Screening selected medicinal plants for antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA)

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ABSTRACT

Methanolic extracts of Vitex negundo (Nochi), Coleus amboinicus (Karpuravalli), Hylocereus polyrhynzus (Dragon fruit) and Andrographis paniculata (Hemptedu Bumi) were evaluated in an effort to identify antibacterial compounds against clinical isolates of Methicillin-Resistant Staphylococcus aureus (MRSA). Coleus amboinicus crude methanolic (CACM) leaves extract inhibited MRSA growth completely at lower concentration compared to other extracts. This is the first time that the CACM leaves extract was tested and reported to be effective against MRSA. Hence, CACM leaves extract was chosen for determination of minimum inhibitory concentration (MIC), growth profile of MRSA, bioautographic and toxicity test against brine shrimp. The MIC value of CACM leaves extract against MRSA was 200µg.ml⁻¹. CACM leaves extract resulted complete inhibition growth of the MRSA in growth profile and bioautography assay. The CACM leaves extract tested showed cytotoxicity activity against the Artemia salina. Therefore, CACM leaves extract could be used as a potential antibacterial agent from the natural plant sources for the treatment of MRSA infection.

Key words: Methicillin-Resistant Staphylococcus aureus; Coleus amboinicus; antibacterial activity; Vitex negundo; Hylocereus polyrhynzus; Andrographis paniculata

Introduction

Nature has been a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to that disease all over the world. Many of modern drugs have been isolated from natural sources are based on their use in traditional medicine. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Over 50% of all modern clinical drugs are of natural product origin (Stuffiness and Douros, 1982) and natural products play an important role in drug development programs in pharmaceutical industry.

Staphylococcus aureus is commonly associated with hospital and community-acquired infections. It causes superficial skin lesions such as boils, styes and furunculosis with more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. In 1980s, methicillin resistant cases ascend and vancomycin has been used as a last resort. The prevalence of MRSA isolates in several hospitals in Malaysia in 1996 was even higher at 40% (Rohani et al., 2000). In general the frequency of S. aureus nasal carriage from such settings in Malaysia varies from 45% to 76%. Antibiotics resistance has emerge as a serious problem in Malaysia hospitals and Methicillin-resistant Staphylococcus aureus (MRSA) are reported to be the major pathogen since 1995 (Malaysia Medical
Association, 2002). Therefore there is an urgent need to develop alternative antimicrobial drugs for the treatment of infectious diseases.

In order to search antibacterial activity against MRSA strains, four different plants such as *Andrographis paniculata* (Humped bumi), *Coleus amboinicus* (Oregano), *Vitex negundo* (Chasteberry) and *Hylocreus polyrhizus* (Dragon Fruit) were selected. *Coleus amboinicus* belongs to the family Lamiaceae. It is also known as oregano, or as Karpuravalli in Tamil. Synonym for this plant is *Coleus aromaticus*. *Coleus amboinicus* Benih, commonly known as an Indian Borage, is a medicinal plant and several medicinal properties are attributed to this plant in the Indian system of medicine. It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia (Morton, 1992). *Andrographis paniculata* or known as Humped Bumi is from the family Acanthaceae. *Andrographis paniculata* is also commonly known as Kalmegh is an important medicinal plant, occurring wild in India, and is used both in Ayurveda and Unani system of medicine (Chadha, 1985). The dried herb is a remedy for a number of ailments related to digestion, hepatoprotection, vermicidal, antiacne, analgesic, anti-inflammatory, antibacterial, antitphoid, antibiotic activities, hypoglycemic, besides immune enhancement (Saxena et al., 2000). *Vitex negundo* is from family Verbenaceae and known as Chastetree. The highly dissected leaves of Cut-leaf Chastetree are shaped like Cut-leaf Japanese Maple and were once believed to have sedative effects. The preparation of leaves used in catarral fever and applied to sinuses and scrofulous sores. Aqueous extract and oil of seeds possessed anti-oxidant and anti-inflammatory property. *Hylocreus polyrhizus* a tropical fruit popular in Southeast Asia belongs to the climbing cacti (Cactaceae) family. Dragon fruits reputedly improve eyesight and prevent hypertension. The seeds of the fruit supposed help in controlling blood glucose levels in people with non-insulin-dependent hyperglycaemic conditions and it is also used to treat stomach and endocrine problems.

