



Antibacterial and biofilm inhibition activities of *Melastoma malabathricum* stem bark extract against *Streptococcus mutans*

Rohazila Mohamad Hanafiah^{1,2}, Wan Syaidatul Aqma¹,
Wan Ahmad Yaacob³ Zulfahmi Said² and Nazlina Ibrahim^{1*}

¹School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

²Faculty of Dentistry, Universiti Sains Islam Malaysia, 55100 Pandan Indah, Selangor Malaysia.

³School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

Email: nazlina@ukm.edu.my

ABSTRACT

Aim: This study was to determine the antibacterial activity of *Melastoma malabathricum* stem bark acetone extract (MMSBAE) against *Streptococcus mutans*.

Methodology and results: Antibacterial activity of the extract was determined by minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), biofilm formation, adherence inhibition, time kill studies and effect on *S. mutans* membrane integrity. MIC and MBC values of MMSBAE were 1.25 and 5 mg/mL, respectively. Time kill studies showed that reduction of colony forming unit in treated cells is 3 log₁₀ after 10 h of treatment ($p < 0.05$). The extracts reduced 50% biofilm and adherence activity of *S. mutans* at 1.88 mg/mL. The effect on *S. mutans* membrane integrity after exposure to MMSBAE for 90 and 120 min was determined by measuring leakage of cell content at 2 different wavelengths of 260 nm and 280 nm. In leakage assay, the percentage of absorbance (260 nm) in treated cell material showed 57% at 90 min and 60% at 120 min which is higher than negative control (<20%) but less than positive control (100%). The percentage absorbance of treated cell material (280 nm) was 61% at 90 min and 63% at 120 min. Identification of compound in MMSBAE was done by gas chromatography mass spectrometry (GCMS). Ten compounds were identified in the MMSBAE with some of them important in antimicrobial activity such as ethyl ester, undecene, and gamma sitosterol.

Conclusion, significance and impact of study: MMSBAE showed excellent bactericidal and antibacterial activities against *S. mutans*. The antibacterial mode of action of MMSBAE is suggested to be the disruption of the *S. mutans* membrane structure. The MMSBAE significantly inhibited biofilm and adherence activities of *S. mutans* in dose dependent manner ($p < 0.05$). MMSBAE has potential in the development of antibacterial agent with anti-biofilm and anti-adherence activities.

Keywords: Antibacterial, biofilm, *Melastoma malabathricum*, *Streptococcus mutans*

INTRODUCTION

Dental caries is a major problem in dental health in most developing countries. Although the health status of people in the developing nations is getting better, but there is an increment in dental caries among children and adults (Bragramian *et al.*, 2009). The situation is getting worse due to antibiotics and chemotherapeutics commonly used for treatment that are resistant to the pathogenic bacteria. For example, antibiotics such as penicillin, cephalosporin, erythromycin and tetracycline, which are commonly used to treat oral infections, are less effective against oral bacterial (Bidault *et al.*, 2007). The emergence of resistant bacteria is due to misuse and overuse of antibiotics. Besides, some mouthwashes used to prevent dental caries contained high concentration of alcohol and can

lead to oral cancer (Lachenmeier, 2008; McCullough and Farah, 2008). This problem has led researchers and the current study to discover new alternative medicines for prevention and treatment of oral diseases especially dental caries.

The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established. Many bacterial species were found in the oral cavity and a number of these are implicated in oral diseases. *Streptococcus* sp., *Staphylococcus* sp. and *Lactobacillus* sp. are microorganisms that can be found in caries lesions (Palombo, 2011). Among this group, *Streptococcus mutans* is the bacteria most often found in dental caries and is able to form biofilms. Biofilms are multiplex microbial communities consisting of microcolonies

*Corresponding author

assembled in a matrix of self-produced polymer material (Yang *et al.*, 2012). *S. mutans* has the virulence property to form biofilm known as dental plaque on tooth surfaces. Dental plaque is formed in three major steps. Firstly, the bacteria will form a film or acquire pellicle on tooth enamel. Then, the bacteria are strongly attached to the tooth surface, and become a primary colonizer. Finally, the colonies will use quorum sensing molecule to interact with other oral bacteria such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Actinomyces* sp. (Baswaraj and Devi, 2011).

Alternative products and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals for antimicrobial use. *Melastoma malabathricum* is a small shrub usually discovered in wasteland throughout the Southeast Asian countries, including Malaysia (Valkenberg and Bunyapraphatsara, 2001). The aqueous root extract of *M. malabathricum* was used to relieve toothaches and the leaf extract has been reported to possess anti-inflammatory, anti ulcerogenic and hypotensive effects (Susanti *et al.*, 2002; Zakaria *et al.*, 2006). The leaves of *M. malabathricum* were reported to have anti-bacterial activity against *Pseudomonas* sp. and methicillin resistant *Staphylococcus aureus* (Alwash *et al.*, 2013). In Malay traditional practice, the stem bark is used to relieve toothache (Joffry *et al.*, 2012). Therefore, this study aimed to evaluate the antibacterial activity of *Melastoma malabathricum* stem bark acetone extract (MMSBAE) against *S. mutans*. The biofilm formation and adherence activity of the treated cell were also evaluated. Finally, the mode of action of MMSBAE on membrane integrity was determined.

