Send Orders for Reprints to reprints@benthamscience.net

Current Traditional Medicine, XXXX, *XXX*, e22150838298401

RESEARCH ARTICLE

Antibacterial, Antibiotic-Potentiating, and Antiviral Activities of Selected Endemic Primary Rainforest Plants of Peninsular Malaysia

Masyitah Zulkipli[1], Mazdida Sulaiman[2], Layane Elbehairy[2], Khoo Teng Jin[1], Nor Hayati Abdullah[3], Chandramathi Samudi Raju[4], Gheetanjali Kathirvalu[4], Veeranoot Nissapatorn[5], Nor Azizun Rusdi[6], Fiffy Hanisdah Saikim[6], Vladimir Zarubaev[7], John Kenny Rafon[8], Jhonnel Villegas[9] and Christophe Wiart[6,*]

[1]School of Pharmacy, University of Nottingham Malaysia Campus, 56000 Semenyih, Selangor, Malaysia; [2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; [3]Forest Research Institute of Malaysia, Kepong, 52109 Kuala Lumpur, Selangor, Malaysia; [4]Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia;[5]School of Allied Health Sciences, World Union for Herbal Drug Discovery (WUHeDD), Research Excellence Center for Innovation and Health Products (RECIHP), Walailak University, Nakhon Si Thammarat 80160, Thailand; [6]Institute for Tropical Biology & Conservation, University Malaysia Sabah, 88400 Kota Kinabalu, Malaysia; [7]Pasteur Institute of Epidemiology and Microbiology, 14 Mira str, 197101 St. Petersburg, Russia; [8]Teacher Education Department, Davao de Oro State College, 8803 Davao de Oro, Philippines; [9]Davao Oriental State University 8200 Davao Oriental, Philippines

ARTICLE HISTORY

Received: March 04, 2024
Revised: May 21, 2024
Accepted: June 05, 2024

DOI:
10.2174/0122150838298401240924105857

Abstract: ***Background*:** There is a need to identify original molecules to develop drugs for the treatment of microbial infections. Such chemical entities could be found in secondary metabolites of rainforest plants that are not so well-known. This study examines the antibacterial and antibiotic-potentiating effects, and antiviral activities of six rainforest plants endemic to the primary rainforest of Malaysia.

***Methods*:** Leaves, bark, fruits, and wood of *Burkillanthus malaccensis*, *Cleistanthus bracteosus*, *Diospyros hasseltii*, *Kibatalia maingayi*, *Knema retusa*, and *Litsea spathacea* were extracted successively with hexane, chloroform, and methanol, and tested against six human pathogenic bacteria species by disc diffusion and broth microdilution. The extracts were tested against influenza virus A/Puerto Rico/8/34 (H1N1) using MDCK cells.

***Results*:** Of the 42 extracts tested, the hexane extract of fruits of *D. hasseltii* inhibited the growth of *E. coli* with the MIC value of 39 µg/mL. The chloroform extract of leaves of *C. bracetosus* potentiated the activity of levofloxacin against *P. aeruginosa*. The strongest antiviral activity was observed with the chloroform extract of leaves of *C. bracteosus* with the IC_{50} value of 6.3 µg/mL. The chloroform extract of bark of *B. malaccensis* with the IC_{50} value of 0.6 µg/mL was the most cytotoxic.

***Conclusion*:** Preserving the primary rainforest of Malaysia is a means to preserve natural products with the ability to be developed as antimicrobial leads. In particular, *D. hasseltii*, *C. bracteosus*, and *B. malaccensis* could be examined for their active antimicrobial constituents.

Keywords: Antibacterial, antibiotic potentiator, antiviral, *Burkillanthus malaccensis*, *Cleistanthus bracteosus*, *Diospyros hasseltii*, *Kibatalia maingayi*, *Knema retusa*, *Litsea spathacea*.

1. INTRODUCTION

In Malaysian hospitals, bacterial resistance to β-lactam antibiotics has become critical; for example, there was reported an increased resistance rate of *Pseudomonas aeruginosa* to imipenem, increasing from 9.9 to 20.6% between 2005 and 2009 [1, 2]. However, the rates of antibiotic resistance in *P. aeruginosa* are increasing worldwide. In fact, the increase in Multidrug-resistant (MDR) bacteria is considered one of the most urgent threats to global human health [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 2% of *S. aureus* infections in the US intensive care units in 1974, which increased to 22% in 1995 and 64% in 2004 [4]. MRSA is now a leading cause of healthcare-associated infections and one of the most important antibi-

* Address correspondence to this author at the Institute for Tropical Biology & Conservation, University Malaysia Sabah, 88400 Kota Kinabalu, Malaysia; E-mail: christophewiart@ums.edu.my

otic-resistant bacteria in the United States [5]. Nosocomial Infections (NIs) are defined as hospital-acquired infections developing at least 48-72 hours after admission [6]. Currently, there is a growing number of patients infected with Gram-positive or Gram-negative multidrug-resistant nosocomial bacteria who are not treatable [7].

The high prevalence of MDR bacteria pinpoints an urgent need for the development of improved policies, surveillance, and infection control, as well as the development of new antibiotics specifically targeting these pathogens [8]. To help control bacterial resistance, the World Health Organization (WHO) recommends developing an economic case for sustainable investment that takes into account the needs of all countries and increases investment in new medicines [9]. In fact, the development of new and effective antibacterial drugs to treat MDR bacteria is recognized as one of the most urgent health problems in the world [10, 11]. Interestingly, the number of new antibiotics approved by the FDA is decreasing [12]. An emerging alternative to traditional antibiotics is compounds and biologicals collectively called "non-traditional", which indirectly have antibacterial activity [13]. We can cite for instance efflux pump inhibitors and other principles targeting antibiotic-targeting enzymes. These include clavulanic acid, isolated from a bacterium, which potentiates the antibacterial effects of β-lactam antibiotics by inhibiting β-lactamase and has mild antibacterial effects [14, 15]. Also, natural products derived from plants, such as plant phenols, like eugenol [16], increase sensitivity to antibiotics by membrane disruption [17]. Thus, there is precedence for potentiators of natural product-derived antibiotics.

The primary rainforest of Malaysia was one of the richest plant biospheres on Earth until intense burning (deforestation) gobbled up huge areas to make way for massive palm oil and rubber tree plantations [18]. Worldwide deforestation between 2000 and 2012 was comprehensively reported by Hansen *et al.* (2013), whose analysis registered Malaysia with the highest level of forest loss in relation to land area [19]. Along with deforestation, residential and commercial developments have caused additional stress to the ecosystems. It is estimated that within 50 years, if nothing is done, this rainforest will disappear completely [20-22]. Many species used in traditional medicine are now vulnerable or in danger [23, 24]. Nonetheless, local rainforest medicinal plants have been used by the indigenous tribes in Peninsular Malaysia for centuries, and these have not yet been studied for their biomedical properties [25]. So far, few reports exist on the isolation of antibiotic-potentiators from Malaysian plants. Among these plants, there are members of the genus *Cleistanthus* Hook. f. ex Planch. (family *Phyllanthaceae*) [26, 27], including *Cleistanthus bracteosus* Jabl., a magnificent tree growing to 45 m tall with bright red capsules and oblong leaves. *Burkillanthus malaccensi*s (Ridl.) Swingle. (family Rutaceae) known to the inhabitants of the Malaysian Peninsula as "limau hantu" and commonly termed Burkill's lime is a tree with thorny stems, trifoliate leaves, large white flowers, and large lemon-like fruits, containing numerous seeds covered with yellow resin (personal observation). *Litsea spathacea* Gamble (Family Lauraceae), *Diospyros hasseltii* Zoll. (Family Ebenaceae), *Kibatalia maingayi* Hook. f. Woodson (Family Apocynaceae), and *Knema retusa* (King) Warb (Family Myristicaceae) are other species common in the lowlands and the hills of the forest of the Northern Peninsula of Malaysia. These are locally known in the Malay language as "derahan", "meranga", "jelutong pipit", and "pala hutan", respectively [28-33].

