Advanced Journal of Microbiology Research Vol. 2016
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Lack of antimicrobial activities of *Dicranopteris linearis* extracts and fractions

Z. A. Zakaria[1]*, A. Mat Desa[2], K. Ramasamy[2], N. Ahmat[3], A. S. Mohamad1, D. A. Israf1 and M. R. Sulaiman1'

[1]Department of Biomedical Sciences, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Faculty of Pharmacy, University Technology MARA, 40450 Shah Alam, Selangor, Malaysia.
[3]Faculty of Applied Sciences, University Technology MARA, 40450 Shah Alam, Selangor, Malaysia.

Accepted 3 December, 2015

The aim of the present study is to determine the antimicrobial activity of the aqueous, chloroform and methanol extracts of the leaves of *Dicranopteris linearis* (Gleicheniaceae) using the micro-broth dilution method. The leaves of *D. linearis* were soaked separately in distilled water (dH_2O), chloroform and methanol in the ratio of 1:20 (w/v) for 72 h at room temperature, while some part of the methanol extracts was also partitioned with hexane, chloroform and methanol. The target microbe used were *Staphylococcus aureus* 25923, *S. aureus* 33591 (a methicillin-resistant *S. aureus* (MRSA) isolate), *S. aureus* 700699 (a vancomycin-intermediate resistant *S. aureus* (VISA) isolate), *S. aureus* 156 (a vancomycin -resistant *S. aureus* (VRSA) isolate), *Escherichia coli* 35218 and *Candida albicans* 10231. The results obtained show that the methanol extract was the most active in antimicrobial testing with the MIC/MBC values of 625 µg/ml. Based on this result, fractionation was carried out on the methanol extract and yielded eleven fractions. Of these, only B5, B6 and B11 fractions were found to be effective against *S. aureus* 33591 and *S. aureus* 25927 with MIC/MBC values ranging between 1250 - 2500 µg/ml. In conclusion, the *D. linearis* possess mild antibacterial activity against the selected panel of microbes, which explained the lack of claimed on the plant antimicrobial activity.

Key word: *Dicranopteris linearis*, Gleicheniaceae, micro-broth dilution method, mild antibacterial, *Stapylococcus aureus*.

INTRODUCTION

There are on average of 4 - 6 new antibiotics introduced into medical practice each year. There is constant need for new antibiotics because of the continuing problem of bacterial resistant to antibiotics (Vahidi et al., 2002). The numbers of resistant strains of microbial pathogens are growing and have caused a major problem throughout the world (Austin et al., 1999). Therefore, the development of new compounds and antimicrobial agents for the treatment of microbial infections is of increasing interest (Trivedi and Hotchandani, 2004).

Many plants are being use in the traditional medicine because they produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Tanaka et al., 2006). *Dicranopteris linearis* (Gleicheniaceae), known locally to the Malay's as Rasam', has been used in the Malay's traditional medicine as a cooling drink and also to reduce fever (see Zakaria et al., 2006). Scientifically, *D. linearis* extracts have been reported to possess antinociceptive, anti-inflammatory and antipyretic activities (Zakaria et al., 2006, 2007a). Despite the reported antistaphylococcal activity of various extracts of *D. linearis* (Zakaria et al., 2007b), the present study was carried out using the activity-guided fractionation of the most effective extract, which is the methanol extract, followed by isolation and identification of active fractions on the basis of the strong need to find new antimicrobial drugs mentioned above.

*Corresponding author. E-mail: dr_zaz @yahoo.com.

MATERIALS AND METHODS

Plant materials

The leaves of *D. linearis* were collected around Seksyen 7, Shah Alam, Selangor between January and February, 2008. The plant

Table 1. The MIC and MBC values for the crude aqueous, chloroform and methanol extract of *D. linearis*.

Extracts	Assay	Inhibitory Potential MIC/MBC (µg/ml)		
		S. aureus	***E. coli***	***C. albicans***
Aqueous	MIC	5000	5000	>5000
	MBC	5000	5000	>5000
Chloroform	MIC	2500	>5000	5000
	MBC	2500	>5000	5000
Methanol	MIC	1250	>5000	2500
	MBC	1250	>5000	2500

has been previously identified by a certified botanist at Universiti Putra Malaysia (UPM), Serdang Selangor, Malaysia and a voucher specimen (SK 855/05) was preserved at the Herbarium of the Laboratory of Natural product, Institute of Bioscience, UPM, Serdang Selangor.

The extraction of *D. linearis* leaves

The leaves of *D. linearis* were air-dried on the laboratory bench at room temperature for 2 weeks. The stems were removed and the leaves were ground into powder form using a sterile electric grinder. The air -dried powder leaves (1 kg) were soaked separately in distilled water (dH_2O), chloroform, methanol in the ratio of 1:20 (w/v) for 72 h at room temperature. To remove solid plants material, the supernatant was first filtered using cotton wool followed by the filter paper (Whatman No.1). The filtrates of methanol and chloro-form were concentrated by evaporation under reduced pressure at 40°C while the aqueous extract was subjected to the freeze-drying process. All extracts were then assayed for antimicrobial activity.