MATERIALS AND METHODS

Plant material

The specimen used in this study was stem barks of *M. malabathricum*. A voucher specimen deposited in the herbarium at Universiti Kebangsaan Malaysia [Rohazila 2014-1 (UKMB)]. The specimen was collected at Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia (2°55'39.8" N101, 46°33.0"E) in an exposed area of roadside bank.

Preparation of *M. malabathricum* stem bark acetone extract (MMSBAE)

The stem barks of *M. malabathricum* were dried at room temperature in a ventilated room, milled to a fine powder in a grinder (Model C14, Kesmac Sdn. Bhd., Malaysia) and stored in closed containers. The stem barks (800 g) were extracted with 1 L of acetone. The mixture was vigorously shaken for 2 h on an orbital shaker (LM-530RD, Taiwan) and the extract was filtered using Whatman No. 1 filter paper. The collected solvent filtrate was evaporated using a rotary evaporator (Laborota 4000, Germany). The stock solution concentration of MMSBAE was 100 mg/mL in 10% dimethyl sulfoxide (DMSO).

Bacterial strain

Bacterial strain used in this study was *S. mutans* ATCC25175 and cultured in Brain Heart Infusion (BHI) Broth (Oxoid, England) at 37 °C under anaerobic condition. Glycerol stock of the bacteria was kept at -80 °C.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was performed using the two fold serial dilution method described by Alwash *et al.*, (2013). MIC was done in a sterile 96-well plate. Test bacteria (100 µL) at 10⁸ CFU/mL was added to various concentrations of MMSBAE (0.04-20 mg/mL) diluted in Mueller Hinton Broth (MHB) to a final volume of 200 µL/well. DMSO 10% (v/v) was used as negative control and penicillin (0.004-0.06 mg/mL) was used as positive control. Following incubation at 37 °C under anaerobic condition for 24 h, MIC value was determined as the lowest concentration that inhibits the visible growth of bacteria. Minimum bactericidal concentration (MBC) was determined by culturing a 5 µL aliquot from wells that exhibited no bacterial growth in MIC wells onto sterile nutrient agar and incubated overnight at 37 °C. The MBC was defined as the lowest concentration preventing bacterial growth.

Time kill assay

The time kill assay of the MMSBAE was assessed using the method described by Raja *et al.* (2011). *S. mutans* ATCC 25175 was grown in Mueller Hinton Broth (MHB) at 37 °C for 24 h. The turbidity of the bacteria culture was adjusted to 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/mL) in sterile normal saline. Two hundred microliters of this suspension were used to inoculate 20 mL of MHB in conical flasks containing MMSBAE extract in the concentration of 3 x MIC. The flasks were incubated at 37 °C under anaerobic condition. A 100 µL aliquot was removed at 0, 1, 2, 4, 6, 8, 10, and 24 h and viable counts were determined in triplicate by plating on Tryptic soy agar (TSA). Killing curves were constructed by plotting the log₁₀ CFU/mL versus time over 24 h.

Biofilm formation assay

The assay was performed using the method from Khan *et al.* (2011). *S. mutans* ATCC 25175 was grown in Mueller Hinton Broth (MHB) at 37 °C for 24 h under anaerobic condition. After incubation, the bacteria culture (1 mL) was transferred to 10 mL preheated BHI (37 °C) broth and grown at 37 °C under anaerobic condition to be mid-exponential phase (OD₅₉₅ ≈ 1). The culture was then diluted 1:100 in preheated BHI media. Cell suspensions (150 µL) were added into each well of a 96-well plate (flat-bottom). Then, 50 µL of MMSBAE at different

concentrations (0.24 to 30 mg/mL) was added. Negative control well contained the untreated cells. Positive control well contained the cells that treated with penicillin (0.032 mg/mL). After inoculation, all plates were incubated at 37 °C for 24 h anaerobically. The culture medium was then decanted, and the plates were gently washed twice with 200 µL sterile distilled water to remove planktonic and loosely bound cells. The adherent bacteria were stained with 50 µL of 0.1% (w/v) crystal violet (Oxoid, England) for 15 min. After rinsing twice with 200 µL of water, the bound dye was extracted from the stained cells by addition of 200 µL of 99% (w/v) ethanol (Sigma, Germany). Biofilm formation was then quantified by measuring the absorbance of the solution at 595 nm in a microplate reader (Model 680, Bio-Rad).

Adherence inhibition

Adherence inhibition assay was performed using the method from Khan *et al.* (2011). BHI (5 mL) containing 0.25% sucrose and MMSBAE (0.24 to 30 mg/mL) was added to a glass tube (20 mL capacity) containing 1 mL of *S. mutans* (OD₅₉₅ ≈ 1). All tubes were inclined at 30° and incubated at 37 °C for 24 h. Adhered cells were separated from non-adherent cells by gentle rotation of the tube and bacterial suspension was removed. Cells that adhered to the glass tube were then collected by washing with sodium hydroxide (0.5 mol) and resuspended in saline. Turbidity of adhered cells was determined by using the spectrophotometer at 600 nm wavelength. All determination was performed in triplicates using untreated BHI medium as negative control. *S. mutans* that was treated with penicillin (0.032 mg/mL) was used as positive control.

