The Journal of Phytopharmacology 2018; 7(3): 225-229
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(3): 225-229
May- June
Received: 22-02-2018
Accepted: 27-04-2018

Nur Amira Mohd Shamsuddin
Biomedical Science Programme, Centre of Health and Applied Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Dayang Fredalina Basri
Biomedical Science Programme, Centre of Health and Applied Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz 50300 Kuala Lumpur, Malaysia

Correspondence:
Dayang Fredalina Basri
Biomedical Science Programme, Centre of Health and Applied Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz 50300 Kuala Lumpur, Malaysia
Email: dayang[at]ukm.edu.my

Anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of an acetone extract from the leaves of *Canarium odontophyllum* (Miq.)

Nur Amira Mohd Shamsuddin, Dayang Fredalina Basri

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health concern that has caused nosocomial and community infections over the past decade. The emergence of multi-drug resistant strains and limitations of present antimicrobial drugs have led to continuous search for natural products as curative agents for MRSA infections. *Canarium odontophyllum* Miq., locally known as dabai, has been considered an alternative phytotherapeutic treatment for MRSA. The aim of this study was to evaluate the bacteriostatic activity of an acetone extract from *C. odontophyllum* leaves against MRSA. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against the ATCC 33591 and Mu50 strains were determined using the broth microdilution method, and a time-kill assay was employed to assess the type of bacteriostatic action of the extract against the Mu50 strain only. The MIC and MBC values of the extract against Mu50 were 312.5 µg/ml and 625 µg/ml, respectively, whereas the MIC and MBC values for ATCC 33591 were 625 µg/ml and 1,250 µg/ml, respectively, confirming the bacteriostatic effect against both MRSA strains. A time-kill assay showed that the acetone extract of *C. odontophyllum* leaves exhibited concentration-dependent bacteriostatic action against the Mu50 strain at 1/2× MIC, 1× MIC and 2× MIC. However, the extract was bactericidal only at the highest concentration (4× MIC) with a reduction in cell viability of more than 3 $\log_{10}$ within 24 hours. These findings confirm that an acetone extract from *C. odontophyllum* leaves inhibited growth of MRSA at low concentration and could be utilised as an alternative anti-MRSA agent in immune uncompromised hosts.

Keywords: bacteriostatic, *C. odontophyllum*, MBC, MIC, MRSA, TKA.

INTRODUCTION

Transmission of infectious diseases is a significant burden to the economy and to community health. However, the majority of pathogens that are involved in the transmission of infectious diseases represent multi-drug resistant strains [1]. Pathogenic bacterial infections are the major cause of increased mortality and morbidity in hospitals [2]. *Staphylococcus aureus* frequently colonises the surface of the outer skin and upper respiratory tract, especially the nasal tract [3]. As a virulent pathogen, *S. aureus* accounts for the high mortality rates in patients with pneumonia, endocarditis, sepsis and urinary tract infections [4].

In the 1950s, methicillin was introduced to treat *S. aureus* infections. Unfortunately, after several years, resistance of *S. aureus* to methicillin was discovered [5]. Methicillin is a β-lactam antibiotic that interferes the penicillin-binding proteins needed for synthesis of peptidoglycans for *S. aureus* [6]. The presence of MecA gene that encodes for PBP2a where used as a benchmark to detect the presence of methicillin-resistant *S. aureus* [7].

The emergence of MRSA infections cannot be underestimated, as treatments are ineffective and it is associated with increased morbidity, mortality, hospital admissions and healthcare costs [8]. MRSA also shows high resistance rates against tetracycline, clindamycin, cotrimoxazole, rifampicin, macrolides and fluoroquinolones [9].

Therefore, various studies have been carried out to identify alternative treatments to curb the problem of MRSA resistance, especially the use of natural products. Plants offer a diverse reservoir of biologically active components as potential therapeutic agents, including antimicrobials. *Canarium odontophyllum* or locally known as 'Borneo olive' or 'dabai' belongs to the Burseraceae family [10] and the genus *Canarium L.* [11]. The tree can be found in Sumatra, Borneo and the Philippines [10]. Secondary metabolites contained in an extract of *C. odontophyllum* leaves, such as tannins, flavonoids, terpenoids, saponins and

phenols, have antibacterial [12] and anti-MRSA activities [13].

