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Literature Review

A REVIEW ON THE CHEMISTRY AND PHARMACOLOGY OF *Rennellia elliptica* KORTH

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ABSTRACT

Rennellia elliptica, popularly dubbed as Malaysian Ginseng, is widely used in traditional medicine among the local Jakun community in Endau-Rompin State Park, Pahang, Malaysia. The decoction of the roots is traditionally taken for treatment of body aches, as postpartum tonic, as aphrodisiac and for the treatment of jaundice. In the effort of searching new botanical drugs and drug candidates from tropical rainforest, the team from this laboratory had conducted a sizeable phytochemical and biological screening program of tropical plant at Endau Rompin State Park, Pahang with the help from the indigenous people. R. elliptica showed strong antiplasmodial activity in vitro with the IC_{50} value of 4.04µg/mL. The comprehensive study on the root extract of R. elliptica in this laboratory yielded seventeen compounds from four different classes, including 2 new pyranoanthraquinones, one new anthraquinone, eleven known anthraquinones, one lactone triterpenoid, one coumarin and one phenolic acid. The chemical profile of the root extract was established using HPLC and the selected marker compounds were used as external standards and quantified using standard calibration curve. Nordamnacanthal **5**, *damnacanthal* **7**, *2-formyl-3-hydroxy-9,10-anthraquinone* **6**, *2-methyl-3-hydroxy-9,10-anthraquinone* **11** *and 1,2-dimethoxy-6-methyl-9,10-anthraquinone* **3** *were determined at 3.57, 10.32, 4.47, 12.18 and 4.09 µg/g, respectively. Owing to the toxicity of dichloromethane, the extraction of the desired marker compounds was attempted using accelerated solvent extraction and soxhlet extraction using ethanol and water at different compositions. R. elliptica root extract and the isolated anthraquinones showed potential antiplasmodial activity, and the active compounds were probed for their mode of action. In addition, the dichloromethane root extract of R. elliptica and the selected anthraquinones were screened for anticancer, antioxidant, and α-glucosidase inhibitory activities as well as toxicity study in vitro. The review summarizes the findings on Rennellia elliptica which includes phytochemistry, toxicity and its biological activities. The chemotaxonomic significance of Rennellia elliptica is also discussed.*

Keywords: *Rennellia elliptica, anthraquinone, Rubiaceae, malaria, antiplasmodial*

INTRODUCTION

Malaysia is the 12th most biodiversed nation in the world[1] and is mainly covered by tropical rainforests. It was reported in 1953, there are about 550 genera of tropical plants containing over 1300 species possessing medicinal values in Peninsular Malaysia alone.[2] Tropical rainforests are rich source of flora and fauna, though they only cover 12% of earth's land area, tropical rainforests are the home of 50–90% of world species. At least 25% of all modern drugs were discovered from rainforests even though less than 1% of tropical rainforests in the world are investigated for pharmacologically active metabolites.[3] The great biodiversity of Malaysian flora provides an immense source of chemically diverse bioactive metabolites for new lead candidates.

Malaysia is blessed with plethora of tropical plant species as well as indigenous knowledge on the traditional uses of medicinal plants. The uses of exotic tropical plants in traditional medicine are mostly confined within the local communities especially in the remote areas and are lost when the elders pass on. The destruction of tropical rainforests threatens the survival of tropical plants and without proper documentation and study, the knowledge of the traditional uses of these plants will be lost forever. Thus, extensive phytochemical and biological assessments of our plant

species are of utmost importance to preserve the knowledge of our natural heritage for the next generation.

In the effort for searching new botanical herbs and new drug candidates, large random plant collection program have been initiated by the Malaysian government through various forms of funding at local research institutions and universities. Various biodiversity centers have been established such as Sarawak Biodiversity Centre and Pahang Biodiversity Institute to conserve the flora and fauna as well as to promote research on the biological, pharmaceutical, medicinal and other applications of tropical rainforests. In conjunction with the national effort, the team from this laboratory had conducted a sizeable phytochemical and biological screening program of tropical plant at Endau Rompin State Park, Pahang with the help from the indigenous people in the search for new potential medicinal plants. *Rennellia elliptica* was one of the most promising plants, and phytochemical and biological studies were carried out to assess its potential as new botanical herbs.

