Antifungal and cytotoxic activities of selected medicinal plants from Malaysia

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Abstract: This study was conducted to investigate the antifungal potential and cytotoxicity of selected medicinal plants from Malaysia. The extracts from the stem of *Cissus quadrangularis* and the leaves of *Asplenium nidus, Pereskia bleo, Persicaria odorata* and *Sauropus androgynus* were assayed against six fungi using *p*-iodonitrotetrazolium-based on colorimetric broth microdilution method. All the plant extracts were found to be fungicidal against at least one type of fungus. The strongest fungicidal activity (minimum fungicidal concentration=0.16 mg/mL) were exhibited by the hexane extract of *C. quadrangularis*, the hexane, chloroform, ethanol and methanol extracts of *P. bleo*, the hexane and ethyl acetate extracts of *P. odorata*, and the water extract of *A. nidus*. In terms of cytotoxicity on the African monkey kidney epithelial (Vero) cells, the chloroform extract of *P. odorata* produced the lowest 50% cytotoxic concentration (100.3 ± 4.2 μg/mL). In contrast, none of the water extracts from the studied plants caused significant toxicity on the cells. The water extract of *A. nidus* warrants further investigation since it showed the strongest fungicidal activity and the highest total activity (179.22 L/g) against *Issatchenkia orientalis*, and did not cause any toxicity to the Vero cells.

Keywords: Cytotoxicity, extraction, fungistatic, fungicidal, Vero cell, medicinal plants.

INTRODUCTION

Mycoses or fungal infections are significant causes of morbidity and mortality. Infections caused by fungi range from superficial, cutaneous or subcutaneous in healthy individuals to deeply invasive in immunocompromised patients. Over the past two decades, there has been a rise of invasive mycoses in cancer patients receiving chemotherapy, organ and bone marrow transplant patients, burns patients and extremely aged persons, apart from those with acquired immunodeficiency syndrome (AIDS) (Kriengkauykiat *et al*., 2011). The overall mortality for invasive mycoses caused by *Candida* spp. and *Aspergillus* spp. is 30-50% (Denning and Hope, 2010). It has been estimated that up to 625,000 deaths from meningitis, caused by *Cryptococcus* spp., occurred in AIDS patients each year (Park *et al*., 2009).

With the increase in mycoses, this resulted in the expanded use of antifungal drugs for treatment that unsurprisingly accelerated the development of drug resistance. Although armamentarium of antifungal drugs in clinical use may appear large, in fact only a limited number of drugs derived from five antifungal classes are available: polyenes, pyrimidine analogues, allylamines, azoles and echinocandins. Amphotericin B, a polyene antifungal drug was the standard therapy for many mycoses, but a high frequency of nephrotoxicity and infusional toxicity has limited its use (Denning and Hope, 2010). Newer antifungal drugs, such as azoles and echinocandins, however, have a narrow spectrum of activity and resistances against these drugs have also been reported clinically (Pfaller, 2012).

The limitations of the therapies currently available and the escalation in the emergence of drug-resistant fungal strains have resulted in an increase in the hunt for new antifungal agents from natural resources. Medicinal plants as sources of medicines have been in vogue since antiquity. World Health Organization (WHO) estimates that 80% of the world population presently uses herbal medicines for some aspects of primary health care (WHO, 1993). The medicinal values of plants lie in a diverse array of secondary metabolites produced as part of plant's defense mechanisms against predation by microorganisms, insects and herbivores, and thus medicinal plants represent a valuable source of new infective agents.

Asplenium nidus L. belongs to the family Aspleniaceae and is known by its common name as Bird's Nest Fern. It is used in traditional medicine as a remedy for hypertension, to kill lice and as a contraceptive (Bourdy and Walter, 1992; Yumkham and Singh, 2011). The leaf extracts of *A. nidus* have been reported to exhibit inhibitory activity against pathogenic bacteria (Lai *et al*., 2009) and herpes simplex virus (HSV) type 1 (Tahir *et al*., 2014).

