ANTIVIRAL, ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF THE CHINESE MEDICINAL PLANTS, *HOUTTUYNIA CORDATA*, *LOBELIA CHINENSIS* AND *SELAGINELLA UNCINATA*

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Abstract. This study was conducted to evaluate the extracts of *Houttuynia cordata* (Saururaceae), *Lobelia chinensis* (Campanulaceae) and *Selaginella uncinata* (Selaginellaceae) for their antimicrobial activities against Chikungunya virus, selected yeasts, filamentous fungi, and gram-positive and gram-negative bacteria of medical importance. Fresh preparations of the studied plants were sequentially extracted to obtain six different extracts. The extracts were examined for cytopathic inhibition effect on African monkey kidney epithelial (Vero) cells co-infected with Chikungunya virus. The chloroform extract of *L. chinensis* had the highest cytopathic inhibition effect with mean (±SD) cell viability of 66.5% (± 2.1)% at 40 μg/ml. The mean (± SD) 50% effective concentration and selectivity index for this extract were 28.4 (± 0.8) μg/ml and 7.5, respectively. A colorimetric broth microdilution assay revealed the hexane extract of *H. cordata* was the only extract with broad spectrum fungistatic activity against all six species of fungi evaluated, with mean minimum inhibitory concentration (MIC) values ranging from 0.08 to 1.25 mg/ml. As for antibacterial activity, the chloroform and ethyl acetate extracts of *S. uncinata* exhibited broad spectrum bacteriostatic activity against all six species of bacteria, with mean MIC ranges of 0.63-1.25 and 0.63-2.50 mg/ml, respectively. The antimicrobial activities of the extracts were found to be dependent on the species of microorganisms, testing concentration, and extractant used. The leaf extracts of *H. cordata* and *S. uncinata* are potential sources of new antifungal or antibacterial agents with broad spectrum activity. The chloroform extract of *L. chinensis* warrants isolation of the compounds active against Chikungunya virus due to its potency.

Keywords: chikungunya virus, cytopathic effect, fungistatic, fungicidal, bacteriostatic, bactericidal, Campanulaceae, Saururaceae, Selaginellaceae

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INTRODUCTION

Medicinal plants have been used in many cultures as a source of medicine. They constitute one of the main components of traditional medicine which has

long been used for prevention and treatment of various diseases. In recent years, medicinal plants have drawn the attention of scientific research due to their accessibility, therapeutic effects and minimum side effects (Kong *et al*, 2008).

Houttuynia cordata Thunb. is a perennial plant native to China, Japan, Korea, and Southeast Asia. It belongs to the Saururaceae family and is commonly known as *Yu-Xing-Cao* in Chinese, which literally means "fishy-smell plant". *Houttuynia cordata* has a long history of use in traditional Chinese medicine as an antiseptic, febrifuge, diuretic and deobstruant (Zheng *et al*, 1998). The plant is also used by traditional Chinese medicine practitioners in Malaysia as a component in the recipes to treat bronchitis, lung abscesses, lung cancer, mastitis, pneumonitis and urticaria (Lee, 2003; Yang, 2007). Several studies have reported a variety of pharmacological properties: antimutagenic, anti-inflammatory, antiviral, antiobesity, anticancer, antiallergic, antidiabetic, antiplatelet aggregation and antioxidant activities (Fu *et al*, 2013; Kumar *et al*, 2014).

Lobelia chinensis Lour., commonly known as 'Chinese Lobelia', is a small flowering perennial plant that belongs to the Campanulaceae family. The plant is distributed widely in East Asian countries, including China, Korea and Japan. It has been used in traditional Chinese medicine as an antidote for snake bite, as a diuretic, a hemostat and to treat diarrhea and jaundice (Shibano *et al*, 2001; Yang *et al*, 2014). It has been used by traditional Chinese medicine practitioners in Malaysia to treat gastric, colon and liver cancers (Yang, 2007). The plant has been reported to inhibit the α-glucosidase enzyme (Shibano *et al*, 2001), to have anti-inflammatory activity, to treat herpes simplex virus infection (Kuo *et al*, 2011) and to treat lung (Yang *et al*, 2014) and colorectal cancers (Han *et al*, 2013).

