

Antibacterial effects of the ethanolic leaf extracts of *Musa paradisiaca*, *Musa acuminata* and *Musa sapientum* against MRSE and MSSE

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Abstract

The extracts from the stem, fruit peels and leaves of banana plants are being investigated for their antibacterial effects. The number of effective drugs against resistant bacteria including methicillin-resistant *Staphylococcus epidermidis* are reducing with time. Thus far, there are no studies done on the antibacterial effects of the ethanolic leaf extracts of banana leaves against MRSE. Thus, the aim of this study was to investigate the antibacterial effects of 80% ethanolic leaf extracts of *Musa paradisiaca*, *Musa acuminata* and *Musa sapientum* against MRSE. The ethanolic leaf extracts were extracted and concentrated using a soxhlet extractor and a rotary evaporator. The antibacterial effects of the extracts against the isolates were analyzed using the agar well diffusion assay. The ethanolic extracts of *M. paradisiaca* showed the highest zone of inhibition of 30.3 mm against MRSE isolate S15B/A. Inhibition was also observed with methicillin-sensitive *S. epidermidis* (MSSE). Although there were differences in the diameters of the zones on the inhibition, the post-hoc test revealed that the extracts of *M. paradisiaca* and *M. acuminata* were equally effective ($p \leq 0.05$) in inhibiting the growth of MRSE isolates at 2.86g/mL and 3.33g/mL concentration respectively. These extracts also produced significantly larger zones of inhibition compared to clindamycin. The presence of salvipisone and aethiopinone diterpenoids in the extracts of *M. paradisiaca* and *M. acuminata* could be the reason for the inhibition against MRSE. These findings strongly suggest that the ethanolic leaf extracts of *Musa paradisiaca* and *Musa acuminata* have the potential to be developed as effective antibacterial agents against MRSE and MSSE.

Keywords

Resistant bacteria, leaf extracts, antibacterial activity, inhibition

Introduction

Phytochemicals with known antimicrobial properties have a prominent importance in therapeutic treatments (Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L., 2000). The bioactive compounds are usually extracted using aqueous and organic solvents such as ethanol and methanol

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(Nascimento, et. al., 2000). In a previous study, extracts from 122 plant species inhibited the growth of *Staphylococcus aureus*, *Aspergillus niger* and *Escherichia coli* (Nascimento, et. al., 2000).

The banana plant (*Musa paradisiaca*) is widely cultivated in many countries including Malaysia (Green Herbology, 2017). It has many antibacterial activities due to the presence of many secondary metabolites such as alkaloid, tannin, flavonoids, terpenoids and saponins. The ethanolic extract of *Musa paradisiaca* showed antibacterial activity against several bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Naikwade, P. V., Gaurav, S., Sharayu, D., & Kailas, J., 2014). The aqueous leaf extract of *Musa paradisiaca* showed antibacterial activity against *Staphylococcus* and *Pseudomonas* species in which, the former was found to be more susceptible towards the extract compared to the latter (Alisi, C. S., Nwanyanwu, C. E., Akujobi, C. O., & Ibegbulem, C. O., 2008).

Many common bacteria are becoming resistant towards antibiotics (Lacey, 1983). This includes the common skin flora *Staphylococcus aureus* and *Staphylococcus epidermidis* which are becoming resistant to beta-lactam antibiotics such as methicillin. Several antibacterial agents are being used to control their growth including daptomycin, linezolid, and clindamycin (Wade & Benjamin, 2011). However, with the increasing traits of multiple-resistance, plant extracts are being analyzed as alternatives to antibiotics and synthetic drugs. Since there are no studies done to investigate the antibacterial effects of banana leaves against MRSE, this study was aimed to investigate the antibacterial effects of ethanolic extracts of *Musa paradisiaca*, *Musa sapientum* and *Musa acuminata* against isolates of MRSE and MSSE.

Methodology

Inocula Preparation and Confirmation

Three MRSE isolates previously obtained from the nasal and axillae of 3 healthy individuals were grown on nutrient agar (OXOID). Methicillin-susceptible *S. epidermidis* was also used in this study as the control group. These single colonies of these isolates were subjected to gram staining and the catalase test. The bacteria were also subcultured onto Mannitol Salt Agar. Their resistance towards cefoxitin (30 µg) was determined using the Kirby Bauer disk diffusion assay according to the Clinical Laboratory Standards Institute.

