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# Antibacterial potential of Malaysian ethnomedicinal plants against methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

This study aimed to evaluate the antibacterial activities of 61 plant extracts from 49 Malaysian ethnomedicinal plants and to investigate the interaction of the active plant extracts in combination with synthetic antibiotics against the MSSA and MRSA strains. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts were determined using a microdilution method against MSSA and MRSA strains. The interaction between active plant extracts and the antibiotics was assessed using the checkerboard method. The total fractional inhibitory concentration (∑FIC) indices from the combination were calculated to determine the nature of the interaction. Out of the 61 plant extracts tested against the MSSA strain, 7 plant extracts (~11%) showed MIC values of less than 200 μg/mL, 17 extracts (~28%) showed MIC between 200 and 800 μg/mL and seed extracts of *Areca catechu* showed MBC values of 400 μg/mL. The seed extract of *A. catechu* showed MIC and MBC of 400 μg/mL against the MRSA strains while leaf extract of *Cocos nucifera* showed MIC of 400 μg/mL against MRSA NCTC 12493. When the active plant extracts (MIC ≤ 200 μg/mL for MSSA, and ≤ 400 μg/mL for MRSA) were tested in combination with vancomycin and ciprofloxacin, they showed no interaction against both MSSA and MRSA with ∑FIC between 1.06 and 2.03. These findings provide a preliminary overview of the anti-MSSA and anti-MRSA properties of Malaysian ethnobotanical plants to combat *Staphylococcal* infections. Further research is needed to establish an antibacterial profile of the tested plant extracts.


## 1. Introduction

*Staphylococcus aureus*, a Gram-positive bacterium, has become one of the leading causes of clinical bacterial infections. It causes pyogenic skin and soft tissue infections especially among children (Sarmiento et al., 2011). The infection occurs mainly due to the ability of *S. aureus* to form biofilm and acquire antibiotic resistance (Quelemes et al., 2015). Empirical treatment for *Staphylococcal* infections are mostly penicillins such as oxacillin, and cephalosporins such as cefazolin (Paul et al., 2011). In severe *S. aureus* infections, patients will be administered with parenteral penicillinase-resistant penicillin, such as nafcillin, or first- or second-generation cephalosporins, such as cephalexin, combined with clindamycin or quinolone. Other antibiotic choices are doxycycline, trimethoprim-sulfamethoxazole (TMP-SMX) and rifampin (Baorto and Baorto, 2019).

Penicillins and cephalosporins are no longer adequate for *Staphylococcal* infections due to the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. MRSA often results from the horizontal acquisition of *mecA* gene by the methicillin-sensitive *Staphylococcus aureus* (MSSA). The gene is encoded for penicillin-binding protein (PBP2a) that confers resistance to all β-lactam antibiotics (Bloemendaal et al., 2010). Initially, MRSA infections were considered as solely nosocomial; however, the global prevalence of community-acquired MRSA

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cases are now showing a rising trend, particularly in developing countries. For instance, the number of MRSA cases in healthcare settings increased from 17.7% to 19.4% from 2012 to 2018 in Malaysia. Vancomycin is a widely used first-line treatment for MRSA infections, and an alternative to vancomycin is linezolid. However, the emergence of MRSA strains with reduced sensitivity and increased resistance to vancomycin and linezolid has limited their usage in the clinical setup. This consequently has led to the administration of second and third-line antibiotics in combinations, of which, are often costly and more toxic (MOH, 2019). Hence, an intensive search for new drugs that are capable of acting on these bacteria or targeting their virulence mechanism is very crucial.

Plants or more specifically herbs are well-known sources of new drugs due to their lower cost, higher accessibility and less adverse effects relative to the synthetic agents (Calixto, 2000). Besides using plants for nutritional purposes, herbs had been extensively utilized for clinical conditions or health improvement since ancient times. Even the novel scholars from the Greek and Roman era, including Hippocrates, Celsus and Theophrastus had described numerous herbs with therapeutic effects (Paulsen, 2010). Plants contain a variety of natural phytochemicals which proved to exhibit pharmacological effects such as antibacterial and anti-inflammatory properties (Złotek et al., 2016). All parts of the plant, including stems, peels, leaves, roots, flowers, seeds, fruits, etc., contain phytochemicals. These phytochemical compounds, which are bio-active compounds synthesized by plants, usually in the form of secondary metabolites, have the potential to be explored for the treatment of MSSA and MRSA infections.