Cissus quadrangularis L. (Vitaceae) or common name "Edible Stemmed Vine" is a common perennial climber which has been reported to have potent fracture healing

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properties. The stem is also used for the treatment of skin infections, gastritis, constipations, eye diseases, piles, anemia, asthma, irregular menstruation, burns and wounds traditionally (Mishra *et al*., 2010; Sen and Dash, 2012). The stem has become the subject of studies for antibacterial property by Kashikar and George (2006), Saikia *et al*. (2013) and Shabi Ruskin *et al*. (2014). It has also been reported to possess inhibitory activity against herpes simplex virus type 1 and 2 (Balasubramaniana *et al*., 2010).

Pereskia bleo (Kunth) DC., also known as rose cactus or leaf cactus is from the Cactaceae family. The leaves have been traditionally used to treat cancer, hypertension, diabetes, headache, gastric pain, ulcers, hemorrhoids, inflammatory conditions like rheumatism and asthma, and for rejuvenating the body (Lee, 2003; Zareisedehizadeh *et al*., 2014). The antibacterial activity of *P. bleo* has extensively been researched in Malaysia (Philip *et al*., 2009; Wahab *et al*., 2009; Appalaraju *et al*., 2013) and the antiviral activities on herpes simplex virus and human immunodeficiency virus (HIV) have also been studied (Hattori *et al*., 1995; Matsuse *et al*., 1998).

Persicaria odorata (Lour.) Sojak (Syn. *Polygonum odoratum* Lour.) (Polygonaceae) belongs to a group of fresh culinary herbs collectively known as "cilantro". In Malaysia and Singapore, the shredded leaf is an essential ingredient of the spicy soup "laksa", thus it is also called laksa leaf or "daun kesom" (Quynh *et al*., 2009). The leaves are also used to reduce fever, swellings and nausea, to ameliorate acne, hair and skin conditions, to aid digestion and stomach complaints, as a diuretic and as an overall health tonic (Shavandi *et al*., 2012). It has been reported that organic extracts from the leaves exhibit antibacterial property (Nanasombat and Teckchuen, 2009; Ridzuan *et al*., 2013; Saad *et al*., 2014).

Sauropus androgynus (L.) Merr. (Phyllanthaceae), known as "katuk" or sweet leaf by locals, is one of the most popular leafy vegetables in South and Southeast Asia countries. The leaves are used to increase lactation, to relieve cough, and to treat hypertension, diabetes, nose ulceration, eye ailments and earache (Ong *et al*., 2011). The leaves, stem and root are claimed by the Central Highlands and Mekong Delta villagers in Vietnam to be diuretic and also relieve fever and fungal infection of the tongue (Ogle *et al*., 2003). The plant has popularly been studied for its antibacterial property over the past few years (Paul and Beena Anto, 2011; Gayathramma *et al*., 2012; Ariharan *et al*., 2013).

Despite the extensive documentation of antimicrobial studies of these five medicinal plants, very little information is available about their biological activity against human fungal pathogens. Thus it is the aim of this study to investigate the antifungal activities of these plants against fungi of medical importance as well as their cytotoxicity on African monkey kidney epithelial (Vero) cells.

MATERIALS AND METHODS

Plant materials

In this study, fresh stem of *C. quadrangularis* and leaves of the other four plants were used. The plant samples (approximately 0.8-1.6kg for each plant) were collected between 27th of September and 9th of October 2011 from different locations in Perak state, except for *P. bleo* which was obtained from Lukut, Negeri Sembilan state. Botanical identification of the plant samples was performed by Professor Hean Chooi Ong, an ethnobotanist from University of Malaya, Malaysia. Vouchers of plant specimens were prepared (UTAR/FSC/11/008 for *A. nidus*; UTAR/FSC/11/007 for *C. quadrangularis*; UTAR/FSC/11/011 for *P. bleo*; UTAR/FSC/11/009 for *P. odorata*; UTAR/FSC/11/010 for *S. androgynus*) and deposited at the Faculty of Science, Universiti Tunku Abdul Rahman, Perak, Malaysia.