Leakage of bacterial cell content material

The release of cell content due to membrane damage was determined by spectrophotometer at 260 and 280 nm as described by Raja *et al.*, (2011) and Cox *et al.*, (2000). MMSBAE was added at a concentration of 3 x MIC to the bacterial suspension (≈ 1 x 10⁹ CFU/mL) and incubated for 90 and 120 min at 37 °C respectively. For complete release of cell content as control, the bacterial suspension was treated with lysozyme (100 µg/mL) at 37 °C for 90 and 120 min respectively, followed by sonication. Cell supernatants were obtained by centrifugation (10,000 x g for 10 min). The absorbance of cell supernatant at 260 and 280 nm was determined by spectrophotometer (UV-Vis, Shimadzu Japan). Background leakage rates (no extracts added) was used as untreated control. The percentage of cell leakage at 260 and 280 nm were calculated as follows:

$$\text{Cell leakage percentage \%} = \frac{\text{Absorbance cell treated with MMSBAE}}{\text{Absorbance cell treated with Lysozyme}} \times 100\%$$

Gas Chromatography Mass Spectrometry (GCMS)

The bioactive component of MMSBAE was determined by GCMS (Shimadzu, model QP5050A) equipped with a HP5 (50 m) capillary column. Helium (1.0 mL /min) was used as the carrier gas. Temperature was programmed from 60 °C to 280 °C at the rate of 60 °C for 2 min and held at 280 °C for 12 min. Then, injector was maintained at 245 °C and the electron impact ion source was maintained at 295 °C. Electron impact spectra were recorded at 70 eV. The compounds were identified by comparing the GC retention indices with the mass spectra provided by National Institute Standard and Technology (NIST) database.

Statistical analysis

All experiments were carried out in triplicates in at least three different occasions. The differences between the two means were evaluated by the Student's *t*-test. The data were analysed by one-way ANOVA for comparison of multiple means. The level of significance for all statistical tests was set at *p* < 0.05.

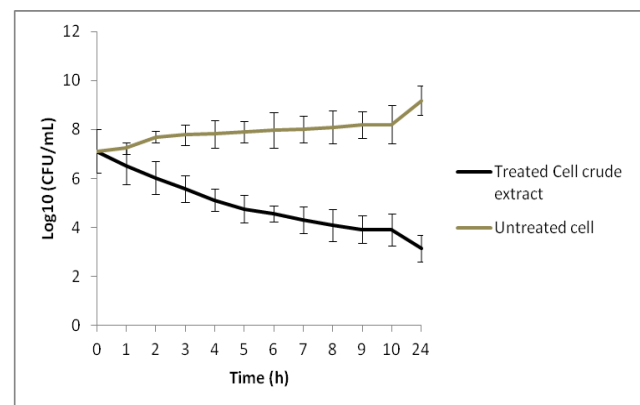


Figure 1: Effects of MMSBAE on cell viability of *S. mutans* ATCC 25175. Untreated cell served as a control group. Each time point represents the mean of three different experiments performed in duplicate. The CFU value of treated cells was significantly different (*p* < 0.05) when compared to CFU value of untreated cells.

RESULTS

The mass of MMSBAE successfully prepared from the stem bark of *M. malabathricum* was 1.4 g with yield percentage of 0.16% (w/w). The MIC value was determined to be 1.25 mg/mL. The MIC value of positive control (penicillin) was 0.008 mg/mL. MBC value of MMSBAE extract was 5 mg/mL, a higher value than the MIC value. The MBC value of penicillin against *S. mutans* was 0.032 mg/mL.

Time kill kinetic studies of MMSBAE showed decrease in the colony forming unit (CFU) of treated cells as opposed to increase in the CFU of untreated cells within 24 h of incubation (Figure 1). From the assay, CFU in

treated cells was reduced 3 log₁₀ times after 10 h of treatment. The CFU value of treated cells was significantly different ($p < 0.05$) when compared to CFU value of untreated cells.

In this study, untreated *S. mutans* formed biofilm after 24 h of growth. MMSBAE effectively inhibited the formation of *S. mutans* biofilms with a dose dependent manner (Figure 2). The biofilm formation in treated cells at lower concentration of 1.88 mg/mL of MMSBAE was significantly inhibited (50%) as compared to the untreated cells ($p < 0.05$) (Figure 2). The results also showed that the percentage of biofilm was significantly reduced to 70 % in cells treated with MMSBAE (30 mg/mL), compared to untreated cell. The results also showed that treatment with 0.032 mg/mL of penicillin significantly reduced the biofilm formation to 84% compared to untreated cells. All MMSBAE at all tested concentration effectively inhibited the *S. mutans* biofilm formation ($p < 0.05$).

Effect on adherence of *S. mutans* with different concentrations of MMSBAE is given in Figure 3. The reduction in adherence activity was found to be in a dose dependent manner. The concentration that inhibited 50-78% adherence was 1.88-30 mg/mL of MMSBAE. The highest reduction in adherence activity was detected in cell that treated with 0.032 mg/mL penicillin. The penicillin was inhibited 80% of the adherence activity of *S. mutans*. Significant ($p < 0.05$) reduction in adherence activity by *S. mutans* was observed in all tested concentrations.