As antibiotic resistance increases, the search for a new source of antimicrobial agents is vitally important as the WHO has pointed out that "no action today means no cure tomorrow." As 7411 plant species have been recorded previously in Malaysia, it is important to identify sources of plant species that may exhibit antibacterial effects against MSSA and MRSA (Abu Bakar et al., 2018). Therefore, the objective of the study was to investigate the anti-MSSA and anti-MRSA activities of 61 extracts from 49 ethnobotanically important plants in Malaysia that are often traditionally used for relieving various ailments, not limited to wound healing and bacterial infections. These 49 plants with their local uses and scientifically reported pharmacological activities are as tabulated in Table 1 in the supplementary material. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using microdilution assay against MSSA and MRSA strains. In addition, the interactions between the active extracts and the synthetic drugs, vancomycin and ciprofloxacin, were determined via checkerboard methodology. To the best of our knowledge, this is a pioneer study reporting on the anti-MSSA and anti-MRSA properties of a large pool of Malaysian medicinal plants. Thus, this study provides new knowledge on the antimicrobial potential of Malaysian medicinal plants.

## 2. Materials and methods

### 2.1. Materials

Bacterial cultures of MSSA ATCC 12600 and MRSA strains (NCTC 12493 and ATCC 43300) were purchased from American Type Culture Collection, USA. Trypticase Soy Agar (TSA), Trypticase Soy Broth (TSB), Mueller Hinton Agar (MHA) and Muller Hinton Broth (MHB) were purchased from BD Difco™, USA. Dimethyl sulfoxide (DMSO), glycerol, Iodonitrotetrazolium chloride (INT) and phosphate buffer saline (PBS) were purchased from Sigma-Aldrich, USA.

### 2.2. Inoculum preparation

Two or three colonies of freshly grown MSSA or MRSA on the MHA were transferred into 5 mL of MHB and incubated in a shaking incubator (N-BIOTEK, Korea) for three hrs at 150 rpm and 37 °C to obtain bacterial cultures at exponential phase. After incubation, the turbidity of the culture was adjusted to McFarland 0.5. The bacterial suspension was further diluted to achieve an optical density of approximately ~ $10^6$ cfu/mL.

### 2.3. Plant extraction and working solution preparation

All plant samples were collected from various areas in Penang Island, Malaysia. The samples were authenticated and deposited at the Herbarium of School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. The work was assisted by the assistant science officer, Mr. Shunmugam Veloosamy, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Further details of voucher specimens can be found at https://ush.mybis.gov.my/specimen.php. The plant samples were air-dried, ground into powder and macerated in 80% methanol for 7–14 days at room temperature (25–30 °C). The extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator (Eyela, Japan) and stored at 4 °C until further use. The pre-weight extracts and antibiotics (vancomycin and ciprofloxacin) were dissolved in DMSO and further diluted in a fresh MHB medium to achieve a working solution with the concentration of 1600 μg/mL and 4 μg/mL, respectively. The concentration of DMSO in the working solution was controlled to be below 1% (v/v).

### 2.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The microdilution assay was performed using 96-well plates as described by Eloff, 1998 with slight modifications (Eloff, 1998). Briefly, 100 μL of fresh MHB was added into wells in row B to H in column 1 to 11 and 200 μL of fresh media at column 12 in row A to D as a blank control. 100 μL plant extract working solutions were added in row A and B in column 1 to 9 in triplicate and 100 μL antibiotic at column 10 to 11. A two-fold serial dilution was performed by transferring 100 μL from row B to C in column 1 to 11 using a multichannel pipette. The same step was performed until row H and the excess 100 μL mixture from row H was discarded. 100 μL of diluted standardized bacterial suspension was added into the wells of row A to H in column 1 to 11 as well as in column 12 from row E to H as a growth control. The final volume in each well was 200 μL. The concentration of the extracts was ranged between 800 and 6.25 μg/mL. The final volume in the wells was 200 μL and the DMSO concentration was less than 1% (v/v).