Preparation of plant extracts

The fresh plant samples were rinsed under running tap water, cut into smaller pieces and blended using a blender (Model 38BL54, Waring Commercial, Torrington, CT, USA). The blended plant materials were macerated in six solvents of increasing polarity, i.e. hexane, chloroform, ethyl acetate, ethanol, methanol and water sequentially. The maceration process was carried out with agitation (140 rpm) using an orbital shaker (TS-525D, Yihder Technology, New Taipei City, Taiwan) at room temperature. Two cycles of maceration were performed for each solvent. The filtrates were pooled and concentrated to dryness at 40°C using a rotary evaporator (Buchi Rotavapor R-200, Flawil, Switzerland) while water extracts were lyophilized using a freeze-dryer (Martin Christ Alpha 1-4, LD Plus, UK). The dried extracts were stored at -20°C prior to antifungal and cytotoxicity assays.

Microorganisms and media

Six species of fungi including four yeasts such as *Candida albicans* (ATCC®90028™), *Candida parapsilosis* (ATCC®22019™), *Issatchenkia orientalis* (ATCC®6258™) and *Cryptococcus neoformans* (ATCC® 90112™) and two molds viz., *Aspergillus brasiliensis* (ATCC®16404™) and *Trichophyton mentagrophytes* (ATCC®9533™) were obtained from the American Type Culture Collection (ATCC) for the study. The yeasts were maintained on Sabouraud dextrose agar (SDA), while molds were maintained on potato dextrose agar (PDA).

Antifungal assay

For preparing broth medium, antibiotic and inoculum suspensions for antifungal assay were based on the Clinical and Laboratory Standards Institute (CLSI)

guidelines (CLSI, 2002a; CLSI, 2002b). The colorimetric broth microdilution method of Liu et al. (2007) with *p*-iodonitrotetrazolium chloride as the growth indicator was adopted with modifications to evaluate the minimum inhibitory concentration (MIC) of each plant extract. The stock solution of each plant extract was prepared at a concentration of 10 mg/mL in a methanol-distilled water mixture (2:1, v/v) and sterilized by filtration through 0.45 µm syringe filters. The assay was performed with two-fold serial dilution of the plant extracts in 96-well round bottom microplates (Cellstar®, Greiner Bio-One, Germany). The final concentrations of the plant extracts ranged from 0.02 to 2.50 mg/mL. Amphotericin B (Bio Basic Inc., Markham, Canada) was used as the positive control in the assay. Growth (fungal inoculum only), negative (plant extracts only) and sterility (medium only) controls were also included in each micro plate. The micro plates were incubated at 35°C for 48 h for *Candida* spp. and *I. orientalis*; 72h for *C. neoformans* and *A. brasiliensis* and at 28°C for 7 days for *T. mentagrophytes*. The MIC of each extract is defined as the lowest concentration which inhibited the growth of tested fungi. After MIC determination, content (20 µL) from the wells that shown no evidence of growth was inoculated on SDA/PDA agar and incubated. The lowest concentration at which fungal growth was not observed on the agar plates was recorded as the minimum fungicidal concentration (MFC) of that extract. The assays were performed in triplicates.

Cell culture

The African monkey kidney epithelial (Vero) cell line was obtained from the American Type Culture Collection (ATCC®CCL-81™). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS), 10,000 units penicillin and 10 mg streptomycin/mL, and 44 mM sodium bicarbonate at pH 7.4. The cells were maintained at 37°C in a humidified 5% CO_2 incubator (Binder, Tuttlingen, Germany).

Cytotoxicity assay

The viability of Vero cells was determined using neutral red uptake assay, as described by Repetto *et al*. (2008) with modifications. One hundred microliter of eight concentrations (640 to 5 µg/mL) of plant extracts, obtained by two-fold serial dilution in maintenance medium (DMEM with 1% FBS) was added into 96-well microplates seeded with 4 x 10^4 Vero cells/well. The cells were then incubated for 72 h at 37°C with 5% CO_2. After incubation, the medium was removed from each well following which each well was washed with 150 µL of phosphate buffered saline once, followed by the addition of 100 µL of neutral red solution (40 µg/mL in maintenance medium) into each well. The microplates were further incubated for 2 h to promote dye uptake by lysosomes. The mixture was then removed and the incorporated neutral red dye was extracted by adding 150 µL of neutral red destain solution (ethanol: glacial acetic acid: water, 50:1:49, v/v/v) into each well. The absorbance value was read at 540 nm using a microplate reader (Infinite M200, Tecan Austria GmbH, Grodig, Austria). Medium and cell controls were also incorporated in each microplate. The results were obtained from three independent experiments.