Therefore, *in vitro* antibacterial activity and a time-kill assay (TKA) analyses were conducted to elucidate the antibacterial activity of *C. odontophyllum*. An acetone extract of *C. odontophyllum* leaves has been shown to exhibit bacteriostatic action with a prolonged and persistent antimicrobial effect against MRSA ATCC 33591 [14]. Therefore, in this present study, different concentrations of the extract were used to investigate the pharmacodynamics of the active compounds in the extract, and to give insight into the mode of action of the acetone extract of *C. odontophyllum* leaf against MRSA.

MATERIALS AND METHODS

Plant material

The leaves of *C. odontophyllum* were purchased from Kuching, Sarawak with voucher specimen number UKMB 40052. The whole leaf was used to prepare the extract [12]. The stock solution was prepared by completely dissolving the residue of the acetone extract of *C. odontophyllum* leaves in absolute acetone using a vortexer. A stock solution of the test material was prepared at a concentration of 20 mg/ml and stored at 4°C. The working solution was prepared by calculating the appropriate dilution of the stock solution. The working solution was sterilised using a 0.45µm pore size membrane filter before the test was conducted.

Chemicals

Vancomycin and linezolid powdered reagents were obtained from Sigma Aldrich (St. Louis, MO, USA). The antibiotic stock solutions were prepared at a concentration of 10 mg/ml. The vancomycin powder was dissolved in sterile distilled water, and linezolid was dissolved in 10% DMSO and stored at 4°C. The working solution was prepared by calculating the appropriate dilution of the stock solution, and the solution was sterilised using a 0.2 µm pore size membrane filter prior to the test.

MRSA strains

A reference strain MRSA ATCC 33591 and clinical strain MRSA Mu50 were obtained from the collection of Biomedical Sciences Programme, Faculty of Health Sciences, UKM and stored in the Microbiology Laboratory and Medical Centre, Universiti Kebangsaan Malaysia (PPUKM). The stock cultures were grown on Muller–Hinton agar (MHA) and incubated at 37°C for 24 hours to obtain isolated colonies. The bacteria inoculates were prepared by transferring one or two single colonies of the same morphology into Muller–Hinton broth (MHB) using a sterile wire loop followed by a 24 hours incubation at 37°C. The estimated concentration of 10^8 CFU/ml was determined by at an optical density of 0.08 at a wavelength of 625 nm. The inoculation solution was adjusted to obtain 10^6 CFU/ml. The bacterial suspension was used within 30 mins.

Determination of the minimum inhibitory concentration (MIC)

The MIC value of the extract was determined using the broth microbroth serial dilution method, with a final inoculation of bacteria of approximately 10^6 CFU/ml. First, 50 µl MHB was added to each well of a 96-well microtiter plate. Then, 50 µl of the working solution was added to the first well and diluted two-fold. Finally, 50 µl of bacteria was added to each well so that the final volume in each well was 100 µl. Negative controls were wells containing only MHB and the compound, whereas the positive control wells contained only MHB and the bacterial suspension. The MIC value was lowest concentration of the compound that did not show any growth of the bacteria after incubation at 37°C for 24 hours [15]. For confirmation, 20 µl triphenyl tetrazolium chloride was added to each well. Wells that appeared pink were interpreted as positive for bacterial growth, whereas colourless wells were interpreted as negative for no growth of bacteria [16]. The test were conducted in triplicate.

Determination of minimum bactericidal concentration (MBC)

The MBC value of the compound is the lowest concentration that did not show any growth in subculture agar. The wells that showed no visible growth of bacteria on the microtiter plate were transferred to MHA, and the plate was incubated for 24 hours at 37°C [16]. The test was conducted in triplicate.

TKA analysis

TKA analysis was conducted using the broth macrodilution technique. A universal bottle that contained 10 ml of bacteria with approximately 10^6 CFU/ml of inoculum was treated with the antimicrobial agents at different concentrations. Untreated bacteria were used as a growth control. Next, viable counts were performed at 0, 2, 4, 6, 8 and 24 hours after adding the treatment agent throughout incubation of 37°C. In each subsequent hour, 100 µL of sample will be taken from the universal bottle and serially diluted ten-fold with normal saline (0.9% NaCl). Then, 50µl was dispensed into five evenly spaced, 10 µl per drop onto the designated quadrant of the petri plate in duplicate. After the drop on the agar was completely dry, the plates were incubated at 37°C for 24 hours. Bacterial colony count between 3–30 CFU/cm^2 for each drop was determined [17]. Time-kill curves were constructed by plotting the log $_{10}$ CFU/cm^2 on the x-axis and time (hours) on the y-axis. A compound was considered as bactericidal if it reduced bacterial concentration by 3log$_{10}$ CFU/cm^2 during the incubation period or killed 99.9% of the bacteria in the starting inoculum [18].

