Chemicals Constituents Isolated from Cultivate *Alpinia conchigera* Griff. and Antimicrobial Activity

Authors:

Mohamad Nurul Azmi Mohamad Taib*, Nursyazwani Anuar, Khayriyyah Mohd Hanafiah, Aeman Ali Kudayr Al-Shammary, Mardiana Saaid and Khalijah Awang

*Correspondence: mnazmi@usm.my

DOI: https://doi.org/10.21315/tlsr2020.31.1.10

Highlights

- Phenylpropanoids were successful isolate as the major components in *Alpinia conchigera*.
- 1'S-1'-acetoxychavicol acetate exhibited good antimicrobial activity can be further evaluated as a potential candidate for treatment against MRSA.
- The results provide some evidence to support the traditional application of this herb in treatment of skin infection as reported in Kelantan, East Coast Malaysia.
Chemicals Constituents Isolated from Cultivate *Alpinia conchigera* Griff. and Antimicrobial Activity

1Mohamad Nurul Azmi Mohamad Taib*, 1Nursyazwani Anuar, 2Khayriyyah Mohd Hanafiah, 2Aeman Ali Kudayr Al-Shammary, 1Mardiana Saaid and 3Khalijah Awang

1School of Chemical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia
2School of Biological Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia
3Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Publication date: 7 April 2020
To cite this article: Mohamad Nurul Azmi Mohamad Taib, Nursyazwani Anuar, Khayriyyah Mohd Hanafiah, Aeman Ali Kudayr Al-Shammary, Mardiana Saaid and Khalijah Awang. (2020). Chemicals constituents isolated from cultivate *Alpinia conchigera* Griff. and antimicrobial activity. *Tropical Life Sciences Research* 31(1): 159–178. https://doi.org/10.21315/tlsr2020.31.1.10
To link to this article: https://doi.org/10.21315/tlsr2020.31.1.10

Abstract: *Alpinia conchigera* Griff. is a plant species from the family Zingiberaceae. Colloquially known as wild ginger, *Alpinia conchigera* Griff. is used as food condiment and for traditional treatment of skin diseases. Isolation studies to identify bioactive compounds of rhizomes of *Alpinia conchigera* yielded seven compounds; 1'S-1'-acetoxychavicol acetate (1), trans-p-coumaryl diacetate (2), p-hydroxycinnamyl acetate (3), 1'S-1'-hydroxychavicol acetate (4) p-hydroxybenzaldehyde (5), stigmasterol (6) and β-sitosterol (7). Compounds 1, 2 and 5 were evaluated for antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Among the compounds tested, Compound 1 showed good antimicrobial activity against the strain of MRSA with minimum inhibition concentration (MIC) value of 0.5 mg/mL. Meanwhile, Compounds 2 and 5 exhibited moderate activity with MIC value between 1.0 and 2.0 mg/mL. These findings indicate antimicrobial potential of 1'S-1'-acetoxychavicol acetate (1), compound derived from rhizome of *Alpinia conchigera* Griff. against MRSA, which warrant further investigation.

Keywords: *Alpinia conchigera* Griff., Zingiberaceae, Antimicrobial, Methicillin Resistant *Staphylococcus aureus*, MRSA

Abstrak: *Alpinia conchigera* Griff. merupakan spesies tumbuhan daripada famili Zingiberaceae. Lebih dikenali sebagai lengkuas liar, *Alpinia conchigera* Griff. sering digunakan sebagai perasa makanan dan rawatan tradisional penyakit kulit. Pemencilan ke atas rizom *Alpinia conchigera* telah menghasilkan tujuh sebatian; 1’S-1’-asetoksikhavikol acetat (1), *trans-p*-koumaril diacetat (2), p-hidroksisinamil asetat (3), 1’S-1’-hidroxychavicol asetat (4) p-hidroksibenaldhid (5), stigmasterol (6) dan β-sitosterol (7). Sebatian 1, 2

*Corresponding author: mnazmi@usm.my

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and 5 has been evaluated for antimicrobial activity against *Staphylococcus aureus* iaitu MRSA. Among the evaluated substances, substance 1 shows strong antimicrobial activity against MRSA with MIC value of 0.5 mg/mL. While substances 2 and 5, respectively, show simple antimicrobial activity with MIC values ranging from 1.0 to 2.0 mg/mL. These findings indicate the potential of *S*-1'-acetoxykhavikol acetate (1) from the rhizome of *Alpinia conchigera* Griff. terhadap MRSA that needs to be further studied.