Rennellia elliptica Korth. is a tropical shrub of about 1-2 m tall and can be found in lowland to hill forest to c. 500m above sea level. *R. elliptica* is locally known as 'mengkudu rimba' or 'segemuk' and popularly dubbed as Malaysian ginseng probably due to the appearance of its yellow roots. Among various Malaysian ethnics, this plant is also known as 'kayu penawar apow' (Dusun), 'mengkudu hutan' (Iban), 'akar bumi', 'urap gondor' (Sakai), 'mengkudu gajah', 'lempedu tanah' and 'sekemang' (Jah Hut, Semelai). *R. elliptica* is native to South East Asia and widely distributed in Peninsular Malaysia, Southern Thailand, Borneo and Indonesia.[4] The decoction of *Rennellia elliptica* is traditionally taken for the treatment of jaundice[5] and body aches, as postpartum tonic and as aphrodisiac.[6] During the random screening of selected Malaysian tropical plants for antiplasmodial activity, *R. elliptica* showed promising activity (4.04 µg/ml) which warranted further investigation. Following the screening program, extensive phytochemical study was carried out on the root extract yielding 17 compounds from four different classes in which four of them were found to possess strong antiplasmodial activity with IC_{50} values of less than 1 µM.[7] In order to establish the use of *R. elliptica* root extract of as potential herbal drug for the treatment of malaria, optimization of extraction methods, qualitative and quantitative HPLC analyses of the extract as well as the investigation of the extract toxicity and possible mechanism of actions were warranted. The chemotherapeutic targets selected were inhibition against β-hematin formation *via* lipids and HRP2 catalyses. The anticancer, antioxidant, and antidiabetic activities of the crude extract and selected chemical compounds were also discussed.

TAXONOMY OF *Rennellia elliptica*

R.elliptica Korth. was also previously known as *R. elongata* (King & Gamble) Ridl. Recent phylogeny study revealed that *R. elliptica* and *R. elongata* are different species and are not synonym.[8] This plant is a shrub of about 1-2 m tall. The leaf is somewhat narrow and rounded apex with tapering ends. It has leathery surface and slightly waxy with greyish-blue bloom below. The veins are purplish in colour when fresh. The inflorescences are terminal, consisting of head of flowers arranged along a rachis. The flower is violet in colour while the fruit is subglobose and unstalked.[4,9] This shrub can be found in lowland to hill forest to c. 500m above sea level. *R. elliptica* is widely distributed from Southern Myanmar to West Malaysia. The pictures of several parts of *R. elliptica* are shown in Figure 1.

PHYTOCHEMISTRY OF *Rennellia elliptica* Korth

Chemical Constituents Isolated from The Root Extract of *R. elliptica*

The air-dried powdered roots of *R. elliptica* were extracted successively with *n*-hexane, dichloromethane and methanol and dried giving brown coloured crude extract (27g). The dichloromethane root extract was fractionated using column chromatography (60 cm x 5 cm) eluted with

Figure 1. *Rennellia elliptica* Korth

various compositions of solvents of increasing polarity (*n*-hexane-dichloromethane, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 100 CH_2Cl_2 v/v; dichloromethane-methanol, 99:1, 95:5, 9:1 v/v) to give six fractions. Purification using repeated column chromatography and preparative column chromatography yielded compounds **1-14,** and **18.** [7,10]

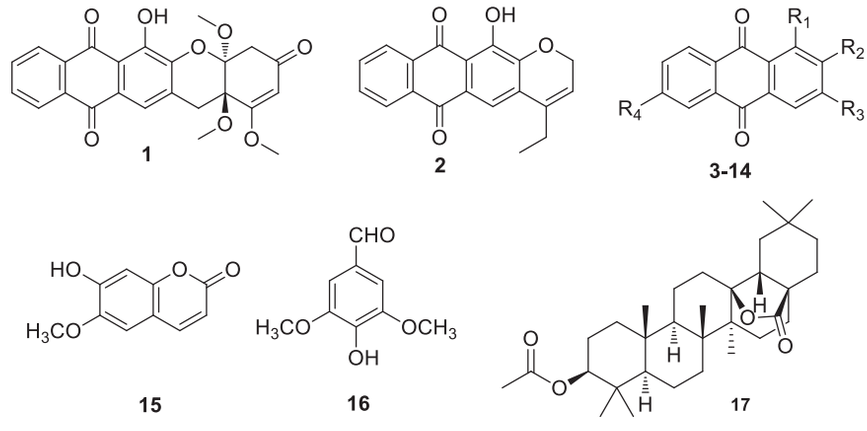
Subjecting the dichloromethane root extract (3g) to second fractionation using MPLC packed with lichroprep (RP-18, 40-63µm) eluted with stepwise gradient (water: aceonitrile, 100% water, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 100% acetonitrile) using about 100 ml solvent for each solvent system to give nine fractions. Fraction B (2-3) was further fractionated using radial chromatography (2 mm, F_{254}, 40-60 mesh) eluted isocratically using dichloromethane to give 31 fractions. Fractions $B2_{7-11}$ was purified using semi-preparative HPLC [Sunfire, C-18 column (250 mm x 5µm x 10 mm i.d.); water: acetonitrile (4:6→100% acetonitrile); flow rate 4.73 ml/min in 60 minutes; formic acid (0.01%) was added to mobile phase] to give compound **14** (1.5 mg), **16** and **17**.

