

## Article

# Phytochemicals from *Rhizophora mucronata* Propagules, Its *In Vitro* Anti-Cancer and *In Silico* Drug-Likeness Potential

Nurhanan Murni Yunos <sup>1,\*</sup>, Sui Kiong Ling <sup>2</sup>, Asiah Osman <sup>1</sup>, Zunoliza Abdullah <sup>2</sup> and Nor Jannah Sallehudin <sup>1</sup>

<sup>1</sup> Bioactivity Programme, Natural Products Division, Forest Research Institute Malaysia, Kepong 52109, Selangor, Malaysia; asiah@frim.gov.my (A.O.); norjannah@frim.gov.my (N.J.S.)

<sup>2</sup> Phytochemistry Programme, Natural Products Division, Forest Research Institute Malaysia, Kepong 52109, Selangor, Malaysia; lingsk@frim.gov.my (S.K.L.); zunoliza@frim.gov.my (Z.A.)

\* Correspondence: hanan@frim.gov.my; Tel.: +60-3-6279-7659

**Abstract:** This is the first report to identify the presence of 3-*O*-caffeoyl quinic acid (1), 4-*O*-caffeoyl quinic acid (2), 5-*O*-caffeoyl quinic acid (3), epi-catechin (4), and procyanidin B2 (5) in the young propagules of *Rhizophora mucronata*. Compounds 2–5 were purified and then treated against breast, colorectal, and ovarian cancer cell lines for 72 h and the results of the Sulphorhodamine-B (SRB) assay were evaluated for percent cell viability and IC<sub>50</sub> values. Epi-catechin, 4-*O*-caffeoyl quinic acid, 5-*O*-caffeoyl quinic acid and procyanidin B2 showed strong to moderate inhibitory effects when treated on breast (T47D), colorectal (HT29), and ovarian (A2780, SKOV3) cancer cell lines with IC<sub>50</sub> values ranging from 16.77 ± 0.58 to 28.28 ± 0.89 µg/mL. *In silico* evaluation was performed to evaluate the drug-likeness and toxicological effects of these compounds using Molinspiration calculation and OSIRIS program. It was found that compounds 2, 3, and 4 have the potential to be orally active and have a low risk in exerting the mutagenic, tumorigenic, irritant, and reproductive effects.

**Keywords:** *Rhizophora mucronata*; phenolic compounds; cancer cell lines; sulphorhodamine B assay; *in silico*



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## 1. Introduction

*Rhizophora mucronata* (Rhizophoraceae) is one of the mangrove plant species that can be found in the tropical and subtropical coastal areas and river estuaries from the east coast of Africa to Asia and Australia, and its hardy wood has been popularly used as fuel and charcoal [1]. The bark, leaves, and fruits consist of tannin [2] along with other phytochemicals that were later scientifically discovered. Mangrove plants are not only ecologically important in protecting the coastal area from soil erosion and strong tidal waves, but they also have various medicinal uses. The bark and leaves of *R. mucronata* are used to treat diarrhea, dysentery, fever, malaria, leprosy inflammation, and angina disorders [1,3]. The plant has also been reported to be used to treat tonsillitis, pharyngitis, hemorrhoids, and burns [4]. The bark is claimed to have astringent and anti-diabetic properties [2].

Secondary plant metabolites are known to be the main source for the development of new therapeutics. Alkaloids, triterpenoids, flavonoids, and steroid compounds have reportedly been isolated and characterized from various plant parts of *R. mucronata*. The leaves contain sterol compounds, namely sitosterol, campesterol, stigmasterol, and 28-isofucosterol and stigmast-7-en-3 $\beta$ -ol [5], 1-hydroxy-5-oxobicyclo [6.4.0] dodecane along with lupeol, betulin, and palmitic acid [6], alkaloids such as amalicine, vindoline, catharanthine, and serpentine [7], and luteolin [8]. The lupeol and betulin have been reported to have antimalarial and antiviral effects [9]. Other studies have shown the presence of endophytic fungi belonging to *Pestalotiopsis* sp. that grows on the leaves of *R. mucronata* and was identified by Hemberger et al. in 2013 [10]. Chemicals that were detected in

this fungus were novel hybrid sesquiterpene–cyclopaldic acid metabolites with an unusual carbon skeleton, named pestalotiopensA and B, and also phytotoxin altiloxinB. Two new coumarins ((*Z*)-apetatolide and mixture of isomers *E* and *Z* of new coumarin) and a new xanthone (2,4,5-trihydroxy-3-(2-hydroxy-3-methylbut-3-enyl)-xanthone), together with 14 known compounds that include eight coumarins (methoxyinophyllum P, calocoumarin B, calophyllolide, brasimarin C, inophyllum C, isocalophyllic acid, inophyllum E, and calophyllic acid), three xanthenes (6-deoxy-jacareubin, jacareubin, and 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)xanthone), a benzoic acid, and two flavonones (amentoflavone and naringenin) have been isolated from the methanol extract of the leaves of *R. mucronata* [11].

