Thailand, Tonga, Trinida and Tobago and Vietnam (Mathivanan et al., 2005).

1.3.4 Uses in traditional medicine

*M. citrifolia* has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, analgesic, hypotensive, anti-inflammatory, antidiabetic and immune enhancing effects (Wang et al., 2002).

Dixon et al. (1999) and Morton (1992) reported the fruit of *M. citrifolia* was traditionally used to treat gum disorder, tuberculosis, anthelmintic and as purgative. The fruit pulp can be used as an insecticidal shampoo. The flower was applied to treat sore eye. The leaves of the plant was used to treat wounds, ulcers, ringworm, rheumatic pain, inflammation, liver diseases, internal bleeding, abdominal swollen, fever, headache, cough and cold. The bark was used to treat malaria and finally the root was consumed to cure hypertension.

Nelson (2003) reported the teas from the leaves were used as treatment for malaria and analgesic in Africa. All parts of the plants are useful laxative. Decoctions of the stem bark are consumed to treat jaundice and the extract of the leaves is for hypertension. Other usages such as to treat sprains, deep bruising, toothaches, fractures, diabetes, loss of appetite, urinary tract ailments, abdominal swelling,
hernias, stings from stonefish and human vitamin A deficiency were also reported by the author.

1.3.5 Literature survey

Yong et al. (2006) studied the free-radical-scavenging activity of *M. citrifolia* juice and powder in processing and storage. The authors proposed the fresh juice *M. citrifolia* possessed free-radical-scavenging activity (RSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), at 140 mg equivalent ascorbic acid/100 mL and total phenols at 210 mg gallic acid/100 mL. Fermentation of the fruit for 3 months resulted in a loss of more than 90% of RSA. Dehydration at 50 °C produced a loss of 20% of RSA. Storage of fresh fruit juice at 24°C for 3 months reduced RSA more than 90%. Storage of fruit juice or powder at -18°C and 4°C for 3 months decreased RSA by 10–55%. For maintenance of the substantial antioxidant properties of *M. citrifolia* fruit products, processing of fruit powder or fresh frozen fruit juice rather than fermented fruit juice is recommended.

Pawlus et al. (2005) isolated a new anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone from the fruit of *M. citrifolia*. The compound was reported to have potent quinone reductase (QR) induction activity, which is 40 times more potent than a positive control, L-sulforaphane. Furthermore, this compound demonstrated no discernible cytotoxicity at the highest dose tested. QR is a phase II metabolizing enzyme. The induction of phase II enzymes is
considered cancer chemopreventive in that potential oxidative and electrophilic molecules can be more readily metabolized and excreted before they can interact with cellular macromolecules such as DNA. QR is also responsible for maintaining the reduced states of antioxidants such as R-tocopherol and coenzyme Q10. Hence, QR inducers are sometimes referred to as “indirect antioxidants”, and this activity is considered protective at the initiation stage of carcinogenesis. The chemical structures of 2-methoxy-1,3,6-trihydroxyanthraquinone together with a few known isolated compounds were shown in Figure 1.8.

Taskin et al. (2009) investigated the apoptosis-inducing effects of M. citrifolia and doxorubicin on the Ehrlich ascites tumor in Balb-c mice and also combined it with a potent anti-cancer agent, doxorubicin. The first group of animal received M. citrifolia fruit only, the second group of animal received doxorubicin, and the third group of animal received both M. citrifolia fruit and doxorubicin for 14 days after the inoculation of cells. The control group received 0.9% NaCl only. The result found that short and long diameters of the tumor tissues were about 40–50% smaller and the proliferation was decreased, compared to those in control group. This anti-growth effect resulted from the induction of apoptosis. The authors concluded M. citrifolia fruit may be useful in the treatment of breast cancer either on its own or in combination with doxorubicin.
Figure 1.8 Isolated compounds by Pawlus et al. (2005)