**Materials and Methods**

**Identification of bacterial strains:**

Twenty three clinical isolates of *Staphylococcus aureus* were obtained from Hospital Besar, Alor Star, Kedah, Malaysia and positive control Methicillin Resistant *Staphylococcus aureus* (MRSA) strain was obtained from Universiti Kebangsaan Malaysia (UKM). Viability tests for each isolate were carried out by resuscitating the organism in nutrient agar. These clinical isolates were reconfirmed by Gram staining plating on Mannitol Salt Agar which is the selective media for *Staphylococcus aureus*. Then followed by the specific biochemical test for *Staphylococcus aureus* such as catalase and coagulate tests were carried out.

**Collection of plant materials:**

*Coleus amboinicus* (Karpuravalli), *Andrographis paniculata* (Humped bumi), *Vitex negundo* (Nochi), and *Hylocreus polyrhizus* (Dragon Fruit) were collected from Kedah. These plant materials were identified and classified by a botanist in the Biotechnology Department of AIMST University.

**Preparation of plant extract:**

*Coleus amboinicus*, *Andrographis paniculata*, and *Vitex negundo* leaves were collected. The leaves were washed with tap water and allowed to air dry. This was done to reduce the microbial load of plant material due to handling and transportation. The plant materials were dried in shade for three to four weeks. Direct sunlight should not be given to the plant materials because the sun light radiation can destroy the active compounds of plant leaves. After drying, the plant leaves were ground finely. This is to increase the surface area of the plant materials when exposed to solvent. The ground plant materials were weighed and stored in separated container. The *Hylocreus polyrhizus* was cut into four pieces and the skin was removed. The fruits were ground by using blender. Methanol was used for crude extraction purpose using maceration method. The mixture is kept for ninety six hours with periodic stirring. After four days, the mixtures were filtered using Whatman No.1 filter papers. The precipitates were discarded and the supernatant was collected. Each extract was concentrated using rotary evaporator (EYELA, AS). These extractions are primarily used for preliminary screening antibacterial activity.

**Antibiotic Susceptibility Test:**

Antibiotic susceptibility test was performed to identify the Methicillin Resistant *Staphylococcus aureus*.
(MRSA) from these 23 clinical isolates. Mueller Hinton Agar was used for this test. Each isolate was spread on the medium and Methicillin antibiotic disc (5µg) was placed on the middle of the culture. It was then incubated at 37°C for 24 hours. Similar procedures were followed for oxacillin and vancomycin antibiotics.

**Disc Diffusion Assay:**

The disk diffusion (Kirby-Bauer) technique, which is of the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS), was used for antimicrobial test. An overnight suspension culture of MRSA was standardized according to no. 0.5 McFarland Standard ($1 \times 10^5$ cell ml$^{-1}$) and spread on Mueller-Hinton agar (MHA) media. All the sterilized discs were placed on agar media. The disc was impregnated with the CACM leaves extract at 100 mg/ml. Methanol was used as negative control and standard antibiotic vancomycin (30 µg/ml) as positive reference to determine the sensitivity of the strain. The inoculated plates were incubated at 37°C for 24 h (Karaman et al. 2003). The antimicrobial activity was evaluated by measuring diameter of the inhibition zone (mm) around the disc.

**Determination Minimum Inhibitory Concentration (MIC):**

The inoculum of MRSA was prepared from 18 h Nutrient broth culture and adjusted to no. 0.5 McFarland standard turbidity. The CACM leaves extract was dissolved in methanol. Then serial twofold dilutions of test samples were prepared in a concentration range from 6.25 to 400 µg/ml in 10 ml sterile test tubes containing Mueller Hinton (MH) broth. The positive control was MH broth with standard reference antibiotic and inoculums and negative control was the MH broth and inoculums. The test tubes were vortex gently to mix the content and incubated at 37°C for 24 h (Roland et al. 2007). The MIC value was determined as the lowest concentration of CACM leaves extract in the broth medium that inhibit the growth of MRSA.

**Determination Minimum Bactericidal Concentration (MBC):**

For the determination of minimum bactericidal concentration (MBC), one hundred microliter of the culture from the diluted sample of MIC assay was transferred into nutrient agar. The liquid was spread plate and incubated at 37°C for 24 h. The MBC was recorded as the lowest concentration of the extract that gave complete inhibition of colony formation for the test bacteria at latter cultivation (Ronald et al., 2007).