Selaginella uncinata (Desv. ex Poir.) Spring, belonging to the family Selaginellaceae, is a type of fern commonly known as blue spikemoss or peacock moss. The plant is native to China and is used in traditional Chinese medicine to treat jaundice, dysentery, edema and rheumatism (Jiangsu New Medical College, 1986). It is also used by traditional Chinese medicine practitioners in Malaysia to treat jaundice, acute and chronic nephritis and hemoptysis (Yang, 2007). Pharmacognostic and phytochemical studies of *S. uncinata* have reported the plant to have antiviral (Ma *et al*, 2003), antianoxic (Zheng *et al*, 2008) and antitumor (Li *et al*, 2014) properties.

Despite the reported pharmacological properties of these three plants, there are few studies of their efficacy against infectious diseases. The lack of specific treatment for Chikungunya virus infection, the escalation of invasive fungal infections in immunocompromised or immunosuppressed patients, the development of resistance against antimicrobial drugs and the significant side effects of chemotherapy (Denning and Hope, 2010; Lo Presti *et al*, 2014; Ciorba *et al*, 2015) have driven a search for newer and safer anti-infective agents among natural resources such as medicinal plants. This study was thus carried out to evaluate the extracts of *H. cordata*, *L. chinensis* and *S. uncinata* for antimicrobial activities against Chikungunya virus, selected yeasts, filamentous fungi, and gram-positive and gram-negative bacteria of medical importance.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *Houttuynia cordata* (472.5 g) and *Selaginella uncinata* (741.0 g)

were collected from a nursery at Kampar, Perak, and from a forest at Bukit Mertajam, Penang, Malaysia, respectively. The fresh aerial parts of *Lobelia chinensis* (81.1 g) were harvested from an herb garden in Endau, Johor, Malaysia. The species identity of the plant samples was authenticated by Professor Hean Chooi Ong, an ethnobotanist of the Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. Specimen vouchers of the plants were prepared, coded (UTAR/FSC/13/004 for *H. cordata*; UTAR/FSC/12/007 for *L. chinensis*; UTAR/FSC/12/006 for *S. uncinata*) and deposited at the Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, Perak, Malaysia.

Preparation of plant extracts

The respective collected plant materials were cleaned, cut and blended prior to extraction. The blended plant materials were sequentially extracted using hexane, chloroform, ethyl acetate, ethanol, methanol and sterile distilled water at room temperature and with agitation at 140 rpm. All the organic solvents used were of analytical grade. Three cycles of maceration were performed for each solvent. The filtrate for each organic solvent was pooled and evaporated *in vacuo* while the water extract was lyophilized. All the dried extracts were kept at -20°C prior to bioassay.

Culture of Vero cells

African monkey kidney epithelial (Vero) cell line (ATCC®CCL-81™), a normal mammalian cell line, was used to propagate the Chikungunya virus, and to conduct the cytotoxicity and antiviral assays. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St Louis, MO) supplemented with 5% fetal bovine serum (FBS), 1% penicillin (10,000 units/ml), 1% streptomycin (10 mg/ml) and 3.7 g/l of sodium bicarbonate at pH of 7.4; the cells were incubated at 37°C in a humidified 5% CO_2 incubator.

Cytotoxicity assay

A stock solution of each plant extract was prepared in a dimethyl sulfoxide-ethanol mixture (60:40, v/v) at a concentration of 512 mg/ml, and subsequently diluted two-fold serially in maintenance medium (DMEM with 1% FBS) to obtain eight final concentrations ranging from 5 to 640 μg/ml for the cytotoxicity assay. The working solutions (100 μl) were introduced into the respective wells in 96-well microplates containing a confluent monolayer of Vero cells and incubated at 37°C in a 5% CO_2 atmosphere for 72 hours. Medium control and cell control were incorporated in each microplate. The viability of the cells after incubation was assessed using a neutral red uptake assay (Repetto *et al*, 2008). The percent cell viability was expressed as a mean (± standard deviation) for the three replicates. The 50% cytotoxic concentration (CC_{50}) of the extract was determined from the plot of the percent cell viability against the concentration of the extract.