The purification of the dichloromethane root extract yielded two new pyranoanthraquinones, one new anthraquinone, and eleven known anthraquinones along with a coumarin, a phenolic compound and a lactone triterpenoid from *R. elliptica.*[7,10] Their structures were elucidated as rennellianone A **1** and rennellianone B **2**, 1,2-dimethoxy-6-methyl-9,10-anthraquinone **3**, nordamnacanthal **4**, 2-formyl-3-hydroxy-9,10-anthraquinone **5**, damnacanthal **6,** 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone **7**, lucidin-ω-methyl ether **8**, 3-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone **9**, rubiadin **10**, 3-hydroxy-2-methyl-9,10-anthraquinone **11**, rubiadin-1-methyl ether **12**, 3-hydroxy-2-hydroxymethyl-9,10-anthraquinone **13,** alizarin-1-methyl ether **14**, scopoletin **15**, 4-hydroxy-3,5-dimethoxybenzaldehyde **16** and 3b-acetateoleanan-13b, 28-lactone **17.**[11-14] The triterpenoid lactone is reported for the first time from the family Rubiaceae. Figure 2 illustrates the chemical structure of compounds isolated from the root of *R. elliptica*.

Chemical Profiling of *R. elliptica* Root Extract using High Performance Liquid Chromatography

Preparation of standardized extract is an authentication of herbal preparation as means of controlling the quality of plant material used for product manufacturing. The standardized extract should have an acceptable content of bioactive metabolites and safe from toxic impurities.[15]

The dichloromethane root extract showed promising antiplasmodial and antioxidant activities. Thus, the dichloromethane extract was profiled over Waters 600 HPLC on the Sunfire column (C-18, 250 mm 5µm x 4.6 mm i.d.) to establish the chemical profile of the root extract. Several combinations of mobile phases were attempted in order to obtain good chromatographic profile. The best



	R_1	R_2	R_3	R_4
3	OCH_3	OCH_3	H	CH_3
4	OH	OCH_3	H	CH_3
5	OH	CHO	OH	H
6	H	CHO	OH	H
7	OCH_3	CHO	OH	H
8	OH	CH_2OCH_3	OH	H
9	H	OCH_3	OH	CH_3
10	OH	CH_3	OH	H
11	H	CH_3	OH	H
12	OCH_3	CH_3	OH	H
13	H	CH_2OH	OH	H
14	OCH_3	OH	H	H

Figure 2. Chemical contituents isolated from the roots of *Rennellia elliptica* Korth.

chromatogram was achieved with combinations of water (solvent A) and acetonitrile (solvent B) buffered with 0.1 % formic acid (FA). The mobile phase was programmed consecutively in a linear gradient as follows: 0-20 min, 60-35 % A; 21-40 min, 35-5 % A; 41-45 min, 5–0 % A; 46-60 min, 0 % A at a flow rate of 1.0 mL/min. The chromatogram of dichloromethane extract is given in Figure 2.

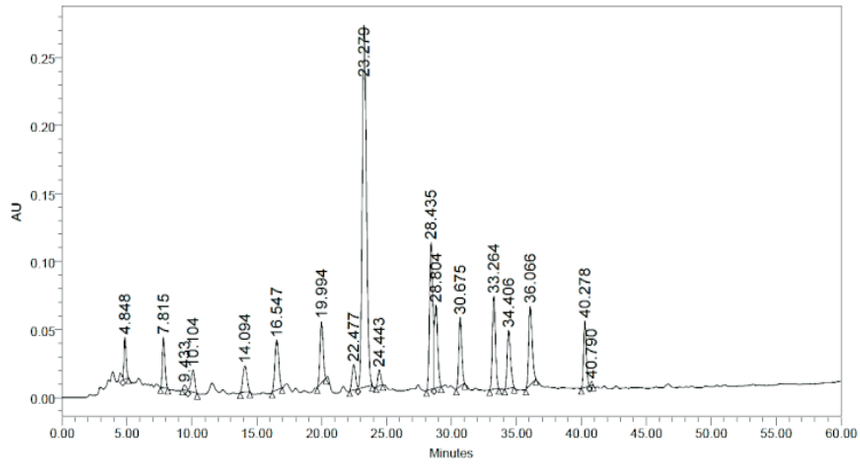
Ten chemical constituents purified using conventional chromatographic techniques were used as external standard for qualitative peaks identification in the chromatogram. The known constituents were used as external standards to qualitatively distinguish the known constituents from unknown constituents. Four compounds, 3-hydroxy-2-hydroxymethyl-9,10-anthraquinone **13**, rubiadin-1-methyl ether **12**, rennellianone A **1** and Rennellianone B **2** were not analyzed as standards due to limited compound availability. Thus the unidentified peaks may belong to these metabolites.

