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Research Letter

Antifungal and antibacterial properties of three medicinal plants from Malaysia

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ABSTRACT: **Introduction:** The increase of opportunistic fungal infections and the escalation of bacterial resistance have seriously reduced the efficacy of chemotherapeutic agents available. Thus the search for new antimicrobial agents from natural sources such as medicinal plants becomes necessary. **Methods:** The aerial parts of *Diplazium esculentum* and *Sechium edule*, and the fruits of *Solanum muricatum* were used, and extracted sequentially using hexane, chloroform, ethyl acetate, ethanol, methanol, and water. The extracts were then evaluated, in triplicate, against a panel of 12 medically-important microorganisms for microbiostatic and microbiocidal activities using colorimetric broth microdilution methods. **Results:** The total percentage yield obtained were 1.20%, 1.84% and 3.53% (w/w, based on fresh weight) for *D. esculentum*, *S. edule* and *S. muricatum*, respectively. All plant extracts showed antifungal activity with 66% and 49% of the bioassays demonstrating fungistatic and fungicidal activity, respectively. Two yeasts, *Cryptococcus neoformans* and *Issatchenkia orientalis* were found to be susceptible to all extracts. The lowest minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was exhibited by the hexane extracts of *S. edule* and *S. muricatum* against *C. neoformans*, both with values of 0.08 mg/mL. In the antibacterial screening assays, 49% of the bioassays exhibited bacteriostatic activity while only 21% of them showed bactericidal activity. The lowest MIC and minimum bactericidal concentration (MBC) was recorded for the hexane extract of *S. muricatum* against *Bacillus cereus* and *Klebsiella pneumoniae*, both with values of 0.31 mg/mL. The susceptibility of bacteria towards the plant extracts evaluated was species-dependent, with the susceptibility indices ranging from 0% for *Escherichia coli* to 72% for *Pseudomonas aeruginosa*. **Conclusions:** The results from this study show that extracts from these plants have significant antimicrobial activity, which corroborates their use in traditional medicine.

KEYWORDS: bacteriostatic, bactericidal, broth microdilution, extraction, fungistatic, fungicidal, *Diplazium esculentum*, *Sechium edule*, *S. muricatum*

INTRODUCTION

For centuries, herbal remedies derived from medicinal plants have been a major source of medicine for the treatment and prevention of ailments.[1] For example, the use of pumpkin (*Cucurbita moschata*) seeds and cranberry (*Vaccinium macrocarpon*) juice to treat urinary tract infections, while species such as garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are used as broad-spectrum antimicrobial agents.[2,3]

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The increase of opportunistic fungal infections and the escalation of bacterial resistance, particularly multi-drug resistance have seriously reduced the efficacy of many chemotherapeutic agents. The clinical usefulness of some antibiotics may be diminished within a short time due to the over-prescription and misuse of antibiotics.[4] Fungal infections remain the fourth-leading cause of life-threatening infections in hospitals, in part as the result of alterations in immune status associated with Acquired Immune Deficiency Syndrome (AIDS) epidemic, cancer chemotherapy and organ or bone marrow transplantation.[5] The most common fungal infection agents are those ubiquitous colonizers such as *Candida* spp., *Cryptococcus* and *Aspergillus* spp. with an overall mortality for invasive diseases of 25–50%.[6–9]

In Malaysia, the 'vegetable fern' (*D. esculentum*) and 'chayote' (*S. edule*), are usually eaten cooked in various dishes,

while the fruits of "pepino" (*S. muricatum*) are mostly consumed as a dessert.[2,10,11] *D. esculentum* (vegetable fern) is used traditionally to treat expectoration of blood, fever, dermatitis, measles, coughs and taken as a tonic by woman after childbirth.[10,12] *S. edule* (chayote) is used as a folk medicine in the treatment of arteriosclerosis, calcifications in the urinary system, hypertension and fever. The flesh of the chayote fruit is applied as a poultice on inflammations and wounds, while the decoction and juice are taken for their diuretic effect, and to treat hypertension and pulmonary ailments.[2,12,13] *S. muricatum* (sweet pepino) is used as a diuretic, and for the treatment of hypotension.[14,15]

This study was conducted to evaluate the antimicrobial activities of vegetable fern, chayote and sweet pepino against a panel of pathogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*) and fungi (*Candida albicans*, *Candida parapsilosis*, *Issatchenkia orientalis*, *Cryptococcus neoformans*, *Aspergillus brasiliensis* and *Trichophyton mentagrophytes*).