The plate was incubated for 18 to 22 hrs at 37 °C. After the incubation period, 50 μL of freshly prepared INT solution was added into all wells except column 3, 6, and 9. The MIC plate was then re-incubated for two hrs at 37 °C. The MIC is defined as the lowest drug or extract concentration that prevented a colour change of the INT from colourless to pinkish. The pinkish colour indicated bacterial growth. On the other hand, 10 μL of the mixture from column 3, 6, and 9 were inoculated into MHA plates to determine the MBC. The plates were incubated at 37 °C for 18 to 22 hrs in an incubator (Memmert, Germany). MBC was determined as the lowest concentration of drug or extract which did not produce any bacterial colonies on the agar plate. The MIC and MBC were determined from three independent experiments performed in triplicates.

**Table 1**
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Malaysian ethnomedicinal plants, vancomycin and ciprofloxacin.

| No. | Scientific name | Local name | Plant Part | MSSA ATCC 12600 | | MRSA ATCC 43300 | | MRSA NCTC 12493 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | MIC (μg/mL) | MBC (μg/mL) | MIC (μg/mL) | MBC (μg/mL) | MIC (μg/mL) | MBC (μg/mL) |
| 1. | *Allium cepa* L. | Bawang merah | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 2. | *Allium sativum* | Bawang putih | rhizome | >800 | >800 | >800 | >800 | >800 | >800 |
| 3. | *Aloe vera* | Lidah buaya | leaf | 800 | >800 | >800 | >800 | >800 | >800 |
| 4. | *Alstonia scholaris* (L.) R. Br. | Pulai | leaf | 200 | >800 | >800 | >800 | >800 | >800 |
| 5. | *Angiopteris evecta* | Paku gajah | leaf | 800 | >800 | >800 | >800 | >800 | >800 |
| 6. | *Archidendron pauciflorum* | Jering | fruit | 50 | >800 | >800 | >800 | >800 | >800 |
| 7. | *Areca catechu* | Pokok pinang | seed | 400 | 400 | 400 | 400 | 400 | 400 |
| 8. | *Artocarpus heterophyllus* Lam. | Nangka | leaf | 200 | >800 | >800 | >800 | >800 | >800 |
| 9. | *Artocarpus heterophyllus* Lam. | Nangka | stem | >800 | >800 | >800 | >800 | >800 | >800 |
| 10. | *Averrhoa carambola* | Belimbing manis | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 11. | *Azadirachta indica* A. Juss | Mambu | leaf | 100 | >800 | >800 | >800 | >800 | >800 |
| 12. | *Azadirachta indica* A. Juss | Mambu | stem | 100 | >800 | >800 | >800 | >800 | >800 |
| 13. | *Borassus flabellifer* | Pokok kelapa laut | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 14. | *Cananga odorata* | Kenanga | stem | >800 | >800 | >800 | >800 | >800 | >800 |
| 15. | *Cananga odorata* | Kenanga | leaf | 800 | >800 | >800 | >800 | >800 | >800 |
| 16. | *Cassia fistula* | Cassia | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 17. | *Centella asiatica* | Pegaga | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 18. | *Chrysanthemum morifolium* | Chrysanthemum | flower | >800 | >800 | >800 | >800 | >800 | >800 |
| 19. | *Citrofortunella microcarpa* | Limau kasturi | rind | >800 | >800 | >800 | >800 | >800 | >800 |
| 20. | *Citrus aurantifolia* | Limau nipis | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 21. | *Citrus aurantifolia* | Limau nipis | skin | >800 | >800 | >800 | >800 | 800 | 800 |
| 22. | *Citrus hystrix* | Limau purut | skin | >800 | >800 | >800 | >800 | >800 | >800 |
| 23. | *Citrus hystrix* | Limau purut | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 24. | *Citrus limon* | Lemon | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 25. | *Citrus maxima* | Limau bali | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 26. | *Citrus maxima* | Limau bali | skin | >800 | >800 | >800 | >800 | >800 | >800 |
| 27. | *Cocos nucifera* | Pokok kelapa | leaf | 800 | >800 | >800 | >800 | 400 | 800 |
| 28. | *Costus speciosus* | Malay ginger | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 29. | *Costus speciosus* | Malay ginger | stem | 200 | >800 | >800 | >800 | >800 | >800 |