Plant Extracts Preparation

The banana leaves of *M. paradisiaca*, *M. sapientum* and *M. acuminata* were rinsed with water, followed by 70% ethanol (v/v) and deionised water. The leaves were air-dried for 24-h and dried at 60°C for another 24-h. The dried leaves were ground using a micro fine multipurpose powder dry medicine blender (Himitzu) and sieved with a 40-mesh sieve. Ethanol (Molecular grade, Merck) was used as the extraction solvent at a ratio of 1:6 of leaf material to solvent. 20 g of the

ground leave powder was extracted with 120 mL of 80% ethanol at 78 °C. The extracts were concentrated using a rotary evaporator (Yamamoto) at 60°C.

Phytochemical Screening

The ethanolic leave extracts were screened for phytochemicals using previously described methods (Table 1).

Table 1. Phytochemical Tests

Phytochemical (s)	Standard procedure
Alkaloid	Add 2 drops of 2% HCL into 2 ml extract and followed by 3 drops of Wagner's reagent.
Carbohydrate	4 drops of benedict solution was added into test tube containing 1 ml of extract and shaken for 2 min. From the sides of the test tube, add 1 ml of concentrated sulphuric acid slowly.
Flavonoid	Add 3 drops of 5% sodium hydroxide (NaOH) solution and 3 drops 4% of diluted sulphuric acid into 1 ml extract.
Glycoside	Add 1ml of extract and 2 ml of glacial acetic acid in a test tube. Then, add 1 drop of 5% of FeCl ₃ and 0.5 mL of concentrated sulphuric acid.
Saponin	Add 5 mL of deionised water into 2 ml extract and mix vigorously
Tannin	Add 2 drops of 5% FeCl ₃ into 1 ml of the plant extract.
Terpenoid	About 2 mL of chloroform added into a test tube containing 2 mL of extract. Then, add 1 mL of concentrated H ₂ SO ₄ .

Antibacterial Activity Test

The antibacterial activities of the leave extracts were detected using the agar-well diffusion assay adapted from Perez, C., Pauli, M. and Bazerque, P. (1990). Overnight bacterial culture of 50 µL was lawned onto MHA. Three wells were made onto the agar. Each wells were filled with 150 µL of the leave extracts. Clindamycin (OXOID, 20 µg) and 80% ethanol were used as positive and negative controls respectively. The agar was incubated at 37°C for 24 hours. This assay was done in triplicates. The zone of inhibition (mm) was measured and analyzed using the one-way ANOVA followed by post hoc test.

Results and Discussion

Figure 1 shows the inhibition of MRSE isolates by *M. paradisiaca* and *M. acuminata*. The zones of inhibition was larger than the ones produced by clindamycin. Although, there was no significant difference between the inhibition zones produced by clindamycin and the extracts, diameter of the zones of inhibition suggests that these extracts are as affective as clindamycin. The antibacterial activity of these extracts were mainly due to the presence of phytochemicals such as alkaloid, flavonoids, glycoside, terpenoids, tannins and saponins. The extracts were also likely to contain salvipisone and aethiopinone, types of diterpenoids. Studies show that these diterpenoids have antibacterial and anti-biofilm activities against various gram negative and gram positive bacteria (Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M., 2017). Combinations of salvipisone and aethiopinone with oxacillin, vancomycin and

linezolid synergistically inhibited the growth of MRSA and MRSE (Walencka, E., Rozalska, S., Wysokinska, H., Rozalski, M., Kuzma, L., Rozalska, B., 2007). The main mode action of many diterpenoids is through the disruption of bacterial cell membrane (Urzúa, A., Rezende, M., Mascayano, C., & Vásquez, L. 2008). Flavonoids such as myricetin, datiscetin, kaempferol, quercetin and flavones inhibit the growth of resistant staphylococci by inhibiting cell metabolism, the function of DNA gyrase as well as the function of cell membrane (Barbieri, et. al., 2017). Hence, the collective actions of various phytochemicals that target various bacterial structures and functions could be the main reason for the antibacterial activity towards the MRSE isolates. These findings can be used to investigate the future potential of these leaf extracts so that they can be developed into effective antibacterial agents in the future.

