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Synthesis of 1′-Acetoxychavicol Acetate (ACA) Analogues and their Inhibitory Activities against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT: *A series of 1′-acetoxychavicol acetate analogues were synthesised and evaluated for their antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA) using broth microdilution technique. The minimum inhibitory concentration (MIC) was used to determine whether the compounds had potential as inhibitory agents against the MRSA ATCC 43300, and the compounds with antimicrobial potential (<2000 μg ml^{-1}) were tested for minimum bactericidal concentration (MBC). Based on this assay, compound* ***1*** *exhibited potent antimicrobial activity with MIC value of 250 μg ml^{-1}. Meanwhile, compounds* ***2*** *and* ***13*** *showed the moderate activity with MIC values of 500 μg ml^{-1}, respectively.*

Keywords: 1′-acetoxychavicol acetate analogues, antimicrobial activity, methicillin-resistant *Staphylococcus aureus*, minimum inhibitory concentration, minimum bactericidal concentration

1. INTRODUCTION

The infectious diseases caused by antibiotic resistance pathogens have been increasing significantly over the last few decades due to the widespread use of antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been known as an important antibiotic-resistant pathogen and has become endemic in most shared facilities such as hospitals, healthcare facilities and nursing homes.[1] The number of MRSA cases has extensively increased especially in Asia, Malta as well as North and South America.[2] In Malaysia, the prevalence of MRSA infections increased from 18.0% in 2016 to 19.8% in 2017, and it is listed as one of the seven pathogens associated with high mortality.[3,4] Recently, a review of surveillance data reported that the prevalence of resistance for the older line of antibiotics has largely decreased over the past three decades, with only clindamycin resistance showing increasing trend.[4]

Currently, treatments for MRSA infection are limited because MRSA strains are often resistant towards first-line beta-lactam antibiotics such as methicillin, oxacillin, penicillin and amoxycilin.[5] Since the antibiotic resistance has reached an alarming level, there is great need to develop new antibacterials.[6] In the search for new antimicrobial compounds, medicinal plants are valuable sources of potent drugs due to the presence of a variety of active chemical substances.[7]

An example of such a plant is *Alpinia conchigera* Griff., also locally known as *cengkenam*, *lengkuas kecil*, *lengkuas padang*, *lengkuas ranting* or *lengkuas genting*.[8] This species belongs to the Zingiberaceae family, and the rhizomes of this species is commonly used as spice or ginger substitutes for flavouring food.[8] In Peninsular Malaysia, the young shoots of rhizomes are often used in vegetarian dishes, post-partum treatment and treatment of fungal infections as part of traditional medicine.[9] There have been a few studies on the chemical constituents from rhizome of *Alpinia conchigera*. Awang et al. and Hasima et al. reported the presence of 1′*S*-1′-acetoxyeugenol acetate and 1′*S*-1′-acetoxychavicol acetate in the rhizome which were found to induce apoptosis in human breast cancer cells (MCF-7) and oral squamous carcinoma cells (HSC-4), respectively.[10,11] Meanwhile, Aziz et al. reported seven known phenylpropanoids: chavicol acetate, *p*-hydroxycinnamaldehyde, 1′-acetoxychavicol acetate, *trans*-*p*-coumaryl diacetate, 1′-acetoxyeugenol acetate, 1′-hydroxyxhavicol acetate and *p*-hydroxycinnamyl acetate from Malaysian *Alpinia conchigera*.[12] Among them, 1′-acetoxychavicol acetate (ACA, **1**) was found as the major constituent that exhibited various biological activity, including anti-inflammatory activity, anti-human immunodeficiency virus (HIV) activity, the inhibition of nitric oxide (NO), antimicrobial activity and the inhibition of xanthine oxidase (XO).[13]

In the present study, we describe the synthesis and antimicrobial evaluation of ACA analogues against MRSA (Figure 1). The structural activity relationship of ACA were observed by comparing the inhibition activities with structural features of analogues.