| 30. | *Cuminum cyminum* | Cumin | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 31. | *Curcuma longa* | Turmeric | rhizome | 800 | >800 | >800 | >800 | >800 | >800 |
| 32. | *Cynometra cauliflora* | Katak puru @ Nam-Nam | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 33. | *Durio zibenthinus* Linn. | Durian | leaf | 800 | >800 | >800 | >800 | >800 | >800 |
| 34. | *Elaeis guineensis* | Oil palm | mesocarp | >800 | >800 | >800 | >800 | >800 | >800 |
| 35. | *Elaeis guineensis* | Oil palm | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 36. | *Eurycoma longifolia* | Tongkat ali | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 37. | *Illicium verum* | Star anise | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 38. | *Ipomoea pes-caprae* | Tapak kuda | leaf | > 800 | > 800 | > 800 | > 800 | > 800 | > 800 |
| 39. | *Magnolia champaca* | Cempaka | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 40. | *Manihot esculenta* Crantz | Ubi kayu | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 41. | *Manihot esculenta* Crantz | Ubi kayu | stem | >800 | >800 | >800 | >800 | >800 | >800 |
| 42. | *Millettia pinnata* (L.) Panigrahi | Mempari | leaf | 800 | >800 | >800 | >800 | >800 | >800 |
| 43. | *Momordica charantia* L. | Peria katak | fruit | 800 | >800 | >800 | >800 | >800 | >800 |
| 44. | *Moringa oleifera* Lam. | Murunggai | stem | >800 | >800 | >800 | >800 | >800 | >800 |
| 45. | *Moringa oleifera* Lam. | Murunggai | leaf | 800 | >800 | >800 | >800 | >800 | >800 |
| 46. | *Murraya koenigii* (L.) Spreng. | Kari | stem | 400 | >800 | >800 | >800 | 800 | >800 |
| 47. | *Murraya paniculata* L. | Kemuning | leaf | 800 | >800 | 800 | >800 | 800 | >800 |
| 48. | *Passiflora edulis* | Markisa | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 49. | *Passiflora edulis* | Markisa | skin | >800 | >800 | >800 | >800 | >800 | >800 |
| 50. | *Piper nigrum* | Black pepper | seed | >800 | >800 | >800 | >800 | >800 | >800 |
| 51. | *Piper sarmentosum* | Kaduk | fruit | >800 | >800 | >800 | >800 | >800 | >800 |
| 52. | *Pluchea indica* (L.) Less. | Beluntas | stem | 200 | >800 | >800 | >800 | >800 | >800 |
| 53. | *Plumeria obtuse* | Kemboja putih | flower | >800 | >800 | >800 | >800 | >800 | >800 |
| 54. | *Psidium guajava* L. | Jambu | leaf | 25 | >800 | >800 | >800 | >800 | >800 |
| 55. | *Psidium guajava* L. | Jambu | stem | 25 | >800 | >800 | >800 | >800 | >800 |
| 56. | *Sindora siamensis* Miq. | Sepetir | leaf | 25 | >800 | >800 | >800 | >800 | >800 |
| 57. | *Sindora siamensis* Miq. | Sepetir | stem | 100 | >800 | >800 | >800 | >800 | >800 |
| 58. | *Syzygium aromaticum* | Clove | fruit | 800 | 800 | >800 | >800 | >800 | >800 |
| 59. | *Tabernaemontana coronaria* (L.) Willd. | Akar susur kelapa | leaf | >800 | >800 | >800 | >800 | >800 | >800 |
| 60. | *Trigonella foenum-graecum* | Fenugreek | seed | >800 | >800 | >800 | >800 | >800 | >800 |
| 61. | *Zingiber officinale* | Ginger | rhizome | >800 | >800 | >800 | >800 | >800 | >800 |
| 62. | Vancomycin | – | – | 1 | 1 | 1 | 1 | 0.5 | 0.5 |
| 63. | Ciprofloxacin | – | – | 0.312 | 0.625 | 0.25 | 2 | 0.25 | 0.25 |

### 2.5. Checkerboard plate preparation

The checkerboard microdilution method had been employed to study the interaction between the active extract and antibiotics using 96-well plates (Eliopoulos and Moellering Jr, 1991). Row A to H and Column 1 to 8 were used for the combination testing while the remaining four rows served as controls for the result validation. Column 9 and 10 contained plant extract alone and

vancomycin or ciprofloxacin alone, respectively. Column 11 and 12 functioned as the negative control which contained MHB alone and MHB with untreated bacteria, respectively.