MATERIALS AND METHODS

Chemicals and reagents

The following chemicals and reagents were used: Hexane (Mallinckrodt Chemicals, USA), chloroform (System, USA), ethyl acetate (R&M, UK), ethanol (PROCHEM, USA), methanol (RCI Labscan, Thailand), amphotericin B and p-iodonitrotetrazolium violet (Sigma-Aldrich, USA), chloramphenicol (Amresco, USA), potato dextrose agar (PDA) and sodium hydroxide pellets (Merck, Malaysia), Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) (Scharlau Microbiology, Spain), RPMI-1640 medium supplemented with glutamine and phenol red, without bicarbonate (MP Biomedicals, France) and 3-(N-morpholino)propanesulfonic acid (MOPS) (Calbiochem, Germany).

Strains tested

Bacterial strains. *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11778), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218) and *Acinetobacter baumannii* (ATCC 19606) were purchased commercially from ATCC. The cultures were maintained on MHA at 4°C.

Fungal strains. *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019), *Issatchenkia orientalis* (ATCC 6258), *Cryptococcus neoformans* (ATCC 90112), *Aspergillus brasiliensis* (ATCC 16404) and *Trichophyton mentagrophytes* (ATCC 9533) were purchased commercially from ATCC. The microorganisms were maintained on PDA at 4°C.

Plant materials

The aerial part of *S. edule* and the fruits of *S. muricatum* were purchased from a marketplace in Cameron Highlands, Malaysia while the aerial part of *D. esculentum* was obtained from a wet market in Kampar, Malaysia. The identification of these plants was ascertained by a co-author of this paper, who is a botanist (H.C. Ong). Voucher specimens of *D. esculentum* (UTAR/FSC/10/023) and *S. edule* (UTAR/FSC/10/022) were prepared and deposited at the Faculty of Science, Universiti Tunku Abdul Rahman. No voucher specimen was prepared for the fruit of *S. muricatum*.

Preparation of extracts

Fresh plant materials were washed thoroughly using tap water. The collected parts of fresh plant samples were blended and immersed in the appropriate solvent. Samples of *D. esculentum* (1214 g), *S. edule* (1200 g) and *S. muricatum* (1000 g) were sequentially extracted with hexane, chloroform, ethyl acetate, ethanol, methanol and distilled water at room temperature with agitation (120 rpm) using an orbital shaker (IKA- Werke KS 501, Germany). Two cycles of extractions were performed for each solvent. The solvent was filtered, evaporated in a rotary evaporator (BUCHI Rota-vapor R205, Switzerland) at 40°C. The water extracts were lyophilized using a freeze-dryer (Martin Christ Alpha, UK). Yields of extracts are presented in Figure 1. For bioassay, the extracts were re-dissolved in methanol: water solution (2:1, v/v) at a concentration of 10 mg/mL, filtered using 0.45 µm nylon syringe filters and stored at –20°C prior analyses.

Antimicrobial screening

A colorimetric broth microdilution method using 96-well round bottom microplates was employed for antimicrobial activities screening of the extracts with modifications.[16] The test was conducted in serially by two-fold descending

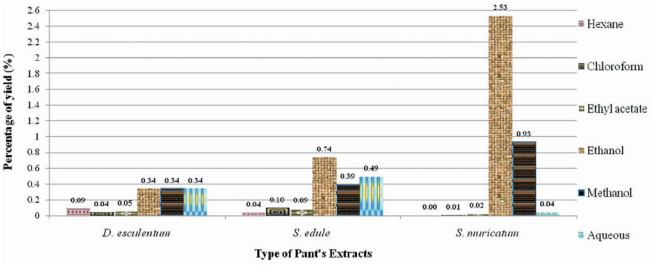

Figure 1. The percentage of yield of various extracts from three medicinal plants, *D. esculentum*, *S. edule* and *S. muricatum*.