The ethanol and methanol extracts from the leaves of *R. mucronata* have been found to have anti-microbial properties in which these extracts inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* [12], while the ethyl acetate extract inhibited the growth of *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella typhi*, and *Bacillus subtilis* [13]. The leaves have also been reported to have anti-oxidative [14], antinociceptive [8,15], and anti-HIV activities [16]. A compound from the leaves of *R. mucronata* identified as quinizarin showed anti-microbial activity against *Bacillus cereus*, *Klebsiella aerogenes*, anti-oxidant activity via the DPPH radical scavenging assay, and cytotoxic activity against cervical HeLa and MDA-MB231 breast cancer cell lines [17]. Taniguchi et al. 2018 [11] reported that a xanthone (jacareubin) compound isolated from the leaves showed cytotoxic potential against HeLa and HL60 cancer cell lines.

Screening of the phytochemical components in the ethanolic extract of *R. mucronata* bark revealed the presence of alkaloids, triterpenes, flavonoids, tannins, catechin, anthraquinone, phenols, sugars, and proteins [18]. Triterpenoids (3 $\beta$ -O-(*E*)-(4-methoxy) cinnamoyl-15  $\alpha$ -hydroxyl  $\beta$ -amyrin, adian-5-en 3-ol and lupeol) have been isolated from the chloroform extract of the stem bark [19]. Diterpenoids (rhizomucronol A and B) have been isolated from the acetone extract of the stem [20]. Extracts from the bark of *R. mucronata* have anti-diabetic (inhibition of the  $\alpha$ -glucosidase enzyme) effect [21], moderate antibacterial effects against *Escherichia coli* and *Staphylococcus epidermidis* [22], and antiplasmodial activities [18]. Alkaloids such as ajmalicine and serpentine, which have been investigated *in silico* for their binding effects on the cyclooxygenase 2 receptor, have shown strong anti-inflammatory activity [23], while the bark polysaccharide has shown anti-HIV activity [24].

Fewer phytochemical and bioactivity studies have been reported for other parts of the plant, including roots and fruits or propagules. Beyerane diterpenoid (Rhizophorin B) and isopimarane diterpenoids have been isolated from the ethyl acetate extract of *R. mucronata* roots [25], while sesquiterpene (mucronatone) and pentacyclic triterpenoid esters (3 $\beta$ -*E*-caffeoyltaraxerol and 3 $\beta$ -*Z*-caffeoyltaraxerol) have been isolated from *R. mucronata* fruits [26]. The extract from *R. mucronata* stilt root has *in vitro* antioxidant properties [27] and larvicidal activity [28]. To our knowledge, however, scientific studies on the medicinal properties of fruits and propagules of *R. Mucronata* are still lacking. Therefore, the aim of this study was to isolate and identify the chemical constituents present in the propagules of *R. mucronata* and to evaluate their anti-cancer activities *in vitro* against some cancer cell lines with their drug-likeness potential.

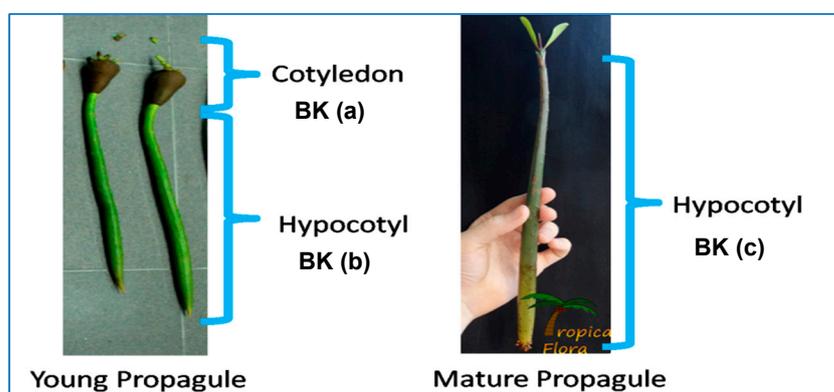
## 2. Materials and Methods

Chemicals and general methods: All reagents and solvents used were of analytical or gradient grade for liquid chromatography.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in ppm ( $\delta$ ) in  $\text{CD}_3\text{OD}$  and acetone- $d_6$  employing a Bruker DRX 300 spectrometer operating at 300 MHz for  $^1\text{H}$  and 75 MHz for  $^{13}\text{C}$ , respectively. Column chromatography was performed with MCI gel CHP 20P (75–150  $\mu\text{m}$ , Supelco, Sigma-Aldrich, St. Louis, MO, USA), Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical Ltd., Aichi, Japan) and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden). HPLC analysis was carried out on a Waters Delta 600 system, equipped

with an in-line degasser, a quaternary pump, an auto sampler, and a diode array detection (DAD) system, connected to an analytical column (Phenomenex LUNA PFP(2)). Data collection was performed using Empower software (Waters). The water used for all the solutions and dilutions was prepared with an ELGA Purelab Classic water purification system. Branson Series ultrasonic bath was used for extraction. TLC was performed on precoated silica gel 60 F<sub>254</sub> plates (0.2 mm thick, Merck) with CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (9:1:0.1 or 8:2:0.1 or 7:3:0.5 or 6:4:1), and spots were detected by UV illumination and by spraying with 10% H<sub>2</sub>SO<sub>4</sub> solution followed by heating or spraying with 2% FeCl<sub>3</sub> solution followed by drying with a stream of cold air.