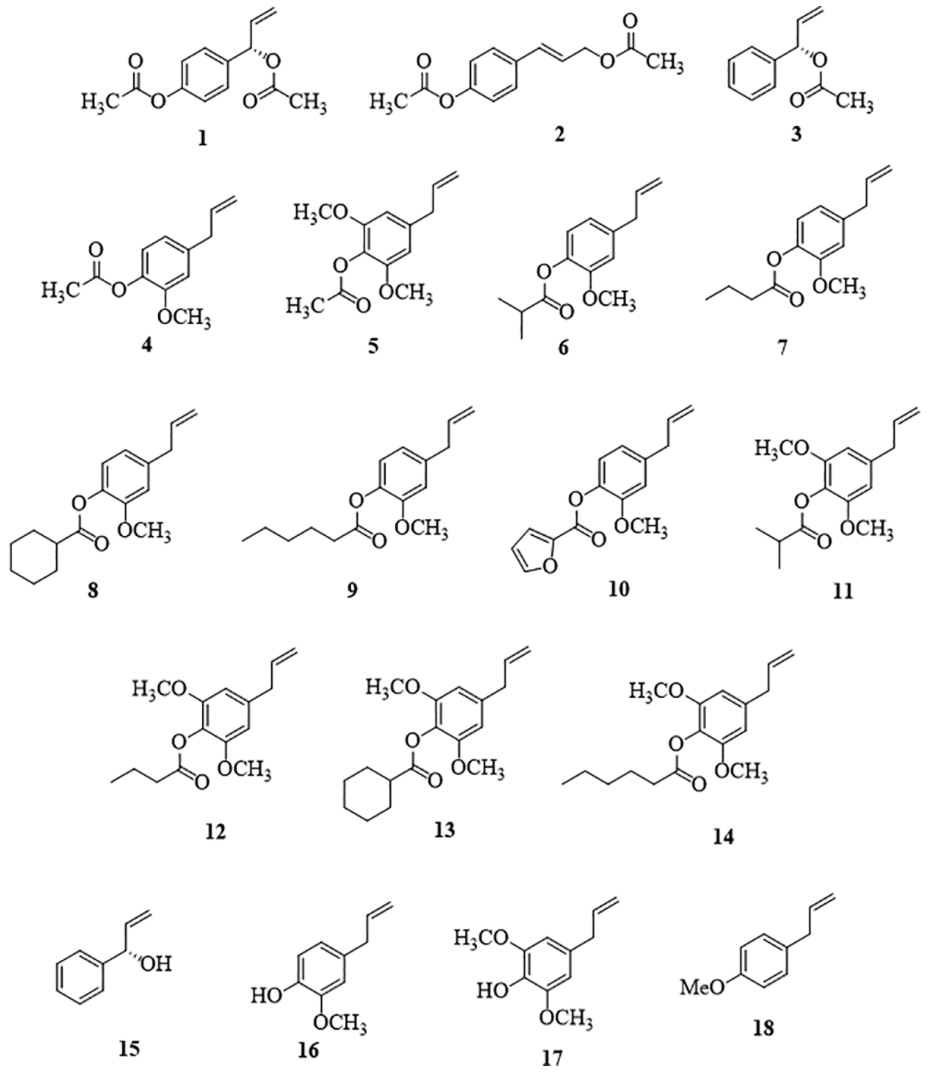


Figure 1: Structures of ACA and its analogues.

2. EXPERIMENTAL

2.1 Chemistry

All reagents used were obtained from Sigma-Aldrich Co. and Merck Chemical Co. without further purification. All reactions were carried out in heat-dried glassware under a dry nitrogen atmosphere unless otherwise stated. All liquid transfers were conducted using syringe. The reactions were monitored by thin layer chromatography (TLC) and were visualised under UV 254 nm. Infrared spectra were recorded using a Perkin Elmer Spectrum 1000 Fourier transform infrared (FTIR) spectrometer. All mass spectra were obtained from GC-MS (Agilent Technology 7890 A) on HP-5 column. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR were recorded in $CDCl_3$-D1 by using Bruker Advanced 500 spectrometers (Bruker Bioscience, Billerica, MA, United States).

2.1.1 General procedure for synthesis of analogues 3–5

The preparation of compounds **3–5** were reported previously using reaction of compounds **15–17** with acetic anhydride (Scheme 1).[14] Dichloromethane (25 ml), acetic anhydride (2.0 equiv.) and 4-dimethylaminopyridine (2.2 equiv.) were added to an alcohol solution (1.0 equiv.). The reaction mixture was stirred under ice bath. The ice bath was removed, and the mixture was allowed to warm at room temperature. After consumption of starting material and product formation, the reaction was quenched with saturated aqueous NH_4Cl (10 ml) solution and extracted with dichloromethane (3 × 15 ml). The organic layers were dried to obtain the desired product. For spectroscopic data, compounds **3–5** were compared with reported data.[14–16]