**Kata Kunci:** *Alpinia conchigera* Griff., Zingiberaceae, Antimikrob, Methicilin Resistant *Staphylococcus aureus*, MRSA

**INTRODUCTION**

*Alpinia conchigera* (Fig. 1) is a Malaysian wild-ginger and also known locally as “lengkuas ranting”, “lengkuas kecil”, “lengkuas padang”, “lengkuas genting” or “chengkenam” (Ibrahim et al. 2008; Henderson 1954; Ridley 1909). It is a herbaceous perennial 2 to 5 feet tall and usually found in eastern Bengal and southwards to Peninsular Malaysia and Sumatera (Burkhill 1966; Smith 1990). Traditionally, all parts of plants such as rhizomes, seeds, flowers and leaves are used medicinally and claimed to have a range effect to treat many physical illnesses. In some states of Peninsular Malaysia, the rhizome of *Alpinia conchigera* is consumed to treat skin disease (Ibrahim et al. 2009) and also used as food condiment (Victorio 2011). Besides that, it is also advocated for the treatment of diabetes in Thailand (Wongsatit & Ampol 2003).

There are few studies reporting on the chemical constituents from *Alpinia conchigera* Griff. Aziz et al. (2013) reported seven known phenylpropanoids isolated from *Alpinia conchigera*; chavicol acetate, *p*-hydroxycinnamaldehyde, 1'S-1'-acetoxychavicol acetate, trans-*p*-coumaryl diacetate, 1'S-1'-acetoxyeugenol acetate, 1'-hydroxychavicol acetate and *p*-hydroxycinnamyl acetate. Of these, 1'S-1'-acetoxychavicol acetate was found as the major constituent and has been reported to bear a variety of important biological activities such as antitumor, anti-inflammatory, antifungal, antioxidative, anti-human immunodeficiency virus (HIV) and xanthine oxidase inhibitory activity (Yasuhara et al. 2009). Besides that, the presence of 1'S-1'-acetoxychavicol acetate and 1'S-1'-acetoxyeugenol acetate in the rhizome were found to induce apoptosis in oral squamous carcinoma cells (HSC-4) and human breast cancer cells (MCF-7) (Awang et al. 2010; Hasima et al. 2010).

For the past few decades, antimicrobial resistance has become a serious threat to global public health as the number of infectious diseases by antibiotic resistance pathogens increased significantly. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the drug resistant form of *Staphylococcus aureus*, which are normal skin flora that can opportunistically infect skin and mucous tissues. Traditionally known for its role in nosocomial infections, MRSA is major cause of morbidity and mortality in hospital worldwide (Lakhundi & Zhang 2018). However, increasing prevalence of community-acquired MRSA has also been reported in
recent years. As MRSA is resistant to the first-line beta-lactam antibiotics, including methicillin, oxacillin, penicillin and amoxycilin (Baba et al. 2016), patients often need to be treated with last-resort drugs such as vancomycin, which possess higher toxicity. Hence, there is an urgent need to explore new antimicrobial compounds that may have lower toxicity such as naturally derived compounds. In the work reported herein, we investigate the chemical constituents isolated from cultivated Alpinia conchigera Griff. and their antimicrobial activity against MRSA.