The effect of MMSBAE on membrane integrity of *S. mutans* was determined by the leakage of cell content that was determined at 260 nm and 280 nm (Figure 4). The absorption value of cell content determined at 260 nm is indicative of nucleic acid leakage, while absorption at 280 nm is indicative of protein leakage (Raja *et al.*, 2011). The percentage of nucleic acid leakage when the cells were treated with MMSBAE was 57% at 90 min and 60 % for 120 min. The percentage of protein leakage at cell treated

with MMSBAE was 61% for 90 min and 63% for 120 min. The absorption value of untreated cell at 260 nm and 280 nm in 90 and 120 min were <20%, respectively.

In the GCMS analysis, ten compounds were identified in the MMSBAE and 3 compounds have been reported to have antimicrobial properties including ethyl ester (Farhan and Saour, 2013), undecene (Ambang *et al.*, 2010) and gamma sitosterol (Venkata *et al.*, 2012) (Table 1). The analysis showed the MMSBAE contain 54.92% of ethyl ester, 7.41% of undecene and 4.76% of gamma sitosterol compounds (Figure 5).

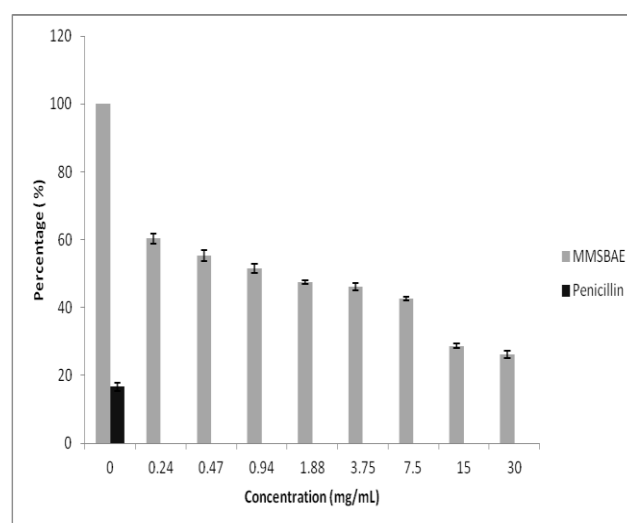


Figure 2: Biofilm formation of *S. mutans* when treated with MMSBAE (0-30 mg/mL) and penicillin (0.032 mg/mL). Significant ($p < 0.05$) reduction in biofilm activity by *S. mutans* was observed in all tested concentrations.

Table 1: GCMS analysis of phytochemicals identified from MMSBAE.

| No | Retention Time | Compound | Molecular Formula | Molecular weight | Peak area (%) |
|----|----------------|---------------------------------------|--|------------------|---------------|
| 1 | 7.533 | Dihexadecyl phosphate | C ₃₂ H ₆₇ O ₄ P | 546 | 3.08 |
| 2 | 16.692 | dl-2,3-Bis [hexadecyloxy] iodopropane | C ₃₅ H ₇₂ IO ₂ | 650 | 2.23 |
| 3 | 21.233 | 3-Octanone | C ₈ H ₁₆ O | 128 | 1.86 |
| 4 | 25.33 | Chloroacetic acid | C ₂ H ₃ ClO ₂ | 95 | 2.78 |
| 5 | 36.875 | Gammasitosterol | C ₂₉ H ₅₀ O | 414 | 4.76 |
| 6 | 37.050 | Cyclopropane | C ₃ H ₆ | 42 | 13.79 |
| 7 | 37.225 | Ethyl ester | C ₁₇ H ₂₈ O ₃ | 280 | 54.92 |
| 8 | 38.20 | Undecene | C ₁₁ H ₂₂ | 154.29 | 7.41 |
| 9 | 38.942 | Bicyclo[2.2.1]heptane | C ₇ H ₁₀ | 94.15 | 1.76 |
| 10 | 39.50 | 9-octadecenoic acid | C ₁₈ H ₃₄ O ₂ | 282 | 5.41 |

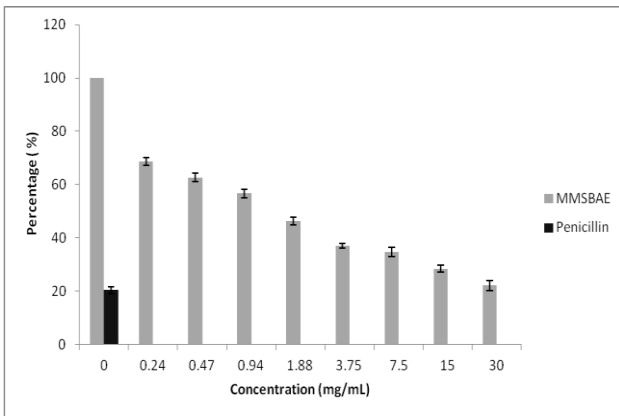


Figure 3: Adherence activity of *S. mutans* when treated with MMSBAE (0-30 mg/mL) and penicillin (0.032 mg/mL). Significant ($p < 0.05$) reduction in adherence activity by *S. mutans* was observed in all tested concentrations.