2.1.2 General procedure for synthesis of analogues 6–14

Compounds **6–14** were synthesised by modifying Abd. Rahim et al.'s procedure.[17] Compounds **6–10** were synthesised from **16**, while compounds **11–14** were synthesised from **17** (Scheme 2). The target compounds were prepared by dissolving the respective 4-allyl phenol (10.0 equiv.) in dichloromethane (20 ml). The mixture was added with 4-dimethylaminopyridine (0.1 equiv.) and triethylamine (20.0 equiv.). The solution was stirred under nitrogen in ice bath. After that, the excess amount of acyl chloride (11.0 equiv.) was added drop wise in a 15 min period. The solution was stirred for 24 h and the progress of reaction was monitored by TLC. After consumption of starting material and product formation, the reaction is worked up by initially adding water and extracted with dichloromethane (3 × 15 ml). The organic layer was dried to give

the desired product. For spectroscopic data, compounds **6–9** were compared with reported data.[17–19] For compounds **10–14**, the spectroscopic data are attached in supplementary information.

2.2 Antimicrobial Activity

2.2.1 Preparation of bacterial inoculum

Staphylococcus aureus subsp. *aureus* (ATCC® 43300TM) from American Type Culture Collection (ATCC, Manassas, VA, United States) was cultured in Brain Heart Infusion Broth overnight in a rotary shaker at 37°C and centrifuged at 200 rpm. The cultures then were suspended in an equal volume of phosphate buffer solution (PBS) solution and the optical density was adjusted to 0.05 at 600 nm, corresponding to approximately 1×10^7 colony forming units (CFU) per ml.

2.2.2 Minimum inhibitory concentration (MIC) assay

Antimicrobial activity of ACA and its analogues (**1–18**) were measured using broth micro dilution technique on 96-well microtiter plates following published protocol.[20] Briefly, all the tested compounds were dissolved in 5% dimethyl sulfoxide (DMSO), to produce a starting concentration of 4000 μg ml^{-1}, which was added to plates containing 100 μl of double strength Muller Hilton broth (since the addition of the inoculum dilutes the medium to 1:2). The test compounds were serially diluted to concentration of 31.25 μg ml^{-1} by mixing 100 μl of the test compound solution and broth in the first well of each microtiter row; then transferring 100 μl from the preceding well to the subsequent well until the seventh well, after which 100 μl of the mixture solution was discarded to leave the eighth well in each row as an antimicrobial-free well.

Then, 5 μl of bacterial suspension adjusted to a 0.5 McFarland turbidity standard were inoculated into each well containing test compound/antibiotic and control (antimicrobial free) wells. Plates were wrapped loosely with a cling film to avoid suspension dehydration and was incubated at 35°C, under aerobic conditions, for 24 h. At the end of the incubation period, 50 μl tetrazolium solution at 5 mg ml^{-1} was added into all wells and the plates were re-incubated for 1 h at RT. The MIC was determined based on a colour change from yellow to purple, whereby a purple colour indicated microbial growth. The MIC is recorded based on the last well before colour change.

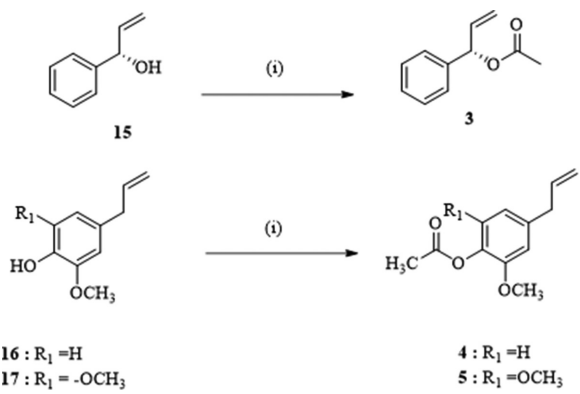
2.2.3 Minimum bactericidal concentration (MBC) assay

MBC was measured by transferring 50 µl aliquots of the suspension from each well (without growth indicator) that did not show any turbidity or visible growth after being incubated during MIC assay, onto nutrient agar plate. Plates that have been inoculated with the suspension were incubated at 37°C for 18 h. The MBC values were determined at the lowest concentration of the tested compound which kills > 99.9% of bacteria based on number of CFU.