Antiviral assay

The Chikungunya virus used was the Bagan Panchor strain and belongs to the Asian genotype. It was obtained from Professor Shamala Devi (Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia) and propagated in Vero cells. The virus titer was determined using the method of Reed and Muench (1938); the multiplicity of infection of 1 was used in the assay. Each plant extract was evaluated at six concentrations: two-fold serially diluted in maintenance medium (DMEM with 1% FBS) starting from the highest concentration (with cell viability ≥ 90%)

based on the results obtained from the cytotoxicity assay. The Vero cells ($4x10^4$ cells/well) were incubated with the plant extract and Chikungunya virus at 37°C in 5% CO_2 for 72 hours. Controls in each microplate included medium (DMEM only), virus (cells with virus only) and cell (cells with medium only). Chloroquine diphosphate (MP Biomedicals, Illkirch, France; purity > 99.9%) with a concentration range of 0.39 to 12.4 μM was used as the positive control. Following incubation, cell viability was assessed using a neutral red uptake assay (Repetto *et al*, 2008). The percent cell viability was expressed as the mean (± standard deviation) for three replicates. The 50% effective concentration (EC_{50}) of an extract was determined from the plot of the percent cell viability against the concentration of the extract. The selectivity index was calculated as the ratio of CC_{50} to EC_{50}.

Fungal cultures

Four species of yeast (*Candida albicans* ATCC®90028™, *Candida parapsilosis* ATCC®22019™, *Candida krusei* ATCC®6258™ (teleomorph) and *Cryptococcus neoformans* ATCC®90112™) and two species of filamentous fungi (*Aspergillus fumigatus* ATCC®204305™ and *Trichophyton mentagrophytes* ATCC®9533™) were used in the study. Prior to inoculum preparation, the yeast species were subcultured on Sabouraud dextrose agar (SDA) (Merck, Darmstadt, Germany) and the filamentous fungi were subcultured on potato dextrose agar (PDA) (Merck, Darmstadt, Germany). The fungal inocula were prepared for antifungal assay according to the guidelines published by Clinical Laboratory Standards Institute (CLSI, 2002a, b).

Antifungal assay

A colorimetric broth microdilution method described by Eloff (1998) was used with some modifications. A stock solution of each plant extract was prepared at 10 mg/ml in a methanol-water mixture (2:1, v/v). The stock solution was two-fold serially diluted with RPMI-1640 medium in 96-well microplates to achieve final concentrations ranging from 0.02 to 2.50 mg/ml. Amphotericin B (Bio Basic, Ontario, Canada; purity >75%) with a final concentration range of 0.06 to 8 μg/ml was used as the positive control. Fifty microliters of the prepared fungal inoculum was added into the wells, making the total volume of each well 100 μl. Sterility (medium only), growth (fungus only) and negative (extract only) controls were included in each microplate. The microplates were incubated at 35°C for 48 hours for the *Candida* spp; 35°C for 72 hours for *C. neoformans* and *A. fumigatus*, and 28°C for 7 days for *T. mentagrophytes*. After incubation, 20 μl of the growth indicator, *p*-iodonitrotetrazolium chloride (INT, 0.4 mg/ml; Sigma-Aldrich, St Louis, MO; purity ≥98%) was added to each well. Observation of the color change of the indicator was made and a minimum inhibitory concentration (MIC) was recorded. Twenty microliters of the content from wells with inhibitory activity (the indicator remained colorless) was inoculated onto SDA/PDA and incubated accordingly. Formation of the fungal colony on the agar plate was observed and the minimum fungicidal concentration (MFC) was determined. The MIC and MFC values were reported as the mean of three replicates.