The study also included the establishment of the plant metabolites chemical profile of root extract using HPLC analyses. Nordamnacanthal **5**, damnacanthal **7**, 2-formyl-3-hydroxy-9,10-anthraquinone **6**, 2-methyl-3-hydroxy-9,10-anthraquinone **11** and 1,2-dimethoxy-6-methyl-9,10-anthraquinone **3** were selected as marker compounds due to their potent antiplasmodial activity [7]. In order to determine the composition of each biomarker in the root extract, external calibration curves were constructed using five point concentrations. The concentration of compounds **5**, 7, **6**, **11**, and **3** were determined at 3.57, 10.32, 4.47, 12.18 and 4.09 µg/g, respectively, with acceptable standard deviation (SD < 0.2) and coefficient of variance (CV<5%). It was evident from the chromatogram (Figure 3), the marker anthraquinones present as major compounds in the root extract, thus it is submitted that the antiplasmodial action of the root extract is potentially due to the action of these metabolites.

The potential antiplasmodial agents, 2-formyl-3-hydroxy-9,10-anthraquinone **6**, nordamanacanthal **5,** damnacanthal **7**, and 2-methyl-3-hydroxy-9,10-anthraquinone **11** were the major constituents in the dichloromethane root extract. Thus, it is postulated that the antiplasmodial action of the root extract is potentially due to the action of these metabolites. These metabolites can be used as biomarkers for standardization of the root extract as antiplasmodial agent.

Optimization of Extraction

Dichloromethane root extract of *R. elliptica* showed promising antiplasmodial activity, however, dichloromethane is not a suitable extraction solvent for herbal preparation owing to the toxic properties of the solvent. Thus, the extraction of the dried root powder was attempted using ethanol and water in cold extraction, Soxhlet and accelerated solvent extraction (ASE). Dichloromethane root extract was also prepared as a control to compare the presence of selected marker compounds. The extracts obtained from soxhlet and ASE extractions were then analyzed for the presence of selected biomarkers using Waters HPLC system. The accelerated solvent extraction (20: 80, Water: Ethanol; 100°C) gave the comparable amount and quality of marker anthraquinones in the root extract as compared to dichloromethane root extract (Table 1). The use of ethanol in cold and soxhlet extraction did not successfully extract the desired biomarkers compounds. The



*The chromatogram was extracted at 276 nm.
Note: Rubiadin (4.848), Alizarin-1-methyl ether (7.815), 2-Hydroxy-3-methoxy-6-methyl-9,10anthraquinone (22.477), 1-Hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (23.279), 3-Hydroxy-2-methyl-9,10-anthraquinone (24.443), 2-formyl-3-hydroxy-9,10-anthraquinone (28.804), Damnacanthal (28.435), Lucidin-ω-methyl ether (34.406), 1,2-dimethoxy-6-methyl-9,10-anthraquinone (36.066), Nordamna-canthal (40.278). The unknown peaks at 10.104, 14.094, 16.547, 19.994 and 30.676 could be due to as rennellianone A and rennellianone B, scopoletin, 4-hydroxy-3,5-dimethoxybenzaldehyde and 3b-acetateoleanan-13b, 28-lactone [10].
Source: Osman et al. (2017)

Figure 3. HPLC chromatogram of dichloromethane extract of *R. elliptica* Korth.

ASE can reduce the polarity of water and ethanol because high pressure and temperature will reduce the dielectric constant of water, which lowers its polarity and assists the extraction of more non-polar compounds.[16, 17]

BIOLOGICAL ACTIVITY of *Rennellia elliptica* Korth

Antiplasmodial Activity

The methanol and dichloromethane root extracts were screened against chloroquine sensitive *P. falciparum* (3D7). The methanol root extract displayed a stronger antiplasmodial activity (IC_{50}=0.73 µg/ml) compared to the dichloromethane root extract (IC_{50} = 4.04 µg/ml). Crude extracts with IC_{50} values of more than 50 µg/ml are considered effective as antiplasmodial agents.[18] The percent inhibitions and IC_{50} values of root extracts of *R. elliptica* against *P. falciparum* are tabulated in Table 2.

The screening of dichloromethane and methanol root extracts for antiplasmodial activity *in vitro* showed promising activity. Thus, the antiplasmodial activity of the extracts was further evaluated using rodent malaria, *P. berghei* (ANKA strain) in animal model. The dichloromethane root extract displayed stronger activity than the methanol root extract with an ED_{50} value of 1.23 µg/ml. Methanol root extract also showed strong antiplasmodial activity with ED_{50} value of 27.57 µg/ml BW. The weaker activity of the methanol root extract was most potentially due to degradation of principle bioactive metabolites in the digestive tract.