**Time-Killing Profile of MRSA:**

The time-killing of MRSA with half, one and two fold MIC concentration over time was plotted to assess the bactericidal effect. The CACM leave extract was added to an aliquot of 25 ml nutrient broth in an amount which would achieved the concentration of 0.5, 1 and 2 fold MIC. After 18 hours, MRSA culture randomly chosen was harvested and adjusted by using no. 0.5 McFarland standard. One milliliter of adjusted culture transferred into CACM leave extract and nutrient broth and nutrient broth alone (control). The samples were incubated on the water bath at 37°C. After the addition of the adjusted culture, 1 ml of the test sample was transferred onto nutrient agar and spread plates. The plates were incubated at 37°C for 24 h and determined the CFU ml$^{-1}$. The growth of MRSA was measured every 4 h for 48 h (Yoga Latha et al., 2007).

**Brine Shrimp Toxicity Assay:**

Brine shrimp eggs, *Artemia salina*, hatch in artificial seawater. Thus artificial sea water was prepared by dissolving 38g of salt in 1 L of distilled water. A total of 0.5g *Artemia salina* were transferred into artificial sea water and incubated for 24 hours under room temperature with oxygen supplement and light intensity. MRSA extract was prepared in various concentrations for toxicity test. One milliliter from MRSA working solution was aliquot and dissolved in 10 ml of 2% dimethylsulphoxide (DMSO) to obtained 10 mg/ml concentration of extract. Then 1 ml of diluted extract was aliquot and transferred into 10 ml of artificial sea water to obtain 1 mg/ml. This method was followed by two fold serial dilution until to 0.008 mg/ml. 15 *Artemia salina* was transferred into each dilute extracts and incubated for 12 hours in light intensity condition. The mortality rate of *Artemia salina* and lethal concentration at 50% (LC$_{50}$) were calculated (Carballo et al., 2002).

**Bioautography assay:**
Thin Layer Chromatography (TLC):

TLC was performed on a silica gel aluminum plate (10 x 20 cm, Silica gel 60 F254, Merck) to fractionate active compound of 100 mg/ml CACM leave extract. The TLC plates were cut into 2 cm x 8 cm and dried into oven at 90°C for 10 minutes. This is to activate the TLC plates by absorbing the moisture content from the plates. Then the extract was spotted on the bottom of the plates. Then the TLC plates were transferred into beaker which contains separating solvent. The plates were placed in ascending direction in a beaker with chloroform: methanol: distilled water (25:3.5:0.5) solvent as mobile phase. The plates were observed under UV light. The separated spots were marked and the R value was calculated.

Bioautography technique:

Bioautographic method is another technique that is used in screening antibacterial activity. Bioautographic method is basically to localize the antibacterial compound from crude extract into chromatogram. This is a supportive and quick search method for antibacterial activity compound. The TLC plates that were used to separate active compounds in CACM leave extract were directly mounted on to seed culture medium. The culture plate was kept for five hour in room temperature to make sure separated compound diffuse into the medium. Then the TLC plates were taken out and incubated at 37°C for 24 hours (Paul Cos et al., 2006.)

Statistical analysis:

Each experiment was carried out three times. The data in this study was computed and expressed as mean ± standard deviation and also in SEM. Statistical analysis (ANOVA) was performed using GraphPad Prism Software version 4.0. P<0.05 was considered statistically significant for all comparisons.

Results and discussion

The methanol yield extracts of the Andrographis paniculata, Coleus amboinicus, Vitex nigundo and Hylocereus polyrhizus were presented in Table 1. The highest quantity of material was extracted from Coleus amboinicus (5.28%), while the lowest quantity was obtained from Hylocereus polyrhizus (1.22%). In Masoko’s antifungal analysis, it was revealed that organic solvent methanol is the best extractant in quantitatively and qualitatively. The methanol extract is competent to yield greater amount of extract and also contain higher polar compounds and tannins (Masoko et al., 2007) which are generally act as antimicrobial agents.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Extractive values (g)</th>
<th>% Yield (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coleus amboinicus (leave)</td>
<td>4.78</td>
<td>5.28</td>
</tr>
<tr>
<td>Andrographis paniculata(leave)</td>
<td>7.43</td>
<td>2.33</td>
</tr>
<tr>
<td>Vitex nigundo (leave)</td>
<td>7.12</td>
<td>5.01</td>
</tr>
<tr>
<td>Hylocereus polyrhizus (fruit)</td>
<td>5.34</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Identification of Bacterial Strains:

Hospital isolates bacterial strains were identified as MRSA using standardized microbiology technique. The colonial appearance of nasal cavity clinical isolates was identical and typical. All colonies were golden yellow, opaque, circular, smooth and creamy colour. Gram-staining revealed that Staphylococcus aureus isolates are Gram positive cocci in grape like clusters (Fig. 1). Qualitative biochemical coagulase test confirmed that all the isolates are positive for S. aureus. Among these 23 isolates, only 4 isolates showed a positive catalase reaction and the remaining 19 were catalase negative (Table 2). Recently few catalase-negative Methicillin Resistant Staphylococcus aureus strain have been reported to cause outbreaks in Brazilian hospitals (Del’ Alamo et al., 2007). Therefore 19 clinical isolates of catalase-negative Staphylococcus aureus were used for antibiotics susceptibility test to identify methicillin resistant strains.

Antibiotic Susceptibility Assay:

Antibiotic susceptibility test was performed to identify the MRSA strains. In general nosocomial MRSA is multi-drug resistant. During 1944, the first reports of penicillin-resistant S. aureus had appeared and today all strains of S. aureus are resistant to natural penicillins, aminopenicillins, and antipseudomonal penicillins (Chambers et al., 2001; Carballo et al., 2002). Therefore the antibiotic test was done for the ten catalase
negative *S. aureus* with methicillin (5 µg) and oxacillin (1 µg). Only ten nasal cavity clinical isolates shows positive results to methicillin and oxacillin antibiotics. Vancomycin antibiotic is used to treat MRSA infection (Stevens et al., 2002). Therefore vancomycin 30 µg antibiotic discs were used to test against all the ten isolates which are showing MRSA positive. As a result, all the ten isolates were susceptible to vancomycin antibiotics (Table 3).

**Table 2:** Qualitative biochemical and antibiotic tests against *Staphylococcus aureus* for the identification of Methicillin Resistant *Staphylococcus aureus* (MRSA).

<table>
<thead>
<tr>
<th>Isolated strains</th>
<th>Biochemical tests</th>
<th>Antibiotics</th>
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<tbody>
<tr>
<td></td>
<td>Coagulase</td>
<td>Catalase</td>
</tr>
<tr>
<td><em>PC1</em></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><em>PC2</em></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><em>PC3</em></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><em>PC4</em></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>3</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>4</td>
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<td>17</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>18</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Note: R: Resistant towards the antibiotics; S: Susceptible towards the antibiotics. PC1, 2, 3, and 4 strains resemble as the Positive Control of *Staphylococcus aureus* from University Kebangsaan Malaysia (UKM). The numerical numbers emphasis the isolates strains from hospitalized respiratory infected patients. * Marked strains were used for further antibacterial screenings.

**Table 3:** Four different plants including *Coleus amboinicus* (Karpuravalli), *Vitex nigundo* (Nochi), *Andrographis paniculata*, *Hylocereus polyrhizus* (Dragon fruit) were used for the antibacterial screening against MRSA clinical isolates.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Remarks</th>
<th>Inhibition zone (diameter, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>MRSA strain</td>
<td><em>E₁</em> (mm)</td>
</tr>
<tr>
<td>5</td>
<td>MRSA strain</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>MRSA strain</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>MRSA strain</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>MRSA strain</td>
<td>16</td>
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<tr>
<td>10</td>
<td>MRSA strain</td>
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<td>11</td>
<td>MRSA strain</td>
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<td>MRSA strain</td>
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<td>14</td>
<td>MRSA strain</td>
<td>13</td>
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<tr>
<td>15</td>
<td>MRSA strain</td>
<td>9</td>
</tr>
<tr>
<td>16</td>
<td>MRSA strain</td>
<td>16</td>
</tr>
</tbody>
</table>

Notes: *E₁*: *Andrographis paniculata* (Hem pedu bumi); *E₂*: *Coleus amboinicus* (Karpuravalli); *E₃*: *Vitex nigundo* (Nochi); *E₄*: *Hylocereus polyrhizus* (Dragon fruit); Control: Methanol. The inhibition zone more than 6 mm is sensitive towards the extract.