concentrations of extracts and antibiotics, with the concentration ranging from 2.50 to 0.02 mg/mL for the plant extracts, 128 to 1 µg/mL for chloramphenicol and 8 to 0.06 µg/mL for amphotericin B. Growth, sterility (medium only) and negative (extracts only) controls were included. The colorimetric indicator, p-iodonitrotetrazolium violet (INT) was prepared in distilled water at the concentration of 0.4 mg/mL. To indicate the antimicrobial activity, INT was added after incubation. A colour change (from colourless to red) was indicative of a positive result. The concentration of extract at which the colour remains clear was recorded as the minimum inhibitory concentration (MIC) value. The minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was obtained by inoculating 20 µL of the preparation that showed no evidence of bacterial or fungal growth during the MIC determination assays on MHA or PDA, respectively. The lowest concentration of extract which inhibited was recorded as the MBC or MFC value. The test was performed in triplicate.

Screening for antibacterial activity

The standard method was used to prepare broth medium and bacterial culture. Three to five healthy colonies of bacteria grown on MHA (24 h old, 37°C) were transferred to MHB.[17] The bacterial concentration was adjusted to the optimal absorbance value (OD = 0.08 to 0.10) at 625 nm, and subsequently diluted with MHB to 1×10^6 CFU/mL. The final inoculum (100 µL) of bacteria in each well of microplate was 5×10^5 CFU/mL. The microplates were covered and incubated for 24 h at 37°C.

Screening for antifungal activity

Preparation of broth medium and inoculum suspensions were based on the CLSI/NCCLS guidelines.[18,19] Inoculum suspensions were prepared from fresh, mature cultures (48 h for *Candida* spp.; 72 h for *C. neoformans*; 7-day-old for *A. brasiliensis* and *T. mentagrophytes*) grown on PDA. The suspensions were mixed for 15 s and adjusted to the optimal absorbance value (OD = 0.12–0.15 for *Candida* spp. and *C. neoformans*; OD = 0.09–0.11 for *A. brasiliensis* and OD = 0.15–0.18 for *T. mentagrophytes*) at 530 nm. Further dilution in sterile distilled water was performed in order to obtain the required final working inoculum ($1–5 \times 10^3$ CFU/ml for *Candida* spp.; $1–5 \times 10^4$ CFU/ml for *C. neoformans*; $0.4–5 \times 10^4$ CFU/ml for *A. brasiliensis* and $1.2–6 \times 10^4$ CFU/ml for *T. mentagrophytes*). After addition of the plant extracts and antibiotics into the 96-well microplates using the same dilution technique, the microplates were incubated at 35°C for 48 h for *Candida* spp.; 72 h for *C. neoformans* and *A. brasiliensis*; and at 28°C for 7 days for *T. mentagrophytes*.

RESULTS

The percentage yields (w/w, based on fresh weight) obtained from the sequential extraction of *D. esculentum*, *S. edule* and *S. muricatum* are presented in Figure 1. *Solanum muricatum* showed the highest total percentages of yields (3.53%) followed by *S. edule* (1.84%), while the lowest yield was obtained from *D. esculentum* (1.20%).

Eighteen extracts from three medicinal plants were tested for antimicrobial activity against two Gram-positive bacteria, four Gram-negative bacteria, four yeasts and two molds using colorimetric broth microdilution methods. The results of the antibacterial and antifungal activities are shows in Tables 1 and 2, respectively. In the antibacterial screening assays, 49% of the assays exhibited bacteriostatic activity while only 21% of them showed bactericidal activity. The lowest MIC and MBC were recorded for the hexane extract of *S. muricatum* against *Bacillus cereus* and *K. pneumoniae*, both with values of 0.31 mg/mL. The water extract of *D. esculentum*, the methanol extract of *S. edule*, and the ethanol and methanol extracts of *S. muricatum* showed selective inhibitory activity against *P. aeruginosa*, with the MIC range of 0.31 to 1.25 mg/mL. None of the extracts showed inhibitory activity against *E. coli*. All the plants showed antifungal property with 66% and 49% of the bioassays demonstrated fungistatic and fungicidal activities, respectively. The lowest MIC and MFC values (both 0.08 mg/mL) obtained from the hexane extracts of *S. edule* and *S. muricatum* against *C. neoformans*. The ethanol, methanol and water extracts of *S. edule* and *S. muricatum*, and the water extract of *D. esculentum* exhibited selective inhibitory activity against *C. neoformans* and *I. orientalis*, with MIC values ranging from 0.16–2.50 mg/mL.