RESULTS

Determination of MIC and MBC

The MIC and MBC values of the acetone extract against MRSA ATCC 33591 and MRSA Mu50 are shown in Table 1. The MIC and MBC values of the acetone extract of *C. odontophyllum* against MRSA Mu50 were 312.5 µg/ml and 625 µg/ml, whereas those against MRSA ATCC 33591 were 625 µg/ml and 1,250 µg/ml respectively. Table 2 shows the MIC and MBC values of vancomycin against MRSA ATCC 33591 and MRSA Mu50. Vancomycin had equal MIC and MBC values, which were 3.91 µg/ml against MRSA Mu50 and 0.98 µg/ml against MRSA ATCC 33591. The MIC and MBC values of linezolid against MRSA ATCC 33591 and MRSA Mu50 were 1.56 µg/ml and 6.25 µg/ml, respectively (Table 3).

TKA analysis

The time-kill curves are shown in Figure 1. MRSA Mu50 treated with 1/2× MIC of the acetone extract of *C. odontophyllum* leaves showed a slight increase in bacterial colony count but lower than the growth control after a 24 hours incubation period. MRSA Mu50 showed inhibited growth after the first 8 hours in response to 1× MIC;

however, the reduction was < 3 log_{10} CFU/cm^2. The 2× MIC resulted in < 3 log_{10} CFU/cm^2 colony count throughout 24 hours incubation. However, the acetone extract of *C. odontophyllum* leaf at 4× MIC showed a bactericidal effect by a 3 log_{10} CFU/cm^2 reduction after exposure to MRSA Mu50 for 24 hours. Linezolid and vancomycin show static curves reductions of < 3 $1og_{10}$ during the incubation period.

Table 1: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the acetone extract of *Canarium odontophyllum* leaves against MRSA ATCC 33591 and MRSA Mu50.

Concentration of Acetone Extract (µg/ml)	MIC Test Result		Control		MBC Test Result		Control	
	ATCC 33591	Mu50	Positive	Negative	ATCC 33591	Mu50	Positive	Negative
5000	-	-	+	-	-	-	+	-
2500	-	-	+	-	-	-	+	-
1250	-	-	+	-	-	-	+	-
625	-	-	+	-	+	-	+	-
312.5	+	-	+	-	+	+	+	-
156.25	+	+	+	-	+	+	+	-
78.13	+	+	+	-	+	+	+	-
39.06	+	+	+	-	+	+	+	-
19.53	+	+	+	-	+	+	+	-
9.77	+	+	+	-	+	+	+	-

(+) Presence of bacterial growth, (-) absence of bacterial growth, positive control: Muller–Hinton broth (MHB) and bacterial suspension, negative control: MHB and acetone extract

Table 2: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of vancomycin against MRSA ATCC 33591 and MRSA Mu50.

Concentration of Vancomycin (µg/ml)	MIC Test Result		Control		MBC Test Result		Control	
	ATCC 33591	Mu50	Positive	Negative	ATCC 33591	Mu50	Positive	Negative
250	-	-	+	-	-	-	+	-
125	-	-	+	-	-	-	+	-
62.5	-	-	+	-	-	-	+	-
31.25	-	-	+	-	-	-	+	-
15.63	-	-	+	-	-	-	+	-
7.81	-	-	+	-	-	-	+	-
3.91	-	-	+	-	-	-	+	-
1.95	-	+	+	-	-	+	+	-
0.98	-	+	+	-	-	+	+	-
0.49	+	+	+	-	+	+	+	-
0.24	+	+	+	-	+	+	+	-
0.12	+	+	+	-	+	+	+	-

(+) Presence of bacterial growth, (-) absence of bacterial growth, positive control: Mueller–Hinton broth (MHB) and bacterial suspension, negative control: MHB and vancomycin

Table 3: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of linezolid against MRSA ATCC 33591 and MRSA Mu50.