Bacterial cultures

Two species of gram-positive bacteria (*Bacillus cereus* ATCC®11778™ and *Staphylococcus aureus* ATCC®6538™) and four species of gram-negative bacteria (*Acinetobacter baumannii* ATCC®19606™, *Escherichia coli* ATCC®35218™, *Klebsiella pneumoniae*

Table 1
Fifty percent cytotoxic concentrations (CC_{50}) of various extracts of Chinese medicinal plants against African monkey kidney epithelial (Vero) cells.

Extracts	50% Cytotoxic concentration (μg/ml)		
	Houttuynia cordata	*Lobelia chinensis*	*Selaginella uncinata*
Hexane	198.5 ± 4.8	256.3 ± 6.8	365.1 ± 50.7
Chloroform	107.2 ± 4.3	213.8 ± 13.3	135.6 ± 10.8
Ethyl acetate	506.9 ± 9.0	132.8 ± 12.4	39.7 ± 3.3
Ethanol	126.1 ± 2.7	-	255.9 ± 17.9
Methanol	227.2 ± 6.4	-	440.5 ± 22.8
Water	-	-	-

"-" no significant toxicity observed at the highest concentration (640 μg/ml).

ATCC®13883™ and *Pseudomonas aeruginosa* ATCC®27853™) were evaluated in the study. All the bacteria were subcultured on Mueller-Hinton agar (MHA) (HiMedia Laboratories, Mumbai) at 37°C for 24 hours prior to inoculum preparation. The bacterial inocula were prepared according to Clinical Laboratory Standards Institute guidelines (CLSI, 2005).

Antibacterial assay

We used a colorimetric broth microdilution method to determine the minimum inhibitory concentration (MIC) of the tested plants (Eloff, 1998). A stock solution of each plant extract (10 mg/ml) was prepared in a methanol-water mixture (2:1, v/v). The stock solution was two-fold serially diluted with Mueller-Hinton broth (MHB) (HiMedia Laboratories, Mumbai) in 96-well microplates to achieve final concentrations ranging from 0.02 to 2.50 mg/ml. Chloramphenicol (Nacalai Tesque, Kyoto, Japan; purity ≥98%) or tetracycline (Sigma-Aldrich, St Louis, MO; purity ≥95%) with a final concentration range of 1 to 128 μg/ml was used as the positive control. Fifty microliters of the prepared bacterial inoculum was added into the wells, making a total volume in each well of 100 μl. Sterility (medium only), growth (bacteria only) and negative (extract only) controls were included in each test. The microplates were incubated at 37°C for 24 hours. Following incubation, 20 μl of INT at 0.4 mg/ml was added to each well. The color of each well was observed and the MIC was determined and recorded. Twenty microliters of the content from the wells with inhibitory activity (the indicator remained colorless) was inoculated onto MHA and incubated at 37°C for 24 hours. Formation of the bacterial colony on the agar plate was observed and the minimum bactericidal concentration (MBC) was determined. The MIC and MBC values were reported as the mean of three replicates.

Data analysis

The susceptibility index for a microorganism is calculated as 100 x number of extracts effective against the microorganism strain divided by number of total extracts (Bonjar, 2004). The percentages of cell viability at the different concentrations of plant extract to evaluate for cytotoxicity and antiviral assays were analyzed with one-way analysis of variance using SPSS®, version 20 (IBM, Armonk, NY). Signifi-

Table 2
Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Chinese medicinal plants against medically-important fungi.