MATERIAL AND METHODS

General

NMR spectra were obtained using Bruker Advance 500 (500 MHz for $^1$H NMR, 125 MHz for $^{13}$C NMR) spectrometer system. Data were analysed via Top Spin software package. Spectra were referenced to TMS or residual solvent (CDCl$_3$ = 7.26 ppm in $^1$H NMR and 77.0 ppm in $^{13}$C NMR). $^1$H NMR spectroscopic data is reported as follows: chemical shift (relative integral, multiplicity [s – singlet, d – doublet, dd– doublet of doublets, spin – spin coupling constant ($J$ in Hertz, Hz), identifier. Mass spectra were carried out on Perkin Elmer GC-MS spectrometer (Perkin Elmer Clarus 600T GCMS System). Fourier transform infrared spectra were obtained using a Perkin-Elmer Spectrum 1000 series Fourier Transform IR spectrometer with Universal Attenuated Total Reflectance (Diamond) sampling accessory. Absorption maxima are expressed as wave numbers (cm$^{-1}$).

Material

The rhizome of Alpinia conchigera Griff. was collected in Jeli, Kelantan and cultivate in Ulu Langat, province of Selangor, west-coast of Peninsular Malaysia and the series number was given as KL5049 (Fig. 1). This species was identified by Professor Dr. Halijah Ibrahim from Institute of Biological Sciences, University of Malaya and a voucher specimen were identified and deposited in the herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur.

Gas Chromatography (GC)

GC analysis was performed on Agilent Technologies 7890A GC System, fitted with 30 m $\times$ 0.32 mm i.d $\times$ 0.25 µm HP-5 capillary column, using nitrogen as the carrier gas. The oven temperature was programmed from 60°C to 280°C at 20°C/min and the end temperature was held for 1 min.
Gas Chromatography Mass Spectrometry (GCMS)

Analyses were carried out on Perkin Elmer Clarus 600T mass spectrometer, directly coupled to Perkin Elmer Clarus 600 gas chromatograph with 30 m × 0.25 mm i.d × 0.25 µm HP-5MS capillary column, using helium as the carrier gas. The oven temperature was programmed from 60°C to 280°C at 20°C/min and the end temperature was held for 1 min.

**Figure 1:** *Alpinia conchigera* Griff.
Extraction and Isolation

The dry and ground rhizomes (1.5 kg) were extracted using maceration method with \( n \)-hexane, dichloromethane (DCM) and methanol (MeOH) successively. The extracts were dried \textit{in vacuo} using rotary evaporator. The \( n \)-hexane and DCM were further subjected to chemical isolation and antibacterial studies. These extracts were subjected to column chromatography (CC) on silica gel using stepwise gradient system (\( n \)-hexane to ethyl acetate).

The crude extracts of \( n \)-hexane (1.95 g) was fractionated using silica gel CC. The extract was dry packing on celite and loaded on a 100 x 15 cm glass column packed with 50 g of silica gel. The column was eluted using a \( n \)-hexane: ethyl acetate gradient (1:0 to 5:5 v/v) to give 71 fractions. The eluate was collected in the test tube and combined based on the basic of TLC profile. Combination of eluent from fraction 1 (FrA1) gave compound 1 (0.5 g, yellowish oil), compound 5 (1.3 mg, yellowish oil) and compound 3 (9.0 mg, yellowish oil). Fraction 3 (FrA1.3) was re-chromatographed using 60 x 8 cm glass column packed with 5 g silica gel and eluted with \( n \)-hexane: ethyl acetate (1:0 to 6:4 v/v). From this process 68 fractions were obtained. Combination of eluent from fraction FrA1.3.2 gave compound 2 (0.2 g, yellowish oil). Meanwhile, the crude extracts of DCM (9.60 g) was subjected to CC. The eluate was collected and combined into four fractions (FrB1 to FrB4). Re-column fractions FrB2 gave compound 1 (2.2 g, yellowish oil) and compound 2 (0.2 g, yellowish oil). While analysis of fraction FrB3 gave mixture of compounds 6 and 7 (0.1 g, white solid) and fraction FrB4 gave a compound 4 (1.0 mg, yellow oil), respectively.

All compounds were successfully characterised using spectroscopic methods and by comparing the data analysis with the literature values. The list of chemical compounds isolated from \textit{Alpinia conchigera} Griff. was shown in Fig. 3 and Tables 2–5.