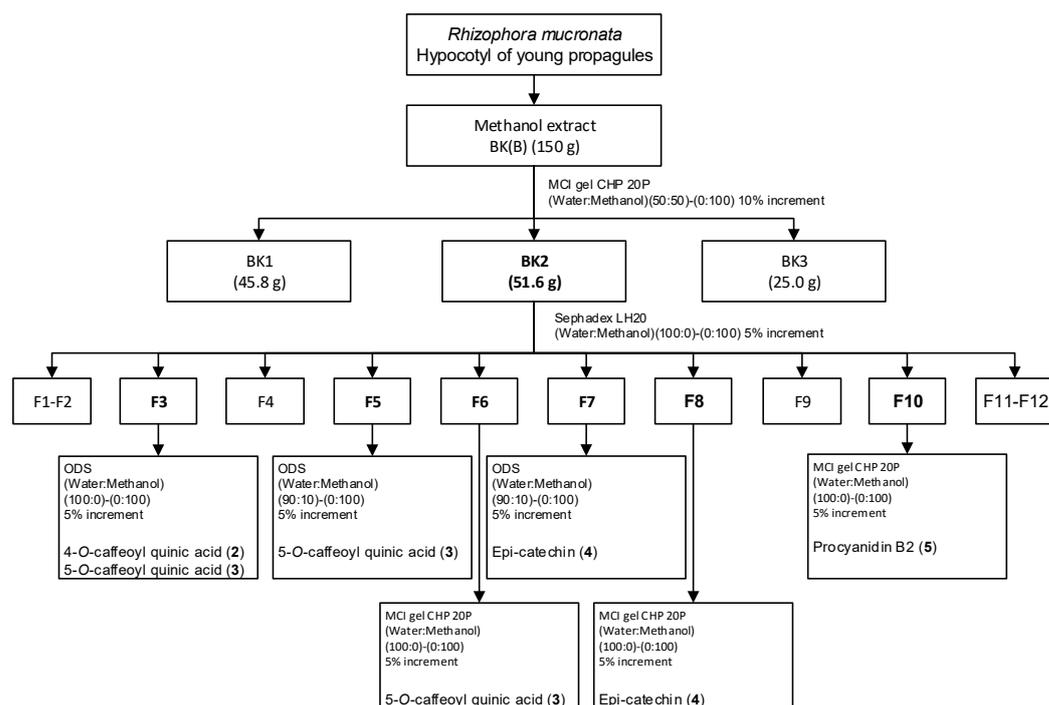
Plant material: Young and old propagules of *R. mucronata* were collected from Carey Island, Selangor, in Malaysia and the samples were coded as BK(A), BK(B), and BK(C) (Figure 1). The voucher specimen was prepared and deposited with number NMY1-KEP 245125 in the herbarium of FRIM, Kepong.



**Figure 1.** Plant parts of young and mature propagules of *R. mucronata*: (a) cotyledon, (b) young hypocotyl, (c) mature hypocotyl.

Preparation of the crude plant extracts: The sliced fresh samples were immediately extracted by soaking in methanol (fresh weight/volume, 1 g/10 mL) for 3 days at room temperature. After that, the solvent was filtered, and the residue was extracted again using methanol with the same weight/volume ratio for another 3 days at room temperature. The solvent was evaporated under reduced pressure using a rotary evaporator with a bath temperature of 45 °C. The extraction process was repeated for the third time. The combined extracts were then stored at –20 °C for further analysis.

Isolation, purification, and characterization of pure compounds: The concentrated methanol extract (150 g) of the hypocotyl of young propagule coded as BK(B) was fractionated through liquid column chromatography employing MCI gel CHP 20P. The resulting fractions of eluent were combined to three fractions based on the thin layer chromatographic profiles. Fraction 2, encoded as BK(2), which had the most visible spots on thin layer chromatography, was further fractionated and purified, employing a combination of Sephadex LH-20, Chromatorex ODS, and MCI gel CHP 20P to yield four thin layer chromatographically homogeneous compounds (compounds 2–5) as shown in Figure 2. The identities and structures of these compounds were determined by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic analyses and by comparison with values in the literature or standard compounds to be 4-*O*-caffeoyl quinic acid (2), 5-*O*-caffeoyl quinic acid (3), Epi-catechin (4), and procyanidin B2 (5).