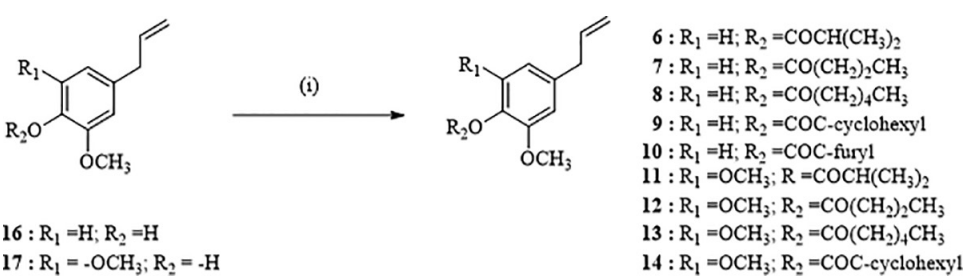
3. RESULTS AND DISCUSSION

3.1 Chemical Compounds

1'-acetoxychavicol acetate (**1**) and *trans-p*-coumaryl diacetate (**2**) were isolated from *Alpinia conchigera*. In addition to that, the syntheses strategy of ACA derivatives (**3–14**) were outlined in Schemes 1 and 2, respectively.



Scheme 1: Reagents and conditions: (i) Ac_2O, DMAP, DCM, ice-bath.



Scheme 2: Reagents and conditions: (i) acyl chloride, DMAP, TEA, DCM, ice-bath.

3.2 Minimum Inhibition and Bactericidal Concentration of ACA Derivatives

Antimicrobial screening of ACA analogues against MRSA ATCC 43300 was performed using the broth micro dilution technique to determine the MIC of each compound. Compounds with lower MIC values indicate that a lower concentration of the drug would be required for inhibiting growth of the organism and would thus pose lower risk of toxicity. Therefore, the antimicrobial activity is more effective when lower MIC values were recorded. According to studies, the antibacterial activity of compounds is considered to be significant when MIC values are below 100 μg ml^{-1}, moderate when MIC values between 100 μg ml^{-1} and 625 μg ml^{-1}, and weak when MIC more than 625 μg ml^{-1}.[21,22] Although the MIC for the compounds suggest they had low antimicrobial potential against MRSA the compounds with < 2000 μg ml^{-1} MIC were selected for MBC testing. The MIC and MBC values obtained are shown in Table 1.

Table 1: Antimicrobial activity of ACA analogues against strain of MRSA.

Compound	MRSA ATCC 43300 MIC (μg ml^{-1})	MBC (μg m^{-1})
1	250	500
2	500	1000
3	4000	NB
4	4000	–
5	4000	–
6	4000	–
7	2000	4000
8	4000	–
9	4000	–
10	2000	4000
11	2000	4000
12	2000	4000
13	500	1000
14	1000	2000
15	4000	–
16	2000	4000
17	NI	–
18	4000	–
Vancomycin	2	–
Tetracycline	0.5	4

Based on the results obtained (Table 1), the range of MIC values for all tested compounds is between 250 μg ml^{-1} and 4000 μg ml^{-1}. Compound **1** showed the highest inhibition against MRSA with MIC value of 250 μg ml^{-1}. Meanwhile, compounds **2** and **13** exhibited moderate antimicrobial activity against MRSA with MIC value of 500 μg ml^{-1}, respectively. Compound **14** exhibited weak antimicrobial activity with MIC value of 1000 μg ml^{-1}, while the remaining compounds tested had MIC values of 2000 μg ml^{-1} and above. Both positive controls vancomycin and tetracycline demonstrated strong antimicrobial activity with MIC values of 2.0 μg ml^{-1} and 0.5 μg ml^{-1}, corresponding to values recorded by ATCC.

MBC values for compounds tested were one-fold higher concentration in comparison to their MIC value, compared to the MBC for tetracycline which was eight-fold higher than its MIC (4.0 μg ml^{-1}). An antimicrobial agent is considered bactericidal if its MBC value is not more than fourfold of the MIC value, hence it can be concluded that although the natural compounds may not have high MIC values, they may have relatively higher bactericidal potential.[23]