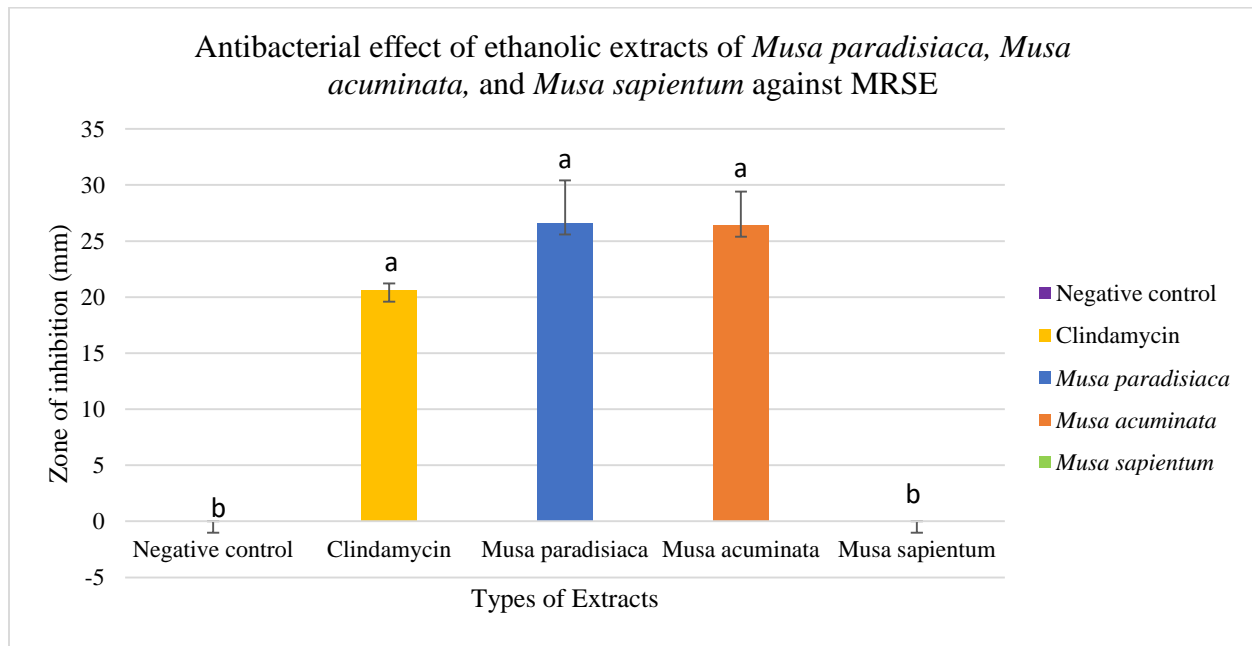


Figure 1. Antibacterial effect of ethanolic leaf extracts of *Musa paradisiaca*, *Musa acuminata*, *Musa sapientum*, on MRSE.

Note: MRSE: *Methicillin-resistant Staphylococcus Eepidermidis*, Negative control: 80% ethanol, Positive control: Clindamycin

The ethanolic extracts of *M. sapientum* did not show any zone of inhibition against MRSE isolates even though it contained the same groups of phytochemicals as *M. acuminata* and *M. paradisiaca* (Figure 1). It is likely that the leaf extracts of *M. sapientum* contained specific compounds such as piperine, reserpine and thymol that are non-effective against staphylococci. Terpenoids such as thymol, cinnamaldehyde, and trans-cinnamaldehyde have the ability to inhibit the growth of gram negative bacteria such as *E. coli*, *S. typhimurium* and *L. monocytogenes* (Barbieri, et. al., 2017). In another study, extracts from the flower, unripe fruit and roots of *M. sapientum* showed antimicrobial activity against several bacteria including *S. aureus* (Imam, M. Z., & Akter, S., 2011). However, the extract of *M. sapientum* used in this study was from the leaves and thus, it probably lacked the bioactive compounds against MRSE. Since, there are no

studies on the banana leaves against MRSE, more isolates of MRSE and phytochemical screening must be done using to confirm the lack of antibacterial effect of *M. sapientum* against MRSE.