Microorganisms tested

Microorganisms tested in this study were those in the collection of Forest Research Institute of Malaysia (FRIM) and belong to *Staphylococcus aureus* 25923, *S. aureus* 33591 (a methicillin-resistant *S. aureus* (MRSA) isolate), *S. aureus* 700699 (a vancomycin-intermediate resistant *S. aureus* (VISA) isolate), VRSA 156 (a vancomycin-resistant *S. aureus* (VRSA) isolate), *Escherichia coli* 35218 and *Candida albicans* 10231.

Antimicrobial screening

The *in vitro* antimicrobial activity of the plant's extracts was tested by liquid micro-dilution method as described by the Society of Japanese Chemotherapy (1990) with slight modification to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Stock solutions of each extract were prepared by dissolving the extract in a defined quantity of dH_2O or dimethylsulfoxide (DMSO), respectively. The 96-well plates were prepared by dispensing into each well (A1- A9) 10 µl of test extracts, 90 µl of Muller Hinton broth (MHB) (A1 - A9) and 50 µl of the inoculum. A number of wells were reserved in each plate for sterility control, inoculum viability (no extract added) and the DMSO inhibitory effect. The final volumes in wells were 100 µl. A standard MHB was employed for the bacterial assays. The microtitre plates were incubated at 35°C for 24 h. The growth of the microorganisms was determined by turbidity. Clear well indicated absence of bacterial growth. The MIC of the preparations was the lowest concentration in the medium that completely inhibited the visible growth. The MBC was determined by testing the plate with methyl thiazolyl tetrazolium chloride (MTT). The lowest concentration showing no growth was identified as the MBC. All the experiments were run in triplicate.

Fractionation of the most effective extract

The methanol extract which showed the most active antimicrobial activity was fractionated by solvent partitioning with hexane, chloroform and methanol as the solvent systems. The resulting fractions were evaporated to dryness and assayed for antimicrobial activity. The most active fraction was further fractionated by silica vacuum liquid chromatography (VLC).

The methanol fraction, which shows positive antimicrobial activity, was subjected to the vacuum liquid chromatography (VLC) using silica gel 60 (1.07747 Merck, Germany) and eluting with solvents of increasing polarity from (100% chloroform to 100% methanol). The solvent system CH_2Cl_2/MeOH in ratios of 99:1 and 98:2 (v/v), respectively, was used to elute compounds in the VLC. Fifty four fractions were collected and analytical thin layer chromatography (TLC) on silica gel 60 F_{254} plates (Merck, Germany) was used to identify the similar fractions. The fractions having the same chromatograms were combined and 11 fractions (B1 - B11) were obtained. The combined fractions was subjected to the micro-broth dilution method

RESULTS AND DISCUSSION

Preliminary antibacterial testing showed that the aqueous extract was not effective against the whole microbial strains because the MIC and MBC values were not detected up to 5000 µg/ml (Table 1). On the other hand, the range of MIC and MBC for methanol and chloroform extracts against *S. aureus* 33591, *E. coli* 35218 and *C. albicans* 10231 were 1250 - 5000 and 2500 - 5000 µg/ml, respectively, indicating that the methanol extract was the most effective antimicrobial compared to the other extracts.

Based on the results obtained, the methanol extract was most effective against *S. aureus* 33591. The methanol extract was further partitioned in sequence with hexane, chloroform and methanol solvents to separate the polar from non-polar compounds. The antimicrobial screening of the three partitions indicate that the methanol partition was effective against *S. aureus* 25923 and *S. aureus* 33591 with the recorded MIC as well as MBC values of 625 µg/ml followed by the chloroform par-

Table 2. The MIC and MBC values for the hexane, chloroform and methanol partitions of the crude methanol extract of *D. linearis*.

Target microbe	Assay	MIC/MBC ((µg/ml)		
		Hexane(HP)	Chloroform(CP)	Methanol(MP)
S. aureus ATCC 25923	MIC	>5000	2500	625
	MBC	>5000	2500	625
S. aureus ATCC 33591	MIC	5000	2500	625
	MBC	5000	2500	625
S. aureus ATCC 700699	MIC	>5000	>5000	>5000
	MBC	>5000	>5000	>5000
VRSA 156	MIC	>5000	>5000	>5000
	MBC	>5000	>5000	>5000
E. coli ATCC 35218	MIC	>5000	>5000	>5000
	MBC	>5000	>5000	>5000
C. albicans ATCC 10231	MIC	2500	2500	>5000
	MBC	2500	2500	>5000