**Figure 2.** Isolation of compounds from the hypocotyl of young propagules.

**HPLC analysis:** The chromatographic separation of the compounds in the extract was carried out with a Phenomenex Luna PFP(2) column (250 × 4.6 mm, 5 µm, Phenomenex, USA) at room temperature. The solvent system consisted of mixtures of water with 0.1% formic acid (solvent A), acetonitrile (solvent B), and methanol (solvent C). Samples for HPLC analysis were filtered through a 0.45 µm membrane filter. The optimized HPLC condition was as follows: 95–70% A, 5–20% B, and 0–10% C (0–50 min), 70% A, 20% B, and 10% C (50–55 min), 70–90% A, 20–10% B, and 10–0% C (55–60 min), 90% A and 10% B (60–65 min). The flow rate was 1.0 mL. The injection volume was 10 µL with the extract dissolved in methanol at 20 mg/mL. Signal was monitored at 280 and 330 nm. Standards of 3-*O*-caffeoyl quinic acid (CAS 202650-88-2), 4-*O*-caffeoyl quinic acid (CAS 905-99-7), 5-*O*-caffeoyl quinic acid (CAS 906-33-2), and epi-catechin (CAS 490-46-0) were used for the identification of major peaks in the chromatogram.

**Cancer cell lines, cell culture maintenance, and treatments:** Ovarian (SKOV-3), breast (T47D), and colorectal (HT-29) cell lines were purchased from American Type Culture Collections, USA, and A2780 ovarian cancer cell line was purchased from European Collection of Cell Cultures (ECACC), UK. The cell lines were cultured and sub-cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 1% penicillin-streptomycin, 0.25% amphotericin B, and 1% gentamycin. Approximately 4000 to 6000 cells were seeded in each well of the 96 well plates and incubated in a humidified incubator at 37 °C and 5% carbon dioxide in air for 24 h. Each cell line was then treated with the extracts and compounds obtained from *R. mucronata* at five different concentrations (0.1, 1, 10, 20, and 100 µg/mL) in triplicate. Cisplatin, a known chemo-drug, was also treated on these cell lines in different concentrations (0.25, 1, 5, 25, and 50 µg/mL) as in the comparative studies. The treated cells were then incubated in the same incubator with the mentioned conditions for 72 h. The experiment was repeated at least three times.

**In vitro anti-cancer activities assessed via Sulphorhodamine B (SRB) assay:** The 72 h treatments were stopped by performing Sulphorhodamine B (SRB) assay [29,30]. Briefly, 50 µL of ice cold trichloroacetic acid (TCA) was added to each well and allowed to stand for 30 min at room temperature, followed by rinsing each well with tap water. Then, 100 µL of 0.4% SRB was added to each well to stain living cells for 30 min followed by a rinse with 1% acetic acid. Finally, 100 µL of Tris buffer was added to each well and the optical

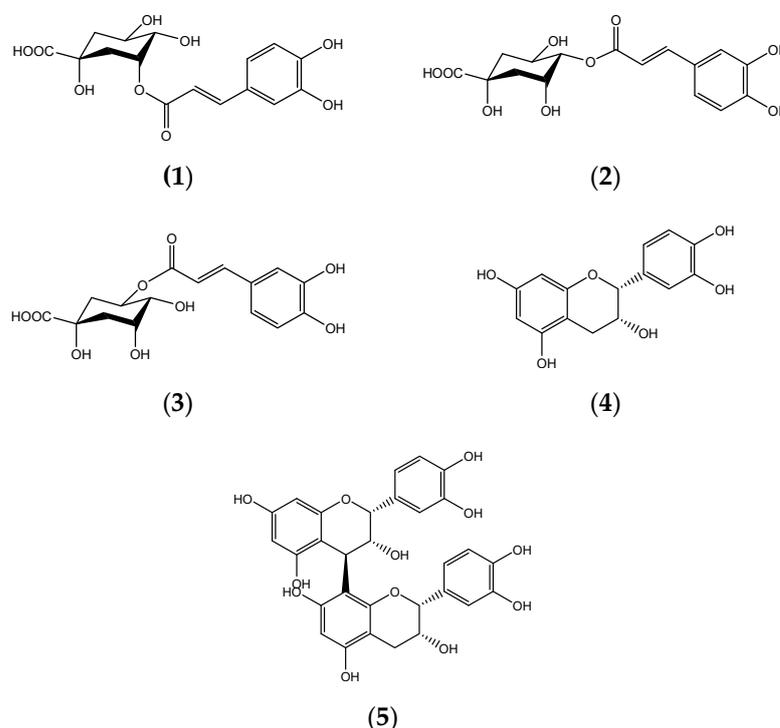
density (OD) of the treated and non-treated cells were read at 492 nm with a Magellan V.4 microtiter plate reader (Tecan, Switzerland). The percentage of cell viability was calculated based on  $(OD_{492nm} \text{ of the treated cells} / OD_{492nm} \text{ of the non-treated cells}) \times 100$ . The  $IC_{50}$  values were determined from the dose–response curve of percentage of cell viability versus the concentration of the extracts ( $\mu\text{g/mL}$ ). Cell viability assays for each treatment were performed in triplicate in at least three independent experiments, and the  $IC_{50}$  values are given as the mean  $\pm$  S.D.

*In silico* analysis: *In silico* analysis of drug-likeness and ADMET properties were performed based on Lipinski's Rule of Five (ROF) using a free online server. Prediction of drug-likeness and bioactivity score was conducted using Molinspiration (Molinspiration Cheminformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic) and OSIRIS property explorer for calculation of the physicochemical properties ([www.organicchemistry.org/prog/peo/](http://www.organicchemistry.org/prog/peo/), accessed on 9 May 2019). Prediction on ADMET of the compounds was performed using vNN-ADMET [31] and OCHEM [32].