The bacterial susceptibility index (BSI) and the fungal susceptibility index (FSI) are shown in Figures 2 and 3, respectively. The susceptibility of bacteria towards the plant extracts

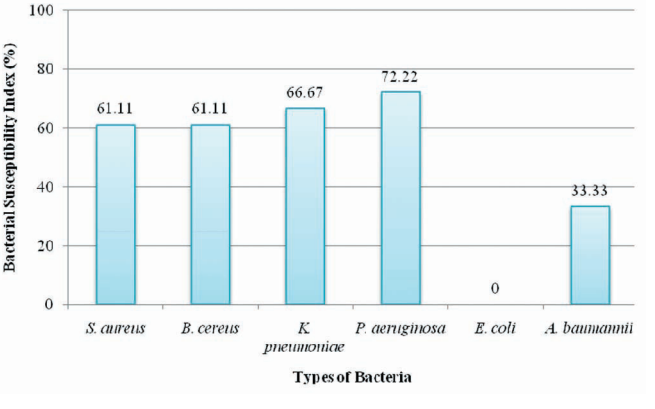

Figure 2. Bacterial susceptibility index (BSI) of tested bacteria towards various extracts of three medicinal plants, *D. esculentum*, *S. edule* and *S. muricatum*.

Table 1: MIC and MBC values of various extracts from three medicinal plants, *D. esculentum*, *S. edule* and *S. muricatum* against bacteria

Plant species	Extracts	Microorganisms tested											
		MIC (mg/mL)						MBC (mg/mL)					
		Gram-positive		Gram-negative				Gram-positive		Gram-negative			
		S.a	B.c	K.p	P.a	E.c	A.b[z]	S.a	B.c	K.p	P.a	E.c	A.b[z]
D. esculentum (aerial part)	Hexane	1.25–2.50	0.63–1.25	1.25	NA	NA	NA	NA	1.25	2.50	–	–	–
	Chloroform	0.31–0.63	0.31	0.31–0.63	0.63–1.25	NA	1.25	NA	0.63	NA	NA	–	2.50
	Ethyl acetate	1.25	1.25	1.25	0.63	NA	1.25	NA	1.25	1.25	NA	–	2.50
	Ethanol	0.31–0.63	0.31–0.63	0.31–0.63	0.63	NA	1.25	NA	1.25	NA	NA	–	NA
	Methanol	0.63–1.25	0.63–1.25	0.63–1.25	0.63–1.25	NA	1.25	NA	2.50	NA	NA	–	2.50
	Water	NA	NA	NA	0.63–1.25	NA	NA	–	–	–	NA	–	–
	Antibiotic	0.004	0.004	0.004	0.064	0.032	0.064						
S. edule (aerial part)	Hexane	0.63–1.25	0.31–0.63	0.31–0.63	1.25	NA	NA	NA	0.63	0.63	NA	–	–
	Chloroform	1.25	0.31–0.63	0.63–1.25	0.63–1.25	NA	2.50	NA	0.63	1.25	NA	–	NA
	Ethyl acetate	1.25–2.50	0.63	0.63	1.25	NA	NA	NA	0.63	0.63	NA	–	–
	Ethanol	NA	NA	2.50	0.63–1.25	NA	NA	–	–	2.50	NA	–	–
	Methanol	NA	NA	NA	0.31–0.63	NA	NA	–	–	–	NA	–	–
	Water	NA	NA	NA	NA	NA	NA	–	–	–	–	–	–
	Antibiotic	0.004	0.004	0.004	0.064	0.032	0.064						
S. muricatum (fruit)	Hexane	0.63	**0.31**	**0.31**	NA	NA	NA	NA	**0.31**	**0.31**	–	–	–
	Chloroform	0.63–1.25	0.63	0.31–0.63	NA	NA	NA	NA	1.25	0.63	–	–	–
	Ethyl acetate	1.25	0.63	0.31–0.63	1.25	NA	2.50	NA	0.63	0.63	NA	–	NA
	Ethanol	NA	NA	NA	1.25	NA	NA	–	–	–	NA	–	–
	Methanol	NA	NA	NA	1.25	NA	NA	–	–	–	NA	–	–
	Water	NA	NA	NA	NA	NA	NA	–	–	–	–	–	–
	Antibiotic	0.004	0.004	0.004	0.064	0.032	0.064						

Mean or range of triplicates; NA, no activity; "–", not tested since no MIC value was obtained; S.a, *S. aureus*; B.c, *B. cereus*; K.p, *K. pneumonia*; P.a, *P. aeruginosa*; E.c, *E. coli*; A.b$_z$, *A. baumannii*.