Data analysis

The MIC and MFC values were tabulated as the mean of three consistent replicated values. Total activity of an extract is expressed as the quantity of the extract obtained from 1 g of plant material divided by its MIC value. The total activity indicates the degree to which the active compounds in one gram of plant material can be diluted and still inhibit the growth of the tested microorganism (Eloff, 2004). Fungal susceptibility index (FSI) is calculated as (number of extract effective against each fungal strain/total number of extracts) x 100%. For cytotoxicity assay, the cell viability was calculated as [(x - y) / (z - y)] x 100%, where x, y and z were average absorbance of cells treated with extract, average absorbance of blank medium, and average absorbance of cell control, respectively. Fifty percent cytotoxic concentrations (CC_{50}) of the extracts were determined from the plots of cell viability against concentration. The data were analyzed with one-way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows software (Version 20, IBM Corp, Armonk, NY, USA). The significance level was set at $p< 0.05$.

RESULTS

Interest in finding alternative antifungal agents from medicinal plants to replace the ineffective ones has increased in recent years. In this study, the antifungal properties of different extracts obtained from five medicinal plants were assessed and their fungistatic and fungicidal activities, which are expressed as MIC and MFC values respectively, are depicted in table 1.All the fungi used in this study were susceptible to the positive control (Amphotericin B). The mean MIC values were 1-2 µg/mL for *C. albicans*, 2-4 µg/mL for *C. parapsilosis* and *I. orientalis*, 0.0625-0.125 µg/mL for *C. neoformans* and 8 µg/mL for both of the molds.

Considering one extract against one type of fungus as one bioassay, 68.9% of the bioassays showed fungistatic activity; whereas, only 60.0% of the bioassays produced fungicidal results. The water extract of *A. nidus* exhibited the strongest fungistatic activity with the lowest MIC value of 0.08 mg/mL against *I. orientalis*, thus giving the highest total activity of 179.22 L/g (table 2). On the other hand, several extracts demonstrated the strongest fungicidal activity and they were hexane extract of *C. quadrangularis*, hexane, chloroform, ethanol and methanol extracts of *P. bleo*, hexane and ethyl acetate