Since there is an urgent need to find original chemical frameworks to develop antimicrobial leads, we examined the antibacterial, synergistic, and antiviral activities of the six plants. Since not much study has been conducted to investigate the biological activities of these six plants, we aimed to examine their antibacterial, antibiotic-potentiating, antiviral, and cytotoxicity properties.

2. MATERIALS AND METHODS

2.1. Plant Collection

The chemotaxonomical collection of plants was performed in February 2017. The survey was done in Manong and Kuala Kangsar primary rainforest, State of Perak, Malaysia (4.7746° N, 100.9520° E). The survey allowed for the selective collection of six plant species, identified by botanists at the National Herbarium of the Forest Research Institute of Malaysia (FRIM). Voucher herbarium specimens with vernacular names, collection locations, and dates of collection were deposited at the Department of Pharmacy, Faculty of Science, University of Nottingham Malaysia Campus. These plants have been found to belong to the families *Rutaceae*, *Ebenaceae*, *Lauraceae*, *Phyllanthaceae*, and *Myristicaceae*. Taxonomical data of specimens, location, and date of collection, parts collected, Malay common names, and traditional uses are given in Table **1**.

2.2. Preparation of Plant Extracts

The leaves, bark, wood, and fruits collected were separated and air-dried at room temperature for two weeks. The dried materials were then finely pulverized by grinding using an aluminum collection blender (Philips, Shanghai, China), and the powders obtained were weighed with a top loading balance (Sartorius AG, Göttingen, Germany). The dried plant powders (200g) were successively soaked at room temperature with hexane, chloroform, and methanol for selective extraction of non-polar, mid-polar, and polar compounds, respectively [34]. Each extraction was performed using the maceration technique with a powder-to-solvent ratio of the plant of 1:5 (w/v) for three days at room temperature in three successive repetitions. The respective liquid extracts were subsequently filtered through qualitative filter papers No. 1, (Whatman International Ltd. Maidstone, UK), using an aspirator pump (EW-35031-00, 18 L/min, 9.5 L Bath, 115 VAC), and the filtrates were concentrated to dryness under reduced pressure at 40°C using the rotary evaporator (Buchi Labortechnik AG Flawil, Switzerland). The dry extracts obtained were weighed and stored in tightly closed glass scintillation vials (Kimble, USA) at -20°C until further use.

Table 1. Plants collected in Manong and Kuala Kangsar primary rainforest.

FAMILY Genus, Species, Authority	Voucher No.	Date of Collection	Location	Common Name	Part Collected	Traditional Uses
APOCYNACEAE *Kibatalia maingayi* (Hook. f.) Woodson	NB0328	23/3/2017	4 °.69', 100 °.82'	Jelutong pipit	Leaves, Barks	None
EBENACEAE *Diospyros hasseltii* Zoll	NB0245	12/3/2017	4.73 °, 100. ° 81'	Merangat	Leaves, Barks Fruits, Wood	Seeds eaten
LAURACEAE *Litsea spathacea* Gamble	NB0523	5/3/2017	4.70 °, 100. ° 83'	Derahan	Leaves, Barks Wood	Fever
MYRISTICACEAE *Knema retusa* (King) Warb	NB0314	25/3/2017	4 °.69', 100 °.82'	Pala hutan	Leaves, Barks Wood	None
PHYLLANTHACEAE *Cleistanthus bracteosus* Jabl.	NB125	2/3/2017	4 °.72', 100 °.81'	None	Leaves, Barks	None
RUTACEAE *Burkillanthus malaccensi*s (Ridl.) Swingle.	NB234	2/3/2017	4 °.72', 100 °.84'	Limau hantu	Leaves, Bark Fruits	None

2.3. Tested Bacterial Strains

Stock cultures of bacteria used for this study were kindly provided by the Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia. The following human pathogenic bacteria were used as tested organisms: *Staphylococcus aureus* (ATCC 11632), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25218), *Pseudomonas aeruginosa* (ATCC 10145), *Acinetobacter baumannii* (clinical strain, imipenem-sensitive), and *Acinetobacter baumannii* (clinical strain, imipenem-resistant); they were sub-cultured in nutrient agar. All sub-cultured bacterial specimens were transferred aseptically using an inoculating loop and prepared in 10 mL suspensions of Mueller-Hinton Broth (Oxoid, Hampshire, UK), and they were then used 15 minutes after inoculation. A fraction equivalent to 1 mL of the bacterial suspensions was transferred to a cuvette and analyzed with a spectrophotometer (Biochrom, Cambridge, UK), where the UV absorbance value was monitored to be in the range of 0.008 to 0.10 at 625 nm to be adjusted to 0.5 McFarland turbidity standards (Healthlink, Florida, USA), corresponding to a bacterial cell count of 1.5×10^8 CFU/mL [35].

2.4. Disc Diffusion Assay

The disc diffusion assay was performed according to the guidelines of the Clinical and Laboratory Standards Institute (2012) [36]. Paper discs with a diameter of six millimeters (filter paper No. 1) (Whatman International Ltd., UK) were used in this study. Fifty microliters of each material at a concentration of 20 µg/mL to be tested were loaded on autoclaved discs and left to dry for 24 hours. Bacterial strains were grown for 18–24h at 37°C. Colonies were directly suspended in Cationically Adjusted Müeller-Hinton Broth (CAMHB) and adjusted to OD_{625} 0.08–0.1, corresponding to $1 \sim 2 \times 10^8$ CFU/mL, and spread on the agar plates using sterile swabs. With the help of sterile forceps, each disc was applied aseptically to the agar surface on a plate, which had initially been inoculated with a pure culture from the test organism. After incubation, the growth inhibition zone for each extract was measured. The zones of inhibition (mm) were measured and the results have been reported as mean ± Standard Deviation (SD) of the triplicate experiments. Ampicillin (Sigma-Aldrich) 10 µg/disc was used as a positive control, while a paper disc loaded with 50 µL of 100% DMSO was used as a negative control. Experiments were performed in triplicate.

2.5. Broth Microdilution Assay

The determination of the Minimum Inhibitory Concentration (MIC) was performed according to the guidelines of the Clinical and Laboratory Standards Institute (2012) [36]. Briefly, bacterial strains were grown for 18-24 hours at 37°C. Colonies were directly suspended in Cationically Adjusted Müeller-Hinton Broth (CAMHB) and adjusted to OD_{625} 0.08-0.1, corresponding to $1\sim2\times10^8$ CFU/mL, followed by 10-fold serial dilutions to give 1×10^6 CFU/mL. The bacterial suspension (50 µL) was added to the 96-well round bottom microtiter plates containing an equal volume of extracts or phytoconstituents in different concentrations, and the 96-well plates were incubated for 24 hours at 37°C. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of material tested that has completely inhibited the growth of bacteria. The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing the test dilutions on a sterile agar plate and further incubated for 18-24 hours. The highest dilution that yielded 0% bacterial growth on agar plates was taken as MBC. MIC and MBC values were calculated as the mean of triplicate experiments. Vancomycin and rifampicin were used as positive control antibiotics.

2.6. Synergistic Interaction Assay

The ability of the extract to increase the sensitivity of bacteria to antibiotics was measured by the technique described by Saquib *et al.* (2019) [37]. Antibiotic discs of ampicillin (10 µg/disc), gentamicin (10 µg/disc), imipenem (10 µg/disc), levofloxacin (5 µg/disc), and ciprofloxacin (5 µg/disc) (Sigma-Aldrich) were loaded with 1 mg of plant extract per disc. The inhibition zones measured after overnight incubation were estimated as follows: zone of combined material and antibiotic > zone of material + zone of antibiotic = synergy; zone of combined material and antibiotic = zone of material + zone of antibiotic = additive; zone of combined material and antibiotic < zone of material + zone of antibiotic = antagonism.