1’S-1’-acetoxychavicol acetate (1):

Yellowish oil. IR (cm\(^{-1}\)): 2932, 1760 (C=O ester), 1607, 1370, 1234 (C-O ester), 1204, 1018. \(^1\)H NMR (500 MHz, CDCl\(_3\), \(\delta\) ppm) 2.08 (3H, s, H5’), 2.27 (3H, s, H2”), 5.22 (2H, dd, \(J = 14.6, 10.5\) Hz, H3’), 5.98 (1H, m, H2’), 6.23 (1H, d, \(J = 5.8\) Hz, H1’), 7.03 (2H, d, \(J = 8.8\) Hz, H3, H5), 7.33 (2H, d, \(J = 8.8\) Hz, H2, H6). \(^13\)C NMR (125 MHz, CDCl\(_3\), \(\delta\) ppm) 21.2 (C5’), 21.3 (C2”), 75.6 (C1’), 117.2 (C3’), 121.7 (C3, C5), 128.5 (C2, C6), 136.1 (C2”), 136.5 (C1), 150.5 (C4), 169.4 (C1”), 169.7 (C4”). GCMS m/z (EI) 77, 103, 132, 150, 192, 234 (M\(^{+}\))

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trans-\textit{p}\text{-coulmaryl diacetate (2)}

Yellowish oil. IR (cm\textsuperscript{-1}): 2918, 1705 (C=O ester), 1464, 1229 (C-O ester). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}, δ ppm) 2.10 (3H, s, H2\textsuperscript{r}), 2.29 (3H, s, H5\textsuperscript{r}), 4.70 (2H, d, J = 5.1, H3\textsuperscript{r}), 6.22 (1H, m, H2\textsuperscript{r}), 6.60 (1H, d, J = 15.9 Hz, H1\textsuperscript{r}), 7.03 (2H, d, J = 8.6 Hz, H3, H5), 7.38 (2H, d, J = 8.6 Hz, H2, H6). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}, δ ppm) 20.9 (C2\textsuperscript{r}), 21.0 (C5\textsuperscript{r}), 64.8 (C3\textsuperscript{r}), 121.5 (C3, C5), 121.6 (C2\textsuperscript{r}), 127.5 (C2, C6), 133.0 (C1\textsuperscript{r}), 133.9 (C1), 150.3 (C4), 169.3 (C1\textsuperscript{r}), 170.7 (C4\textsuperscript{r}). GCMS m/z (EI) 121, 133, 149, 150, 192, 234 (M\textsuperscript{+}).

\textit{p}\text{-hydroxycinnamyl acetate (3)}

Yellowish oil. IR (cm\textsuperscript{-1}): 3400 (OH stretch), 1737 (C=O ester). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}, δ ppm) 2.30 (3H, s, 5\textsuperscript{r}), 4.31 (2H, d, J = 5.6 Hz, H3\textsuperscript{r}), 6.32 (1H, m, H2\textsuperscript{r}), 6.58 (1H, d, J = 15.9 Hz, H1\textsuperscript{r}), 7.03 (2H, d, J = 8.7 Hz, H3, H5), 7.38 (2H, d, J = 8.7 Hz, H2, H6). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}, δ ppm) 21.1 (C5\textsuperscript{r}), 63.6 (C3\textsuperscript{r}), 121.7 (C3, C5), 127.4 (C2, C6), 128.7 (C2\textsuperscript{r}), 130.1 (C1\textsuperscript{r}), 134.5 (C4), 150.1 (C1), 169.5 (C4\textsuperscript{r}). GCMS m/z (EI) 77, 94, 107, 150, 192 (M\textsuperscript{+}).