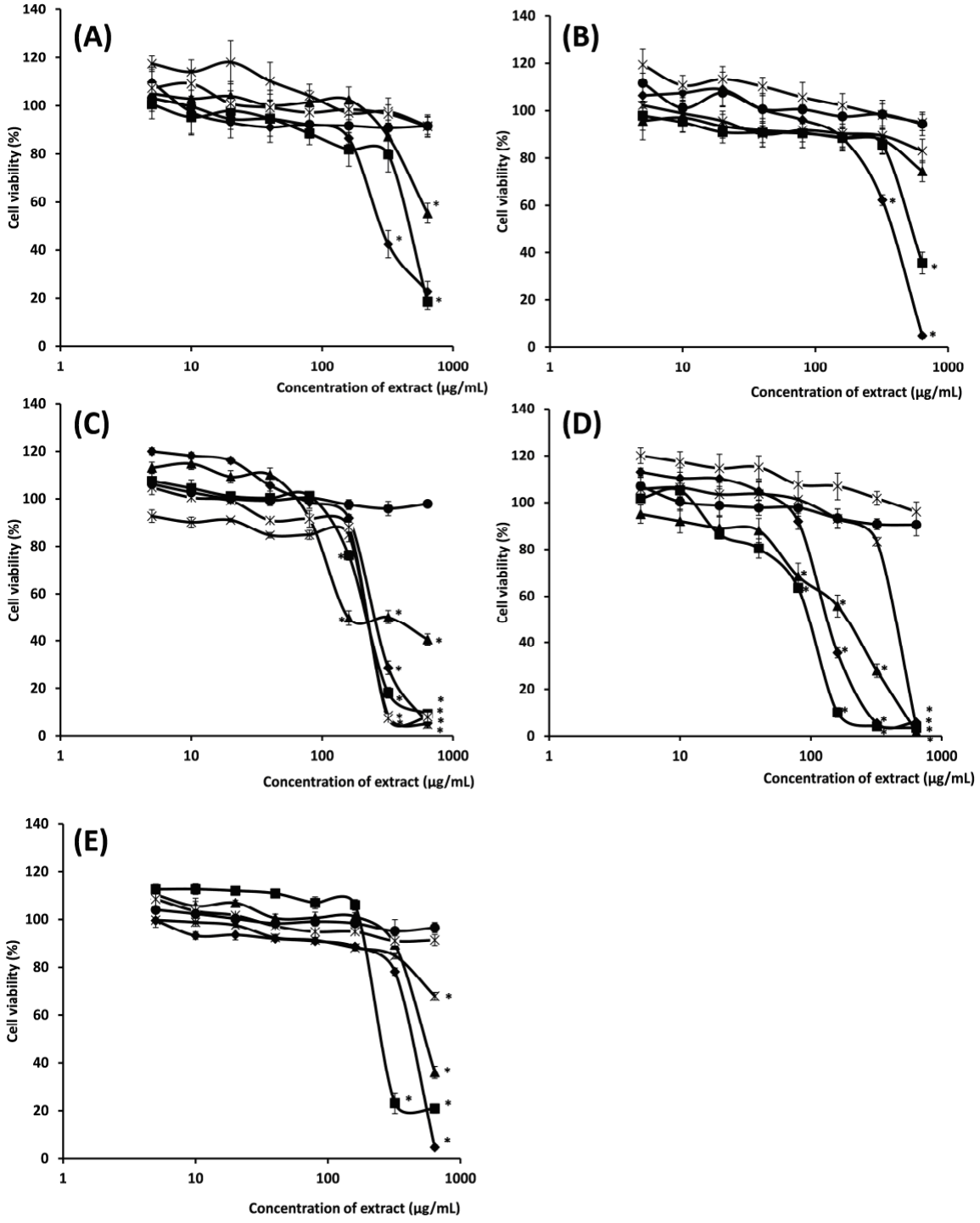

Fig. 1: Viability of African monkey kidney epithelial (Vero) cells treated with various extracts of five medicinal plants. The cell viability was assessed using neutral red uptake assay. The results represent mean ± SEM, $n = 3$. The asterisk mark indicates significant difference ($p < 0.05$) when analyzed with one-way ANOVA test. (A) – *Asplenium nidus*; (B) – *Cissus quadrangularis*; (C) – *Pereskia bleo*; (D) – *Persicaria odorata*; (E) – *Sauropus androgynus.* (♦) – Hexane extract; (■) – Chloroform extract; (▲) – Ethyl acetate extract; (×) – Ethanol extract; (∗) – Methanol extract; (●) – Water extract.

Table 1: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of various extracts obtained from five medicinal plants against fungi of medical importance

