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

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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 mL⁻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 mL⁻¹, respectively whereas for the M. calabura extracts the MIC and MBC range between 1.250-5.000 and 2.500-5.000 μg mL⁻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 mL⁻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,
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
*Cochorus 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 (dH2O), 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 (3.25%),
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* 29213a,
*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 alpha, *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 (Becto™) 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 mL⁻¹. Interestingly, all of the
*M. calabura* extracts were effective only against the
normal *S. aureus* (*S. aureus* 29213a, *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 mL⁻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 mL⁻¹ while the MBC value, determined only
for the methanol and chloroform extracts of *M. calabura*,
were 2.500 μg mL⁻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*. 

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>S. aureus 29213x</th>
<th>S. aureus 33591</th>
<th>S. aureus 700699</th>
<th>VISA</th>
<th>VRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>M</td>
<td>C</td>
<td>A</td>
<td>M</td>
</tr>
<tr>
<td>M. calabura</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D. linearis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. malabathricum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. purpurea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. capsularis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

A: Aqueous extract; C: Chloroform extract; M: Methanol extract; VISA: Vancomycin-intermediate S. aureus; VRSA: Vancomycin-resistant S. aureus; Activity is not seen at 5 μg mL⁻¹. All extracts were tested using a microtiter plate assay once using the single concentration test (at the dose of 5 μg mL⁻¹). Extracts with antibacterial activity at the 5 μg mL⁻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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Value (μg mL⁻¹)</th>
<th>D. linearis</th>
<th>M. calabura</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>A</td>
<td>M</td>
</tr>
<tr>
<td>Staph. aureus 29213x</td>
<td>1.25</td>
<td>5</td>
<td>1.25</td>
</tr>
<tr>
<td>MBC</td>
<td>2.50</td>
<td>5</td>
<td>1.25</td>
</tr>
<tr>
<td>MBC</td>
<td>1.25</td>
<td>5</td>
<td>1.25</td>
</tr>
<tr>
<td>MBC</td>
<td>1.25</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>VISA</td>
<td>1.25</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>VRSA</td>
<td>1.25</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

A: Aqueous extract; C: Chloroform extract; M: Methanol extract; VISA: Vancomycin-intermediate; VRSA: Vancomycin-resistant; Staph. aureus; NA: Not available; Activity is not seen at 5 μg mL⁻¹.

Our earlier findings have demonstrated the presence of flavonoids, triterpenes, sapogenins 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., 2005d). Flavonoids and chalcones, like muntingone and (2S,5S)-5’-hydroxy-7,3’-4’-trimethoxyflavanone and 2’-4’-dihydroxy-3’-methoxyhydrochalcone and (-)-3’-methoxy-2’-4’,β,tetrahydroxychalcone, 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., aeglin, quercetin, isoquercitrin, astragalin, 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-rhamnopyranosyl-(1→6)-beta-D-glucopyranoside and 6S,13S)-6-[6-O-actyl-beta-D-glucopyranosyl-(1→4)-alpha-L-rhamnopyranosyl-(1→3)-[alpha-L-rhamnopyranosyl-(1→4)-beta-D-fucopyranosyl-(1→2)-[3,4,5-6](3,4,5-6)]]-cortex-(3,4,5-6)], (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 porins, causing changes in the tridimensional conformation exposing 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-carboxylfluorescein from phosphatidyl choline liposomes (Iikigai 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.

ACKNOWLEDGMENT

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REFERENCES