Concentration of linezolid (µg/ml)	MIC Test Result		Control		MBC Test Result		Control	
	ATCC 33591	Mu50	Positive	Negative	ATCC 33591	Mu50	Positive	Negative
100	-	-	+	-	-	-	+	-
50	-	-	+	-	-	-	+	-
25	-	-	+	-	-	-	+	-
12.5	-	-	+	-	-	-	+	-
6.25	-	-	+	-	-	-	+	-
3.13	-	-	+	-	+	+	+	-
1.56	-	-	+	-	+	+	+	-
0.78	+	+	+	-	+	+	+	-
0.39	+	+	+	-	+	+	+	-
0.2	+	+	+	-	+	+	+	-

(+) Presence of bacterial growth, (-) absence of bacterial growth, positive control: Mueller–Hinton broth (MHB) and bacterial suspension, negative control: MHB and linezolid

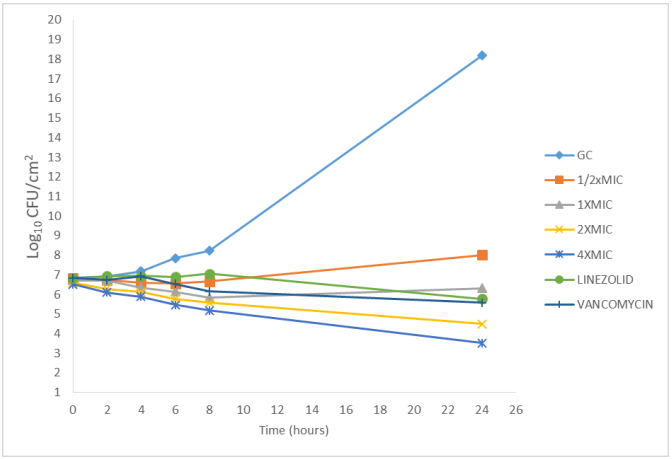

Figure 1: Time-kill curve for acetone extract (1/2×, 1×, 2×, 4×) minimum inhibitory concentration (MIC) of *Canarium odontophyllum* leaves, vancomycin, linezolid and growth control against MRSA Mu50.

DISCUSSION

MRSA are strains of *S. aureus* that are resistant to methicillin and most ß-lactam antibiotics, such as penicillin, cephalosporins and carbapenems [19]. Increased antibiotic resistance has caused a large challenge to treat infectious disease [20]. Therefore, natural products have been used as alternatives to treat infections over the years [21]. Some natural products have antimicrobial potential against MRSA [22, 23].

C. odontophyllum is a candidate phytotherapeutic against MRSA [14]. The leaf extract of *C. odontophyllum* exhibited bacteriostatic activity against MRSA strains with a MBC dilution two-fold higher than the MIC [13]. This finding contradicts that of [14], which reported bactericidal activity against MRSA ATCC 33591. The difference in antibacterial activity might due to differences in MRSA strains or a decrease in susceptibility to the antibacterial agent [14]. The finding that *C. odontophyllum* has helped curb the antimicrobial resistance problem was supported by [25] that some phytochemicals contained in extracts exhibit significant potential to alter antibiotic resistance. Secondary metabolites, such as saponins, terpenoids, tannins, flavonoids and phenolic compounds, contained in *C. odontophyllum* leaves have activity against MRSA [26, 12]. MRSA is a Gram-positive bacterium comprised of a mesh-like peptidoglycan layer that allows permeation [27].

The antibacterial activity assay showed that MRSA Mu50 was more inhibited by the extract than MRSA ATCC 33591. The extract was less potent compared to the standard antibiotics, this may be due to the variety of bioactive compounds that contain in the crude extract compared to standard antibiotic which is the active pure compound [28].

TKA can be used to determine antibacterial activity based on time or concentration. This assay provides this type of interaction and bactericidal activity [29]. In the present study, the drop plate method was used instead of the streak plate method in which $\log_{10}$ CFU/cm^2 was used instead of $\log_{10}$ CFU/ml to count the bacterial colonies [18]. The 0.5× MIC of the C. *odontophyllum* extract showed partial inhibition in colony count. The 1× MIC and 2× MIC inhibited the colony count but did not exhibit any bactericidal activity because the reduction was < 3 log 10 CFU/cm^2. This finding was supported by [14] that an acetone extract of *C. odontophyllum* leaves showed no bactericidal effect at 1× MIC. The 1× MIC and 2× MIC exhibited bacteriostatic activity by maintaining bacterial growth or killing < 99.9% of the bacteria [13, 30]. Interestingly, 4× MIC of the extract reduced the 3 $\log_{10}$ CFU/cm^2 colony count [18].