1\textsuperscript{r}\textit{S}-1\textsuperscript{r}-hydroxychavicol acetate (4)

Yellowish oil. IR (cm\textsuperscript{-1}): 3080 (OH stretch), 1756 (C=O ester), 1201(C-O). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}, δ ppm) 2.32 (3H, s, H2\textsuperscript{r}), 5.18 (2H, dd, J = 17.1, 9.3 Hz, H3\textsuperscript{r}), 5.32 (1H, d, J = 6.7, H1\textsuperscript{r}), 6.01 (1H, m, H2\textsuperscript{r}), 7.05 (2H, d, J = 8.5 Hz, H3, H5), 7.36 (2H, d, J = 8.5 Hz, H2, H6). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}, δ ppm) 21.2 (C2\textsuperscript{r}), 115.4 (C1\textsuperscript{r}), 121.5 (C3, C5), 127.3 (C2, C6), 140.0 (C1), 140.1 (C2\textsuperscript{r}), 150.2 (C4), 169.4 (C4\textsuperscript{r}). GCMS m/z (EI) 55, 65, 74, 95, 107, 121, 133, 150, 192 (M\textsuperscript{+}).

\textit{p}\text{-hydroxybenzaldehyde (5)}

Yellowish oil. IR (cm\textsuperscript{-1}): 3369 (OH stretch), 1683 (C=O aldehyde), 860. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}, δ ppm) 6.97 (2H, d, J = 8.8 Hz, H2, H6), 7.81 (2H, d, J = 8.8 Hz, H3, H5), 9.82 (1H, s, H1\textsuperscript{r}). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}, δ ppm) 116.1 (C2, C6), 129.7 (C1), 132.6 (C3, C5), 161.8 (C4), 191.4 (C1\textsuperscript{r}). GCMS m/z (EI) 65, 74, 93, 110, 122 (M\textsuperscript{+}).
Stigmasterol (6)

White solid. IR (cm$^{-1}$): 3430 (OH stretch), 1653 (C=C) 1463, 1049. $^1$H NMR (500 MHz, CDCl$_3$, $\delta$ ppm) 0.67 (3H, s, H18), 0.78 (3H, d, J = 6.5 Hz, H27), 0.81 (3H, t, J = 7.8 Hz, H29), 0.83 (3H, d, J = 6.5 Hz, H26), 0.92 (1H, m, H9), 1.00 (3H, s, H19), 1.01 (1H, m, H14), 1.01 (3H, s, H21), 1.06 (1H, m, H1b), 1.06 (1H, m, H15b), 1.13 (1H, m, H17), 1.15 (1H, m, H28b), 1.15 (1H, m, H12b), 1.43 (1H, m, H28a), 1.46 (1H, m, H8), 1.50 (1H, m, H2b), 1.50 (1H, m, H7b), 1.50 (1H, m, H11), 1.52 (1H, m, H24), 1.53 (1H, m, H25), 1.56 (1H, m, H15a), 1.65 (1H, m, H16), 1.82 (1H, m, H1a), 1.82 (1H, m, H2a), 1.98 (1H, m, H7a), 1.98 (1H, m, H12a), 2.01 (1H, m, H20), 2.22 (1H, m, H4b), 2.29 (1H, m, H4a), 3.50 (1H, m, H3), 5.03 (1H, dd, J = 15.6, 8.7 Hz, H23), 5.17 (1H, dd, J = 15.6, 8.7 Hz, H22), 5.33 (1H, m, H6). $^{13}$C NMR (125 MHz, CDCl$_3$, $\delta$ ppm) 12.0 (C18), 12.2 (C29), 19.0 (C27), 19.4 (C19), 21.2 (C26), 21.1 (C11), 21.1 (C21), 25.4 (C28), 24.3 (C15), 28.9 (C16), 31.6 (C2), 31.9 (C25), 31.9 (C7), 31.9 (C8), 36.5 (C10), 37.2 (C1), 39.7 (C12), 40.5 (C20), 42.3 (C4), 42.3 (C13), 50.1 (C9), 51.2 (C24), 55.9 (C17), 56.8 (C14), 71.8 (C3), 121.7 (C6), 129.2 (C23), 138.3 (C22), 140.7 (C5). GCMS m/z (El) 119, 145, 173, 199, 271, 300, 327, 369, 412 (M$^+$)