2.7. Cells and Virus

A/Puerto Rico/8/34 (H1N1) obtained from the collection of viruses of the St. Petersburg Pasteur Institute, St. Petersburg, Russia, was used in the study. Prior to the experiment, the viruses were propagated in the MDCK cells (ATCC no. CCL-34) for 48 h at 36C. The infectious titer of the virus was further determined in MDCK cells in 96-well plates in alpha-MEM medium (Biolot, St. Petersburg, Russia) by end-point dilution assay.

2.8. Cytotoxic Assay

The Micro Tetrazolium Test (MTT) was used to study the cytotoxicity of the extracts [19]. Briefly, the extracts were serially diluted threefold (from 300 to 4 µg/mL, with additional lower dilutions for separate experiments, if needed) in a Minimal Essential Medium (MEM). MDCK cells were incubated for 48 h at 37 °C in 5% CO_2 in the presence of the dissolved substances. The degree of destruction of the cell monolayer was then determined by MTT. The cells were washed twice with saline, and a solution of 3-(4,5-dimethylthiazolyl-2) 2,5-diphenyltetrazolium bromide (ICN Biochemicals Inc., Ohio, USA) (0.5 mg/mL) in MEM was added to the wells. After 1h incubation, the wells were washed for 5 min with saline, and the formazan precipitate was dissolved in DMSO (0.1 mL per well). The optical density of cells was then measured on a Multiscan FC reader (Thermo Scientific) at a wavelength of 535 nm and plotted against the concentration of the extracts. Each concentration was tested in three replicates. The optical density was plotted against the concentration, and the 50% cytotoxic concentration (CC_{50}) was calculated from the data obtained.

2.9. Antiviral Assay

The extracts at increasing concentrations (4-300 µg/mL) were dissolved in MEM with trypsin (1 µg/ mL) and incubated with MDCK cells at 36 °C for 1 h. The cell culture was then infected with the corresponding viruses at a multiplicity of infection (moi) of 0.01 and incubated for 1 h at 36 °C in the presence of 5% CO_2. The culture medium was then removed and replaced with fresh medium containing the same concentrations of material to be tested. Plates were incubated at 36 °C in the presence of 5% CO_2 for 24 h, followed by virus titer determination by TCID50 for 48 h. Each extract concentration was tested in quadruplicate. The 50% inhibitory concentration (IC_{50}) and SI (CC_{50}-to-IC_{50} ratio) were calculated from the data obtained. Oseltamivir carboxylate (Hofmann LaRoche, Basel, Switzerland) and rimantadine (Sigma-Aldrich, Missouri, USA) were used as reference antiviral drugs.

3. RESULTS

3.1. Disc Diffusion Assay

Disc Diffusion Assay of the 42 extracts tested against two Gram-positive and four Gram-negative bacteria, the strongest activity was obtained with the methanol extract from the bark of *C. bracteosus*, which inhibited the growth of *S. aureus*, *E. coli*, and *P. aeruginosa*, with the inhibition zone diameters of 15.5±0.2, 13.5±0.2, and 17.1±0.4 mm, respectively (Table **2**). Most extracts were active against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Six extracts were active against *E. coli*, notably the methanol extracts from leaves and bark of *C. bracteosus*, which yielded inhibition zone diameters of 13.3±0.5 and 13.5±0.2 mm, respectively. Nine extracts inhibited the growth of *P. aeruginosa*; the strongest activity was observed with the methanol extract of bark of *C. bracteosus* and the methanol extract of wood of *K. retusa* with the inhibition zone diameters of 17.1±0.4 and 11±0.0 mm, respectively.

The methanol extract from leaves of *C. bracteosus* was active against *A. baumannii* (imipenem-resistant) with a diameter of the inhibition zone of 8.3±0.5 mm. Five extracts of *D. hasseltii* inhibited the growth of *A. baumannii* (imipenem-sensitive), with the methanol extract from the bark being the most active with the diameter of the zone of inhibition of 10±0.0 mm, but it showed no relevant activity against the imipenem-resistant *A. baumannii*. The chloroform extract of leaves from *L. spathacea* showed the most relevant antibacterial activity with a zone of inhibition of 12±0.1 mm.

3.2. Broth Microdilution Assay

We sought to determine the Minimum Inhibitory Concentration (MIC) of 42 extracts from the five plant species by the broth microdilution method (Table **3**) [38-43]. Results of the broth microdilution assay confirmed the Gram-positive bacteria *S. aureus* and *B. subtilis* to be more susceptible to the plant extracts than Gram-negative bacteria (Table **3**). Chloramphenicol and tetracycline were used as positive controls because of their broad-spectrum activities [44]. The hexane extract of the fruits of *D. hasseltii* exhibited noteworthy activity with an MIC value against the Gram-negative bacillus *E. coli* as low as 39.0 µg/mL (Table **3**). The lowest MIC value towards the Gram-positive *S. aureus* was obtained by the methanolic extract of bark from *C. bracteosus* (250 µg/mL). The chloroform extract from leaves of *L. spathacea* was able to inhibit the growth of *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* with the MIC value of 500 µg/mL. Additionally, this extract inhibited the growth of *A. baumannii* (imipenem-sensitive) with an MIC value of 500

µg/mL and MBC above 1000 µg/mL. The methanolic extract of *L. spathacea* leaves was active against *A. baumannii* (imipenem-sensitive) with an MIC value of 1000 µg/mL and MBC value > 1000 µg/mL. None of the extracts tested could inhibit the growth of imipenem-resistant *A. baumanii.*

Table 2. Inhibition zone diameters.

Plant	Part	Solvent	*S. aureus* (ATCC1 1632)	*B. subtilis* (ATCC 6633)	*E. coli* (ATCC 25218)	*P. aeruginosa* (ATCC 10145)	*A. baumannii* (imipenem-sensitive)	*A. baumannii* (Clinical, imipenen-resistant)
B. malaccensis	Leaves	Hexane	6.5 ± 0.6	6.± 0.5	-	6.6 ± 1.1	-	-
	Leaves	Chloroform	7 ± 0.5	6.5 ± 0.0	-	-	-	-
	Leaves	Methanol	7.6 ± 0.5	7.2 ± 0.1	6.6 ± 0.0	-	-	-
	Bark	Hexane	10.6± 0.0	8.3 ± 0.4	-	-	-	-
	Bark	Chloroform	7 ± 0.5	6.5 ± 1.3	-	-	-	-
	Fruit flesh	Chloroform	-	-	-	**11.3 ± 0.0**	-	-
C. bracteosus	Leaves	Hexane	7.3 ± 0.6	-	6.7 ± 0.3	6.6 ± 0.1	-	-
	Leaves	Chloroform	9.3 ± 0.8	6.3 ± 0.5	8.3 ± 0.4	-	-	-
	Leaves	Methanol	13.1 ± 0.5	-	**13.3 ± 0.5-**	10.3 ± 0.2	-	**8.3 ± 0.4-**
	Bark	Hexane	10.5 ± 0.2	-	-	-	-	-
	Bark	Chloroform	9.1 ± 0.4	8.6 ± 0.3	**13.5 ± 0.2**	-	-	-
	Bark	Methanol	**15.5 ± 0.2**	-		**17.1 ± 0.4**	-	-
D. hasselti	Leaves	Hexane	7.0 ± 0.0	7.5 ± 1.5	-	7.5 ± 0.5	8.0 ± 0.4	-
	Leaves	Chloroform	6.8 ± 0.02	-	-	10.1 ± 0.3	-	-
	Leaves	Methanol	7.8 ± 0.2	7 ± 0.5	-	-	8.0 ± 0.2	-
	Bark	Chloroform	-	-	-	-	10 ± 0.0	-
	Bark	Methanol	11.7 ± 0.8	-	8 ± 0.5	6.7 ± 0.3	9.0 ± 0.8	-
	Fruit	Hexane	8.3 ± 0.1	-	-	-	9.0 ± 0.1	-
	Fruit	Chloroform	8.7 ± 0.0	-	7.7 ± 0.2	7.3 ± 0.0	-	-
	Fruit	Methanol					-	
K. retusa	Leaves	Chloroform	6.6 ± 0.2	-	-	-	-	-
	Leaves	Methanol	8.5 ± 0.0	-	-	-	-	-
	Bark	Hexane	8.0 ± 0.8	7.1 ± 0.2	-	-	-	-
	Bark	Chloroform	7.8 ± 0.2	7.0 ± 0.0	-	-	-	-
	Wood	Hexane	8.0 ± 0.0	7.8 ± 0.4	-	10 ± 0.2	-	-
	Wood	Chloroform	12 ± 0.5	9.0 ± 0.2	7.3 ± 0.1	-	-	-
	Wood	Methanol	-	11 ± 0.4	9.0 ± 0.2	9.0. ± 0.0	-	-
L. spathacea	Leaves	Chloroform	7.1 ± 0.2	-	-	-	**12.0 ± 0.1**	-
	Bark	Hexane	7.0 ± 0.07	-	-	-	-	-
	Bark	Chloroform	6.9 ± 0.07	-	-	-	-	-
	Wood	Methanol	-	7.7 ± 0.3	-	**11 ± 0.0**	-	-
Ampicillin (10 µg/disc)			44 ± 0.4	34 ± 2.7	14.5 ± 0.3	22 ± 0 .8	20 ± 0.4	-
Imipenem (5 µg/disc)								10 ± 0.04