Plant extracts	*Candida albicans*		*Candida parapsilosis*		*Issatchenkia orientalis*		*Cryptococcus neoformans*		*Aspergillus brasiliensis*		*Trichophyton mentagrophytes*	
	MIC*	MFC*	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Asplenium nidus												
Hexane	0.63	0.63	1.25	1.25	0.31	0.31	0.31	0.31	NA	-	2.50	NA
Chloroform	0.63	0.63	1.25	2.50	0.31	0.31	0.31	0.31	NA	-	NA	-
Ethyl acetate	1.25	2.50	NA	-	0.63	0.63	NA	-	NA	-	NA	-
Ethanol	1.25	1.25	NA	-	0.31	0.31	2.50	NA	NA	-	NA	-
Methanol	NA	-	NA	-	0.63	0.63	2.50	NA	NA	-	NA	-
Water	0.31	NA	NA	-	0.08	0.16	0.63	NA	NA	-	NA	-
Cissus quadrangularis												
Hexane	0.31	0.31	1.25	1.25	0.16	0.16	0.16	0.31	0.63	0.63	1.25	1.25
Chloroform	0.63	0.63	NA	-	1.25	1.25	0.63	0.63	NA	-	NA	-
Ethyl acetate	0.63	0.63	2.50	2.50	0.31	0.31	0.31	0.31	NA	-	2.50	NA
Ethanol	0.63	0.63	NA	-	0.31	0.31	0.31	0.31	NA	-	NA	-
Methanol	1.25	1.25	NA	-	0.63	0.63	0.31	0.31	NA	-	2.50	NA
Water	NA	-	NA	-	0.63	0.63	1.25	1.25	NA	-	NA	-
Pereskia bleo												
Hexane	1.25	1.25	2.50	2.50	0.63	1.25	0.16	0.16	NA	-	2.50	NA
Chloroform	1.25	2.50	1.25	1.25	0.63	0.63	0.16	0.16	2.50	NA	2.50	NA
Ethyl acetate	1.25	2.50	1.25	1.25	0.63	0.63	0.31	0.31	2.50	2.50	1.25	2.50
Ethanol	0.63	0.63	1.25	1.25	1.25	1.25	0.16	0.16	NA	-	0.31	0.31
Methanol	0.63	0.63	1.25	1.25	1.25	2.50	0.16	0.16	NA	-	0.31	0.63
Water	2.50	2.50	NA	-	NA	-	0.63	2.50	NA	-	1.25	1.25
Persicaria odorata												
Hexane	0.63	0.63	1.25	1.25	0.63	0.63	0.16	0.16	1.25	2.50	0.16	0.63
Chloroform	0.63	0.63	2.50	2.50	0.63	0.63	0.16	1.25	2.50	2.50	0.16	0.31
Ethyl acetate	0.63	0.63	1.25	1.25	0.63	0.63	0.16	0.16	NA	-	0.16	2.50
Ethanol	0.63	0.63	NA	-	1.25	1.25	0.31	1.25	NA	-	0.63	2.50
Methanol	2.50	2.50	NA	-	0.31	0.31	0.63	0.63	NA	-	1.25	NA
Water	NA	-	NA	-	2.50	2.50	0.63	0.63	NA	-	NA	-
Sauropus androgynus												
Hexane	2.50	NA	2.50	2.50	1.25	1.25	1.25	1.25	NA	-	0.31	0.63
Chloroform	2.50	NA	2.50	2.50	1.25	2.50	0.63	0.63	NA	-	2.50	2.50
Ethyl acetate	2.50	NA	2.50	2.50	1.25	1.25	0.31	0.31	NA	-	NA	-
Ethanol	1.25	NA	2.50	2.50	1.25	1.25	0.31	0.31	NA	-	NA	-
Methanol	NA	-	2.50	2.50	2.50	2.50	0.31	0.31	NA	-	NA	-
Water	2.50	NA	2.50	2.50	0.63	0.63	0.63	0.63	NA	-	NA	-

The MIC and MFC values are expressed as mean of three consistent replicates in mg/mL. NA denotes no activity while "-" denotes unavailable due to absence of antifungal activity.

extracts of *P. odorata*, and water extract of *A. nidus*, all with the MFC value of 0.16 mg/mL.

All the plant extracts were found to be active or fungicidal against at least one type of fungus. Four of the 30 extracts obtained from the five medicinal plants exhibited broad-spectrum fungicidal activity against all the six types of fungi, i.e. hexane extract of *C. quadrangularis*, ethyl acetate extract of *P. bleo* and, hexane and chloroform extracts of *P. odorata* with their MIC ranges of 0.16-1.25, 0.31-2.50, 0.16-2.50, and 0.31-2.50 mg/mL respectively. In contrast, the methanol and water extracts of *A. nidus* only showed fungicidal activity against *I. orientalis* with MFC values of 0.63 and 0.16 mg/mL respectively.

In this study, *I. orientalis* was susceptible to all the plant extracts, except the water extract of *P. bleo*. Similarly, *C. neoformans* was susceptible to all the plant extracts, except the ethyl acetate extract of *A. nidus*. Thus both yeasts had the highest fungal susceptibility index (FSI), which was 96.7%. *Aspergillus brasiliensis* was only susceptible to the extracts of *C. quadrangularis* (hexane), *P. bleo* (chloroform and ethyl acetate) and *P. odorata* (hexane and chloroform), thus having the lowest FSI value of 16.7%. The FSI for *C. albicans*, *C. parapsilosis* and *T. mentagrophytes* were 86.7%, 60.0%, and 56.7% respectively.