### 3. Results

#### 3.1. Isolation, Purification, and Identification of Compound

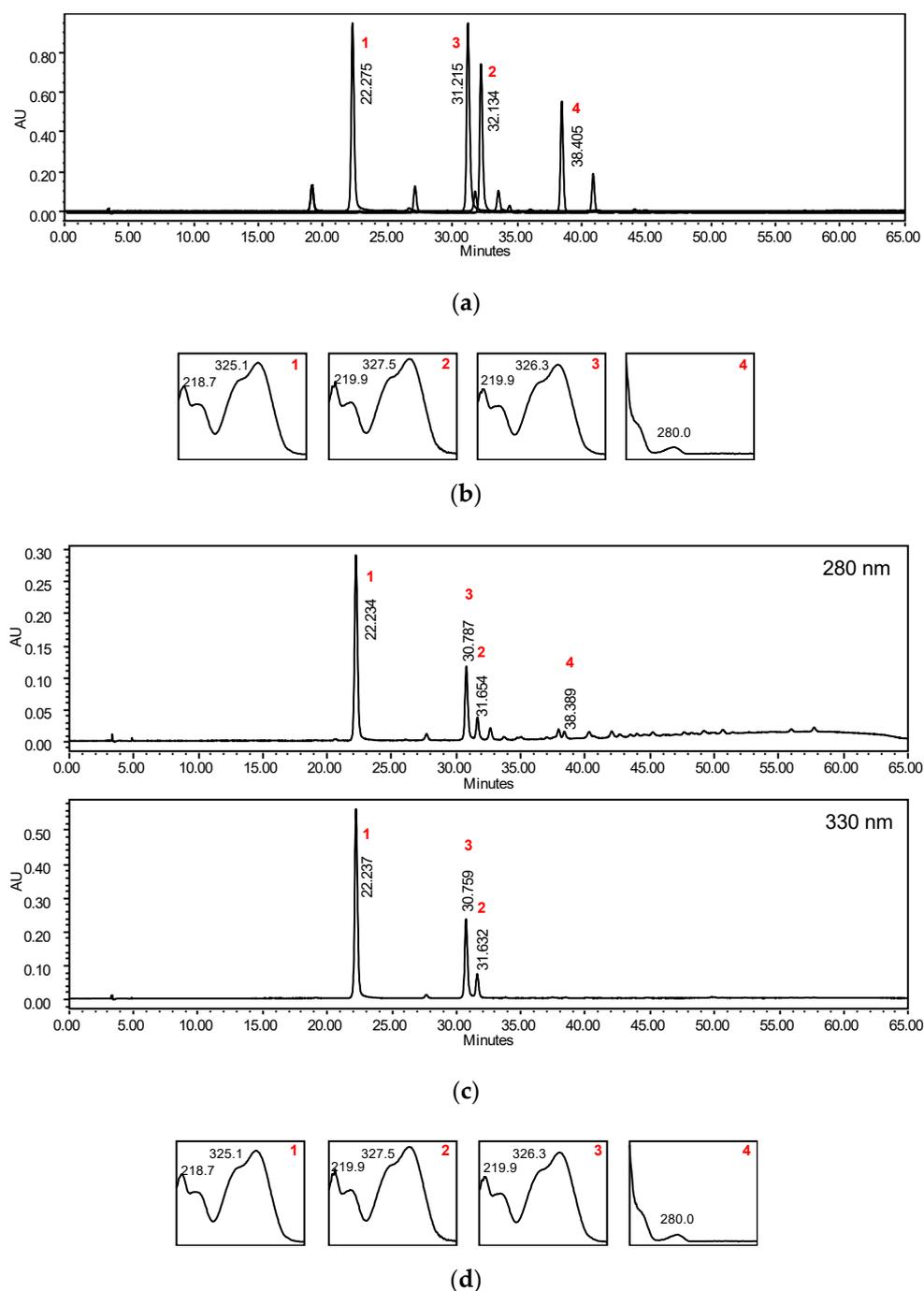
The methanol extract, BK(B), exhibited  $IC_{50}$  values of  $\leq 20 \mu\text{g/mL}$  for three of the cancer cell lines used in the *in vitro* anti-cancer study, indicating very potent anti-cancer effects [33,34]. The isolation and subsequent purification activities of the BK(B) extract identified four compounds known as 4-*O*-caffeoyl quinic acid (2) [35], 5-*O*-caffeoyl quinic acid (chlorogenic acid) (3) [36,37], epi-catechin (4) [38], and procyanidin B2 (5) [39–41] by  $^1\text{H}$  and  $^{13}\text{C}$  spectroscopic analyses and comparison with the values in the literature or standard compounds (Figure 3).



**Figure 3.** Chemical structures of 3-*O*-caffeoyl quinic acid (1), 4-*O*-caffeoyl quinic acid (2), 5-*O*-caffeoyl quinic acid (chlorogenic acid) (3), epi-catechin (4), and procyanidin B2 (5).