6 mm diameter paper discs impregnated with 1 mg of plant extracts; Values are expressed as mean of triplicate experiments; extracts without any activity are not included.

Table 3. Minimum inhibiting concentrations (MIC).

Plant	Part	Solvent	*S. aureus* (ATCC11632)	*B. subtilis* (ATCC 6633)	*E. coli* (ATCC25218)	*P. aeruginosa* (ATCC 10145)	*A. baumannii* (imipenem-sensitive)
B. malaccensis	Leaves	Hexane	1000	-	-	-	-
	Leaves	Chloroform	**250**	**250**	-	-	-
	Leaves	Methanol	500	1000	-	-	-
	Bark	Chloroform	1000	-	-	**250**	-
	Bark	Methanol	**250**	-	500	1000	-
	Fruit flesh	Chloroform	-	-	-	1000	-
	Seed	Hexane	-	-	-		-
C. bracteosus	Leaves	Chloroform	1000	-	-	-	-
	Leaves	Methanol	500	-	500	-	-
	Bark	Hexane	1000	-	-	-	-
	Bark	Chloroform	1000	-	-	-	-
	Bark	Methanol	**250**	-	500	**250**	-
D. hasseltii	Leaves	Hexane	-	-	500	-	-
	Leaves	Chloroform	500	-	-	500	-
	Leaves	Methanol	-	-	500	-	-
	Bark	Chloroform	-	-	500	500	-
	Bark	Methanol	500	-	**250**	1000	-
	Fruit	Hexane	1250	-	**39.0**	-	-
	Fruit	Chloroform	1250	-	-	**250**	-
	Fruit	Methanol	2500	-	2500	2500	-
K. retusa	Leaves	Methanol	**250**	-	-	-	-
	Wood	Hexane	**250**	-	2500	2500	-
	Wood	Chloroform	1250	625	1250	1250	-
	Wood	Methanol	2500	1250	-	1250	-
L. spathacea	Leaves	Chloroform	500	500	500	500	**500**
	Leaves	Methanol	1000	-	-	-	1000
	Bark	Hexane	-	-	500	-	-
	Bark	Chloroform	-	-	500	-	-
	Wood	Hexane	-	1250	2500	-	-
	Wood	Chloroform	625	-	2500	-	-
	Wood	Methanol	2500	-	-	**250**	-
Chloramphenicol			0.03	0.02	0.002	0.01	nt
Imipenem			-	-	-	-	20

MIC (µg/mL), against 6 bacteria by microdilution assay. Extracts without any activity are not included. nt: not tested.

3.3. Synergistic Interaction Assay

Out of the 42 extracts tested with the antibiotics ampicillin, ciprofloxacin, gentamicin, levofloxacin, and imipenem, 6 exhibited synergistic antibacterial effects and, interestingly, most of them increased the sensitivity of the Gram-negative bacilli *E. coli* and *P. aeruginosa* to the β-lactam antibiotic ampicillin. The antibacterial-potentiator results of the plant extracts are summarized in Table **4**. Plant extracts with no recorded synergy have not been included in this table. A stronger antibacterial potentiation was achieved by the chloroform extract of leaves of *C. bracteosus*, which potentiated the effect of ampicillin (44±0.4 mm) against the Gram-positive cocci *S. aureus* with a combined inhibition zone of 50±0.3 mm. This *C. bracteosus* extract increased the sensitivity of *P. aeruginosa* to the antibiotic fluoroquinolone levofloxacin (21 ± 0.4 mm) with a combined zone of inhibition of 35±0.4 mm. Hexane and methanol extracts from the wood of *K. retusa* potentiated the effect of ampicillin against the two Gram-positive bacteria tested, *S. aureus* and *B. subtilis*.

The methanol extract of fruits of *D. hasseltii* potentiated the aminoglycoside antibiotic gentamicin against *E. coli* with an increase in the inhibition zone of 132 mm. The hexane extract of *L. spathacea* leaves increased the sensitivity of *E. coli* towards gentamicin with an increase in zone of inhibition from 19.5±0.0 to 22±0.01 mm. The only plant extract capable of potentiating the fluoroquinolone antibiotic ciprofloxacin was the chloroform extract obtained from the bark of *C. bracteosus* towards *E. coli* bacteria. No plant extract showed a potentiating effect with the antibiotic β-lactam imipenem in relation to the Gram-negative coccobacillus *A. baumannii*.

Table 4. Synergistic activities.

-			***B. subtilis*** **(ATCC 6633)**			***E. coli*** **(ATCC25218)**			***P. aeruginosa*** **(ATCC 10145)**		
I	Ampicillin 44 ± 0.4	I + Ampicillin **50 ± 0.**3	IV	Ampicillin 34 ± 2.7	IV + Ampicillin 34.3 ±0.2	II	Ampicillin 14.5 ± 0.3	II + Ampicillin **16 ± 0.2**	I	Levofloxacin 21 ± 0.4	I + Levofloxacin **35 ± 0.4**
V	Ampicillin 44 ± 0.4	V + Ampicillin 44.7 ± 1.5	-	-	-	III	Gentamicin 19.5 ± 0.0	III + Gentamicin **32.7 ± 0.2**	V	Levofloxacin 21 ± 0.4	VII + Levofloxacin **30 ± 1.0**
-	-	-	-	-	-	VI	Gentamicin 19.5 ± 0.0	VI + Gentamicin **22 ± 0.01**	V	Ciprofloxacin 35 ± 0.02	VII + Ciprofloxacin 36 ± 0.1
-	-	-	-	-	-	II	II 28.0 ± 1.60	II + Ciprofloxacin **32 ± 0.0**		-	-

6 mm diameter paper discs impregnated with 1 mg of plant extracts or antibiotics and extract + antibiotic
I-*C. bracteosus* leaves chloroform (1 mg/disc)
II-*C. bracteosus* bark chloroform (1 mg/disc)
III-*D. hasselti* fruit methanol (1 mg/disc)
IV-*K. retusa* wood hexane (1 mg/disc);
V-*K. retusa* wood methanol (1 mg/disc);
VI-*L. spathacea* wood hexane (1 mg/disc);
VII: Fruit flesh chloroform (1 mg/disc)Ampicillin (10 µg/disc); Ciprofloxacin (5 µg/disc); Gentamicin (10 µg/disc); Levofloxacin (5 µg/disc).
Values are expressed as mean of triplicate experiments
Plant extracts without synergistic activity are not included in this table

3.4. Antiviral Activity

Out of the 42 extracts tested, 17 were able to inhibit the replication of influenza A virus (H1N1) in MDCK cells. Most extracts had IC_{50} values below 10 µg/mL, but they were almost equally cytotoxic, except the chloroform extract of leaves of *L. spathacea*, chloroform extract of bark and leaves methanol extract of *B. malaccensis*, and chloroform extract of bark of *C. bracteosus*, and the chloroform extract of *D. hasseltii* with the selectivity indices of 5, 2, 2, 3, and 2, respectively (Table **5**).