A difference in the antibiotic concentration by one dilution can cause a difference in the antibacterial pattern [31]. We concluded that the acetone extract of *C. odontophyllum* leaves exhibited dose-dependent bacterial killing activity at different concentrations [32]. In the present study, vancomycin and linezolid showed bactericidal activity against MRSA. [33] Previous study reported that time-dependent killing often slows the bactericidal action and vancomycin and linezolid have been proven to be agents that exhibit time-dependent killing [34].

CONCLUSION

The acetone extract from *C. odontophyllum* leaves exhibited a concentration-dependent bacterial killing effect at 4× MIC against MRSA Mu50.

Acknowledgement

This project was funded by the Ministry of Higher Education, Government of Malaysia under Research Code Grant No. FRGS /2/2014/SG05/UKM/02/3.

REFERENCES

1. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. Nature. 2008; 451(7181):990-993.
2. Rice LB. Unmet medical needs in antibacterial therapy. Biochemical pharmacology. 2006; 71(7):991-995.
3. Stapleton PD, Taylor PW. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. Science Progress. 2002; 85(1):57-72.
4. Kollef MH, Morrow LE, Niederman MS, Leepe KV, Anzueto A, Benz-Scott L, *et al*. Clinical characteristics and treatment patterns among patients with ventilator-associated pneumonia. Chest Journal. 2006; 129(5):1210-1218.
5. Jevons MP. "Celbenin"-resistant *Staphylococci*. British Medical Journal. 1961; 1:124-125.
6. Shurland S, Zhan M, Bradham DD, Roghmann MC. Comparison of mortality risk associated with bacteremia due to methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. Infection Control & Hospital Epidemiology. 2007; 28(3):273-279.
7. Rachman A, Mat Azis N, Hui P, Suhaili Z, Amin Nordin S, Othman Z, Mohd Desa MN. Genotypic and phenotypic characterization of methicillin resistance determinants and β-lactamase in *Staphylococcus* species. Malaysian Journal of Microbiology. 2017; (13):308-317.
8. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infection Control and Hospital Epidemiology. 2005; 26(2):166-174.
9. Kaleem F, Usman J, Hassan A, Omair M, Khalid A, Uddin R. Sensitivity pattern of methicillin resistant *Staphylococcus aureus* isolated from patients admitted in a tertiary care hospital of Pakistan. Iranian Journal of Microbiology. 2010; 2(3):143-146.
10. Khoo HE, Azlan A, Ismail A, Abas F. Influence of different extraction media on phenolic contents and antioxidant capacity of defatted dabai (*Canarium odontophyllum*) fruit. Food Analytical Methods. 2012; 5(3):339-350.