Extracts	Yeasts								Filamentous fungi			
	Candida albicans		*Candida parapsilosis*		*Candida krusei*		*Cryptococcus neoformans*		*Aspergillus fumigatus*		*Trichophyton mentagrophytes*	
	MIC[a]	MFC[a]	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Houttuynia cordata												
Hex	1.25	1.25	0.31	0.31	0.31	0.31	0.08	0.16	1.25	NA	0.31	0.63
Chl	NA	-	NA	-	NA	-	0.31	0.63	2.50	NA	1.25	1.25
EA	NA	-	NA	-	NA	-	0.16	NA	1.25	NA	NA	-
EtOH	NA	-	NA	-	NA	-	0.31	NA	NA	-	NA	-
MeOH	NA	-	NA	-	NA	-	0.31	NA	NA	-	NA	-
Water	NA	-	NA	-	NA	-	0.08	NA	NA	-	NA	-
AMB	0.001	-	0.0005	-	0.002	-	0.0001	-	0.004	-	0.002	-
Lobelia chinensis												
Hex	0.31	1.25	0.63	0.63	0.08	0.08	0.04	0.16	NA	-	0.31	NA
Chl	0.31	NA	0.63	1.25	0.16	0.31	0.08	0.16	NA	-	2.5	NA
EA	0.63	NA	1.25	1.25	0.31	0.63	0.16	0.16	NA	-	1.25	NA
EtOH	1.25	2.5	1.25	NA	1.25	NA	0.16	2.5	NA	-	NA	-
MeOH	NA	-	NA	-	NA	-	0.16	0.31	NA	-	NA	-
Water	NA	-	0.63	NA	0.31	0.63	0.16	0.63	NA	-	NA	-
AMB	0.001	-	0.001	-	0.002	-	0.0001	-	0.001	-	0.002	-
Selaginella uncinata												
Hex	0.63	0.63	0.63	NA	0.16	0.16	0.16	0.16	NA	-	0.04	0.16
Chl	2.50	2.50	0.63	NA	0.16	0.16	0.31	0.31	NA	-	0.04	1.25
EA	1.25	NA	0.16	NA	0.16	0.63	0.31	0.31	NA	-	0.08	0.63
EtOH	NA	-	1.25	NA	0.31	0.63	1.25	1.25	NA	-	1.25	2.50
MeOH	0.63	1.25	0.63	NA	0.63	0.63	0.63	0.63	NA	-	0.63	1.25
Water	NA	-	1.25	NA	0.63	2.50	NA	-	NA	-	NA	-
AMB	0.001	-	0.001	-	0.002	-	0.0005	-	0.001	-	0.002	-

[a]The MIC and MFC values are expressed as a mean of three replicates in mg/ml. Hex, hexane extract; Chl, chloroform extract; EA, ethyl acetate extract; EtOH, ethanol extract; MeOH, methanol extract; AMB, Amphotericin B (positive control); NA , no activity; "-" unavailable.

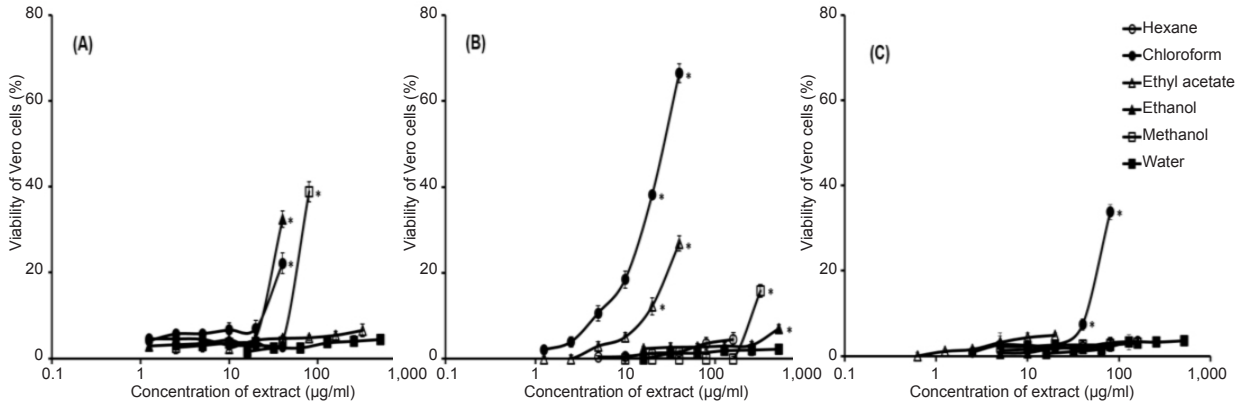

Fig 1–Viability of African monkey kidney epithelial (Vero) cells co-incubated with Chikungunya virus and plant extracts. The cells were incubated at 37ºC with 5% CO_2 for 72 hours. Cell viability was assessed by a neutral red uptake assay. (A) *Houttuynia cordata*, (B) *Lobelia chinensis,* (C) *Selaginella uncinata*. The x-axis is in a log scale.

cance was set at $p < 0.05$. A post hoc test, either using the Tukey's (equal variance assumed) or Dunnett's (equal variance not assumed) test was conducted to determine which concentration of extract produced a significant result.