4. DISCUSSION

Some species of bacteria develop the ability to escape the effects of broad-spectrum antibiotics. This antimicrobial resistance is responsible for significant rates of nosocomial infections (now called Healthcare-associated Infections, HCAIs) worldwide, as well as the commonly called 'superbugs', and weighs highly on global healthcare systems [3, 45]. Therefore, there is an urgent need to identify antibiotic potentiators in response to increased antibiotic resistance. In fact, the WHO has made the research and development of new antibiotics a priority to address the growing global resistance to antimicrobial drugs. Among the list of WHO priority pathogens for R&D of new antibiotics are the bacteria *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [8].

In this context, we have examined the antibacterial and antibiotic potentiator effects of five plants selected from Malaysia's primary tropical rainforest: *Cleistanthus bracteosus*, *Diospyros hasseltii*, *Kibatalia maingayi*, *Knema retusa*, and *Litsea spathacea*. The choice to collect these medicinal plants from this geographical area was because (i) they escaped deforestation and (ii) no antibacterial reports are available on these plants. The plants were selected at a time when fruits or flowers were present to allow absolute botanical identification by FRIM botanists. The plants collected were extracted with hexane, chloroform, and methanol to obtain non-polar, mid-polar, and polar secondary metabolites [34]. The last pockets of the deforestation-threatened primary rainforest of Peninsular Malaysia remain a source of untapped plant resources for drug discovery. Nonetheless, some of these plants are known by local tribes and have been used as traditional medicines by natives [46, 47]. For example, *L. spathacea* is used for the treatment of fever by locals [48], while fruits of *D. hasseltii* are edible [49].

In this study, we have first assessed the antibacterial activities of 42 extracts obtained from leaves, wood, barks, and fruits of 5 species of rainforest trees. These plant extracts were tested against two Gram-positive bacteria, *S. aureus* and *B. subtilis*, and four Gram-negative bacteria, *E. coli*, *P. aeruginosa*, and *A. baumannii* strains being imipenem-sensitive and imipenem-resistant. We used the paper disc diffusion method to test the antibacterial activity of the extracts. Of the 42 extracts tested, the strongest activity was obtained with the methanol extract from the bark of *C. bracteosus*, which inhibited the growth of *S. aureus*, *E. coli*, and *P. aeruginosa*. Plants in the genus *Cleistanthus* Hook. f. ex Planch. (1848) tended to produce antibacterial natural products [30, 50]. Members of this genus have been reported to produce cytotoxic butyrolactone lignans [51], of which justicidin B is antibacterial [52]. In this study, *C. bracteosus* extracts showed low toxicity for MDCK cells *in vitro*. It is necessary to isolate and identify the substance(s) responsible

Table 5. Antiviral activities.

Plant	Part	Solvent	CC_{50}, µg/mL	IC_{50}, µg/mL	Selectivity Index
B. malaccensis	Leaves	Hexane	0.52	>0.3	**2**
	Leaves	Chloroform	0.04	>0.03	**2**
	Leaves	Methanol	5.7	>3.7	2
	Bark	Hexane	2.4	>1	**2**
	Bark	Chloroform	0.6	>0.4	**2**
	Wood	Chloroform	6.8	>3.7	**2**
	Fruit flesh	Chloroform	0.05	0.03	**1**
	Fruit flesh	Methanol	0.17	0.1	**2**
	Seeds	Hexane	18	>10	**2**
	Seeds	Methanol	16.2	>11	**1**
	Peel	Hexane	0.14	0.1	**1**
	Peel	Chloroform	0.05	0.03	**2**
C. bracteosus	Leaves	Hexane	251	>100	3
	Leaves	Chloroform	13.2	>11	1
	Leaves	Methanol	250	43.3	**6**
	Bark	Hexane	61.9	>33	2
	Bark	Chloroform	99	>33	3
	Bark	Methanol	**300**	>79	**4**
D. hasselti	Leaves	Hexane	31.2	>11	3
	Leaves	Chloroform	6.8	>3.7	2
	Leaves	Methanol	19.4	>11	2
	Bark	Chloroform	25	10	3
	Bark	Methanol	170	41	4
	Fruit	Chloroform	75.3	9.8	8
	Fruit	Methanol	20.6	>10	2
K. retusa	Leaves	Chloroform	50.1	>33	2
	Bark	Hexane	8.6	3	3
	Bark	Chloroform	47.5	>33	1
	Wood	Hexane	75.4	>33	2
	Wood	Chloroform	7.4	>11	1
	Wood	Methanol	250	54.4	5
L. spathacea	Leaves	Chloroform	7.1 ± 0.2	-	-
	Bark	Hexane	126	>100	1
	Bark	Chloroform	**158.5**	**30.1**	**5**
	Wood	Chloroform	12	>11	1
	Wood	Methanol	91.3	>33	3
Rimantadine			62±4	12±3	5
Oseltamivir carboxylate			>70	0.088±0.010	795

Inactive extracts are not included
Values are expressed as mean of triplicate experiments

for the antibacterial activity of *C. bracteosus* before it becomes extinct.

Six extracts of *D. hasseltii* inhibited the growth of *A. baumannii* (imipenem-sensitive), with the bark methanol extract being the most active with an inhibition zone diameter of 10 mm. It also showed significant activity against *E. coli*, particularly the hexane extract of its fruits. There are reports on antibacterial and cytotoxic activities from plants in the genus *Diospyros* L. (1753), attributed in general to naphthoquinones [53-55]. One example is plumbagin, which evoked 50% inhibition of growth of *Mycobacterium luteus*, *S. aureus*, *M. smegmatis*, and *M. avium* at concentrations of 80, 100, 300, and 600 µg/mL, respectively [56, 57]. Besides naphthoquinones, other antibacterial principles in this genius are pentacyclic triterpenes and flavonoids [53].

We observed that the methanol extract of leaves *L. spathacea* inhibited *A. baumannii* (imipenem-sensitive) with the largest zone of inhibition. The methanol extract from leaves of this plant was the only active against *A. baumannii* (imipenem-resistant plants in the genus *Litsea* Lam. (1792) have been reported to exhibit antibacterial effects on account of monoterpenes and isoquinolines) [58-61]. Thus, it is necessary to submit this plant to a phytochemical study aimed at identifying its antibacterial principle(s).

After assessing the antibacterial potential of the plant extracts *via* disc diffusion assays, we investigated the Minimum Inhibitory Concentrations (MIC) at which the plant extracts inhibited bacterial growth *via* broth microdilution assays. MIC values below 1000 µg/mL were considered to indicate extracts active at inhibiting the growth of bacteria (two Gram-positive and 4 Gram-negative strains).

According to Krishnan *et al.* (2010), antibacterial extracts or compounds are categorized into two classes: bacteriostatic (MBC/MIC ratio more than 4) and bactericidal (MBC/MIC ratio less than or equal to 4) [62]. Following this classification, the chloroform extract of leaves of *L.*

spathacea was bactericidal against *A. baumannii* (imipenem-sensitive). Again, we had no clue about the type of natural product responsible for this bactericidal activity, but anticipated an alkaloid or a terpene to be responsible for it [63, 64]. We ran LC-MS of the chloroform extract of leaves of *L. spathacea* and observed two major compounds with the formula of $C_{11}H_{14}O_2$ (retention time: 4.888 mins) and $C_{19}H_{13}NO_5$, corresponding to eugenol methyl ether and dicentrinone, but since we had no pure standard compounds, we were unable to ascertain the identity of these 2 peaks. It is noteworthy that eugenol methyl ether and dicentrinone appeared to be antibacterial [65]. Dicentrinone was found to intercalate bacterial DNA [66, 67].