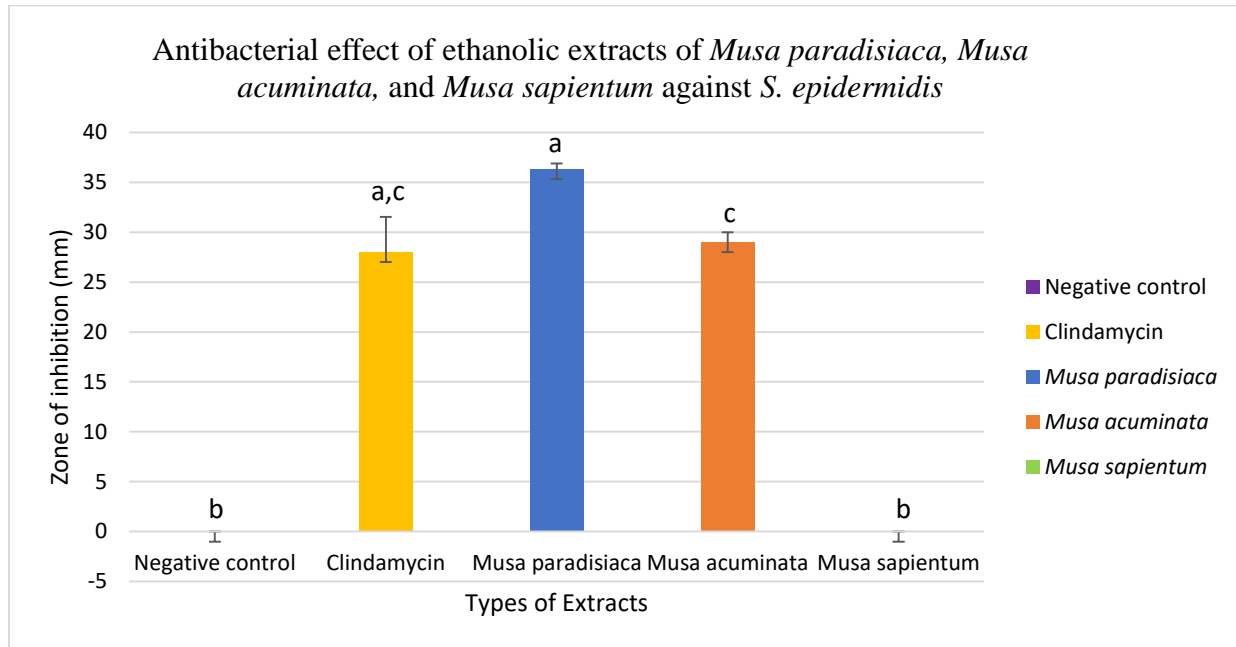


Figure 2. Antibacterial effect of ethanolic leaf extracts of *Musa paradisiaca*, *Musa acuminata*, *Musa sapientum*, against *S. epidermidis*.

Note: SE: *Staphylococcus epidermidis*, Negative control: 80% ethanol, Positive control: Clindamycin

The ethanolic extracts of *M. paradisiaca* had significance difference ($p \leq 0.05$) compared to the extract of *M. acuminata* against methicillin-sensitive *S. epidermidis* (MSSE) producing larger zones of inhibition (Figure 2). This strongly suggest that the former is a stronger antibacterial agent against *S. epidermidis*. This is probably because the extracts of *M. paradisiaca* contains higher amounts of alkaloids and terpenoids. An alkaloid such as berberine has the ability to prevent the formation of biofilm by *S. epidermidis* (Wang, X., Qiu, S., Yao, X., Tang, T., Dai, K., & Zhu, Z. A., 2009). Terpenoids such farnesol, salvipisone and aethiopinone are able to inhibit the growth of *S. epidermidis* (Barbieri, et. al., 2017). The similar zones of inhibition with clindamycin ($p \geq 0.05$) indicates that these leave extracts are equally effective in inhibiting the growth of MSSE. The larger zones of inhibition produced also showed that MSSE was more susceptible to the extracts compared to MRSE isolates (data not shown). This is likely due to the differences in the cell structures.

Conclusions

The MRSE and MSSE isolates used in this study were susceptible to the 80% ethanolic leave extracts of *Musa paradisiaca* (2.86 g/mL) and *Musa acuminata* (3.33 g/mL) but not towards *Musa sapientum* (2.86 g/mL). The ethanolic extracts of *Musa paradisiaca* and *Musa acuminata* also exhibited a similar inhibitory effect as clindamycin indicating that the leave extracts of *Musa*

paradisiaca and *Musa acuminata* have the potential to be developed as effective antibacterial agents against MRSE and MSSE.

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