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In vitro Antistaphylococcal Activity of the Extracts of Several Neglected Plants in Malaysia

[1]Z.A. Zakaria, [2]A.M. Mat Jais, [3]M. Mastura, [4]S.H. Mat Jusoh,
[4]A.M. Mohamed, [4]N.S. Mohd. Jamil, [4]M.S. Rofiee and [2]M.R. Sulaiman
[1]Faculty of Pharmacy, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Department of Biomedical Sciences, Faculty of Medicine and Health Science,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Medicinal Plants Programme, Biotechnology Division,
Forest Research Institute of Malaysia, 52109 Kepong, Selangor, Malaysia
[4]Faculty of Biotechnology and Life Sciences, Universiti Industri Selangor,
Jalan Zirkon A7/A, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia

Abstract: The present study was carried out to evaluate the antibacterial activity of the aqueous, methanol and chloroform extracts of several plants available in Malaysia, namely *Muntingia calabura* (L.), *Melastoma malabathricum* (L.), *Bauhinia purpurea* (L.), *Corchorus capsularis* (L.) and *Dicranopteris linearis* (L.) using the single screening *in vitro* microtiter plate dilution methods. The extracts, at the dose of 5 μg μL^{-1}, were screened against various strains of *Staphylococcus aureus*, namely *S. aureus* 29213α, *S. aureus* 33591, *S. aureus* 700699, vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA). Results: Interestingly, only the methanol extracts of *D. linearis* exhibited an antibacterial activity against all strains of *S. aureus* whereas all extracts of *M. calabura* were effective only against the *S. aureus* 29213α, *S. aureus* 33591 and *S. aureus* 700699. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for *D. linearis* range between 0.625-1.250 and 1.250-2.500 μg μL^{-1}, respectively whereas for the *M. calabura* extracts the MIC and MBC range between 1.250-5.000 and 2.500-5.000 μg μL^{-1}, respectively. Although the other plants gave negative results in this study, their potential antibacterial properties should not be disregarded as the present study was carried out using only one low concentration (5 μg μL^{-1}) and that the activity was determined using crude, but not pure, extracts. The present study demonstrated the potential of chloroform extract of *D. linearis*, which indicate the present of non-polar bioactive compounds, as VRSA antibacterial agents and all extracts of *M. calabura* as a potential source of antibacterial agents for the treatment of normal *S. aureus* infection.

Key words: Antibacterial activity, *Dicranopteris linearis*, *Muntingia calabura*, vancomycin-resistant, *Staphylococcus aureus*

INTRODUCTION

Plant, being a major source of natural therapeutic remedies, has been used in various part of the world to treat various infectious diseases (Vahidi *et al*., 2002). Recent focus of research for new source of safer and more effective antibacterial agents has been shifted towards natural products of plant sources (Nitta *et al*., 2002; Souza *et al*., 2003) as a result of unwanted side effects or limited efficacy (Trivedi and Hotchandani, 2004) reported on some of the available antibiotics. The emergence of resistance among key microbial pathogens, including Staphylococcus aureus, to conventional antimicrobials is a serious problem that scientist face all around the world (Tanaka *et al*., 2006). The effect caused by vancomycin-resistant *S. aureus* (VRSA) on the poultry industry, for example, has increased awareness among scientists to look for new antibiotics against the VRSA.

We have earlier reported on the antinociceptive and anti-inflammatory activities of the extracts of *Muntingia calabura*, *Dicranopteris linearis*, *Melastoma malabathricum*, *Bauhinia purpurea* and *Corchorus capsularis* (Zakaria *et al*., 2007a,b,c, 2006a,b). In addition, we have also reported on the antibacterial activity of *M. calabura* extract (Zakaria *et al*., 2006c,d). Based on the report of the emergence of antibiotic-resistant *S. aureus*,

Corresponding Author: Dr. Zainul Amiruddin Zakaria, Faculty of Pharmacy, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia Tel: 603-55442738 Fax: 603-55442725

particularly the VRSA, it is necessary for us to screen those plants for antistaphylococcal activity. Thus, the aim of the present study was to determine the effect of various extracts of several neglected plants available in Malaysia, namely *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis*, on various strains of *S. aureus* using the *in vitro* single concentration liquid microdilution method.

MATERIALS AND METHODS

Plant materials: The leaves of *Muntingia calabura*, *Dicranopteris linearis*, *Melastoma malabathricum* and *Bauhinia purpurea* were collected from its natural habitat in Shah Alam, Selangor, Malaysia whereas the leaves of *Corchorus capsularis* were collected from its natural habitat in Alor Setar, Kedah, Malaysia, in June 2006. They have been identified by Mr. Shamsul Khamis, a botanist from the Institute of Bioscience, Universiti Putra Malaysia, Malaysia and the respective voucher specimens, SK964/04, SK855/05, SK507/03, SK1095/05 and SK856/05, were deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia as described elsewhere (Zakaria *et al*., 2006a,b, 2007a,b,c).