Anthraquinones isolated from the root of *R. elliptica* were screened for antiplasmodial activity based on the promising screening results of the dichloromethane root extract (IC_{50} = 4.04 µg/mL). The *in vitro* antiplasmodial activity of anthraquinones isolated from *R. elliptica* against chloroquine sensitive strain of *P. falciparum* (3D7) is shown in Table 3. Compound **11** displayed the strongest inhibition activity, with an IC_{50} value of 0.34 µM, followed by compound **6** with an IC_{50} value of 0.63 µM. Sittie et al. (1999) established that an aldehyde group at C-2 and a phenolic hydroxy group at C-3 on the anthraquinone skeleton enhance the activity of anthraquinones against the growth of *P. falciparum*. These results showed that a methyl group at C-2 together with a phenolic hydroxy group at C-3 as in compound **11** also gave significant activity. It should also be noted that both compounds **6** and **11** do not possess hydroxyl substituents at the *peri* positions. The new anthraquinone **3** also exhibited strong inhibition, with an IC_{50} value of 1.1 µM. Interestingly, anthraquinone **4**, which structurally differs only at C-1 (hydroxyl substituent instead of methoxyl substituent) did not show any significant activity. The position of substituents on the anthraquinone skeleton clearly influences the antiplasmodial activity, which warrants further investigation.

One of the principle metabolites, compound **6** was also reported from the root extract of *Morinda lucida* Benth., an African medicinal plant widely used to treat malaria. Many chemical constituents present in *R. elliptica* were also reported in *Morinda lucida*. There is undocumented claim that *R. elliptica* is also taken from indigenous people to treat fever. Thus, the data might support the traditional application of this plant.

One of the important chemotheraupeutic targets in combating malaria infection is its food vacuole. The malaria parasite digests erythrocytes and releases heme[19] along with oxygen.[20] Free heme is toxic owing to its detergent-like properties that destabilizes and lyses membranes.[21,22] as well as inhibits the activity of several enzymes such as cysteine proteases[22] and consequently leads to the death of

Table 1. Optimization of extraction of root extract of *Rennellia elliptica*

Type of Extraction	Solvent/Condition	%Yield
Cold extraction (10g)	Dichloromethane (3 days)	0.97
	Ethanol (3 days)	2.07
Soxhlet Extraction (10 g)	Dichloromethane (2 hours)	0.58
	Ethanol (2 hours)	2.28
Accelerated solvent extraction (1g)	100: 0 (H2O:EtOH), 60oC, 10 min	0.55
	50: 50 (H2O:EtOH), 60oC, 10 min	2.43
	20: 80 (H2O:EtOH), 60oC, 10 min	3.03
	20: 80 (H2O:EtOH), 80oC, 10 min	0.52
	20: 80 (H2O:EtOH), 100oC, 10 min	0.3
	20: 80 (H2O:EtOH), 140oC, 10 min	0.5

Source: Osman et al. (2017)

Table 2. Inhibition against *Plasmodium faciparum* (3D7) growth *in vitro*

Sample	% Inhibition at Different Dosage (µg/mL)					IC50 (µg/mL)
	100	10	1	0.1	0.01	
MeOH extract	100.00	59.18	54.66	23.84	21.78	0.73
DCM extract	92.02	62.32	20.87	9.10	5.60	4.04

Table 3. Antimalarial activity of anthraquinones from *R. elliptica* Korth.

Compounds	R1	R2	R3	R4	IC50 (µM)
3	OCH_3	OCH_3	H	CH_3	1.10
4	OH	OCH_3	H	CH_3	na†
5	OH	CHO	OH	H	72.46
6	H	CHO	OH	H	0.63
7	OCH_3	CHO	OH	H	51.28
8	OH	CH_2OCH_3	OH	H	2.10
9	H	OH	OCH_3	CH_3	nt‡
10	OH	CH_3	OH	H	na†
11	H	CH_3	OH	H	0.34
12	OCH_3	CH_3	OH	H	na†
13	H	CH_2OH	OH	H	nt‡
Chloroquine Diphosphate					6.30[a]

Each sample was tested in duplicate; The IC_{50} values were obtained from average values of percent inhibition within a series of concentration; Notes: na† –no activity; nt‡ – not tested; [a] unit in nM.