3.3 Structural Activity Relationship (SAR) Analysis of ACA Derivatives

As shown in Table 1, the analogues **1–18** were examined for the inhibitory effects against MRSA. Firstly, the contribution of carbonyl ester moiety that is directly attached to the phenyl ring at position C-4 and C-1′ is highly responsible for antimicrobial activity against MRSA. As an example, compound **1** exhibited good activity compared to analogue **3** which is lacking the carbonyl ester moiety at C-4. In addition, the introduction of methoxyl group at C-3 and C-5 (analogues **13–14**) also exhibited potent activity. Furthermore, substitution of ester group with long chain carbon such as isobutyryl (**11**), butyryl (**12**), cyclohexane (**13**) and hexanoyl (**14**) showed higher activity compared to the analogue **5**, which has less carbon chain and gave less activity. Next, the contribution of 2′-3′ double bond of **1** to the activity was evaluated and as a result, compound **2** was less active than **1**. These results suggested that the double bond position C2′-C3′ are essential. Another factor that give effect to the activity is the presence of ester moiety at C-4 position. The higher the alkyl chain attached to the carbonyl ester (C=O), the higher the antimicrobial activity against MRSA. Finally, the structure requirements of ACA and its derivatives for antimicrobial activity were summarised as follows (Figure 2).

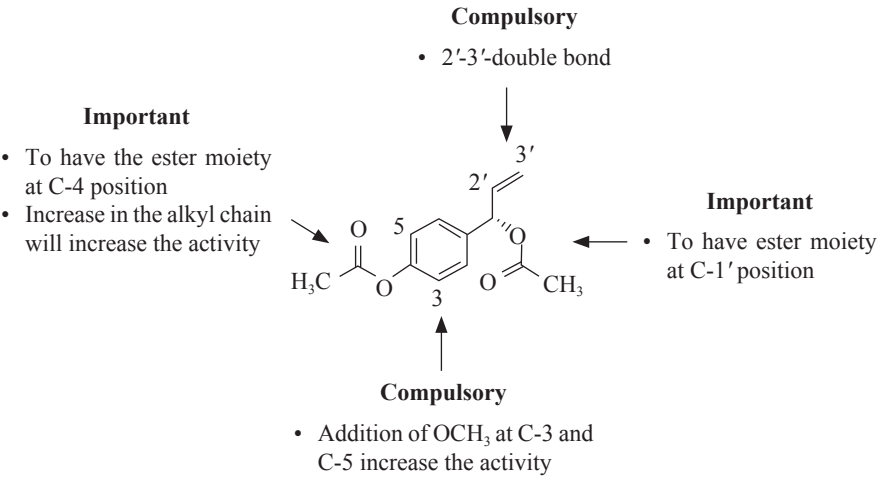


Figure 2: SAR of ACA analogues.

4. CONCLUSION

In conclusion, we synthesised the derivatives based on the ACA (**1**) skeleton and revealed their SAR against MRSA. The result obtained concluded that the natural compounds **1** and **2** showed potent and moderate antimicrobial activity against MRSA with MIC value of 250 µg m^{-1} and 500 µg m^{-1}, respectively, while synthetic analogue 13 also demonstrated antimicrobial potential with MIC value of 500 µg m^{-1}.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

1. Celik, A., Aydinlik, N. & Arslan, I. (2011). Phytochemical constituents and inhibitory activity towards methicillin-resistant *Staphylococcus aureus* strains of *Eryngium* species (Apiaceae). *Chem. Biodivers.*, 8(3), 454–459. https://doi.org/10.1002/cbdv.201000124
2. Stefani, S. et al. (2012). Meticillin-resistant *Staphylococcus Aureus* (MRSA): Global epidemiology and harmonisation of typing methods. *Int. J. Antimicrob. Agents*, 39(4), 273–282. https://doi.org/10.1016/j.ijantimicag.2011.09.030