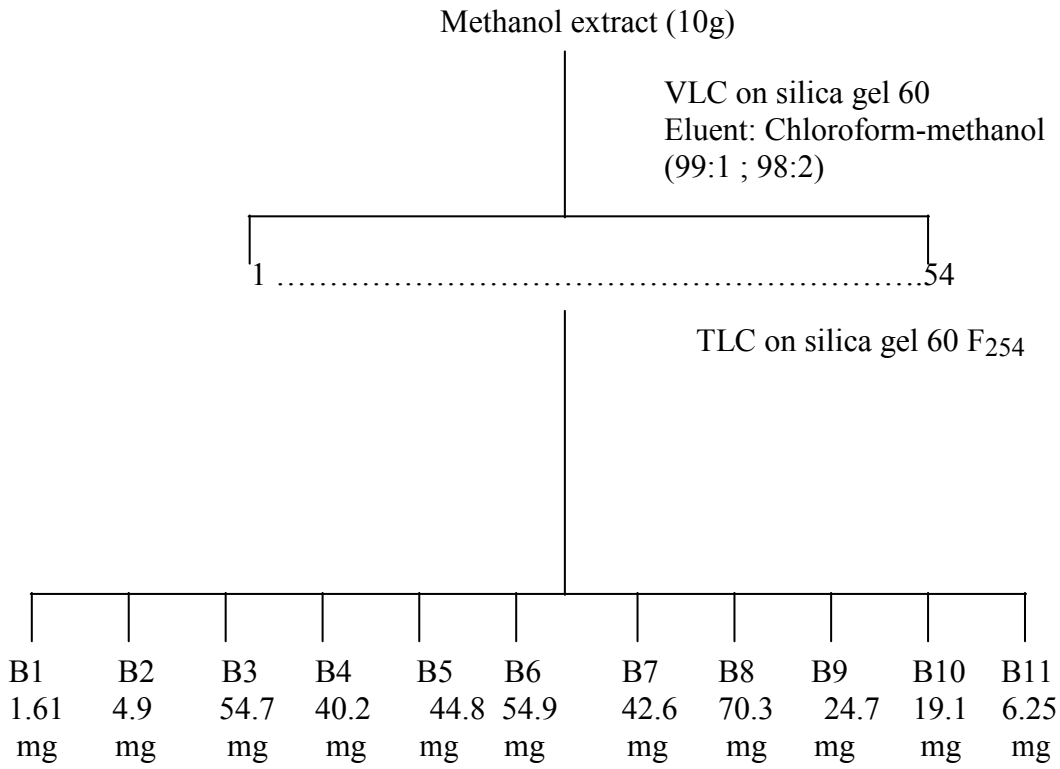

Figure 1. Fractionation of the methanol partition of crude methanol extra0 ct *D. linearis* by vacuum liquid chromatography.

tition with values of 2500 µg/ml (Table 2). In addition, the hexane and chloroform partitions were also effective against *C. albicans* 10231 with the MIC and MBC value of 2500 µg/ml. Based on the results obtained, the metha-nol partition of *D. linearis* shows the most promising antimicrobial activity (with the lowest MIC and MBC values of 625 µg/ml), thus, was subjected to the bioassay guided fractionation.

The fractionation of the methanol extract (10g) by vacuum liquid chromatography (VLC) using silica gel 60 gave fifty four fractions, which were pooled into eleven fractions, labeled as B1, B2, B3, B4, B5, B6, B7, B8, B9, B10 and B11, based on their chemical similarity (Figure 1). The antibacterial activity of these fractions was tested against *S. aureus* 25923 and *S. aureus* 33591. The antimicrobial screening clearly shows that only fractions

Table 3. The MIC and MBC values from various fractions of the methanol partition.

Samples	*S. aureus* ATCC 25923		*S. aureus* ATCC 33591	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
B1	>5000	>5000	>5000	>5000
B2	>5000	>5000	5000	5000
B3	25000	2500	2500	2500
B4	2500	2500	2500	2500
B5	2500	2500	1250	1250
B6	1250	1250	1250	1250
B7	2500	2500	5000	5000
B8	5000	5000	5000	5000
B9	>5000	>5000	>5000	>5000
B10	>5000	>5000	>5000	>5000
B11	1250	1250	1250	1250

B5, B6 and B11 demonstrated antimicrobial activity with MIC and MBC value of 1250 µg/ml compared to the other fractions (Table 3).