100 μL working extract solution was added into wells in row A and B, column 1 to 8, followed by a 2-fold serial dilution from row B until H. The excess 100 μL of the mixture from row H was discarded. On the other hand, 100 μL of drug solution was added at column 1 and 2, and 2-fold serial dilution was performed from column 2 until 8. The excess 100 μL of the mixture from column 8 was discarded. This creates unique combinations of plant extract and antibiotic. Finally, 100 μL of standardized bacterial suspension was added into all wells in the microplate except column 11 and incubated for 18 to 22 hrs at 37 °C. Upon incubation, 50 μL of freshly prepared INT solution were added. The microplate was then re-incubated for two hrs at 37 °C. The MIC values of antibiotics and plant extracts were recorded as mentioned earlier.

The fractional inhibitory concentration (FIC) was calculated using the formula below to determine the nature of the interaction.

$$\Sigma FIC = \frac{MIC\ of\ extract\ in\ combination}{MIC\ of\ extract\ alone} + \frac{MIC\ of\ drug\ in\ combination}{MIC\ of\ drug\ alone}$$

∑FIC value below 0.5 shows synergistic interaction between plant extract and the drug tested. ∑FIC value between 0.5 and 4 shows no interaction, while ∑FIC value above 4 indicates antagonistic interaction (Odds, 2003).

## 3. Results

### 3.1. MIC and MBC values for MSSA and MRSA strains

MIC and MBC values of 61 plant extract tested against MSSA ATCC 12600, MRSA ATCC 43300 and MRSA NCTC 12493 are as shown in Table 1. Out of the 61 plant extracts tested against MSSA, 7 plant extracts (~ 11%) showed MIC values of below than 200 μg/mL, 17 extracts (~ 27%) showed MIC between 200 and 800 μg/mL, while the remaining plant extracts (~ 62%) showed MIC values of>800 μg/mL. The lowest MIC of 25 μg/mL against the MSSA isolate was shown by stem and leaf extracts of *Psidium guajava L.* and leaf extract of *Sindora siamensis Miq.* The second lowest MIC against MSSA strain reported was at 50 μg/mL by fruit extract of *Archidendron pauciflorum*. Other plant extracts such as stem and leaf extracts of *Azadirachta indica A. Juss* and stem extract of *S. siamensis* Miq. also showed effective inhibition on MSSA strain with MIC values of 100 μg/ml. Besides that, stem extracts of *Costus speciosus* and *Pluchea indica (L.) Less*, leaf extracts of *Artocarpus heterophyllus* Lam and *Alstonia scholaris* (L.) R. Br. recorded MIC of 200 μg/mL against the MSSA strain. Almost all the plant extracts tested showed MBC of above 800 μg/mL toward the MSSA strain except seed extracts of *Areca catechu* and *Syzygium aromaticum* that recorded an MBC of 400 μg/mL and 800 μg/mL, respectively.

Similarly, when these plant extracts were tested against the MRSA strains (ATCC 43300 and NCTC 12493), seed extract of *A. catechu* (MIC and MBC of 400 μg/mL) and leaf extract of *Cocos nucifera* (MIC of 400 μg/mL and MBC > 800 μg/mL against MRSA NCTC 12493) showed promising results compared to other plant extracts. The remainder 97% (59 out of 61) showed MIC and MBC values of above 800 μg/mL against both MRSA strains. The MIC and MBC of the control drugs, ciprofloxacin and vancomycin were in the range of the previously reported values (Amada et al., 1997; Alós et al., 2008; Lamaming et al., 2015) which proved the validity of our assay.

### 3.2. Interaction between active plant extracts and standard antibiotics

The active plant extracts with MIC of ≤ 200 μg/mL and ≤ 400 μg/mL against MSSA and MRSA, respectively, were chosen for the combination test with standard antibiotics, vancomycin and ciprofloxacin. The results are tabulated in Table 2. The active extract of *C. nucifera* was omitted from the combination assay as the pinkish colour of the extract masks the colour change of the dye which makes the interpretation of the results not reliable. All the active plant extracts showed no interactions with vancomycin and ciprofloxacin against the MSSA and both MRSA strains. The ∑FIC values were in the range of 1.06–2.03.