the parasite. The mechanism of heme detoxification can be broadly classified into two types; primarily *via* dimerization into hemozoin and secondarily *via* degradation of heme by gluthatione and hydrogen peroxide.[23] Histidine-rich protein II (HRP2)[20,22] and lipids[23] are proposed to catalyze the reaction but there are other evidences that the hemozoin formation may be spontaneous[24] and autocatalytic.[25] Drugs such as quinine and chloroquine which targeted the prevention of β-hematin formation have a longer lifespan of effective use against malarial parasite. The parasite seems to have difficulties in finding alternative processes for haemoglobin utilization and heme detoxification as compared to other chemotherapeutic targets.[26]

In this study, the biomarkers were probed for their possible mode of action against β-hematin formation. Nordamnacanthal **5** and damnacanthal **7** showed significant inhibition against hemozoin formation via HRP2 and lipids catalyses (Table 4). It is interesting to note that the Nordamnacanthal **5** and damnacanthal **7** showed weaker activity when tested against *Plasmodium falciparum* (3D7 strain) *in vitro* as compared to 2-formyl-3-hydroxy-9,10-anthraquinone **6** and 2-methyl-3-hydroxy-9,10-anthraquinone **11**.[7] 2-Formyl-3-hydroxy-9,10-anthraquinone **6** and 2-methyl-3-hydroxy-9,10-anthraquinone **11** showed the strongest antiplasmodial activity *in vitro* and their mode of action are yet to be discovered.

Toxicity Study

The toxicity study was carried out to determine the selectivity of the dichloromethane root extract and marker compounds against the hepatocyte cell. The dichloromethane root extract showed mild toxicity with CC_{50} value of 318.0 µg/ml (Table 4). For both *in vitro* and *in vivo* studies, the selectivity indexes were determined at 78.7 and 258.3, respectively. The selected biomarkers showed no toxicity except 2-formyl-3-hydroxy-9,10-anthraquinone, nordamnacanthal and damnacanthal which showed moderate toxicity with CC_{50} values of 181.34, 908.96 and 338.65 µM, respectively, with moderate selectivity index (Table 4).

Anticancer Activity

Anthraquinones are known chromophore for anticancer. They act mainly *via* DNA intercalation[27] and induce lipid peroxidation *via* free radical chain reaction and consequently induce oxidative stress on cancerous cells.[28] Oxidative stress can cause permanent modification of genetic material[29] which represents the first step involved in mutagenesis, carcinogenesis and various disorders such as Alzheimer, Hungtinton's disease, diabetes, and Parkinson.[30,31]

In the previous report, nordamnacanthal was found to enhance cytotoxic effect of tamoxifen in treating human

Table 4. The toxicity, β-hematin and HRP2 assays of the root extract and selected compounds.

AQ	Toxicity CC_{50} µM	Antiplasmodial in vitro IC_{50} µM	Selectivity Index	β-hematin IC_{50} µM	HRP2 IC_{50} µM
3	>3546.1	1.1	3225	na	na
5	908.96	72.46	12.5	67.16 ± 0.2	4.37
6	181.34	0.63	285.6	158.73 ± 0.2	nt
7	338.65	51.28	6.6	5.32 ± 0.2	11.77
11	>3968.3	0.34	12,500	138.65+-0.1	nt
Root	318.0†	4.04†	78.7	nt	nt

nt- not tested; na- no activity
† - unit µg/ml

breast cancer MCF7.[32] Damnacanthal **6** enhanced the expression of p21 and caspase-7 subsequently increased apoptosis in human breast cancer MCF7 cell.[33] In the present study, only three other major compounds screened for cytotoxic activities using MCF7 and 4T1 cell lines (Table 5).

The dichloromethane root extract did not show cytotoxic effect against the MCF7 and 4T1 cancer cell lines. The presence of known anticancer against MCF7 and 4T1 cell lines, nordamnacnathal **4** and damnacanthal **6** as major compounds in the root extracts of *R. elliptica* does not contribute its cytotoxicity. 2-Formyl-3-hydroxy-9,10 anthraquinones **5** and 2-methyl-3-hydroxy-9,10-anthraquinones **11** showed moderate cytotoxic activity against human breast cancer MCF7 cell lines. When tested against 4T1 cancer cell, only 2-formyl-3-hydroxy-9,10-anthraquinones **5** showed moderate activities.

Dichloromethane root extract did not show cytotoxicity against 3T3 cell lines when screened at 30 µg/ml. The major compounds from *R. elliptica* were also were also screened for cytotoxic activity against 3T3 cell lines at 30 µg/ml. 2-Formyl-3-hydroxy-9,10- anthraquinones **5** and damnacanthal **6** showed moderate activity with 74.15 % and 67.34%, respectively. Other compounds showed weak cytotoxicity. The cytotoxic activity of the selected anthraquinones was tabulated in Table 5.