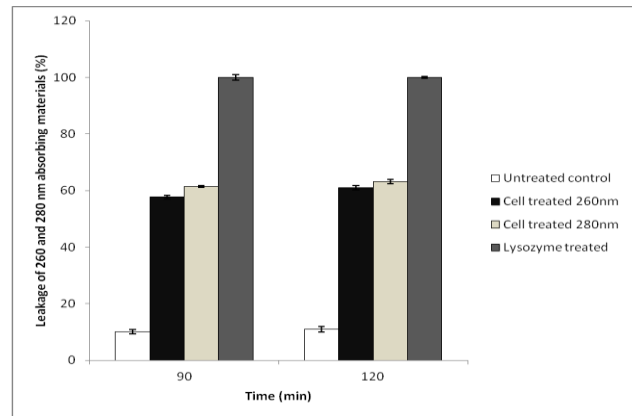


Figure 4: Effects of MMSBAE on cell materials leakage at 90 and 120 min. The percentage of all cell material leakage that treated with MMSBAE and lysozyme were significantly higher as compared to untreated control ($p < 0.05$).

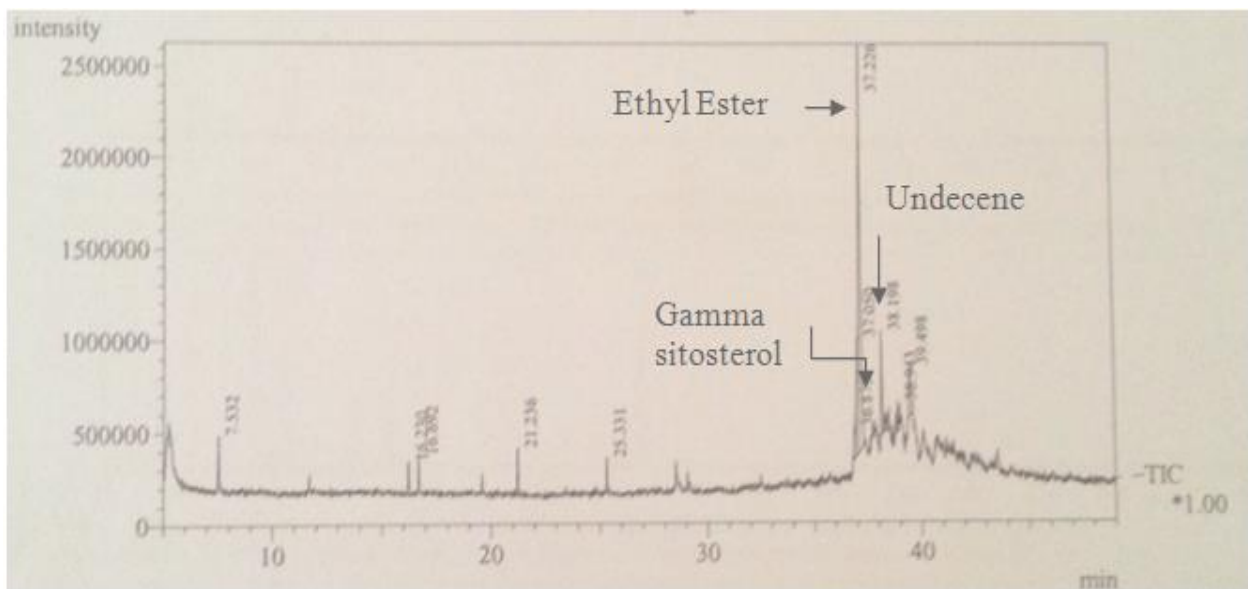


Figure 5: GCMS chromatogram of MMSBAE. The number represents retention time of the compounds. The arrow indicates ethyl ester, undecene and gamma sitosterol peak based on retention time.

DISCUSSION

MMSBAE is a non-polar compound which can be extracted using acetone as a solvent with different polarities. We found that the extract produced using acetone as solvent exhibited good antibacterial activity compared to other extracts using different solvents. MIC is generally the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. MIC determination is important in diagnostic laboratories in order to determine the activity of new antimicrobial agents and also can be used to confirm resistance of

microorganisms to an antimicrobial agent (Andrews, 2001).

In this study, MIC value of MMSBAE (1.25 mg/mL) against *S. mutans* was lower than other plant extracts such as *Breynia nivosus* extract. The MIC value of *Breynia nivosus* extract against *S. mutans* was >25 mg/mL (Amadi *et al.*, 2007) and garlic extract against *S. mutans* was >35.7 mg/mL (Bakri and Douglas, 2005). From this assay, we speculate that MMSBAE has higher activity to inhibit *S. mutans* compared to garlic and *Breynia nivosus* extract because the MIC value of MMSBAE was lower than those extracts. The lower MIC

value indicates that less antibiotic is required for inhibiting growth of the bacteria. Therefore, antibiotic with lower MIC scores are more effective as antimicrobial agents (Andrews, 2001).

The antibacterial agents were usually regarded as having bactericidal activity if the value of MBC was no more than four times the value of MIC (French, 2006). The MBC results showed that the MMSBAE (5.0 mg/mL) had bactericidal activity against *S. mutans*. MBC value can be subject to technical variation and several theoretical limitations (Pankey and Sabath, 2004). The MBC assay is normally performed against bacteria at log growth phase. However, in clinical infections, bacteria grow slowly and the bactericidal activity of some antibacterial agents may be reduced or lost (Eng *et al.*, 1991). Therefore, time kill curve is important in confirming the bactericidal activity of MMSBAE against *S. mutans*.