#### 3.2. Separation and Identification of Chromatographic Peaks in Extract

Figure 4 represents the analytical HPLC chromatogram of BK(B) extract with the identification of 3-*O*-caffeoyl quinic acid, 4-*O*-caffeyl quinic acid, 5-*O*-caffeoyl quinic acid, and epi-catechin. The main peaks were dominated by caffeoyl quinic acids.



**Figure 4.** HPLC chromatogram of standard compounds, 3-O-caffeoyl quinic acid (1), 4-O-caffeoyl quinic acid (2), 5-O-caffeoyl quinic acid (3), and epi-catechin (4); 1-3 extracted at 330 nm, 4 extracted at 280 nm (a), UV spectra of standard compounds 1-4 (b), HPLC chromatograms of BK(B) extract (c), and UV spectra of compounds 1-4 in BK(B) extract (d).

### 3.3. *In Vitro* Anti-Cancer Studies

The *in vitro* anti-cancer activities of extracts, fractions, and compounds of *Rhizophora mucronata* were evaluated on the basis of their  $IC_{50}$  values (Table 1). Phytochemicals that give an  $IC_{50}$  value  $\leq 20$   $\mu\text{g/mL}$  indicate very potent anti-cancer activities [33,34]. The results indicate that the four compounds have inhibitory effects with  $IC_{50}$  values of less than 100  $\mu\text{g/mL}$ . 4-O-caffeoyl quinic acid was found to be the most active of the four compounds in the treatment of ovarian (SKOV3) and colorectal (HT29) cancer cell lines, followed by epicatechin and 5-O-caffeoyl quinic acid, which are also active against ovarian

(A2780) cancer cell lines. The compound procyanidin-B2 showed moderate to almost very potent anti-cancer activities in all cancer cell lines tested.

**Table 1.** IC<sub>50</sub> values (μg/mL) of four compounds isolated from the hypocotyl of young propagules of *R. mucronata*.

Cancer Cell Lines	A2780 (Ovarian Cancer)	SKOV-3 (Ovarian Cancer)	HT-29 (Colorectal Cancer)	T47D (Breast Cancer)
Extracts:				
BK(A) Young cotyledon	14.85 ± 1.79	28.86 ± 2.25	>100	16.59 ± 2.25
BK(B) Young Hypocotyls	12.67 ± 0.40	27.36 ± 0.96	15.85 ± 0.14	15.95 ± 2.52
BK(C) Mature Hypocotyls	22.37 ± 6.00	35.50 ± 0.51	>100	15.63 ± 3.13
Fractions from BK(B):				
BK(B)1	21.91 ± 1.57	25.07 ± 0.79	15.67 ± 0.07	>100
BK(B)2	17.52 ± 0.87	20.98 ± 0.49	15.17 ± 1.32	>100
BK(B)3	18.55 ± 0.90	21.39 ± 0.51	15.39 ± 0.33	>100
Compounds:				
4- <i>O</i> -caffeoyl quinic acid	21.01 ± 0.41	16.77 ± 0.58	18.50 ± 0.89	20.20 ± 1.36
5- <i>O</i> -caffeoyl quinic acid	19.54 ± 1.05	21.68 ± 0.78	28.01 ± 1.08	24.70 ± 0.88
Procyanidin B2	23.11 ± 0.52	23.13 ± 0.34	21.14 ± 0.43	24.44 ± 1.40
Epicatechin	18.22 ± 0.81	22.68 ± 0.89	28.28 ± 2.06	27.11 ± 1.00
Cisplatin	0.71 ± 0.10	0.82 ± 0.04	0.81 ± 0.04	0.86 ± 0.01

Values are the mean ± standard error of three samples, analyzed individually in triplicate.

### 3.4. In Silico Analysis on Drug-Likeness and Toxicity of Selected Compounds

The evaluation of the drug-likeness properties of four compounds isolated from BK(B) showed that all compounds, except procyanidin B2, fulfilled Lipinski's Rule of Five (RoF) (Table 2). Procyanidin B2 violated three of the RoF, namely molecular weight (578.5 > 500 Da), number of hydrogen acceptors (12 > 10), and number of hydrogen donors (10 > 5). The bioactivity score indicated that all compounds were active and moderately active against normal human receptors (Table 3). Interestingly, all of the compounds were active against nuclear receptor ligands, GPCR ligands, protease inhibitors, and enzyme inhibitors. Epicatechin is the only compound that was active against all of the predicted receptors, with the highest score for the nuclear receptor ligand (0.60), but the highest score for this receptor was 5-*O*-caffeoyl quinic acid (0.74). Prediction of toxicity showed that not all compounds have mutagenic, tumorigenic, reproductive, and irritant potential, except procyanidin B2, which displayed a high risk of reproductive effects (Table 4). However, as shown in Table 5, not all compounds were cytotoxic. The results of ADMET prediction are summarized in Table 5. All compounds showed no inhibition of cytochrome P450 metabolism (CYP3A4, CYP2D6, CYP2C19, CYP2C6, and CYP1A2), as predicted by OCHEM and vNN-ADMET.