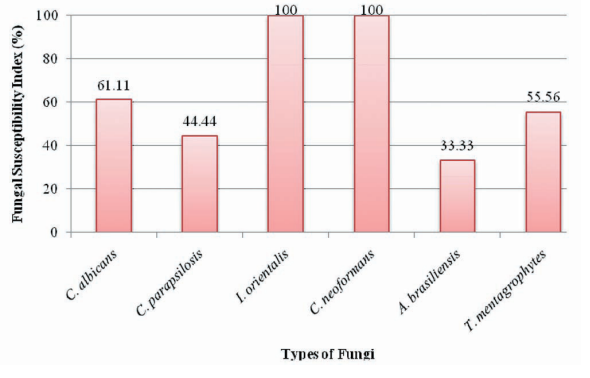

Figure 3. Fungal susceptibility index (FSI) of tested fungi towards various extracts of three medicinal plants, *D. esculentum*, *S. edule* and *S. muricatum*.

evaluated was species-dependent, with the susceptibility indices ranged from 0% for *E. coli* to 72% for *P. aeruginosa*. All the extracts exhibited antifungal activity against at least two strains of fungi. Two yeasts, *C. neoformans* and *I. orientalis* were found to be 100% susceptible to all the plant extracts with MIC values ranging from 0.08 to 2.50 mg/mL. *Aspergillus brasiliensis* was found to be the most insensitive among the tested fungal strains with a susceptibility index of 33%.

DISCUSSION

In this study, a total of 18 extracts isolated from three plants, i.e. *D. esculentum*, *S. edule* and *S. muricatum* were subjected to antimicrobial activity screening. Although these medicinal plants have been traditionally used as herbal remedies, there has been relatively few studies regarding their effects on human pathogens.

The extracts are regarded to have strong inhibitory effects if the MIC values is 0.5 mg/mL and below; moderately inhibitory if the MIC value is between 0.6 and 1.5 mg/mL

Table 2: MIC and MFC values of various extracts from three medicinal plants, *D. esculentum*, *S. edule* and *S. muricatum* against fungi

Plant species	Extracts	Microorganisms tested											
		MIC (mg/mL)						MFC (mg/mL)					
		Molds		Yeasts				Molds		Yeasts			
		A.b[y]	T.m	C.a	C.p	I.o	C.n	A.b[y]	T.M	C.a	C.p	I.o	C.n
D. esculentum (aerial part)	Hexane	NA	1.25	1.25	NA	0.31	**0.08**	–	1.25	NA	–	0.31	0.16
	Chloroform	2.50	0.63–1.25	0.63–1.25	2.50	0.31	0.08–0.16	NA	1.25	NA	NA	0.31	0.31
	Ethyl acetate	NA	NA	1.25	NA	0.31	0.16	–	–	NA	–	0.31	0.31
	Ethanol	NA	1.25	0.63–1.25	1.25–2.50	0.31	0.08–0.16	–	2.50	2.50	NA	0.31	0.16
	Methanol	NA	2.50	1.25–2.50	NA	0.31	0.16	–	NA	NA	–	0.31	0.31
	Aqueous	NA	NA	NA	NA	0.16–0.31	0.16	–	–	–	–	0.31	0.31
	Antibiotic	0.002	0.008	0.001	0.002	0.004	0.0001						
S. edule (aerial part)	Hexane	1.25	0.31–0.63	0.31–0.63	0.63	0.31	**0.08**	2.50	0.63	NA	NA	0.31	**0.08**
	Chloroform	2.50	0.63–1.25	0.63–1.25	1.25	0.63	0.16	2.50	1.25	2.50	NA	0.63	0.31
	Ethyl acetate	NA	0.63–1.25	0.63–1.25	1.25	0.63	0.16–0.31	–	1.25	2.50	NA	0.63	0.31
	Ethanol	NA	NA	NA	NA	0.63	0.31	–	–	–	–	0.63	NA
	Methanol	NA	NA	NA	NA	0.63	0.31–0.63	–	–	–	–	0.63	NA
	Aqueous	NA	NA	NA	NA	0.63	0.31–0.63	–	–	–	–	0.63	0.63
	Antibiotic	0.002	0.008	0.001	0.002	0.004	0.0001						
S. muricatum (fruit)	Hexane	0.31	0.16	0.63	1.25	0.63	**0.08**	0.31	0.31	0.63	1.25	0.63	**0.08**
	Chloroform	0.63–1.25	0.31–0.63	0.63–1.25	2.50	1.25	0.08–0.16	1.25	0.63	NA	2.50	1.25	0.31
	Ethyl acetate	2.50	0.63–1.25	0.63	1.25	0.63–1.25	0.08–0.16	2.50	1.25	1.25	NA	1.25	0.31
	Ethanol	NA	NA	NA	NA	0.63	0.31–0.63	–	–	–	–	0.63	0.63
	Methanol	NA	NA	NA	NA	0.63	0.63–1.25	–	–	–	–	0.63	NA
	Aqueous	NA	NA	NA	NA	0.63–1.25	2.50	–	–	–	–	1.25	NA
	Antibiotic	0.002	0.008	0.001	0.002	0.004	0.0001						