In the time kill study, bactericidal effect of MBC was confirmed. Bactericidal activity is defined when the antibacterial agents reduced $>3 \log_{10}$ colony forming unit (CFU) from the initial inoculum while bacteriostatic activity is defined when antibacterial agents reduced $<3 \log_{10}$ of CFU from the initial inoculum (NCCLS, 1999). From this assay, MMSBAE displayed bactericidal activity when the extract reduced $>3 \log_{10}$ of CFU from initial inoculum after 10 h.

The ability of natural products to inhibit cell attachment is a promising tool for reducing colonization on various surfaces. The application of anti-biofilm agents appears to be an interesting approach in the prevention of microbial infection. Biofilm formation is a process where microorganisms irreversibly attach to a surface and produce extracellular polymers that facilitate attachment and matrix formation (Donlan, 2001). In this study, the results showed that the biofilm formation for cells treated with MMSBAE significantly reduced compared to untreated cells. MMSBAE inhibited *S. mutans* biofilm formation by inhibiting bacterial growth.

This study is the first report on *M. malabathricum* activity in inhibiting biofilm formation. Other plants that showed biofilm inhibition include *Polygonum cuspidatum* (Song *et al.*, 2006), *Psoralea corylifolia* (Katsura *et al.*, 2001) and *Trachyspermum ammi* (Raja *et al.*, 2011). These reports suggested that inhibitory effects may be mediated by the presence of alkaloids, phenolics and terpenes in the extract (Song *et al.*, 2006). Other than that, terpene (Bakuchiol) isolated from *Psoralea corylifolia* was able to inhibit biofilm and growth of *S. mutans* adhered cell (Katsura *et al.*, 2001). In this study, MMSBAE contained among others ethyl ester, undecene and gamma sitosterol compounds. Ethyl ester compound from *Salvadora persica* was reported to show antibiofilm activity against *S. mutans* (Al-Sohaibani and Murugan, 2012). However, undecene and gamma sitosterol compounds have never been reported to have anti-biofilm activity against *S. mutans*. Nevertheless, β -sitosterol glucoside isolated from citrus was reported to have anti-biofilm activity against *Escherichia coli* O157:H7 (Vikram *et al.*, 2013).

Adherence activity of cells is the initial stage in biofilm formation. The adherence activity of bacteria requires nutrients, organic and inorganic molecules to attach onto the tooth surface. Pre-treatment of cells with plant extracts produces unfavourable film that promotes detachment, thereby reducing surface adhesion (Sandasi *et al.*, 2008). In this study, adherence activity of cells treated with MMSBAE was significantly reduced as compared to untreated cell. The inhibition was due to the effect of extract or phytochemicals on the growth of bacteria and the adhesion activity of bacteria towards surface of glass.

The effect of MMSBAE on membrane integrity of *S. mutans* was determined by the leakage of cell contents. According to Miksusanti *et al.* (2008), cell contents such as nucleic acid and protein are absorbed at 260 nm and 280 nm, respectively. The increase in the absorption values observed at 260 nm and 280 nm indicated higher nucleic acid and protein loss from bacteria due to the damaged cell membrane. In this study, lysozyme was used as positive control. It is a catalytic enzyme and responsible for hydrolysis of structural polysaccharides in the cell wall. Hence, the absorption values of cells treated with lysozyme at 260 nm and 280 nm are considered as 100%, respectively.

In the treated cell, cell contents leaked more compared to the untreated cells. This comparison signifies that MMSBAE is able to rupture the bacteria cell wall and paved a way out for the cell contents from the cytoplasm. The prolonged treatment with the extract does not increase the capability in membrane rupture. However, the percentage of cell contents leakage was significantly higher as compared to untreated control ($p < 0.05$).

Research done by Raja *et al.* (2011) suggested that the breakage of cell wall is caused by the reaction of the bioactive compounds that are present in the plant extract. According to Sikkema *et al.* (1995) essential oil and monoterpenoid compound caused membrane expansion, increased membrane fluidity and inhibited a membrane-embedded enzyme of gram positive and negative bacteria. Furthermore, Trombetta *et al.* (2002) speculated that the antimicrobial effect of monoterpene compound might be due to perturbation of the lipid fraction of the bacterial plasma membrane. This situation caused alteration of the membrane permeability and leakage of intracellular materials.

In this study, MMSBAE contained 54.92% ethyl ester. Ethyl ester is a functional group that can assist many active compounds to inhibit bacterial growth. Babayan and McIntyre (1971) have shown that polyglycerol esters encompass a wide range of hydrophilic to lipophilic natures, dependent on fatty acid chain length and glycerol polymer length. When the length of fatty acid is long, it decreases the solubility rate in water towards that polyglycerol ester. Inversely, the longer the glycerol polymer, the higher the solubility rate in the water. When fatty acid is inactive, polyglycerol ester becomes an inhibitor agent. Decrement of antimicrobial activity to the polyglycerol ester substrate that has a high mass molecule size depends on the structural features and also the physical structure. The bigger the molecule structure,

the lower the inhibition rate (Conley and Kabara, 1973). For further analysis, the structure of ethyl ester in MMSBAE should be identified by Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). The structure of ethyl ester in MMSBAE can be used to explore the full potential of the compound and related active lipid derivatives. The information from further analysis can be used to investigate the structural relationships involved with antimicrobial activity.