RESULTS

Antiviral assay

The cytotoxicity of each plant extract against Vero cells was determined prior to the antiviral assay. The water extracts of the three studied plants were not significantly toxic ($p > 0.05$) to the Vero cells, even at the highest concentration of 640 mg/ml. The ethanol and methanol extracts of *L. chinensis* were not significantly toxic to the Vero cells ($p>0.05$). The mean 50% cytotoxic concentrations (CC_{50}) for those extracts which were significantly toxic to the Vero cells are shown in Table 1. The ethyl acetate extract of *S. uncinata* was the most toxic extract having the lowest mean CC_{50} value of 39.7 µg/ml. Concentrations of an extract which were associated with greater than 90% Vero cell viability (compared to untreated Vero cells which is the cell control of the assay) were used in the antiviral assay. Of the 18 extracts evaluated in this study, only the chloroform, ethanol and methanol extracts of *H. cordata*, the chloroform, ethyl acetate, ethanol and methanol extracts of *L. chinensis*, and the chloroform extract of *S. uncinata* exhibited a significant ($p < 0.05$) protective effect for the Vero cells against cytopathic effects caused by the Chikungunya virus. The highest percentage of cell viability was seen with the chloroform extract of *L. chinensis* (66.5% ± 2.1% at 40 µg/ml), followed by the methanol extract of *H. cordata* (38.8% ± 2.4% at 80 µg/ml) and the chloroform extract of *S. uncinata* (33.8% ± 1.8% at 80 µg/ml). In the virus control wells, the Vero cells were completely lyzed (cell viability = 0%) after 72 hours of co-incubation with the virus titer at the multiplicity of infection of 1. The chloroform extract of *L. chinensis* was the only extract that provided greater than 50% protection for the Vero cells against virus infection; the mean 50% effective concentration (EC_{50}) for this extract was

Table 3
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Chinese medicinal plants against medically important bacteria.

Extracts	Gram-positive bacteria				Gram-negative bacteria							
	Bacillus cereus		*Staphylococcus aureus*		*Acinetobacter baumannii*		*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
	MIC[a]	MBC[a]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Houttuynia cordata												
Hex	1.25	2.50	0.63	2.50	NA	-	NA	-	1.25	NA	1.25	2.50
Chl	1.25	NA	NA	-	NA	-	NA	-	1.25	NA	0.63	1.25
EA	2.50	NA	NA	-	NA	-	NA	-	2.50	2.50	0.63	1.25
EtOH	NA	-	NA	-	1.25	NA	NA	-	NA	-	0.63	NA
MeOH	NA	-	NA	-	1.25	NA	NA	-	NA	-	0.63	NA
Water	NA	-	NA	-	NA	-	NA	-	NA	-	1.25	NA
Antibio	0.004	-	0.004	-	0.001	-	0.002	-	0.004	-	0.008	-
Lobelia chinensis												
Hex	0.16	0.16	0.31	0.63	NA	-	NA	-	0.31	0.31	NA	-
Chl	0.31	0.31	1.25	2.50	NA	-	NA	-	1.25	1.25	NA	-
EA	0.63	0.63	1.25	2.50	NA	-	NA	-	1.25	1.25	NA	-
EtOH	0.63	NA	2.50	NA	NA	-	NA	-	1.25	1.25	NA	-
MeOH	1.25	NA	NA	-	NA	-	NA	-	2.5	2.50	NA	-
Water	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-
Antibio	0.004	-	0.004	-	0.001	-	0.002	-	0.002	-	0.004	-
Selaginella uncinata												
Hex	0.16	0.16	0.16	0.31	2.50	NA	NA	-	0.31	2.50	1.25	1.25
Chl	0.63	0.63	0.63	2.50	1.25	NA	1.25	NA	0.63	1.25	1.25	1.25
EA	0.63	1.25	1.25	NA	1.25	NA	2.50	NA	0.63	NA	0.63	0.63
EtOH	NA	-	NA	-	NA	-	NA	-	NA	-	0.63	2.50
MeOH	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-
Water	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-
Antibio	0.004	-	0.004	-	0.002	-	0.004	-	0.004	-	0.008	-