**Table 2.** Physicochemical properties of compounds isolated from BK(B).

Lipinski's Parameters	Compound			
	Epi-Catechin	5- <i>O</i> -Caffeoyl Quinic Acid	4- <i>O</i> -Caffeoyl Quinic Acid	Procyanidin B2
miLogP	1.369	−0.453	−0.671	2.58
Topological polar surface area (Å <sup>2</sup> ) (TPSA)	110.4	164.7	164.7	220.75
No. of atoms	21	25	25	42
Mwt	290.27	354.3	354.3	578.53
No. of hydrogen acceptor (nOH)	6	9	9	12
No. of hydrogen donor (nOHNH)	5	6	6	10
Lipinski's violation	0	1	1	3
No. of rotatable bonds	1	5	5	3
Volume (Å <sup>3</sup> )	244.1	296.3	296.3	475.67

**Table 3.** Bioactivity score of compounds isolated from BK(B).

Parameters	Compound			
	Epi-Catechin	5-O-Caffeoyl Quinic Acid	4-O-Caffeoyl Quinic Acid	Procyanidin B2
GPCR ligand	0.41	0.29	0.19	0.20
Ion channel modulator	0.14	0.14	0.02	−0.33
Kinase inhibitor	0.09	−0.00	−0.10	−0.12
Nuclear receptor ligand	0.60	0.74	0.66	0.16
Protease inhibitor	0.26	0.27	0.14	0.17
Enzyme inhibitor	0.47	0.62	0.49	0.09

**Table 4.** Toxicity prediction of compounds isolated from BK(B).

Toxicity Parameters	Compound			
	Epi-Catechin	5-O-Caffeoyl Quinic Acid	4-O-Caffeoyl Quinic Acid	Procyanidin B2
Mutagenic	None	None	None	None
Tumorigenic	None	None	None	None
Reproductive	None	None	None	High
Irritant	None	None	None	None
Drug score	0.871	0.437	0.697	0.33

**Table 5.** ADMET prediction using OCHEM and vNN-ADMET web servers.

	Epi-Catechin		5-O-Caffeoyl Quinic Acid		4-O-Caffeoyl Quinic Acid		Procyanidin B2	
	OCHEM	vNN	OCHEM	vNN	OCHEM	vNN	OCHEM	vNN
CYP450 Inhibitor (CYP3A4)	No	No	No	No	No	No	No	No
CYP450 Inhibitor (CYP2D6)	No	No	No	No	No	No	No	No
CYP450 Inhibitor (CYP2C19)	No	No	No	No	No	No	No	No
CYP450 Inhibitor (CYP2C9)	No	No	No	No	No	No	No	No
CYP450 Inhibitor (CYP1A2)	No	No	No	No	No	No	Yes	No
AMES	Inactive	No	Inactive	No	Inactive	No	Active	No
DILI	-	Yes	-	Yes	-	Yes	-	No
Cytotoxicity	-	No	-	No	-	No	-	No
HLM	-	Yes	-	Yes	-	Yes	-	Yes
BBB	-	No	-	No	-	No	-	No
P-gp Inhibitor	-	No	-	No	-	No	-	No
P-gp Substrate	-	Yes	-	No	-	No	-	Yes
hERG blocker	-	Yes	-	No	-	No	-	Yes
MMP	-	No	-	No	-	No	-	No
MRTD (mg/day)	-	1762	-	2605	-	3096	-	4066

#### 4. Discussion

To the best of our knowledge, these are the first reported results for all four compounds, namely epi-catechin, 4-O-caffeoyl quinic acid, 5-O-caffeoyl quinic acid, and procyanidin B2, which were isolated from the propagules of *R. mucronata*. Although these compounds have newly been isolated and identified in the species, they can be found in other plant species, including many types of fruits and vegetables. The 4-O-caffeoyl quinic acid and 5-O-caffeoyl quinic acid are chlorogenic acids (CGA) that are found in many species of plants, and the most notable one that is rich in CGAs is the green coffee bean [42]. CGAs are phenolic acids which are obtained from the esterification of cinnamic acids with quinic acid. CGAs exhibit several biological properties, including antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic, and antibacterial activities. 5-O-caffeoyl quinic acid is the main CGA of arabica and robusta green coffee beans [42]. Chlorogenic acids, which include

4-*O*-caffeoyl quinic acid and 5-*O*-caffeoyl quinic acid, and caffeic acid have been shown to have an additive anti-proliferative effect with cisplatin on ovarian cancer cells [43].

Epi-catechin is one of the catechin polyphenols, which is one of the main compounds in green tea and cocoa [44,45]. Cocoa powder, which is rich in epi-catechin, has been found to be active and kill pancreatic cancer cells through apoptosis [46]. However, another *in vitro* anti-cancer study suggested that epi-catechin was less active than other catechins, such as epigallocatechin-3-gallate, when tested in prostate (DU-145) and ovarian cancer (HH639) cells [44].