3. Rohaidah, H. et al. (2017). National surveillance of antimicrobial resistance, Ministry of Health Malaysia. Retrieved 12 August 2020 from https://www.imr.gov.my/images/uploads/NSAR/NSAR_2017/NSAR_report_2017-edited-31.1.2019.pdf.
4. Che Hamzah, A. M. et al. (2019). *Staphylococcus aureus* infections in Malaysia: A review of antimicrobial resistance and characteristics of the clinical isolates, 1990–2017. *Antibiot. (Basel)*, 8(3), 128. https://doi.org/10.3390/antibiotics8030128
5. Ahmad, N. et al. (2009). Characteristics of community- and hospital-acquired methicillin-resistant *Staphylococcus aureus* strains carrying SCC*mec* type IV isolated in Malaysia. *J. Med. Microbiol.*, 58(9), 1213–1218. https://doi.org/10.1099/jmm.0.011353-0
6. Baba, J. et al. (2015). Antibiotic resistance patterns of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from chronic skin ulcer of patients in Kaduna state, Nigeria. *IOSR J. Pharm.*, 5(2), 7–12.
7. Ari, S., Temel, M. & Konuk, M. (2017). An ethnobotanical approach to MRSA (methicillin resistant *Staphylococcus aureus*) in Western Anatolia: A case of Afyonkarahisar. *Ind. J. Trad. Knowl.*, 16(1), 35–43.
8. Burkill, I. H. (1966). *A dictionary of the economic products of the Malay Peninsular* (2nd ed.). Kuala Lumpur: Ministry of Agriculture and Cooperative.
9. Ibrahim, H, Chooi, O. H. & Hassan, R. (2000). Ethnobotanical survey of the ginger family in selected Malay villages in Peninsular Malaysia. *Mal. J. Sci*., 19(1), 93–99.
10. Awang, K. et al. (2010). The apoptotic effect of 1′*S*-1′-acetoxychavicol acetate from *Alpinia conchigera* on human cancer cells. *Mol*., 15(11), 8048–8059. https://doi.org/10.3390/molecules15118048
11. Hasima, N. et al. (2010). 1′S-1′-acetoxyeugenol acetate: A new chemotherapeutics natural compound against MCF-7 human breast cancer cells. *Phytomed.*, 17(12), 935–939. https://doi.org/10.1016/j.phymed.2010.03.011
12. Aziz, A. N. et al. (2013). Antimicrobial compound from *Alpinia conchigera. J. Ethnopharm.*, 145(3), 798–802. https://doi.org/10.1016/j.jep.2012.12.024
13. Misawa, T. et al. (2015). Structure-activity relationships of benzhydrol derivatives based on 1′-acetoxychavicol acetate (ACA) and their inhibitory activities on multiple myeloma cell growth via inactivation of the NF-κB pathway. *Bioorg. Med. Chem. Lett.*, 23(9), 2241–2246. https://doi.org/10.1016/j.bmc.2015.02.039
14. Liew, S. K. et al. (2017). Anti-proliferative, apoptotic induction and anti-migration effects of hemi-synthetic 1′*S*-1′-acetoxychavicol acetate analogs on MDA-MB-231 breast cancer cells. *Drug Des. Devel. Ther.*, 11, 2763–2776. https://doi.org/10.2147/DDDT.S130349
15. Matsuda, H. et al. (2005). Structure-activity relationships of 1′S-1′-acetoxychavicol acetate for inhibitory effect on NO production in lipopolysaccharide-activated mouse peritoneal macrophages. *Bioorg. Med. Chem. Lett.*, 15(7), 1949–1953. https://doi.org/10.1016/j.bmcl.2005.01.070

16. Álvarez-Calero, J. M., Jorge, Z. D. & Massanet, G. M. (2016). $TiCl_4/Et_3N$-Mediated condensation of acetate and formate esters: Direct access to *β*-alkoxy- and *β*-aryloxyacrylates. *Org. Lett*., 18(24), 6344–6347. https://doi.org/10.1021/acs.orglett.6b03233
17. Abd. Rahim, N. H. et al. (2017). Synthesis and antibacterial study of eugenol derivatives. *Asian J. Chem.* 29(1), 22–26. https://doi.org/10.14233/ajchem.2017.20100
18. Díaz-Álvarez, A. E., Crochet, P. & Cadierno, V. (2012). A general route for the stereoselective synthesis of (*E*)-(1-propenyl)phenyl esters by catalytic C=C bond isomerization. *Tetrahedr.*, 68(12), 2611–2620. https://doi.org/10.1016/j.tet.2012.01.083
19. Sadeghian, H. et al. (2008). Design and synthesis of eugenol derivatives, as potent 15-lipoxygenase inhibitors. *Bioorg. Med. Chem*., 16(2), 890–901. https://doi.org/10.1016/j.bmc.2007.10.016
20. Lazarević, J. et al. (2018). Synthesis, antimicrobial activity and *in silico* studies on eugenol eters. *Acta Chim. Slov.*, 65(4), 801–810. https://doi.org/10.17344/acsi.2018.4380
21. Kuete, V. (2010). Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med.*, 74, 1479–1491. https://doi.org/10.1055/s-0030-1250027
22. Kuete, V. & Efferth, T (2010). Cameroonian medicinal plants: Pharmacology and derived natural products. *Front. Pharmacol.*, 1(123), 1479–1491. https://doi.org/10.3389/fphar.2010.00123
23. French, G. L. (2006). Bactericidal agents in the treatment of MRSA infections – The potential role of daptomycin. *J. Antimicrob. Chemother.*, 58(6), 1107–1117. https://doi.org/10.1093/jac/dkl393