[a]The MIC and MBC values are expressed as a mean of three replicates in mg / ml. Hex, hexane extract; Chl, chloroform extract; EA, ethyl acetate extract; EtOH, ethanol extract; MeOH, methanol extract; Antibio, chloramphenicol was used for *B. cereus*, *S. aureus* and *K. pneumoniae* whereas tetracycline was used for *E. coli*, *P. aeruginosa* and *A. baumannii;* NA, no activity; "-" unavailable.

28.4 (± 0.8) μg/ml. The selectivity index for this extract was 7.5.

Antifungal assay

The MIC and minimum fungicidal concentration (MFC) for each tested plant extract are shown in Table 2. Considering one extract against one fungus as one bioassay, 58.3% (63/108) of the bioassays showed fungistatic activity and 38.0% (41/108) of the bioassays exhibited fungicidal activity. The lowest MIC value recorded from this study was 0.04 mg/ml, from the hexane extract of *L. chinensis* against *C. neoformans*, and the hexane and chloroform extracts of *S. uncinata* against *T. mentagrophytes*. The lowest MFC value (0.08 mg/ml) from this study was the hexane extract of *L. chinensis* against *C. krusei*. The hexane extract of *H. cordata* had broad spectrum antifungal activity against all the fungi evaluated in this study, with MIC values ranging from 0.08 to 1.25 mg/ml. The hexane extract of *H. cordata* was the only *H. cordata* extract active against the three species of *Candida* tested. This extract and the chloroform and ethyl acetate extracts of *H. cordata* had fungistatic activity against *Aspergillus fumigatus*. *Houttuynia cordata* is the only plant extract active against this filamentous fungus. *Aspergillus fumigatus* gave the lowest susceptibility index (16.7%) among the fungi tested in this study.

Antibacterial assay

Extracts of the three medicinal plants generally had less antibacterial activity than antifungal activity. Forty-three point five percent of the bioassays showed bacteriostatic activity and 25.9% of the bioassays showed bactericidal activity (Table 3). The hexane extracts of *L. chinensis* and *S. uncinata* had the lowest MIC and MBC values in this study, both with 0.16 mg/ml against *B. cereus*. The chloroform and ethyl acetate extracts of *S. uncinata* had broad spectrum antibacterial activity against both the gram-positive and all four of the gram-negative tested bacteria. Their MIC ranges were 0.63-1.25 and 0.63-2.50 mg/ml, respectively. These two extracts were also the only extracts active against *E. coli* in this study. *Escherichia coli* was the least susceptible bacterium towards the plant extracts tested with a susceptibility index of 11.1%. None of the extracts of *L. chinensis* were active against *A. baumannii* or *P. aeruginosa*.

DISCUSSION

The Vero cell line was used in this study because it is one of the most common and well established normal mammalian cell lines used to assess the effects of chemicals, toxins and other substances, such as plant extracts, on cells at the molecular level (Ammerman *et al*, 2008). This cell line is known to be susceptible to many viruses, such as Chikungunya virus (Sourisseau *et al*, 2007), and has visible cytopathic effects found upon infection.