Fig. 1: *Staphylococcus aureus* Gram positive cocci in grape like clusters
Disc Diffusion Assay:

The antibacterial activity of four different plants including *Andrographis paniculata, Coleus amboinicus, Vitex nigundo* and *Hylocreus polyrhizus* methanolic crude extracts were evaluated through standard disc diffusion method. In the disc diffusion technique, a reservoir containing the test compound at a known concentration is brought into contact with the inoculated medium. The diameter of the clear zone around the reservoir (inhibition diameter) is measured at the end of the incubation period. In order to enhance the detection limit, the inoculated system is kept at lower temperature for several hours before incubation to favour compound diffusion over microbial growth. Thereby it increases the inhibition diameter efficiently (Paul Cos et al., 2006). After 12 hours incubation period, CACM leave extract conferred prominent results compared to *Andrographis paniculata Vitex nigundo* and *Hylocreus polyrhizus* crude extract. Clear and larger inhibition zones were formed surrounding the CACM leave extract (Table 4). This is the first time whereby CACM leave extract has been used for the evaluation of antibacterial activity against MRSA strains. Other two plants methanolic extracts such as *Vitex nigundo* and *Hylocreus polyrhizus* does not show any better inhibition zones. As CACM leave extract gave prominent results compared to other plants extracts, therefore the same plant extract was chosen for further tests against MRSA strains.

<table>
<thead>
<tr>
<th>Clinical Isolates</th>
<th>Remarks</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Extract</td>
<td>Extract</td>
</tr>
<tr>
<td>3</td>
<td>MRSA strain</td>
<td>200</td>
<td>400</td>
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<tr>
<td>5</td>
<td>MRSA strain</td>
<td>200</td>
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<td>6</td>
<td>MRSA strain</td>
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<tr>
<td>15</td>
<td>MRSA strain</td>
<td>200</td>
<td>400</td>
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</table>

Broth Dilution and effect of CACM leave extract against MRSA strains:

Table 4 shows MIC of CACM leave extract against 11 MRSA clinical isolates. MIC is defined as the lowest concentration that is able to inhibit any visible microbial growth and below that concentration which, there is no further inhibition. The minimal bactericidal concentration (MBC) is determined by plating-out samples of completely inhibited dilution cultures and assessing growth (static) or no-growth (cidal) after incubation (Paul Cos et al., 2006). MIC of this extract is 200 µg/ml. However, when this concentration was lower, the broth containing the extracts was turbid. Growth of a MRSA strain in broth culture with CACM leave extract was assessed by determining the optical density at 540 nm in each four hours interval for 48 hours. The time-killing profile of MRSA in the presence of extract was shown in Figure 2. The growth profile of MRSA was inhibited at 4 h for MIC and two fold MIC (400 µg/ml). The log phase of MRSA was reduced from 28h to 20h in half fold MIC value (100 µg/ml). This exhibits that the extract decelerated the reproduction of MRSA. The time killing profile of MRSA with CACM leave extract prove that the effectiveness of the extract within 48 h.

Brine Shrimp Toxicity Assay:

The brine shrimp lethality assay was proposed by Michael et al. 1956; Carballo et al., 2002. The results revealed that LC₅₀ of CACM leave extract was 182 µg/ml. In primary toxicity evaluation of plant extracts by brine shrimp lethality bioassay, LC₅₀ values lower than 1000 µg/ml are considered bioactive (Meyer et al., 1982). Therefore the extract consists relatively high amount of bioactive constituents. Besides this, the extract also might consist of bioactive compound which is cytotoxic to *Artemia salina*. Therefore for more accurate result for toxicity assay, animal models and isolate pure compound should be further evaluated.

Bioautography Assay:

Bioautography localizes antimicrobial activity on a chromatogram using three approaches such as direct bioautography, contact bioautography and agar over lay bioautography (Hamburger and Cordell, 1987,
Rahalison et al., 1991, Paul Cos et al., 2006). Separated fragments were visualized under Ultraviolet (UV) light and the R, values were calculated. The TLC plates had eleven separated spots. Contact bioautography method was used to localize antibacterial activity compounds of CACM leaf extract from TLC plates to an inoculated agar plate through direct contact. Two separated spots which corresponds to R, values 0.347 and 0.292 revealed inhibition zone on the culture medium (Fig. 3). Therefore it can be presumed that these two spots may have antibacterial activity compounds in CACM leaf extract.