In GCMS analysis, ten compounds were identified from the MMSBAE and 3 compounds have been reported to have antimicrobial properties. Farhan and Saour (2013), reported that ethyl ester in benzoxazole heterocyclic moiety has antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes*. Undecene compound was found in *Thevetia peruviana* seeds from ethyl acetate extract as anti-fungal agent against *Phytophthora megakarya* (Ambang *et al.*, 2010). Gamma sitosterol compound from *Eupatorium odoratum* extract contains antibacterial activity against *E. coli*, *Klebsiella pneumonia* and *Streptococcus thermophilus* (Venkata *et al.*, 2012).

CONCLUSION

Acetone extract of *Melastoma malabathricum* stem bark showed excellent antibacterial activity against *S. mutans*. MMSBAE has bactericidal activity with the ability to inhibit biofilm and adherence activities in dose-dependent manner against *S. mutans*. The leakage assay suggested that the extract disrupted the cell membrane of the bacteria. The extract contains a high amount of ethyl ester which may contribute to the anti-microbial activity.

ACKNOWLEDGEMENTS

This study was supported by Universiti Kebangsaan Malaysia (DPP-2014-021) and Universiti Sains Islam Malaysia (PPP/USG-0213/FPG/30/12613). Centre for Natural Products and Drug Research (CENAR) University Malaya is greatly appreciated for HPLC analysis.

REFERENCES

- Al-Sohaibani, S. and Murugan, K. (2012).** Anti-biofilm activity of *Salvadora persica* on cariogenic isolates of *Streptococcus mutans*: *In vitro* and molecular docking studies. *The Journal of Bioadhesion and Biofilm Research* **28(1)**, 29-38.
- Alwash, M. S., Ibrahim, N. and Ahmad W. A. Y. (2013).** Identification and mode of action of antibacterial components from *Melastoma malabathricum* Linn leaves. *American Journal of Infectious Diseases* **9(2)**, 46-58.
- Amadi, E. S., Oyeka, C. A., Onyeagba, R. A., Ugbogu O. C. and Okoli I. (2007).** Antimicrobial screening of *Breynia nivosus* and *Ageratum conyzoides* against dental caries organisms. *Journal of Biological Sciences* **7(2)**, 354-358.
- Ambang, Z., Ngoh Dooh, J. P., Esono, G., Bekolo, N., Checawong, G. and Asseng, C. C. (2010).** Effect of *Thevetia peruviana* seeds extract on *in vitro* growth of four strains of *Phytophthora megakarya*. *Plants Omics Journal* **3(3)**, 70-76.
- Andrews, J. M. (2001).** Determination of minimum inhibitory concentrations. *Journal Antimicrobiology of Chemotherapy* **48(1)**, 5-16.
- Babayan, V. K. and McIntyre, R. T. (1971).** Preparation and properties of some polyglycerol esters of short and medium chain length fatty acids. *Journal of America Oil and Chemistry Society* **48**, 307-309.
- Bakri, I. M. and Douglas, C. W. I. (2005).** Inhibitory effect of garlic extract on oral bacteria. *Archives of Oral Biology* **50(7)**, 645-651.
- Baswaraj, B. and Devi, P. (2011).** Quorum sensing in plaque biofilms: Challenging and future prospect. *The Journal of Contemporary Dental Practice* **12(6)**, 479-485.
- Bidault, P., Chandad, F. and Grenier, D. (2007).** Risk of bacterial resistance associated with systemic antibiotic therapy in Periodontology. *Journal Canadian Dental Association* **8**, 721-725.
- Bragramian, R. A., Godoy, F. R. and Volpe, A. R. (2009).** The global increase in dental caries. A pending public health crisis. *American Journal of Dentistry* **21(1)**, 1-6.
- Conley, A. J. and Kabara, J. J. (1973).** Antimicrobial action of esters of polyhydric alcohols. *Antimicrobial Agents and Chemotherapy* **4(5)**, 501-506.
- Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R. and Wyllie, S. G. (2000).** The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tree oil). *Journal of Applied Microbiology* **88**, 170-175.
- Donlan, R. M. (2001).** Biofilm formation: A clinically relevant microbiological process. *Clinical Infection Disease* **33**, 1387-1392.
- Eng, R. H., Padberg, F. T. and Smith, S. M. (1991).** Bactericidal effects of antibiotics on slowly growing and nongrowing bacteria. *Antimicrobial Agents Chemotherapy* **35**, 1824-1828.
- Farhan, M. S. and Saour, K. Y. (2013).** Synthesis characterization and antimicrobial activity of new benzoxazole amino acid ester derivatives. *International Journal of Comprehensive Pharmacy* **4(2)**, 1-5.
- French, G. L. (2006).** Bactericidal agents in the treatment of MRSA infections: the potential role of daptomycin. *Journal of Antimicrobial chemotherapy* **58**, 1107-1117.
- Joffry, M. S., Yob, N. J., Rofiee, M. S., Meor Mohd Affendi, M. M. R., Suhaili, Z., Othman, F., Md Akim, A., Md Desa, M. M. N. and Zakaria, Z. A. (2012).** *Melastoma malabathricum* (L) Smith ethnomedicinal uses, chemical constituents, and pharmacological properties. *Evidence-Based Complementary and Alternative Medicine* **2012**, 258434-258482.