Damnacanthal and nordamnacanthal are widely reported as anticancer agents and antioxidants, however their abundance presence in the root extract of *R. elliptica* do not contribute to the activity of the extract. The activity of the extract could be a result of synergism between matrices of other components and not on the major components alone.[34]

Antioxidant Activity

The roots extracts were tested for lipid peroxidation inhibition activity using FTC and TBA methods. FTC measures the primary product of lipid peroxidation while TBA method quatifies malondialdehyde (MDA), the secondary product of lipid peroxidation that is commonly found as marker in oxidative stress related diseases.[35] The dichloromethane root extract of *R. elliptica* showed stronger antioxidant activity than quercetin in both FTC and TBA assays with 93.4 % and 90.6 %, respectively.[36] The percent inhibitions of lipid peroxidation were calculated based on the final day of FTC assay when the absorbance of the control drops. The daily absorbance of FTC experiment is plotted in Figure 1. The major anthraquinones from *R. elliptica* such as nordamnacanthal **5**, damanacanthal **7**, rubiadin **10**, rubiadin-1-methyl ether **12** and lucidin-ω-methyl ether **8** were not tested because their antioxidant activities were widely reported.[37,38]

The radical scavenging assay was performed using DPPH radicals. The method was modified from reported literature[38,39] The absorbance values of samples were compared to quercetin as positive standard. The IC_{50} values of quercetin (~ 10-20 µM) were comparable with those reported in literature at same DPPH final concentration of 300 µM.[40,41] DPPH radical was purple in colour and upon reduction *via* hydrogen acceptance; the purple colour is bleached to yellow and pale yellow.[42] However, DPPH assay is often affected by colour of sample solution which lead to underestimation of actual radical scavenging activity.[43]

When tested for the radical scavenging activity against DPPH radicals, the methanol root extract of *R. elliptica* showed stronger activity than dichloromethane extract with IC_{50} values of 39.0 µg/ml and 250 µg/ml, respectively. Based on these observations, the dichloromethane extract showed antioxidative role by inhibiting lipid peroxidation and has potential as a preventive antioxidant. The lipid peroxidation inhibition could be due to the presence of nordamanacanthal[37] and damnacanthal[38] as major compounds in the root extract. On the other hand, the methanol extract may play antioxidative role by competitive

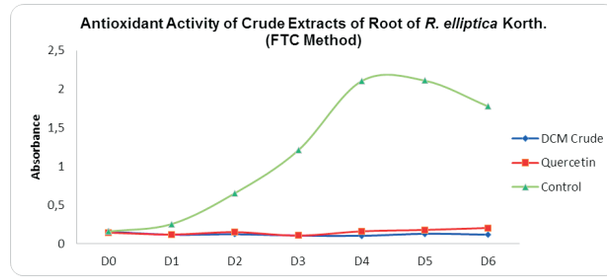


Figure 4. Daily UV absorbance of *R. ellptica* extracts in FTC assay.

Table 5. Cytotoxicity of *R. elliptica* using 3T3, 4T1 and MCF7 cell lines

Compounds	3T3 % Inhibition at 30 µg/ml	4T1 IC_{50} (µg/ml)			MCF IC_{50} (µg/ml)		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1	74.15	3.87	31.21	42.13	33.01	26.31	26.31
2	28.18		nt			nt	
3	67.34		nt			nt	
8	50.40	46.41	45.89	46.29	50.13	na	
10	42.48	50.34	na	44.44	38.70	na	
Dichloromethane extract	nt		na			na	

Each sample was tested in triplicate; na – no activity, nt – not tested

reaction in which antioxidant and substrate compete for radicals in biological system.

Several anthraquinones were screened for DPPH radical scavenging activity. The compounds were screened at the concentration of 100 µg/ml (Table 6). All anthraquinones isolated from the root extract were generally weak radical scavengers. These observations were consistent with the DPPH radical scavenging data reported by several authors[37,38] Anthraquinones showed weak radical scavenging activity probably due to the stability of anthraquinone radicals which could not form uncharged ions with other radicals.[44,45]

α-Glucosidase Activity

When screened for α-glucosidase inhibitory activity at 10 µg/ml, the dichloromethane root extract did not show any activity. The anthraquinones showed weak activity and the moderate activity was shown by 1,2-dimethoxy-6-methyl-9,10-anthraquinone 10 and damnacanthal 3 with 21.3 % and 19.9 %, respectively (Table 6).

There is no correlation observed between antioxidant and antidiabetic activities of anthraquinones from *R. elliptica*. Even though the extract is a good antioxidant, the result does not echo in antidiabetic assay.