Vero cells are one of the most common continuous cell lines used to assess the effects of chemicals, toxins and other substances on mammalian cells at the molecular level (Ammerman et al., 2008). The toxicity of each extract on Vero cells is illustrated in fig 1. The water extract of all the five medicinal plants appeared to be the

Table 2: Total activity of various extracts obtained from five medicinal plants against fungi of medical importance

Plant extracts	Total activity (L/g)*					
	Candida albicans	*Candida parapsilosis*	*Issatchenkia orientalis*	*Cryptococcus neoformans*	*Aspergillus brasiliensis*	*Trichophyton mentagrophytes*
Asplenium nidus						
Hexane	2.83	1.43	5.75	5.75	-	0.71
Chloroform	16.49	8.31	33.51	33.51	-	-
Ethyl acetate	2.45	-	4.86	-	-	-
Ethanol	17.95	-	72.39	8.98	-	-
Methanol	-	-	17.53	4.42	-	-
Water	46.25	-	179.22	22.76	-	-
Cissus quadrangularis						
Hexane	1.02	0.25	1.98	1.98	0.50	0.25
Chloroform	1.86	-	0.94	1.86	-	-
Ethyl acetate	2.86	0.72	5.82	5.82	-	0.72
Ethanol	4.13	-	8.40	8.40	-	-
Methanol	1.20	-	2.38	4.83	-	0.60
Water	-	-	2.07	1.04	-	-
Pereskia bleo						
Hexane	0.38	0.19	0.76	2.98	-	0.19
Chloroform	0.63	0.63	1.26	4.96	0.32	0.32
Ethyl acetate	0.58	0.58	1.15	2.33	0.29	0.58
Ethanol	8.19	4.13	4.13	32.24	-	16.64
Methanol	7.11	3.58	3.58	27.98	-	14.44
Water	1.65	-	-	6.55	-	3.30
Persicaria odorata						
Hexane	5.89	2.97	5.89	23.19	2.97	23.19
Chloroform	8.11	2.04	8.11	31.92	2.04	31.92
Ethyl acetate	3.98	2.00	3.98	15.66	-	15.66
Ethanol	6.14	-	3.09	12.47	-	6.14
Methanol	1.96	-	15.78	7.76	-	3.91
Water	-	-	1.66	6.58	-	-
Sauropus androgynus						
Hexane	1.76	1.76	3.53	3.53	-	14.22
Chloroform	4.44	4.44	8.87	17.60	-	4.44
Ethyl acetate	4.44	4.44	8.89	35.84	-	-
Ethanol	19.85	9.92	19.85	80.04	-	-
Methanol	-	7.34	7.34	59.20	-	-
Water	2.04	2.04	8.08	8.08	-	-

Total activity is calculated as: (the quantity of the extract obtained from 1 g of plant material/mean MIC value). "-" denotes unavailable due to absence of antifungal activity.

Table 3: Fifty percent cytotoxic concentrations (CC_{50}) of various extracts obtained from five medicinal plants on African monkey kidney epithelial (Vero) cells

Plant extracts	50% Cytotoxic concentration (µg/mL)				
	Asplenium nidus	*Cissus quadrangularis*	*Pereskia bleo*	*Persicaria odorata*	*Sauropus androgynus*
Hexane	283.2 ± 12.2	387.3 ± 19.0	266.4 ± 10.0	139.4 ± 5.4	442.3 ± 6.1
Chloroform	482.3 ± 32.9	549.1 ± 45.9	232.2 ± 9.6	100.3 ± 4.2	268.8 ± 8.9
Ethyl acetate	> 640	-	160.5 ± 9.8	184.8 ± 42.8	558.4 ± 19.0
Ethanol	-	-	232.7 ± 6.2	454.9 ± 6.9	> 640
Methanol	-	-	235.2 ± 6.2	-	-
Water	-	-	-	-	-

"-" denotes unable to determine as no significant toxicity was observed at the highest concentration (640 µg/mL).

safest extract, as no significant toxicity was observed even at the highest concentration of 640 µg/mL. However, the chloroform and ethyl acetate extracts of *P. odorata* showed significant toxicity (p<0.05) on Vero cells when the concentration was 80 µg/mL or higher. The chloroform extract of *P. odorata* produced the lowest mean 50% cytotoxic concentration (CC_{50}), i.e. 100.3 µg/mL (table 3). The percent cell viability (mean±s.d.) for the ethyl acetate extract of *A. nidus* and the ethanol extract of *S. androgynus* at 640 µg/mL was 55.39±4.15% and 67.99±1.52% respectively, and their CC_{50}values were estimated to be > 640 µg/mL.