$\beta$-sitosterol (7)

White solid. IR (cm$^{-1}$): 3430 (OH stretch), 1463, 1049. $^1$H NMR (500 MHz, CDCl$_3$, $\delta$ ppm) 0.67 (3H, s, H18), 0.81 (3H, d, J = 6.5 Hz), 0.83 (3H, d, J = 6.5 Hz), 0.85 (3H, t, J = 7.8 Hz), 0.92 (1H, m, H24), 0.92 (1H, m, H9), 0.92 (3H, s, H21), 1.00 (3H, s, H19), 1.01 (1H, m, H14), 1.01 (1H, m, H22b), 1.06 (1H, m, H1b), 1.06 (1H, m, H15b), 1.13 (1H, m, H17), 1.15 (1H, m, H23), 1.24 (1H, m, H28), 1.30 (1H, m, H22a), 1.35 (1H, m, H20), 1.46 (1H, m, H8), 1.50 (1H, m, H2b), 1.50 (1H, m, H7b), 1.50 (1H, m, H11), 1.56 (1H, m, H15a), 1.65 (1H, m, H16), 1.65 (1H, m, H25), 1.82 (1H, m, H1a), 1.82 (1H, m, H2a), 1.98 (1H, m, H7a), 1.98 (1H, m, H12a), 3.50 (1H, m, H3), 2.22 (1H, m, H4b), 2.29 (1H, m, H4a), 5.33 (1H, m, H6). $^{13}$C NMR (125 MHz, CDCl$_3$, $\delta$ ppm) 11.8 (C18'), 12.0 (C29'), 18.8 (C27'), 19.4 (C19'), 19.8 (C26'), 21.1 (C11'), 21.1 (C21'), 23.0 (C28'), 24.3 (C15'), 26.0 (C23'), 28.2 (C16'), 29.1 (C25'), 31.6 (C2'), 31.9 (C8'), 31.9 (C7'), 33.9 (C22'), 36.1 (C20'), 36.5 (C10'), 37.2 (C1'), 39.8 (C12'), 40.2 (C4'), 42.3 (C13'), 45.8 (C24'), 50.1 (C9'), 56.0 (C17'), 56.7 (C14'), 71.8 (C3'), 121.7 (C6'), 140.7 (C5'). GCMS m/z (El) 55, 81, 105, 133, 159, 187, 213, 273, 382, 414 (M$^+$)
Chemical Transformation of 1'S-1'-acetoxychavicol acetate (1) into trans-p-coumaryl diacetate (2)

The chemical transformation of (1) into (2) was done by modification of Azuma's procedure (Azuma et al. 2006). 1'S-1'-acetoxychavicol acetate (1) (0.2 g) and sodium acetate (0.2 g) were dissolved in acetic acid (10 mL). After stirring overnight at 60°C, the mixture was neutralised with saturated aqueous NaHCO₃ and extracted with EtOAc (3 × 10 mL). The organic layer was dried with Na₂SO₄ and the solvents were removed in vacuo using rotary evaporator. The obtained residue was analysed directly without purification and the spectroscopic data was identical to that of natural trans-p-coumaryl diacetate (2).

Preparation of Bacterial Inoculum

MRSA (American Type Culture Collection 43300) was cultured in Brain Heart Infusion Broth overnight in a rotary shaker at 37°C, centrifuged at 200 rpm. The cultures pellet was then resuspended 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 × 10⁷ colony forming units (CFU) per mL.

Antimicrobial Activity

Antimicrobial activity was measured by using broth microdilution technique on sterile 96-well microtiter plates. Briefly, all the tested compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 31.25 µg/mL. 100 µL of double strength Muller Hilton broth was distributed to each row of the wells. Then, 100 µL of each test compound solution was serially diluted across the wells in duplicate, starting from a concentration of 4000 µg/mL to 31.25 µg/mL, the final well of each row left without addition of test compound to serve as a negative control. In addition, tetracycline was used as positives control as comparison for antimicrobial potential.