## 4. Discussion

To the best of our knowledge, there is no cut-off or any well-established reference on the MIC and MBC range to determine if a plant extract is active against *S. aureus*. Most of the researchers interpreted the susceptibility results using their in-house standards. For instance, Fonkeng et al. mentioned active plant extracts generally possessed MIC range of 500 to 1500 μg/mL against the tested microorganism (Fonkeng et al., 2015). However, Aliyu et al., considered MIC as high as 3000 μg/mL to be active (Aliyu et al., 2008). Hence, in our study, we classified MIC and MBC values of 100 μg/mL and below as significantly active, 200 to 800 μg/mL as active, while MIC and MBC values of > 800 μg/mL were considered to be inactive.

There were 7 plant extracts possessing significant anti-MSSA activity, especially stem and leaf extracts of *P. guajava* L., leaf extract of *S. siamensis* Miq. and fruit extract of *A. pauciflorum* with MIC values within 25 to 50 μg/mL. A previous study revealed that tannins in methanolic extract and saponins in aqueous extract of *P. guajava* were effective in inhibiting *S. aureus* (mean zone of inhibition of 12.3 mm) (Biswas et al., 2013). However, another study revealed that MIC of methanolic *P. guajava* leaf extract against *S. aureus* clinical isolates was 1.6 mg/mL (Zahin, 2010) which was higher than the reported MIC in this study. There were no previous antimicrobial studies that have been reported on *S. siamensis* Miq. leaf extracts. Thus, this is a significant finding from this study which suggests further studies on this least-explored plant for its application for the treatment of MSSA infections.

In addition, *A. pauciflorum* extract showed MIC value of 50 μg/mL against the MSSA strain which suggests that the activity might be contributed by the presence of lectins. Charungchitrak et al. reported a MIC value of 56.7 μg/mL from lectin extracted from the seeds of the same species, also known as *A. jiringa*, against *S. aureus* ATCC 25923 (Charungchitrak et al., 2011). On the other hand, the MIC value showed by *A. indica* leaf extract (100 μg/mL) varied from the reported MIC (1000 μg/mL) by a Kenyan study for methanolic leaf extract against *S. aureus* clinical isolates (Fabry et al., 1998). Imran et al. (2017) also reported MIC values ranging from 6.25 mg/mL to 12.5 mg/mL for methanolic *A. indica* leaf extract against five *S. aureus* clinical isolates (Imran et al., 2017). These differences might be due to different bacterial strains or different geographical sources (locality, climate and soil composition) of the plant sample which resulted in different phytochemical content in the extracts (Khattak, 2015). Another interesting finding is that the stem extract of *Cananga odorata* did not show anti-MSSA activity (MIC > 800 μg/mL), however its leaf extract showed active anti-MSSA activity (MIC = 800 μg/mL). Similarly, *Costus speciosus* stem extract showed active anti-MSSA activity (MIC = 200 μg/mL) while the leaf extract (MIC > 800 μg/mL) did not show any activity. Hence, these findings evidently show a variation in the phytochemical content in different plant parts (Tan et al., 2015).

**Table 2**
Interaction between the active plant extracts, vancomycin and ciprofloxacin.

| Bacteria | Local name | Plant part | ∑FIC with vancomycin | Interaction | ∑FIC with ciprofloxacin | Interaction |
|---|---|---|---|---|---|---|
| **MSSA ATCC 12600** | | | | | | |
| *Archidendron pauciflorum* | Jering | fruit | 1.063 | No interaction | 1.063 | No interaction |
| *Areca catechu* | Pokok pinang | seed | 1.016 | No interaction | 1.016 | No interaction |
| *Azadirachta indica* A. Juss | Mambu | leaf | 1.063 | No interaction | 1.063 | No interaction |
| *Azadirachta indica* A. Juss | Mambu | stem | 1.063 | No interaction | 1.500 | No interaction |
| *Psidium guajava* L. | Jambu | leaf | 1.063 | No interaction | 1.063 | No interaction |
| *Psidium guajava* L. | Jambu | stem | 1.031 | No interaction | 2.030 | No interaction |
| *Sindora siamensis* Miq. | Sepetir | stem | 1.031 | No interaction | 1.031 | No interaction |
| *Sindora siamensis* Miq. | Sepetir | leaf | 1.031 | No interaction | 1.031 | No interaction |
| **MRSA ATCC 43300** | | | | | | |
| *Areca catechu* | Pokok pinang | Seed | 1.016 | No interaction | 1.016 | No interaction |
| **MRSA NCTC 12493** | | | | | | |
| *Areca catechu* | Pokok pinang | seed | 1.016 | No interaction | 1.016 | No interaction |