Mean or range of triplicates; NA, no activity; "–", not tested since no MIC value was obtained; C.a, *C. albicans*; C.p, *C. parapsilosis*; I.o, *I. orientalis*; C.p, *C. neoformans*; A.b[y], *A. brasiliensis*, T.m, *T. mentagrophytes*.

and weak inhibitory if the MIC value is 1.6 mg/mL or more.[20] The MIC values obtained in this study indicate that the tested plant extracts are generally more potent against fungi than bacteria. Based on the antimicrobial assays, 23.1% and 2.8% of the bioassays showed strong inhibition against fungi and bacteria, respectively; with MIC values ranging from 0.08 to 0.31 mg/mL (Tables 1 and 2). The antifungal activity of the extracts was more pronounced on the yeast strains, particularly on *C. neoformans* and *I. orientalis*, both with FSI of 100% (Figure 3).

Yeasts (mainly *Candida* spp.) are the third most common cause of intravascular catheter-related infection, with the second highest colonization-to-infection rate and the overall highest crude mortality.[21] *I. orientalis* is commonly implicated in urinary tract infections in immunocompromised patients[22,23] while *C. neoformans* ranks as one of the most common infectious agents that causes human meningoencephalitis (cryptococcosis).[7] The present study shows that the overall antifungal activity screening results are indicative of the potential of these plant extracts as effective medicaments in the treatment of fungal infectious diseases.

The antimicrobial activities of these plant extracts were found to decrease with increasing polarity of the extracts. According to the antibacterial assays, the lowest MIC and MBC was recorded for the hexane extract of *S. muricatum* against *Bacillus cereus* and *K. pneumoniae*, both with values of 0.31 mg/mL. Based on the antifungal assays, the lowest MIC and MFC was exhibited by the hexane extracts of *S. edule* and *S. muricatum* against *C. neoformans*, both with values of 0.08 mg/mL. The hexane extract of *S. muricatum* also exhibited strong inhibition against the filamentous fungi, *A. brasiliensis* and *T. mentagrophytes* with MIC values of 0.31 and 0.16 mg/mL,

respectively. The results indicate that the non-polar compounds (hexane extracts) had greater antimicrobial activity compared to the more polar compounds extracted by ethanol, methanol and water extracts.

Previous studies demonstrated that ascorbic acid, phenolic acids and flavonoids isolated from the fruits of *S. muricatum* (pepino) exhibited anti-oxidative, anti-inflammatory and anti-glycative protection in diabetic mice.[24] The antioxidant activity of the ripe pepino fruit was reported to be largely due to polyphenols.[25] The anti-tumor effect of pepino fruits has been reported by Ren & Tang[26] but the active compounds that are responsible for its anti-tumour activity remains to be identified. Eight flavonoids (vicenin-2, apigenin-6-C-β-d-glucopyranosyl-8-C-β-d-apiofuranoside, vitexin, luteolin-7-O-rutinoside, luteolin-7-O-β-d-glucopyranoside, apigenin-7-O-rutinoside, chrysoeriol-7-O-rutinoside, and diosmetin-7-O-rutinoside) have been isolated from the aerial parts of *S. edule*.[27] *S. edule* leave extracts have been reported to possess broad-spectrum antimicrobial activity against *E. coli, K. pneumoniae, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens, Morganella morganii, A. baumannii, P. aeruginosa, Stenotrophomonas maltophilia, Candida* spp. and *Aspergillus* spp.[13]