DISCUSSION

The antifungal property of *A. nidus* extracts has not been reported prior to this study. The water extract of the leaves of this plant showed the strongest fungistatic and fungicidal activities against *I. orientalis*, produced the highest total activity value and did not cause any toxicity to the Vero cells. *Issatchenkia orientalis* is the teleomorph (sexually reproductive form) of *Candida krusei*, which is one of the key causes of invasive fungal infections in patients undergoing bone marrow or stem-cell transplantation, and in patients with malignant hematological disease (Denning and Hope, 2010). These results have highlighted that the active compounds present in the water extract are potent and highly selective towards yeast.

Srivastava *et al*. (2013) and Saikia *et al*. (2013) reported that the leaf extracts of *C. quadrangularis* possess inhibitory activity against several fungal pathogens including *C. albicans* and *C. neoformans*. The leaf extracts of *C. quadrangularis* have also shown antifungal activity against *Aspergillus* spp. (Gahlaut et al., 2013). However, no documentation of fungicidal activity for the extracts was recorded in these studies. As shown in table 1, it is noted that the stem of *C. quadrangularis* also possesses antifungal property, suggesting that the bioactive compounds in this plant could be distributed in the leaves as well as the stem.

Appalaraju *et al*. (2013) evaluated antibacterial and antifungal activities of chloroform, ethanol and water extracts of the dried leaves of *P. bleo* and reported that only the water extract was active against fungi (5 mg/disc) using the disc diffusion method. In contrast, with the exception of *A. brasiliensis*, the fungi were susceptible to at least five extracts of different polarities from *P. bleo*, despite lower amount of extracts (equivalent to 0.25 mg/well for the highest concentration) was used in the assay. This may be attributed to the difference in plant sample, as fresh leaves were used in this study compared to dried leaves used in the study by Appalaraju *et al*. (2013). Fresh and dried materials of the same plant may produce differences in terms of phytochemical contents and antimicrobial activities (Mondal *et al*., 2007; Alabi *et al*., 2012).

The leaves of *P. odorata* are usually used in the fresh form for cooking and are known to contain many volatile organic compounds such as (Z)-3-hexenal, (Z)-3-hexenol, decanal, dodecanal, 3-sulfanyl-hexanal, 3-sulfanyl-hexan-1-ol and polygodial (Starkenmann *et al*., 2006). In this study, the fresh leaves produced significant antifungal activities in which two of its extracts (hexane and chloroform) showed broad spectrum fungicidal activity. Ridzuan *et al*. (2013) used the oven-dried leaves and reported that none of the hexane, dichloromethane, methanol and water extracts showed antifungal activity against *C. albicans*. The phytochemicals in a plant sample may reduce in quantity or degrade upon drying.

Sauropus androgynus is an inexpensive source of dietary proteins and vitamins. It also contains phytochemicals such as tannins, flavonoids, anthocyanins and carotenoids (Petrus, 2013). These phytochemicals could be responsible for the antifungal activity observed in this study. This further supports the use of *S. androgynus* as a functional food for human. The results from this study justify the use of this plant to treat fungal infection of the tongue by the Central Highlands and Mekong Delta villagers in Vietnam (Ogle *et al*., 2003).

Fungal susceptibility indices revealed that yeasts are more susceptible to the phytochemicals in plant extracts compared to molds. This might be due to the complexity of the structure of molds, which grow as long filaments (hyphae) and form a mat (mycelium); while yeasts grow as single cells (Ray and Ryan, 2010). Thus the bioactive compounds in the plant extracts may affect the membrane of the yeasts more effectively compared to molds.

CONCLUSION

The results showed that there is a wide range of potential antifungal agents present in all of the tested plants. Further isolation and structural elucidation could be performed on those extracts that exhibited high total activity or broad spectrum antifungal activity. In this regard, the water extract of the leaves of *A. nidus* deserves attention due to its potency and selectivity.

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