Most of the plant extracts (~62%) tested in our study were inactive (MIC > 800 μg/mL) against MSSA. For instance, the MIC of *Allium cepa* extract reported in this study was>800 μg/mL. However, a study carried out in Zhe Jiang, China revealed that the essential oil of *A. cepa* has MIC and MBC values of 0.18 mg/mL and 0.54 mg/mL, respectively against *S. aureus* ATCC 25923 (Ye et al., 2013). Another study conducted in Spain reported that ethyl acetic and aqueous extract of *A. cepa* exhibited MIC of 80 μg/mL and > 80 μg/mL, respectively against *S. aureus* CECT 239 (Santas et al., 2010). Therefore, significant differences among MIC and MBC results in our study might be due to the different phytochemical contents yielded from different extraction methods (maceration vs. steam distillation), solvents and tested bacterial strains as well as environmental factors as mentioned earlier.

On the other hand, out of 61 plant extracts tested, only seed extract of *A. catechu* showed MIC and MBC value of 400 μg/mL against MRSA ATCC 43300 and NCTC 12493. Similarly, Nursidika et al. and Baiti et al. reported anti-MRSA activity for water fraction and ethanol extract of *A. catechu* (Nursidika P et al., 2014; Baiti M et al., 2018). However, these studies are anecdotal; hence further studies are crucial to explore the benefit of this plant.

Furthermore, our study showed that both leaf and stem extracts of *Artocarpus heterophyllus* were inactive against MRSA, but another study reported that a flavone, namely artocarpesin, isolated from its fruit extract using acetone, exhibited MIC value as low as 16 μg/mL against various clinical MRSA isolates (Manuel et al., 2012). This suggests that the 80% methanol may not be a suitable solvent for plant extraction if flavone is targeted.

The seven plant extracts with MIC of 100 μg/mL and below against the MSSA strain (*A. pauciflorum* fruit extract, *A. indica* stem and leaf extracts, *P. guajava* stem and leaf extracts, and *S. siamensis* Miq. stem and leaf extracts) and, one extract with MIC of 400 μg/mL against the both MRSA strains (seed of *A. catechu*) were chosen for the interaction study. This was because extracts with lower MIC indicated more substantial antibacterial effect against the bacteria tested, hence were highly possible to produce a synergistic effect with synthetic antibiotics. We found that all tested extracts showed no interactions with vancomycin and ciprofloxacin in reference to their FIC index. Generally, no interaction could be due to the indifferent mechanisms of action of the drugs which is based on the idea that the combined drugs were not interacting, causing only one metabolic pathway to become the growth limiting factor of an organism at a time (Eliopoulos G and Moellering Jr R, 1991). The mechanism of action of vancomycin is via inhibition of cell wall synthesis while ciprofloxacin inhibits the bacterial DNA-gyrase enzyme (Medscape, 2020a; Medscape, 2020b). Hence, the findings from our study suggest that the plant extracts consisting of various components that are either acting on the bacterial cell wall or at the DNA level. This similar targeting mechanism in combination has neither enhanced nor hindered their respective anti-MSSA or anti-MRSA activities. Nonetheless, further studies are being carried out to identify the active compounds in these extracts and their interactions with vancomycin and ciprofloxacin.

## 5. Conclusion

Out of the 61 plant extracts tested, seven extracts showed significant anti-MSSA activity (MIC of 100 μg/mL and below) whereas only one extract exhibited moderate anti-MRSA activity (MIC of 400 μg/mL). These extracts also showed no interactions with vancomycin and ciprofloxacin against MSSA and MRSA strains. These findings provide a preliminary overview of the anti-MSSA and anti-MRSA properties of Malaysian ethnobotanical plants to combat Gram-positive *Staphylococcal* infections. Though some of the plants have been used as folk medicine for the treatment of bacterial infections, further phytochemical and pharmacological studies are still needed to translate these findings into clinical usage, especially the ones that are least scientifically explored.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.06.036.

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