CHEMOTAXONOMIC SIGNIFICANCE

The family Rubiaceae was divided into three subfamilies, 43 tribes and 637 genera with over 13000 plant species. At least 70 genera and 555 species of Rubiaceous plants were reported in Malaysia.[4] The genus *Rennellia* belongs to family Rubiaceae and subfamily Rubiodeae. *Rennellia* comprises of eight species which are native to South East Asia.[4,46] Five species of *Rennellia* are endemic to Peninsular Malaysia.[4] The genus *Rennellia* is characterized by cup-like stipule tube without ridges around the leaf stalk bases and the prominent secondary veins that loop at the leaf margin.[4] The inflorescence is often rather large, but always at the ends of the stems. The calyx tubes are joined together as in *Morinda*, but the head of flower, with only a few in each, set along a spike at the ends of the shoots.[47] *Rennellia* was initially placed in the tribe Morindeae on the basis of morphology[4] and phylogeny,[48] however recent molecular study[8,49] and wood anatomy study[49] support the new placement of *Rennellia* and *Prismatomeris* in the tribe Prismatomerideae. Both genera are distinguished from the tribe Morindeae by the occurrence of solitary vessels and the absence of axial parenchyma bands[49] which is exclusive to the tribe Prismatomerideae. Close investigation of the tribe Morindeae suggested close alliance between four genera, *Morinda*, *Prismatomeris*, *Rennellia* and *Motleya* despite the disputes over their tribal classification in the subfamily Rubiodeae.

To date, no phytochemical report on other species of the genus *Rennellia* has been recorded. This review highlights the chemotaxonomic significance between the genera *Prismatomeris*, *Morinda* and *Rennellia*. Most of the anthraquinones (**5**, **6**, **7**, **6**, **8**, **10**, **12**, **14**) identified from *R. elliptica* are common *Rubia* type anthraquinones that can be found in the genera *Morinda*[50-53] and *Prismatomeris.*[54-57] *Rubia* anthraquinones are characterized by substitution patterns on ring C only and substitutions on ring A are introduced at a later stage of biosynthetic pathways.[58] This finding affirms the close alliance between *Morinda*, *Prismatomeris* and *Rennellia* and support the placement of *Prismatomeris* and *Rennellia* in the tribe Prismatomerideae.

Anthraquinones from the genera *Prismatomeris* and *Morinda* are typically substituted at C-1, C-2, C-3 and C-1 and C-2. Compound **5**, **7**, **10**, and **12** which substituted at C-1, C-2 and C-3 are reported in almost all species from *Morinda* and *Prismatomeris*. These anthraquinones present as major constituents especially plants from the genus

Table 6. Radical scavenging activity of anthraquinones from *R. elliptica* at 100 µg/ml concentration

Compound	DPPH Percent Inhibition (%) at 100 µg/ml	α-Glucosidase Activity Percent Inhibition (%) at 10 µg/ml
5	2.87	9.4
6	3.26	6.6
11	3.99	6.8
7	9.18	19.9
3	1.30	21.3
10	4.87	3.6
12	nt	6.4
8	3.91	na
13	nt	na
4	nt	na
9	nt	na
Quercetin	15[a]	-

Each sample was tested in triplicate; the data was recorded as average percent inhibition at 100 µg/ml and 10 µg/ml. nt - not tested, na = no activity. [a]Unit in µM.

Source: Osman et al. (2017)

Morinda. The anthraquinones are also present abundantly in *R. elliptica*, however the presence of 2,3-disubstituted anthraquinones are more prevalent in this plant.

3-Hydroxy-2-methoxy-6-methyl-9,10-anthraquinone **9** was only reported from *Hedyotis diffusa* (Huang et al., 2008). In addition, the new anthraquinone, 1,2-dimethoxy-6-methyl-9,10-anthraquinone **3** and 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone **4** were also isolated and characterized from the root extract of *R. elliptica*. Anthraquinones **3**, **4** and **9** have a rare methyl substitution at C-6[7] differing from the anthraquinones of *Prismatomeris* and *Morinda* which are typically hydroxyl or methoxy substituted at C-6[58] and often followed by similar substitution at C-5. The presence of these anthraquinones could serve as taxonomic markers for *R. elliptica*.

SUMMARY

Phytochemical study on the root extract of *Rennellia elliptica* yielded 17 compounds from four different classes of natural products. The dichloromethane root extract showed strong antiplasmodial and antioxidant activities. The activities could be contributed by the presence of major compounds in the dichloromethane root extract. However, the dichloromethane root extract did not show significant anticancer activities against 4T1 and MCF breast cancer cell lines despite the major presence of nordamnacantahal and damnacanthal, the potent anticancer agents. The dichloromethane root extract showed mild toxicity will moderate selectivity against hepatocyte cell. The presence of *Rubia* type anthraquinones in *R. elliptica* affirms the close alliance between *Morinda*, *Prismatomeris* and *Rennellia* and support the placement of *Prismatomeris* and *Rennellia* in the tribe Prismatomerideae.

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