We also analyzed, specifically, the ability of these extracts to potentiate the activity of ampicillin, gentamicin, imipenem, levofloxacin, and ciprofloxacin towards *S. aureus, B. subtilis, P. aeruginosa, E. coli, A. baumannii* (imipenem-sensitive), and *A. baumannii* (imipenem-resistant) by synergistic interaction assay. Chloroform extracts of *C. bracteosus* induced the strongest antibacterial and synergic effects. Plants in this genus have been reported to produce lignans and diterpenes [68, 69]. The diterpene 16α-hydroxycleroda-3,13(14)-Z-dien-15,16-olide from the leaves of *Polyalthia longifolia* (Sonn.) Thwaites (family Annonaceae) elicited synergistic effect with norfloxacin, ciprofloxacin, and ofloxacin against several strains of Methicillin-resistant *S. aureus* (MRSA) *via* inhibition of multidrug resistance pumps [70]. While the neolignans (-)-acuminatin, (-)-denudatin B, and puberulin D of *Piper betle* L. (family Piperaceae) had synergistic effect with norfloxacin towards *S. aureus* (strain SA1199B) [71].

Wood hexane and methanol extract from *K. retusa* were found to potentiate ampicillin effects against *S. aureus* and *B. subtilis*, respectively. Members of the genus *Knema* Lour. (1790) are known to produce antibacterial alkyl resorcinols [72]. Further chemical analysis is being conducted in our laboratories to identify and isolate the antibiotic-potentiating constituents in *C. bracteosus* and *K. retusa*. Antibiotic potentiators are needed to be discovered by clinicians [73, 74].

Gram-negative bacteria are particularly resistant to antibiotics due to their outer membrane, lipopolysaccharides, porins, and efflux pumps [75]. Most of the bacteria that responded to our synergy test were Gram-negative, showing Malaysian primary forest plants to be a source of natural products with the potential to be developed as antibiotic potentiators [76, 77]. Our data have also confirmed recently published findings [78].

Finally, we have investigated the *in vitro* toxicities of the plant extracts against MDCK cells as well as their antiviral activity against H1N1. The American National Cancer Institute defines extracts as toxic to canine cells when cytotoxic concentration (CC_{50}) values are below 20 µg/mL [78]. In this context, most extracts were not toxic to MDCK cells. As for the antiviral activity, most extracts had IC_{50} values below 10 µg/mL, but were almost equally cytotoxic (selectivity index close or equal to 1), except the chloroform extract of leaves of *L. spathacea*, chloroform extract of bark and leaves methanol extract of *B. malaccensis*, chloroform extract of the bark of *C. bracteosus*, and the chloroform extract of *D. hasseltii* These antiviral effects have been reported for the first time.

CONCLUSION

In the past few decades, there has been a dramatic decrease in the number of new antibiotics approved by the FDA, while infections with multi-resistant bacteria are on the rise. Resistant bacteria are a source of concern to clinicians due to the small number of effective antibiotics now available to save patients. The development of resistant-modifying agents could be a supplemental strategy to overcome bacterial resistance. Furthermore, the risk of viral pandemics, including influenza virus outbreaks, should stimulate developing countries, including Malaysia, to safeguard what is left of their rainforests, which are home to molecules with the potential to be developed as antiviral agents. The current results present evidence, further demonstrating that the rainforest of Malaysia comprises plants with potential for the development of plant-based material to improve the current treatment strategies for bacterial and viral infections. In addition, most plant extracts studied showed to not be toxic to canine cells, which magnifies the relevance of these plants as antibiotic-potentiating agents. Studies are being conducted to identify the active constituents.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: study conception and design: CW, KTJ, MS, VN, VZ; data collection: MZ, LE, GK; analysis and interpretation of results: NA, CR, NR, draft manuscript: JV, CW.

LIST OF ABBREVIATIONS

MDR	=	Multidrug-resistant
MRSA	=	Methicillin-resistant Staphylococcus Aureus
NIs	=	Nosocomial Infections
WHO	=	World Health Organization
MIC	=	Minimum Inhibitory Concentration
CAMHB	=	Cationically Adjusted Müeller-hinton Broth
MBC	=	Minimum Bactericidal Concentration
MTC	=	Micro Tetrazolium Test
RPMI	=	Rosewell Park Memorial Institute
FBS	=	Fetal Bovine Serum
TCC	=	Total Compound Chromatograms

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article and its supplementary information files.

FUNDING

This research was funded by grant FRGS/1/2018/WAB07/UNIM/02/1 from the Ministry of Education Malaysia (Ministry of Higher Education Malaysia, Malaysia).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Pathmanathan SG, Samat NA, Mohamed R. Antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa from a Malaysian Hospital. Malays J Med Sci 2009; 16(2): 27-32. PMID: 22589655

[2] Rohani MY, Raudzah A, Lau MG, *et al.* Susceptibility pattern of Staphylococcus aureus isolated in Malaysian hospitals. Int J Antimicrob Agents 2000; 13(3): 209-13. http://dx.doi.org/10.1016/S0924-8579(99)00129-6 PMID: 10724026

[3] World Health Organization Antimicrobial resistance. 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed Jun 5, 2020).

[4] Weber DJ, Hoffmann KK, Rutala WA, Pyatt DG. Control of healthcare-associated Staphylococcus aureus: Survey of practices in North Carolina hospitals. Infect Control Hosp Epidemiol 2009; 30(9): 909-11. http://dx.doi.org/10.1086/599772 PMID: 19622048

[5] Centers for Disease Control and Prevention. Invasive Staphylococcus aureus (MRSA/MSSA) infection tracking . Available from: https://www.cdc.gov/hai/eip/saureus.html (accessed Jun 16, 2020).

[6] Vincent JL. Nosocomial infections in adult intensive-care units. Lancet 2003; 361(9374): 2068-77. http://dx.doi.org/10.1016/S0140-6736(03)13644-6 PMID: 12814731

[7] Gray DA, Wenzel M. Multitarget approaches against multiresistant superbugs. ACS Infect Dis 2020; 6(6): 1346-65. http://dx.doi.org/10.1021/acsinfecdis.0c00001 PMID: 32156116

[8] WHO publishes list of bacteria for which new antibiotics are urgently needed. Available from: https://www.who.int/news--room/detail/27-02-2017-who-publishes-list-of-bacteria--for-which-new-antibiotics-are-urgently-needed (accessed Jun 5, 2020).