Preparation of aqueous extract of plants: The leaves of all plants were washed and rinsed with tap water and then oven-dried for 72 h at the temperature of 40°C. The dried leaves were then ground into small particles, weighed (40 g) and sequentially soaked (1:20; w/v) in aqueous (distilled water (dH_2O)), chloroform and methanol for 72 h. The supernatant of each plant was collected and filtered using Whatman No. 1 filter paper and then the aqueous extracts were subjected to the freeze-drying process while the chloroform and methanol extracts were evaporated (Buchi, Germany) to dryness. The weight (and percentage of yield (%) (w/w)) for the crude dried aqueous extracts of *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis* were approximately 2.10 g (5.25%), 2.00 g (5%), 1.98 g (4.95%), 2.06 g (5.15%) and 2.11 g (5.28%), respectively. The weight (and percentage of yield (%)) for the crude dried methanol and chloroform extracts of *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis* were approximately 1.66 g (4.15%), 2.83 g (7.08%), 0.80 g (2%), 0.69 g (1.73%) and 1.08 g (2.7%) and 4.03 g (10.08%), 2.73 g (6.83%), 2.14 g (5.35%), 2.65 g (6.63%) and 1.15 g (2.88%), respectively.

Types of microorganisms: Microorganisms tested in this study were those in the collection of Forest Research Institute of Malaysia (FRIM) and belong to the Staphylococcus aureus strains, namely *S. aureus* 29213α, *S. aureus* 33591, *S. aureus* 700699, vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA).

Antimicrobial assay: The cultures of antibiotic-susceptible *S. aureus* 29213 alfa, *S. aureus* 33591 and *S. aureus* 700699 were grown in Muller Hinton Broth (Difco) while those of VISA and VRSA were grown in Tryptic Soy Broth (Bucto™) at 37°C. The screening procedure for antibacterial activity as well as the MIC and MBC determination was carried out according to the liquid microdilution method described by Society of Japanese Chemotherapy (1990) with slight modifications in which the single concentration test was performed prior to the determination of the MICs and MBCs.

RESULTS AND DISCUSSION

The antibacterial profile of various extracts of *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis* against various strains of *S. aureus* after single concentration screening using the microtiter plate methods is shown in Table 1. Only *M. calabura* and *D. linearis* extracts produced antibacterial activity at the concentration of 5 μg μL^{-1}. Interestingly, all of the *M. calabura* extracts were effective only against the normal *S. aureus* (*S. aureus* 29213α, *S. aureus* 33591 and *S. aureus* 700699) while for *D. linearis*, only its methanol extract was effective against all strains of *S. aureus*, including the VISA and VRSA. All extracts that show positive antibacterial activity were also subjected to the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (Table 2). The range of MIC and MBC for methanol extract of *D. linearis* against normal S. aureus, VISA and VRSA were 0.625-1.250 and 1.250-2.500 μg μL^{-1}, respectively. On the other hand, the range of MIC for the aqueous, methanol and chloroform extracts of *M. calabura* were 5.000, 1.250 and 1.250-2.500 μg μL^{-1} while the MBC value, determined only for the methanol and chloroform extracts of *M. calabura*, were 2.500 μg μL^{-1}.

Based on the results obtained, the methanol extract of *D. linearis* shows the most promising antistaphylococcal activity in which the extract also exhibited an activity against the VRSA and VISA. For the *M. calabura* extracts, the methanol, followed by chloroform and aqueous, was effective only against the antibiotic-susceptible *S. aureus*. As far as our literature search is concerned, this is the first preliminary report on the potential of *D. linearis* as an antibacterial agent with promising activity against VRSA. On the other hand, the potential of *M. calabura* as antibacterial agent has been reported earlier (Zakaria *et al*., 2006c,d), but not against different strains of *S. aureus*.

Table 1: The antibacterial activity of aqueous, chlororofrm and methanol extracts of *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis* determined by the microtiter plate method

	S. aureus 29213α			*S. aureus* 33591			*S. aureus* 700699			VISA			VRSA		
Samples	A	M	C	A	M	C	A	M	C	A	M	C	A	M	C
M. calabura	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
D. linearis	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-
M. malabathricum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. purpurea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. capsularis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A: Aqueous extract; C: Chloroform extract; M: Methanol extract; VISA: Vancomycin-intermediate *S. aureus*; VRSA: Vancomycin-resistant *S. aureus*; >5-Activity is not seen at 5 μg μL^{-1}. All extracts were tested using a microtiter plate assay once using the single concentration test (at the dose of 5 μg μL^{-1}). Extracts with antibacterial activity at the 5 μg μL^{-1} concentration were subjected to the MIC and MBC tests