- Katsura, H., Tsukiyama, R. I., Suzuki, A. and Kobayashi, M. (2001).** *In vitro* antimicrobial activities of bakuchiol against oral microorganisms. *Antimicrobial Agents and Chemotherapy* **45(11)**, 3009-3013.
- Khan, R., Zakir, M., Khanam, Z., Shakil, S. and Khan, A. U. (2011).** Novel compound from *Trachyspermum ammi* seeds with anti biofilm and anti adherence activities against *Streptococcus mutans*: A potential chemotherapeutic agents against dental caries. *Journal Applied Microbiology* **109(6)**, 2151-2159.
- Lachenmeier, D. W. (2008).** Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *Journal of Occupational and Environmental Medicine* **3(26)**, 1-16.
- McCullough, M. J. and Farah, C. S. (2008).** The role of alcohol in oral carcinogenesis with particular reference to alcohol containing mouthwashes. *Australia Dental Journal* **53(4)**, 302-306.
- Miksusanti, Betty, S. L. J., Bambang, P. P., Rizal, S. and Gatot, T. R. (2008).** Mode of action Temu Kunci (*Kaempferia pandurata*) essential oil on *E. coli* K1.1 cell determined by leakage of material cell and salt tolerance assays. *Journal of Biosciences* **15(2)**, 56-60.
- NCCLS (1999).** Methods for determining bactericidal activity of antimicrobial agents; approved guideline, NCCLS document M26-A. Wayne, PA, National Committee for Clinical Laboratory Standards.
- Palombo, E. A. (2011).** Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. *Evidence-Based Complementary and Alternative Medicine* **2011**, 1-15.
- Pankey, G. A. and Sabath, L. D. (2004).** Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram positive bacterial infections. *Clinical Infection Disease* **38**, 864-870.
- Raja, F. A., Ali, F., Khan, I. A., Shawl, A. I., Arora, D. I., Shah, B. A. and Taneja, S. C. (2011).** Antistaphylococcal and biofilm inhibitory activities of acetyl-11-keto-b-boswellic acid from *Boswellia serrata*. *BioMed Central Microbiology* **11**, 54-63.
- Sandasi, M., Leonard, C. M. and Viljoen, A. M. (2008).** The effect of five common essential oil components on *Listeria monocytogenes* biofilms. *Food Control* **19**, 1070-1075.
- Sikkema, J., Bont, J. A. M. and Poolman, B. (1995).** Mechanisms of membrane toxicity of hydrocarbons. *Microbiology Revision* **59**, 201-222.
- Song, J. H., Kim, S. K., Chang, K.W., Han, S. K., Yi, H. K. and Jeon. J. G. (2006).** *In vitro* inhibitory effects of *Polygonum cuspidatum* on bacterial viability and virulence factors of *Streptococcus mutans* and *Streptococcus sobrinus*. *Archives of Oral Biology* **51**, 1131-1140.
- Susanti, D., Sirat, H. M., Ahmad, F., Ali, R. M., Aimi, N. and Kiajima, M. (2002).** Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food Chemistry* **103**, 710-716.
- Trombetta, D., Saija, A. Bisignano, G., Arena, S., Caruso, S., Mazzanti, G., Uccella, N. and Castelli, F. (2002).** Study on the mechanisms of the antibacterial action of some plant unsaturated aldehydes. *Letters Applied Microbiology* **35**, 285-290.
- Valkenberg, J. L. C. H. and Bunyapraphatsara, N. (2001).** *Melastoma malabathricum* L. in Plant Resources of South-East Asian. In: Medicinal and Poisonous Plants 2. Vol. 12. Netherland. Backhuys and Leiden. pp. 365-366.
- Venkata, R., Samuel, L., Pardha, S. M., Narashimha, R. B., Naga, V. K. A., Sudhakar, M. and Radhakrishnan, T. M. (2012).** Antibacterial, antioxidant activity and GCMS analysis of *Eupatorium odoratum*. *Asian Journal of Pharmaceutical and Clinical Research* **5(2)**, 100-106.
- Vikram, A., Jayaprakasha, G. K. and Patil, B. S. (2013).** Inhibition of *Escherichia coli* 0157:H7 motility and biofilm β sitosterol glucoside. *Biochimica et Biophysica Acta* **1830(11)**, 5219-5228.
- Yang, L., Liu, Y., Wu, H., Song, Z., Hoiby, N., Molin, S. and Givskov, M. (2012).** Combating biofilms. *FEMS Immunology Medical Microbiology* **65(2)**, 146-157.
- Zakaria, Z. A., Raden, M. N. R. N. S. and Hanan, K. G. (2006).** Antinociceptive, anti-inflammatory and antipyretic properties of *Melastoma malabathricum* leaves aqueous extract in experimental animals. *Canadian Journal of Psychology and Pharmacology* **84(12)**, 1291-1299.