Despite its phytochemical contents, which include flavanoids, saponins, triterpenes, tannins and steroid (Zakaria et al., 2007a;b), the crude extracts, its partitions and fractions could not be considered as potent antimicrobial agents or, particularly, as potent antistaphylococcal candidates due to the high MIC and MBC values (625 µg/ml). Several plants' extracts that is essential oil of *Ocimum gratissimum* (Nakamura et al., 1999) and ethyl acetate extract of *Acacia sieberiana* (Eldeen et al., 2005) have been reported to possess the MIC and MBC values that are less than 100 µg/ml when tested against *S. aureus*. Nevertheless, the present of moderate antimicrobial activity, at least of the methanol partition of the methanol extract of *D. linearis* leaves (Table 2), should be highlighted as seen with other reports. Vahidi et al. (2002) reported that the MIC value of the ethyl acetate extract of *Croccus sativus* to be between 25 - 50 mg/ml while Nkere and Iroegbu (2005) reported that the MIC for *Picralima nitida* extracts was between 6.25 - 50 mg/ml, both depending on the parts used. Flavonoids and tannins, particularly, have been reported to inhibit the growth of *S. aureus* (Akiyama et al., 2001; Xiao et al., 2005) and could be responsible for the observed antistaphylococcal activity.

We have earlier reported on the antistaphylococcal activity of several neglected plants found in Malaysia, which include *D. linearis* (Zakaria et al., 2007b). Interestingly, the methanol extract of *D. linearis* was also effective against VISA and VRSA with MIC and MBC values of 1.25 and 2.50 g/ml, respectively. However, the extracts, partitions and fractions did not produce the same results against VISA and VRSA in the present study. The reasons for these differences include strain-to-strain differences, physicochemical characteristics of the oil, and even susceptibility testing conditions (Nakamura et al., 1999). Furthermore, the decrease in potency of the methanol fractions (B5, B6 and B11; > 1250 µg/ml) when compared to the methanol partition (625 µg/ml) seems to indicate the loss of synergistic action between any of the phytochemical constituents present in the methanol partition due to its subjection to the separation process. In conclusion, the present study confirm previous report on the moderate antimicrobial and, in particular the anti-staphylococcal activity, of *D. linearis* and directly explain the lack of claim on its medicinal uses asantimicrobial agent within the Malays traditional culture.

ACKNOWLEDGMENT

This study was supported by the research grant from the Ministry of Science, Technology and Innovation, Malaysia.

REFERENCES

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2001) Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother. 48: 487-491.

Austin DJ, Kristinsson KG, Anderson RM (1999) The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc. Natl. Acad. Sci. USA. 96: 1152-1156.

Eldeen IMS, Elgorashi EE, Van Staden J (2005) Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. J. Ethnopharmacol. 102: 457-464.

Nakamura CV, Ueda-Nakamura T, Bando E, Melo AFN, Cortez DAG, Filho BPD (1999) Antibacterial activity of *Ocimum gratissimum* L. essential oil. Mem. Inst. Oswaldo Cruz. 94: 675-678.

Nkere CK, Iroegbu CU (2005) Antibacterial screening of the root, seed and stembark extracts of *Picralima nitida*. Afr. J. Biotech. 4: 522-526.

Society for Japanese Chemotherapy (1990) Standards methods for liquid microdilution antimicrobial susceptibility test. Chemother. 38: 103-106.

Tanaka JCA, Da Silva CC, De Oliveira AJB, Nakamura CV, Dias Filho BP (2006) Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Braz. J. Med. Biol. Res. 39: 387-391.

Trivedi NA, Hotchandani SC (2004) A study of the antimicrobial activity of oil of Eucalyptus. Indian J. Pharmacol. 36: 93-95.

Vahidi H, Kamalinejad M, Sedaghati N (2002) Antimicrobial properties of *Croccus sativus L*. Iranian J. Pharmaceut. Res. 1: 33-35.

Xiao JB, Jiang XY, Chen XQ (2005) Antibacterial, anti-inflammatory and

diuretic effect of flavonoids from *Marchantia convolute*. Afr. J. Tradit. Comp. Alt. Med. 2: 244-252.

Zakaria ZA, Abdul Ghani ZDF, Raden Mohd. Nor RNS, Hanan Kumar G, Sulaiman MR, Fatimah CA (2006) Antinociceptive and Anti-inflammatory Activities of *Dicranopteris linearis* Leaves Chloroform Extract in Experimental Animals. *Yakugaku Zasshi.* 126(11): 1197–1203.

Zakaria ZA, Abdul Ghani ZDF, Raden Mohd. Nor RNS, Hanan Kumar G, Sulaiman MR, Mat Jais AM, Somchit MN, Arifah AK, Ripin J (2007a) Antinociceptive, anti-inflammatory and antipyretic properties of *Dicranopteris linearis* leaves aqueous extract in experimental animals. J. Nat. Med. 62: 179-187.

Zakaria ZA, Mat Jais AM, Mastura M, Mat Jusoh SH, Mohamed AM, Mohd. Jamil NS, Rofiee MS, Sulaiman MR (2007b) In vitro anti-staphylococcal activity of the extracts of several neglected plants in Malaysia. Int. J. Pharmacol. 3: 428-431.