[9] Antimicrobial resistance: Global report on surveillance. 2014. Available from: https://www.who.int/publications/i/item/9789241564748

[10] Butler MS, Paterson DL. Antibiotics in the clinical pipeline in October 2019. J Antibiot 2020; 73(6): 329-64. http://dx.doi.org/10.1038/s41429-020-0291-8 PMID: 32152527

[11] Theuretzbacher U, Outterson K, Engel A, Karlén A. The global preclinical antibacterial pipeline. Nat Rev Microbiol 2020; 18(5): 275-85. http://dx.doi.org/10.1038/s41579-019-0288-0 PMID: 31745331

[12] Kinch MS, Patridge E, Plummer M, Hoyer D. An analysis of FDA-approved drugs for infectious disease: Antibacterial agents. Drug Discov Today 2014; 19(9): 1283-7. http://dx.doi.org/10.1016/j.drudis.2014.07.005 PMID: 25043770

[13] Theuretzbacher U, Piddock LJV. Non-traditional antibacterial therapeutic options and challenges. Cell Host Microbe 2019; 26(1): 61-72. http://dx.doi.org/10.1016/j.chom.2019.06.004 PMID: 31295426

[14] Neu HC, Fu KP. Clavulanic acid, a novel inhibitor of beta-lactamases. Antimicrob Agents Chemother 1978; 14(5): 650-5. http://dx.doi.org/10.1128/AAC.14.5.650 PMID: 310279

[15] Reading C, Cole M. Clavulanic acid: A beta-lactamase-inhiting beta-lactam from *Streptomyces clavuligerus*. Antimicrob Agents Chemother 1977; 11(5): 852-7. http://dx.doi.org/10.1128/AAC.11.5.852 PMID: 879738

[16] Sikkema J, de Bont JA, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 1995; 59(2): 201-22. http://dx.doi.org/10.1128/mr.59.2.201-222.1995 PMID: 7603409

[17] Prakash Singh M. Rapid test for distinguishing membrane-active antibacterial agents. J Microbiol Methods 2006; 67(1): 125-30. http://dx.doi.org/10.1016/j.mimet.2006.03.011 PMID: 16631264

[18] Koh LP, Wilcove DS. Is oil palm agriculture really destroying tropical biodiversity? Conserv Lett 2008; 1(2): 60-4. http://dx.doi.org/10.1111/j.1755-263X.2008.00011.x

[19] Hansen M C, Potapov P V, Moore R, *et al.* High-resolution global maps of 21st-century forest cover change. Science 2013; 342: 850-3. http://dx.doi.org/10.1126/science.1244693

[20] Miettinen J, Shi C, Liew SC. Deforestation rates in insular Southeast Asia between 2000 and 2010. Glob Change Biol 2011; 17(7): 2261-70. http://dx.doi.org/10.1111/j.1365-2486.2011.02398.x

[21] Bryan JE, Shearman PL, Asner GP, Knapp DE, Aoro G, Lokes B. Extreme differences in forest degradation in Borneo: Comparing practices in Sarawak, Sabah, and Brunei. PLoS One 2013; 8(7): e69679. http://dx.doi.org/10.1371/journal.pone.0069679 PMID: 23874983

[22] Cushman SA, Macdonald EA, Landguth EL, Malhi Y, Macdonald DW. Multiple-scale prediction of forest loss risk across Borneo. Landsc Ecol 2017; 32(8): 1581-98. http://dx.doi.org/10.1007/s10980-017-0520-0

[23] Keong CH. A Malaysian Assessment of The World List of Threatened Trees. Selangor, Malaysia: TRAFFIC Southeast Asia 2004.

[24] The IUCN Red List of Threatened Species Available from: https://www.iucnredlist.org (accessed Jun 1, 2020).

[25] Milow P, Malek S, Ramli RM. Medicinal plants of the indigenous tribes in peninsular Malaysia: Current and future perspectives. Active Ingredients from Aromatic and Medicinal Plants. InTech 2017. http://dx.doi.org/10.5772/66658

[26] Singh B, Borthakur SK, Phukan SJ. Cleistanthus Nokrensis (Euphorbiaceae), a New Species from Indian Himalaya. Taiwania 2014; 59: 197-205. http://dx.doi.org/10.6165/tai.2014.59.197

[27] World Conservation Monitoring Centre. Cleistanthus bracteosus. http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T31471A9636486.en

[28] Raghunathan M. Muthuswamy. An Ethnomedicinal Survey of Medicinal Plants Utilized by Folk People of the Thrissur Forest Circle. Eur J Pharm Med Res 2017; 4: 401-9.

[29] Chrispal A. Cleistanthus collinus poisoning. J Emerg Trauma Shock 2012; 5(2): 160-6. http://dx.doi.org/10.4103/0974-2700.96486 PMID: 22787347

[30] Thamburaj S. Bio-Prospecting of cleistanthus collinus and its antibacterial activity. Asian J Pharm Clin Res 2013; 6: 206-9.

[31] Mohanraja R. Anti HIV-1 activity, anti-bacterial activity and phytochemical analysis of leaf extracts from *Cleistanthus Collinus* (Roxb.) Benth. exHook.f. Indian J Tradit Knowl 2018; 17: 770-5.

[32] Panda SK, Padhi L, Leyssen P, Liu M, Neyts J, Luyten W. Antimi-

crobial, anthelmintic, and antiviral activity of plants traditionally used for treating infectious disease in the similipal biosphere reserve, Odisha, India. Front Pharmacol 2017; 8: 658. http://dx.doi.org/10.3389/fphar.2017.00658 PMID: 29109684

[33] Salleh N, Azeman S, Kiew R, Kamin I, Cheng Kong R, Plant R. Plant checklist of the bukit nanas forest reserve, Kuala Lumpur, Malaysia. One Ecosystem 2017; 2: e13708. http://dx.doi.org/10.3897/oneeco.2.e13708

[34] Harborne AJ. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. (3rd ed.), Springer Netherlands 1998.

[35] Darwish AM, Farmer BD, Hawke JP. Improved method for determining antibiotic susceptibility of *Flavobacterium columnare* isolates by broth microdilution. J Aquat Anim Health 2008; 20(4): 185-91. http://dx.doi.org/10.1577/H07-047.1 PMID: 19306607

[36] Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobicallytle, Ninth. Wayne, PA: Clinical and Laboratory Standards Institute 2012.

[37] Saquib SA, AlQahtani NA, Ahmad I, Kader MA, Al Shahrani SS, Asiri EA. Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: An *in vitro* microbiological study. Antibiotics 2019; 8(3): 89. http://dx.doi.org/10.3390/antibiotics8030089 PMID: 31266146

[38] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65(1-2): 55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4 PMID: 6606682

[39] Fabry W, Okemo PO, Ansorg R. Antibacterial activity of East African medicinal plants. J Ethnopharmacol 1998; 60(1): 79-84. http://dx.doi.org/10.1016/S0378-8741(97)00128-1 PMID: 9533435

[40] Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005; 100(1-2): 80-4. http://dx.doi.org/10.1016/j.jep.2005.04.025 PMID: 15964727

[41] Kuete V. Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Med 2010; 76(14): 1479-91. http://dx.doi.org/10.1055/s-0030-1250027 PMID: 20533165

[42] Parthasarathy VA, Chempakam B. Chemistry of Spices. Wallingford, UK: CAB International 2008. http://dx.doi.org/10.1079/9781845934057.0000

[43] Jorgensen JH, Ferraro MJ, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin Infect Dis 2009; 49(11): 1749-55. http://dx.doi.org/10.1086/647952 PMID: 19857164

[44] Lorian V. Antibiotics in Laboratory Medicine. (5th ed.), Philadelphia: Lippincott Williams & Wilkins 2005.

[45] Haque M, Sartelli M, McKimm J, Abu Bakar MB. Health care-associated infections – An overview. Infect Drug Resist 2018; 11: 2321-33. http://dx.doi.org/10.2147/IDR.S177247 PMID: 30532565

[46] Hussain AG, Noor NM, Hussin K. Nature's Medicine: A Collection of Medicinal Plants from Malaysia's Rainforest. (1st ed.), Malaysia: Landskap Malaysia 2015.