**Fig. 2:** Growth profile of MRSA in the presence of *Coleus amboinicus* methanolic crude extract for 48 hours.

**Fig. 3:** Bioautography result of MRSA strain in CACM leaf extract. There is clear inhibition zone on the MRSA at 0.347 and 0.292 R, values.

**Discussion**

MRSA is important nosocomial pathogens in Malaysia and several recent reports have shown that its prevalence as an endemic nosocomial pathogen is increasing. Methicillin-resistant *Staphylococcus aureus* (MRSA) as a hospital pathogen has presented many clinical problems in the University Hospital, Kuala Lumpur, Malaysia since 1978. It was noted that the incidence of MRSA among S. aureus isolated from hospital inpatients had increased from 11.5% in 1979 to 18.8% in 1985 (Rohani et al., 1999).

The identification of hospital isolates bacteria strains is very important to confirm the presence of MRSA infection in patients. During the identification coagulase test was carried out. This is an important test to differentiate *S. aureus* from other species especially from *Staphylococcus epidermidis*. *S. aureus* produces coagulase, an enzyme like protein that clots oxalated or citrated plasma which is generated in both esterase and clotting activities. Generally catalase test is used to differentiate *Streptococcus* from *Staphylococcus*. 

However, now is difficult to verify this hypothesis because there are few cases on catalase-negative MRSA infections. First case of catalase-negative MRSA was reported by Xavier and his colleagues in 2001. This catalase-negative MRSA specimen was isolated from a 70-year-old woman, suffering from an Alzheimer’s disease (Xavier et al., 2001). Therefore, catalase-negative S. aureus strains were chosen for identification of MRSA strains and warrant for the further antibacterial screening. The hospitals isolates catalase negatives S. aureus exhibit methicillin and oxacillin resistant. Resistance to these drugs occurs because of the acquisition of genes that encode drug-inactivating enzymes known as β-lactamases. The expression of the mecA gene encoding low-affinity penicillin-binding protein PB2a confers resistance to other β-lactams in addition to methicillin. Besides that, transformation of a SCCmec type I element into S. aureus strains yielded highly oxacillin-resistant (Utsui et al., 1985; Louis, 2006). The MIC and time killing profile reveal the effectiveness of the CACM leave extract MRSA clinical strains. The MRSA growth inhibition is may due to the active compound from the extract. This statement is pretty true due to the comparison of the control MRSA growth for 48 h with the extract. The reduction in the growth rate may due to the restriction of cell division in the culture. Therefore again it is proven the effectiveness of the extract against MRSA strain. The brine shrimp assay is based on the ability to kill laboratory cultured Artemia salina nauplii. The Artemia salina lethality assay is considered a useful tool for preliminary assessment of toxicity. The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts. The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the microwell scale. In addition, this method is rapid, simple, reproducible and economical. A wide variety of biologically active chemical compounds, in particular cytotoxic agents are toxic to brine shrimps. Bioactive compounds are nearly always toxic in high concentrations and as toxicology can be described as pharmacology at higher doses. This premise has been applied to the screening of medicinal plant extracts in the brine shrimp toxicity test. The TLC two blue colour spots exhibits inhibition area in the bioautography assay. Under UV light these two relative spots expressed blue colour. Generally flavonoid derivatives express blue colour under UV light. Besides that, Alcaraz and colleagues (2000) reported that specific growth rate of MRSA strains linearly decrease with the increase in the concentration of flavonoids in the culture medium.

Therefore, it can be assumed that the antibacterial activity spots in TLC plates may be flavonoids or its derivatives. Further evaluation for the isolation and identification of bioactive compounds from CACM leave extract should be undertaken.

Conclusion

Coleus amboinicus methanolic crude leave extract pronounce promising results against nosocomial MRSA pathogens. In further studies isolation of these two pure compounds which completely inhibit this pathogen should be the next line of research. This can give more promising results on antibacterial activity of Coleus amboinicus methanolic extract against nasal carriage MRSA pathogens.

References