Assessment of the drug-likeness properties of four compounds isolated from young propagules of *R. mucronata* uses the criteria of Lipinski's Rule of Five (RoF). The criteria are often used in drug design and development to predict the oral bioavailability of potential lead or drug molecules. According to Lipinski's RoF, a compound is more likely to be orally active if it has no more than one violation of the following criteria: Log P is less than 5; molecular weight is less than 500 Da; hydrogen bond donor is less than 5; and hydrogen bond acceptor is less than 10 [47]. Among the four compounds, epi-catechin, 5-*O*-caffeoyl quinic acid, and 4-*O*-caffeoyl quinic acid fulfilled Lipinski's RoF, with only one violation being observed for the latter two compounds. Epi-catechin was found to have no violation to the rule, with a TPSA below 160 Å<sup>2</sup>, molecular weight < 500 Da, number of hydrogen donors < 5, number of hydrogen acceptors < 10, and number of rotatable bonds < 10.

Bioactivity score, as shown in Table 3, indicated that all compounds were active and moderately active against normal human receptors. A molecule with a bioactivity score greater than 0.00 is considerably active, while values from −5.00 to 0.00 are moderately active, and if the score is less than −5.00 it is considered to be inactive. Epi-catechin was active against all receptors, i.e., GPCR ligands, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor. 5-*O* caffeoyl quinic acid and 4-*O* caffeoyl quinic acid were also active against these biological targets, but were moderately effective against the kinase inhibitor. Procyanidin B2 was only active against three biological targets, namely GPCR ligand, nuclear receptor, protease inhibitor, and enzyme inhibitor, and moderately active against the other two receptors.

The ADME assessment was conducted using OCHEM and vNN-ADMET webservers. The results are summarized in Table 5. All compounds showed no inhibition of cytochrome P450 metabolism, which was predicted using both servers, with the exception of procyanidin B2, which showed conflicting results for the CYP1A2 inhibitor. Cytochrome P450 enzymes are essential for the metabolism of drugs. While there are more than 50 CYP450 enzymes, CYP3A4, CYP2D6, CYP2C19, CYP2C9, CYP1A2, and CYP3A5 enzymes metabolize 90 percent of drugs [48,49]. The human liver microsomal (HLM) stability assay is commonly used to identify and exclude compounds that are too rapidly metabolized. In order to achieve the effective therapeutic concentrations in the body, the molecule or drug cannot be metabolized too rapidly by the liver. Compounds with half-life of 30 min or longer in an HLM assay are considered to be stable; otherwise, they are considered unstable. All compounds have been classified as stable in the liver.

All compounds were classified as BBB non-permeable. BBB is a highly selective barrier that separates the circulating blood from the central nervous system. Recently, many methods have been explored to improve the permeability of BBB, including strategies in the field of physical, chemical, biological, and various nanoparticle systems [50].

P-glycoprotein (P-gp) is an essential cell membrane protein and plays an important role in the absorption and disposition of drugs. This drug transporter is also involved in the metabolism, distribution, and interaction of drugs. In this study, it was shown that all compounds are not P-gp inhibitors, however epi-catechin and procyanidin B2 were classified as substrates of P-gp. P-gp is not only important in ADMET issues, but is also extended to a field of multidrug resistance cancer [51]. Identification of compounds that are either transported out of the cell by P-gp (substrates) or impair the function of P-gp (inhibitors) is of great interest.

In this study, several toxicity parameters were predicted with OSIRIS, OCHEM, and vNN-ADMET. The prediction of the toxicity of compounds with the OSIRIS software revealed that all compounds have no mutagenic, tumorigenic, reproductive, or irritant potential, however procyanidin B2 has shown a high risk of reproductive effects (Table 4). In terms of cytotoxicity, mitochondrial toxicity (MP), and mutagenicity (AMES test), all compounds were found to be non-toxic, except for procyanidin B2, which was predicted by OCHEM to be active (Table 5). Epi-catechin and procyanidin B2 were classified as hERG blockers, while the other two compounds were classified as non-blockers. In the vNN-ADMET prediction program, compounds with an IC<sub>50</sub> cutoff value of 10 µM or less were classified as blockers, while compounds with an IC<sub>50</sub> greater than 10 µM were classified as non-blockers. It was predicted that only procyanidin B2 did not cause drug-induced liver injury damage (DILI), while the other three compounds were at high risk of causing DILI.

Overall, the drug score for epi-catechin (0.87) is the highest of all, followed by 4-O-caffeoyl quinic acid (0.697), 5-O-quinic acid (0.437) and the lowest is procyanidin B2 (0.33) suggesting that epi-catechin could be developed as a potential cancer drug (Table 4).

## 5. Conclusions

Propagules of *R. mucronata* have anti-cancer properties and four compounds have been identified. 4-O-caffeoyl quinic acid, epi-catechin and 5-O-caffeoyl quinic acid were shown to be active, but procyanidin B2 showed moderate inhibition of ovarian cancer cell proliferation. Of the four compounds, epi-catechin met the ADMET criteria and showed the highest drug score (0.871). Toxicological risk and metabolic prediction show that all four compounds were non-corrosive, with the exception of epi-catechin, which was believed to be irritating or corrosive to the skin. Epi-catechin can be seen as one of the most important potential compounds for the development of new drugs, e.g., as an anti-cancer agent, but efficacy, mechanism of action, and toxicity studies need further validation at the *in vitro* and *in vivo* levels.

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