11. Mogana R, Wiart C. *Canarium L.*: a phytochemical and pharmacological review. Journal of Pharmacy Research. 2011; 4(8):2482-2489.
12. Basri DF, Nor NHM. Phytoconstituent screening and antibacterial activity of the leaf extracts from *Canarium odontophyllum* Miq. American Journal of Plant Sciences. 2014; 5(19):2878-2887.
13. Basri DF, Sandra V. Synergistic interaction of methanol extract from *Canarium odontophyllum* Miq. Leaf in combination with oxacillin against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591. International Journal of Microbiology. 2016; 2016(Art ID 5249534):7 pages.
14. Basri DF, Shamsuddin NAM, Ishak SF. Time-kill assay and post-antibiotic effect of acetone extract of *Canarium odontophylum* leaves against methicillin-resistant *Staphylococcus aureus* (MRSA) International Journal of Current Medical and Pharmaceutical Research. 2016; 2(9):630-635.
15. Amman V, Basri DF, Huyop F. Determination of the post-antibiotic effect (PAE) of combinations of extracts from galls of *Quercus infectoria* with vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA). African Journal of Biotechnology. 2013; 10(79):18274-78.
16. CLSI. M100-S22 Performance Standards For Antimicrobial Susceptibility Testing; Twenty-Second Information Supplement, Pennsylvania: Clinical and Laboratory Standards Institute. 2012; 32(3).
17. Herigstad B, Hamilton M, Heersink J. How to optimize the drop plate method for enumerating bacteria. Journal of Microbiological Methods. 2001; 44(2):121-129.
18. Barber KE, Werth BJ, McRoberts JP, Rybak MJ. A novel approach utilizing biofilm time-kill curves to assess the bactericidal activity of ceftaroline combinations against biofilm-producing methicillin-resistant *Staphylococcus aureus.* Antimicrobial Agents and Chemotherapy. 2014; 58(5):2989-2992.
19. APIC. Guide to the Elimination of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Transmission in Hospital Settings. Washington: Roche Diagnostics Corporation, 2010.
20. Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, Upadhyay G, Chapagain ML. Threat of drug resistant *Staphylococcus aureus* to health in Nepal. BMC infectious diseases. 2014; 14(1):157-161.
21. Wright GD, Sutherland AD. New strategies for combating multidrug-resistant bacteria. Trends in Molecular Medicine. 2007; 13(6):260-267.
22. Razmavar S, Abdulla MA, Ismail SB, Hassandarvish P. Antibacterial activity of leaf extracts of *Baeckea frutescens* against methicillin-*resistant Staphylococcus aureus.* BioMed Research International. 2014; 2014:5.
23. Ishak SF, Ghazali AR, Zin NM, Basri DF. Pterostilbene enhanced anti-methicillin resistant S*taphylococcus aureus* (MRSA) activity of oxacillin. Revista Brasileira de Gestao e Desenvolvimento Regional. 2016; 12(1):1-10.
24. Basri DF, Zainal NH, Santhanam J. The potential of *Canarium odontophyllum* Miq.(dabai) as anti-methicillin resistant S*taphylococcus aureus* agent. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(9):290-293.
25. Monte J, AC Abreu A, Borges LC, Simões M, Simões. Antimicrobial activity of selected against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. Pathogens. 2014; 3(2):473-498.
26. Paiva PMG, Gomes FS, Napoleão TH, Sá RA, Correia MTS, Coelho LCBB. Antimicrobial activity of secondary metabolites and lectins from plants. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. 2010; 1:396-406.
27. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. International Journal of Microbiology. 2013; 2013:7.
28. Gatsing D, Nkeug CF, Nji-Nka BF, Kuiate J, Tchouan FM. Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extract of *Alchornea cordifolia (Euphorbiaceae*). International Journal of Pharmacology International Journal of Pharmacology. 2010; 6(3):173-182.
29. Jacqueline C, Caillon J, Le Mabecque V, Miègeville AF, Donnio PY, Bugnon D, Potel G. *In vitro* activity of linezolid alone and in combination with gentamicin, vancomycin or rifampicin against methicillin-resistant *Staphylococcus aureus* by time–kill curve methods. Journal of Antimicrobial Chemotherapy. 2003; 51(4):857-864.
30. Silva F, Lourenço O, Queiroz JA, Domingues FC. Bacteriostatic versus bactericidal activity of ciprofloxacin in *Escherichia coli* assessed by flow cytometry using a novel far-red dye. The Journal of Antibiotics. 2011; 64(4):321-325.
31. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, *et al*. Synergy testing by E-test, microdilution checkerboard, and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. Antimicrobial Agents and Chemotherapy. 2010; 54(11):4678-4683.
32. Khairon R, Zin NM, Abdul Rahman M, Basri DF. Comparative proteomic analysis of differential proteins in response to aqueous extract of *Quercus infectoria* Gall in methicillin-resistant *Staphylococcus aureus*. International Journal of Proteomics. 2016; 2016(Art ID 4029172):9 pages.
33. Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. Elsevier. Mayo Clinic Proceedings. 2011; 86(2):156-167.
34. Quintiliani R. Pharmacodynamics of antimicrobial agents: time-dependent vs. concentration-dependent killing. Antimicrobe. Org, 2012.

HOW TO CITE THIS ARTICLE

Shamsuddin NAM, Basri DF. Anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of an acetone extract from the leaves of *Canarium odontophyllum* (Miq.). J Phytopharmacol 2018; 7(3):225-229.