Table 2: The MIC and MBC values for extracts of *M. calabura* and *D. linearis*, determined by the microdilution method

		D. linearis		*M. calabura*	
Samples	Value (μg μL^{-1})	M	A	M	C
Staph. aureus 29213α	MIC	1.25	5	1.25	1.25
	MBC	2.50	>5	2.50	2.50
Staph. aureus 33591	MIC	0.62	5	1.25	2.50
	MBC	1.25	>5	2.50	2.50
Staph. aureus 700699	MIC	0.62	5	1.25	2.50
	MBC	1.25	>5	2.50	2.50
VISA	MIC	1.25	NA	NA	NA
	MBC	2.50	NA	NA	NA
VRSA	MIC	1.25	NA	NA	NA
	MBC	1.25	NA	NA	NA

A: Aqueous extract, C: Chloroform extract, M: Methanol extract, VISA: Vancomycin-intermediate, Staph. aureus; VRSA: Vancomycin-resistant Staph. aureus; NA: Not available; >5-Activity is not seen at 5 μg μL^{-1}

Our earlier findings have demonstrated the presence of flavonoids, triterpenes, saponins and steroids in all of the plants with only *M. calabura*, *D. linearis* and *M. malabathricum* contained tannins. However, alkaloids were not detected in any of the plants. Interestingly, *D. linearis* demonstrated the presence of highest content of saponins (as indicated by the presence of thick froth) while *M. calabura* showed the highest presence of flavonoids (as indicated by the presence of strong colouration) contents when compared to the other plants (Zakaria *et al*., 2006d). Flavonoids and chalcones, like muntingone and (2S)-(-)-5'-hydroxy-7,3',4'-trimethoxyflavanone and 2',4'-dihydroxy-3'-methoxydihydrochalcone and (-)-3'-methoxy-2',4',β-trihydroxydihydrochalcone, have been isolated from the leaves of *M. calabura* (Chen *et al*., 2005) while the leaves of *D. linearis* have been reported to contain various types of flavonoids (e.g., like afzelin, quercitrin, isoquercitrin, astragarin, rutin and kaempferol) and flavonoids of the flavonol 3-O-glycosides types (e.g., 3-O-(4-O-p-coumaroyl-3-O-alpha-L-rhamnopyranosyl)-alpha-L-rhamnopy ranoysl-(1→6)-beta-D-glucopyranoside and (6S,13S)-6-[6-O-actyl-beta-D-glucopyranosyl-(1→4)-alpha-L-rhamnopy-ranosyloxy]-13-[alpha-L-rhamnopyranosyl-(1→4)-beta-D-fucopyra nosyloxy]-cleroda-3,14-diene) (Raja *et al*., 1995). Two groups of compounds, flavonoids and tannins, in particular, have been reported to inhibit the growth of *S. aureus* (Xiao *et al*., 2005; Akiyama *et al*., 2001) and interestingly, both types of compounds have also been found in *M. calabura* and *D. linearis*.

Several mechanisms of action could be suggested with regards to the chemical compounds, particularly flavonoids and tannins, presence in the extracts of *M. calabura* and *D. linearis*. According to Alvarez *et al*. (2006), some of the flavonoids that favor polar solutes entry, like rutin and quercetin, bind to the bacteria's structural membrane proteins called porines, causing changes in the tridimentional conformation exposuring the hydrophilic character of the pore, which lead to an easier passage of other polar bioactive compounds via diffusion. Other than that, Guz *et al*. (2001) have also reported on the ability of some antimicrobial-bearing flavonoid-type compounds to inhibit the multidrug resistance (MDR) efflux pump found on the membrane of *S. aureus*. MDR efflux pump have been associated with the emergence of drug-resistance bacteria. The ability of tannins to form chelates with metal ions, particularly iron, which lead to the disruption of the *S. aureus* membrane, could be one of the possible factors that contribute to its antimicrobial activity (Akiyama *et al*., 2001). In addition, tannins, like tannic acid, has a greater relative binding efficiency to iron and may act with iron from the medium to form chelates and, in the end, making iron unavailable to microorganisms. It is well known that aerobic microorganisms require iron to perform a variety of functions, like reduction of the ribonucleotide precursor of DNA and formation of haem (Chung *et al*., 1998). Other than that, tannins of the catechin group have also been shown to exhibit antimicrobial activity via mechanisms that involved damage to the membrane, for example the leakage of 5,6-carboxyfluorescin from phospatidyl choline liposomes (Ikigai *et al*., 1993). In conclusion, the present study has proven that the respective *M. calabura* and *D. linearis* leaves extracts possesses antistaphylococcal activity and thus provide the initial steps for future isolation and identification of the antibacterial compounds from those plants.

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