All the extracts of *D. esculentum*, from non-polar to polar extracts (hexane, chloroform, ethyl acetate, ethanol, methanol and water) showed strong inhibition against *C. neoformans* and *I. orientalis*, with MIC and MFC values ranging from 0.08 to 0.31 mg/mL (Table 2). These results agree well with another study which reported the antifungal activity of the methanolic extract from *D. esculentum*.[28] However, the antifungal activity of *D. esculentum* reported was limited to a few species (*A. niger, Rhizopus stolonifer* and *C. albicans*). The results showed the inhibitory property of the extracts was weak, with MIC values ranging from 50 mg/mL to 100 mg/mL.

In the antibacterial screening assays, 66.7% of the extracts from *D. esculentum* exhibited bacteriostatic activity (Table 1). Sakunpak and Panichayupakaranant[29] reported that extracts of *D. esculentum* did not exhibit inhibitory activity against gastrointestinal pathogenic bacteria, including *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The data reported show that extracts of *D. esculentum* have substantial antimicrobial activity against medically-important microorganisms, which corroborates its use in traditional medicine for the treatment of skin infections such as dermatitis and measles.

The antimicrobial activity profile of the three plants indicated that *E. coli* (BSI = 0%) was the least susceptible bacterium (Figure 2) to the plant extracts evaluated. *E. coli* is one of the most frequent causes of bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection, diarrhoea, neonatal meningitis, and ulcerative colitis.[30–32] The *E. coli* (ATCC 35218) strain used in this study is an ampicillin-resistant strain.[33] *E. coli* is among the Gram-negative bacteria that develop multi-drug resistance.[34]

CONCLUSION

All the plants investigated possessed antifungal and antibacterial properties against human pathogens. The results of this study corroborate the usage of these plants in traditional medicine. Further studies will be carried out to isolate and identify the active compounds.

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REFERENCES

1. Samie A, Obi CL, Bessong PO, Namrita L. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. African Journal of Biotechnology. 2005; 4(12):1443–51.
2. Ong HC. Vegetables for health and healing. Kuala Lumpur: Utusan Publications & Distributors Sdn. Bhd.; 2008.
3. Mendonca-Filho RR. Bioactive phytocompounds: new approaches in the phytosciences. In: Ahmand I, Aqil F, Owais M, Editors. Modern phytomedicine, turning medicinal plants into drugs. Weinheim: Wiley, p. 1–22; 2006.
4. Bush K. Antibacterial drug discovery in the 21st century. Clinical Microbiology and Infection. 2004; 10(4):10–17.
5. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20(1): 133–63.
6. Denning DW, Hope WW. Therapy for fungal diseases: opportunities and priorities. Trends in Microbiology. 2010; 18(5):195–204.
7. Heitman J, Filler SG, Edwards JrJE, Mitchell AP. Molecular principles of fungal pathogenesis. In: Sanglard D, White TC, Editors. Molecular principle of antifungal drug resistance. Washington: ASM Press, 197–209; 2006.
8. Lin X. *Cryptococcus neoformans*: Morphogenesis, infection, and evolution. Infection, Genetics and Evolution. 2009; 9(4):401–16.
9. Perfect JR, Casadevall A. Cryptococcosis. Infectious Disease Clinics of North America. 2002; 16(4):837–74.
10. Ong, HC. Sayuran 2: khasiat makanan dan ubatan. Kuala Lumpur: Utusan Publications & Distributors Sdn. Bhd.; 2011.
11. Prohens J, Anderson GJ, Rodríguez-Burruezo A, Nuez F. Exploiting wild species for the genetic improvement of the pepino (*Solanum muricatum*). Journal of Applied Botany. 2003; 77(1–2):21–7.
12. Roosita K, Kusharto CM, Sekiyama M, Fachrurozi Y, Ohtsuka R. Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. J. Ethnopharmacology. 2008; 115(1):72–81.
13. Ordoñez AAL, Ordoñez RM, Zampini IC, Isla MI. Design and quality control of a pharmaceutical formulation containing natural products with antibacterial, antifungal and antioxidant properties. International Journal of Pharmaceutics. 2009; 378(1–2):51–8.