This study reported for the first time the antiviral activity of *H. cordata*, *L. chinensis* and *S. uncinata* against Chikungunya virus. A literature search revealed *H. cordata* has potential antiviral activity against human (Hayashi *et al*, 1995; Lau *et al*, 2008; Lin *et al*, 2009) and animal (Choi *et al*, 2009) viruses. *Houttuynia cordata* has been reported to have activity against human immunodeficiency virus type 1, herpes simplex virus type 1, influenza virus (Hayashi *et al*, 1995), herpes simplex virus type 2 (Chiang *et al*, 2003), severe acute respiratory syndrome coronavirus (Lau *et al*, 2008), enterovirus-71 (Lin *et al*, 2009), porcine epidemic diarrhea virus (Choi *et al*, 2009), dengue virus type 2 and mouse hepatitis virus (Chiow *et al*,

2016). The methanol extract of *L. chinensis* has been found to have *in vitro* and *in vivo* inhibitory activity against herpes simplex virus type 1 (Kuo *et al*, 2008). Two compounds, uncinoside A and B, isolated from *S. uncinata* have been reported to have antiviral activity against respiratory syncytial virus and parainfluenza type 3 virus (Ma *et al*, 2003). The ethyl acetate extract of *S. uncinata* has been found to have antiviral activity against herpes simplex virus type 1 and coxsackie B3 virus (Jiang *et al*, 2008). In our study, we found *L. chinensis* had stronger antiviral activity against Chikungunya virus than *H. cordata* and *S. uncinata*.

This study also reported the antifungal activity of *H. cordata* and *S. uncinata* for the first time. The hexane extract of *H. cordata* had broad spectrum antifungal activity against all six fungi tested in the study. Extracts of *S. uncinata* also had antifungal activity against all the tested fungi except *A. fumigatus*. All the extracts of *L. chinensis* had antifungal activity against at least one species of fungus. In a study of 58 traditional Chinese medicinal plants, Zhang *et al* (2013) found the ethanol extract of *L. chinensis* had no activity against *C. albicans* or *A. fumigatus*, even at the highest concentration of 1.0 mg/ml. Their findings agree with our findings: the ethanol extract of *L. chinensis* had a MIC value of 1.25 mg/ml against *C. albicans* but had no inhibitory activity against *A. fumigatus* even at a dose of 2.50 mg/ml.

The broad spectrum antibacterial activities of *S. uncinata* were reported for the first time in this study. These activities were only found in extracts derived from less polar solvents, such as hexane (except against *E. coli*), chloroform and ethyl acetate. The different extracts of *H. cordata* had different antibacterial activities. Both gram-positive bacteria (*B. cereus* and *S. aureus*) and *K. pneumoniae* were susceptible to the non-polar or less polar extracts (hexane, chloroform or ethyl acetate) while *A. baumannii* was only susceptible to the ethanol and methanol extracts, which are polar in nature. *Escherichia coli* was resistant to all extracts examined, but *P. aeruginosa* was susceptible to all the extracts of *H. cordata*. This plant has also been reported to have antibacterial activity against acne-inducing bacteria (*Propionibacterium acnes* and *Staphylococcus epidermidis*) (Chomnawang *et al*, 2005), typhoid fever-causing bacteria (*Salmonella typhimurium*) (Kim *et al*, 2008) and methicillin-resistant *Staphylococcus aureus* (Sekita *et al*, 2016). These results support the traditional use of *H. cordata* as an antiseptic (Zheng, 1998). The antibacterial activity of *L. chinensis* was also reported by Zhang *et al* (2013) using the ethanol extract of *L. chinensis*.

We found resistance to the extracts of the studied plants by some microorganisms, such as the filamentous fungus *A. fumigatus* and *E. coli* bacterium. *Aspergillus fumigatus* and *E. coli* are regarded as nosocomial pathogens among humans. Resistance to antimicrobials has made treatment difficult or even impossible (von Baum and Marre, 2005; Arendrup, 2014). The search for medicinal plants against these microorganisms needs to continue.

The antimicrobial activities of the extracts of *H. cordata*, *L. chinensis* and *S. uncinata* were found to be dependent on the species of microorganism, tested concentration and type of solvent used for extraction. The chloroform extract of *L. chinensis* was the most potent extract against Chikungunya virus; this warrants further investigation to identify the active compounds and mechanisms. The plant extracts of *H. cordata* and *S. uncinata* could

be potential sources of new antifungal or antibacterial agents with broad spectrum activity.

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