[47] Hossan MS, Jindal H, Maisha S, *et al.* Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh. Pharm Biol 2018; 56(1): 201-8. http://dx.doi.org/10.1080/13880209.2018.1446030 PMID: 29529970

[48] Johnson T. CRC Ethnobotany Desk Reference. CRC Press 2019. http://dx.doi.org/10.1201/9781351070942

[49] Milow P, Malek SB, Edo J, Ong HC. Malaysian species of plants with edible fruits or seeds and their valuation. Int J Fruit Sci 2014; 14(1): 1-27. http://dx.doi.org/10.1080/15538362.2013.801698

[50] Gobalakrishnan R, Kulandaivelu M, Bhuvaneswari R, Kandavel D, Kannan L. Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrata* L. J Pharm Anal 2013; 3(6): 460-5. http://dx.doi.org/10.1016/j.jpha.2013.07.001 PMID: 29403856

[51] Liu Y, Young K, Rakotondraibe LH, *et al.* Antiproliferative Compounds from *Cleistanthus boivinianus* from the madagascar dry forest1. J Nat Prod 2015; 78(7): 1543-7. http://dx.doi.org/10.1021/np501020m PMID: 26091020

[52] Hemmati S, Seradj H, Justicidin B. Justicidin B: A Promising bioactive lignan. Molecules 2016; 21(7): 820. http://dx.doi.org/10.3390/molecules21070820 PMID: 27347906

[53] Rashed K, Ćirić A, Glamočlija J, Soković M. Antibacterial and antifungal activities of methanol extract and phenolic compounds from Diospyros virginiana L. Ind Crops Prod 2014; 59: 210-5. http://dx.doi.org/10.1016/j.indcrop.2014.05.021

[54] Odelola HA, Okorosobo VI. Preliminary investigation of *in-vitro* antimicrobial activity of two *Nigerian Diospyros* species (Ebenaceae). Afr J Med Med Sci 1988; 17(3): 167-70. PMID: 2845757

[55] Bagla VP, Lubisi VZ, Ndiitwani T, Mokgotho MP, Mampuru L, Mbazima V. Antibacterial and antimetastatic potential of *Diospyros lycioides* extract on cervical cancer cells and associated pathogens. Evid Based Complement Alternat Med 2016; 2016: 1-10. http://dx.doi.org/10.1155/2016/5342082 PMID: 27239210

[56] Gu JQ, Graf TN, Lee D, *et al.* Cytotoxic and antimicrobial constituents of the bark of Diospyros maritima collected in two geographical locations in Indonesia. J Nat Prod 2004; 67(7): 1156-61. http://dx.doi.org/10.1021/np040027m PMID: 15270571

[57] Lajubutu BA, Pinney RJ, Roberts MF, Odelola HA, Oso BA. Antibacterial activity of diosquinone and plumbagin from the root of *Diospyros mespiliformis* (Hostch) (Ebenaceae). Phytother Res 1995; 9(5): 346-50. http://dx.doi.org/10.1002/ptr.2650090508

[58] Hu W, Li C, Dai J, Cui H, Lin L. Antibacterial activity and mechanism of Litsea cubeba essential oil against methicillin-resistant Staphylococcus aureus (MRSA). Ind Crops Prod 2019; 130: 34-41. http://dx.doi.org/10.1016/j.indcrop.2018.12.078

[59] Su YC, Ho CL. Essential oil compositions and antimicrobial activities of various parts of *Litsea cubeba* from Taiwan. Nat Prod Commun 2016; 11(4): 1934578X1601100. http://dx.doi.org/10.1177/1934578X1601100425 PMID: 27396208

[60] Kuspradini H, Wulandari I, Putri AS, Tiya SY, Kusuma IW. Phytochemical, antioxidant and antimicrobial properties of *Litsea angulata* extracts. F1000 Res 2018; 7: 1839. http://dx.doi.org/10.12688/f1000research.16620.1 PMID: 30774930

[61] Wang H, Liu Y. Chemical composition and antibacterial activity of essential oils from different parts of Litsea cubeba. Chem Biodivers 2010; 7(1): 229-35. http://dx.doi.org/10.1002/cbdv.200800349 PMID: 20087994

[62] Krishnan N, Ramanathan S, Sasidharan S, Murugaiyah V, Mansor SM. Antimicrobial activity evaluation of cassia spectabilis leaf extracts. Int J Pharmacol 2010; 6(4): 510-4. http://dx.doi.org/10.3923/ijp.2010.510.514

[63] Devika M, Nalini MS. *In vivo* evaluation of anxiolytic activity of aqueous and ethanolic extracts of Litsea floribunda (Bl.) Gamble - Lauraceae. Asian J Pharm Pharmacol 2018; 4(1): 31-7. http://dx.doi.org/10.31024/ajpp.2018.4.1.6

[64] Wang YS, Wen ZQ, Li BT, Zhang HB, Yang JH. Ethnobotany, phytochemistry, and pharmacology of the genus Litsea: An update. J Ethnopharmacol 2016; 181: 66-107. http://dx.doi.org/10.1016/j.jep.2016.01.032 PMID: 26812679

[65] Joshi RK. Chemical composition, *In vitro* antimicrobial and antioxidant activities of the essential oils of *Ocimum gratissimum*, *O. sanctum* and their major constituents. Indian J Pharm Sci 2013; 75(4): 457-62. http://dx.doi.org/10.4103/0250-474X.119834 PMID: 24302801

[66] Udvardy A, Miskovics A, Sipos A. Antibacterial porphinoids – Progress and perspectives based on structure-activity analysis. Int Bull Drug Res 2014; 4: 1-34.

[67] Tan KH, Nishida R. Methyl eugenol: Its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. J Insect Sci 2012; 12(56): 1-60. http://dx.doi.org/10.1673/031.012.5601 PMID: 22963669

[68] Anjaneyulu ASR, Ramaiah PA, Row LR, Venkateswarlu R, Pelter A, Ward RS. New lignans from the heartwood of *cleistanthus collinus*. Tetrahedron 1981; 37(21): 3641-52.

http://dx.doi.org/10.1016/S0040-4020(01)98893-3
[69] Candy H A, Pakshong J M, Pegel K H. Pimarane diterpenes from *cleistanthus schlechteri*. J Chem Soc C Org 1970; 1970(18): 2536. http://dx.doi.org/10.1039/j39700002536
[70] Gupta VK, Tiwari N, Gupta P, *et al.* A clerodane diterpene from *Polyalthia longifolia* as a modifying agent of the resistance of methicillin resistant *Staphylococcus aureus*. Phytomedicine 2016; 23(6): 654-61. http://dx.doi.org/10.1016/j.phymed.2016.03.001 PMID: 27161406
[71] Sun ZL, He JM, Wang SY, *et al.* Benzocyclohexane oxide derivatives and neolignans from Piper betle inhibit efflux-related resistance in *Staphylococcus aureus*. RSC Advances 2016; 6(49): 43518-25. http://dx.doi.org/10.1039/C6RA10199B
[72] Kozubek A, Tyman JHP. Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. Chem Rev 1999; 99(1): 1-26. http://dx.doi.org/10.1021/cr970464o PMID: 11848979
[73] Alanis AJ. Resistance to antibiotics: Are we in the post-antibiotic era? Arch Med Res 2005; 36(6): 697-705. http://dx.doi.org/10.1016/j.arcmed.2005.06.009 PMID: 16216651
[74] Corona F, Blanco P, Alcalde-Rico M, *et al.* The analysis of the antibiotic resistome offers new opportunities for therapeutic intervention. Future Med Chem 2016; 8(10): 1133-51. http://dx.doi.org/10.4155/fmc-2016-0027 PMID: 27304087
[75] Poole K. Outer membranes and efflux: the path to multidrug resistance in Gram-negative bacteria. Curr Pharm Biotechnol 2002; 3(2): 77-98. http://dx.doi.org/10.2174/1389201023378454 PMID: 12022261
[76] Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. J Pharm Anal 2019. http://dx.doi.org/10.1016/j.jpha.2019.11.002 PMID: 32923005
[77] Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: A mechanistic viewpoint. Antimicrob Resist Infect Control 2019; 8(1): 118. http://dx.doi.org/10.1186/s13756-019-0559-6 PMID: 31346459
[78] Zulkipli M, Mahbub N, Fatima A, *et al.* Isolation and characterization of *werneria chromene* and dihydroxyacidissimol from Burkillanthus malaccensis (Ridl.) swingle. Plants 2022; 11(11): 1388. http://dx.doi.org/10.3390/plants11111388 PMID: 35684161