14. Redgwell RJ, Turner NA. Pepino (*Solanum muricatum*): Chemical composition of ripe fruit. Journal of the Science of Food and Agriculture. 1986; 37(12):1217–22.
15. Sánchez-Vega, I. Andean Fruits: Pepino (*Solanum muricatum*). In: Hernández-Bermejo JE, León J, Editors. Neglected crops: 1492 from a different perspective. Rome, Italy: FAO, p. 181–91;1994.
16. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 1998; 64:711–4.
17. National Committee on Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial susceptibility testing; approved standard, NCCLS document M100-S9, M2-A6. Pennsylvania: Wayne; 1999.
18. National Committee on Clinical Laboratory Standards (NCCLS). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, NCCLS document M27-A2, 2nd edition. Pennsylvania: Wayne; 2002a.
19. National Committee on Clinical Laboratory Standards (NCCLS). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, NCCLS document M38-A, 2nd edition. Pennsylvania: Wayne; 2002b.
20. Aligiannis N, Kalpotzakis E, Mitaku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agri. and Food Chemistry. 2001; 40:4168–70.
21. Crump JA, Collignon PJ. Intravascular catheter-associated infections. Eur. J. Clin. Microbiol. Infect. Dis. 2000; 19(1):1–8.
22. Muñoz P, Guinea J, Rojas L, Bouza E. New antifungal agents for the treatment of candidaemia. International Journal of Antimicrobial Agents. 2010; 36S:S63–S69.
23. özÇelik B, Çitak S, Cesur S, Abbasoglu U, IÇli F. *In vitro* susceptibility of *Candida* spp. to antifungal agents. Drug Metab. Drug Interact. 2004; 20(1–2):5–8.
24. Hsu CC, Guo YR, Wang ZH, Yin MC. Protective effects of an aqueous extract from pepino (*Solanum muricatum* Ait.) in diabetic mice. Journal of the Science of Food and Agriculture. 2011; 91(8):1517–22.
25. Sudha G, Sangeetha PM, Indhu SR, Vadivukkarasi S. Antioxidant activity of ripe pepino fruit (*Solanum muricatum* aiton). International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(3):257–61.
26. Ren W, Tang DG. Extract of *Solanum muricatum* (Pepino/CSG) inhibits tumor growth by inducing apoptosis. Anticancer Res. 1999; 19(1A): 403–8.
27. Siciliano T, Tomasi ND, Morelli I, Braca A. Study of flavonoid of *Sechium edule* (Jacq) Swartz (Cucurbitaceae). Different edible organs by liquid chromatography photodiode array mass spectrometry. J. Agric. Food Chem. 2004; 52:6510–5.
28. Zakaria Z, Sanduran S, Sreenivasan S. Antifungal activity of the edible ferns: Application for Public Health. The International Journal of the Humanities. 2010; 8(8):113–8.
29. Sakunpak A, Panichayupakarananta P. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. Food Chemistry. 2012; 130(4):826–31.
30. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA. Yolken RH. Manual of Clinical Microbiology. 8th edn. Washington: ASM Press; 2003.
31. Brooks GF, Butel JS, Morse SA. Jawetz, Melnick, and Adelberg's medical microbiology. 23rd edn. USA: The McGraw-Hill Companies; 2004.
32. Rolhion N, Darfeuille-Michaud A. Adherent-invasive *Escherichia coli* in inflammatory bowel disease. Inflammatory Bowel Diseases. 2007; 13(10):1277–83.
33. Lamp, K.C. and Vickers, M.K. Pharmacodynamics of ampicillin-sulbactam in an *in vitro* infection model against *Escherichia coli* strains with various levels of resistance. Antimicrobial Agents and Chemotherapy. 1998; 42(2):231–5.
34. Sader HS, Jones RN, Silva JB. Skin and soft tissue infections in Latin American Medical Centers: four-year assessment of the pathogen frequency and antimicrobial susceptibility patterns. Diagn. Microbiol. Infect. Dis. 2002; 44(3):281–8.