5 µL of bacterial suspension adjusted to a 0.5 MacFarland turbidity standard was inoculated into each antimicrobial-containing and control (antimicrobial free) wells. Plates were wrapped loosely with a cling film to avoid suspension dehydration and was incubated at 37°C, under aerobic conditions, for 24 h. At the end of the incubation period, 50 µL tetrazolium solution was added into all wells and the plates were re-incubated for an additional hour. The minimum inhibitory concentration (MIC) determined as the last well before the tetrazolium color changes from yellow to purple indication microbial growth.
RESULTS AND DISCUSSION

Maceration extraction of the rhizomes yielded 1.95 g (0.13% w/w) of \(n\)-hexane extracts, 9.60 g (0.64% w/w) of DCM extracts and 11.7 g (0.78% w/w) of MeOH extracts (Table 1). The \(n\)-hexane and DCM crudes were injected into a GC system to give the chromatograms (Fig. 2), in which compound 1 gave a strong peak at \(t_R = 7.00\) min. From these chromatograms, it showed that compound 1 was the major component in \textit{Alpinia conchigera} Griff.

Table 1: Weight and percentage of crude extracts from the rhizomes of \textit{Alpinia conchigera} Griff.

| Plant material | Crude extract | Weight of crude extract (g) | Yield of crude extract (%) |
|---------------|--------------|----------------------------|---------------------------|
| Rhizomes      | \(n\)-hexane| 1.95                       | 0.13                      |
|               | Dichloromethane | 9.60                      | 0.64                      |
|               | Methanol      | 11.7                       | 0.78                      |

Figure 2: Gas chromatography chromatograms for \(n\)-hexane and dichloromethane extracts.
Seven compounds were successfully isolated from the rhizomes of *Alpinia conchigera* Griff (Fig. 3 and Table 2). The isolation of *n*-hexane crude extract afforded four phenylpropanoids compounds which known as 1'S-1'-acetoxychavicol acetate (1), *trans*-*p*-coumaryl diacetate (2) and *p*-hydroxycinnamyl acetate (3), and one phenolic compound; *p*-hydroxybenzaldehyde (5). Meanwhile, the DCM crude extract gave three phenylpropanoids; 1'S-1'-acetoxychavicol acetate (1), *trans*-*p*-coumaryl diacetate (2) and 1’S-1’-hydroxychavicol acetate (4) and a mixture of triterpenes; stigmasterol (6) and β-sitosterol (7). The NMR spectra results are presented in Table 3–5, respectively.

![Chemical structures of compounds 1–7](image)

**Figure 3**: The structure of compounds 1–7 isolated from the rhizomes of *Alpinia conchigera* Griff.

**Table 2**: Chemical compounds isolated from the rhizomes of *Alpinia conchigera* Griff.

| Crude extracts    | Name of compound                  | % yield of compounds |
|-------------------|-----------------------------------|----------------------|
| *n*-hexane        | 1'S-1'-acetoxychavicol acetate (1) | 25.64                |
|                   | *trans*-*p*-coumaryl diacetate (2) | 7.91                 |
|                   | *p*-hydroxycinnamyl acetate (4)   | 0.05                 |
|                   | *p*-hydroxybenzaldehyde (5)       | 0.07                 |
| Dichloromethane   | 1’S-1’-acetoxychavicol acetate (1) | 23.11                |
|                   | *trans*-*p*-coumaryl diacetate (2) | 2.20                 |
|                   | 1’S-1’-hydroxychavicol acetate (4) | 0.01                 |
|                   | Stigmasterol (6)                   | 5.21                 |
|                   | β-sitosterol (7)                    |                      |
Compound 1 was obtained as a yellowish oil. The IR spectrum of compound 1 showed absorption bands indicative of an ester functionality at 1760 cm\(^{-1}\) (C=O ester) and 1234 cm\(^{-1}\) (C-O ester). The GC-EIMS of compound 1 displayed a molecular ion peak at m/z 234 and six important fragment ions at m/z 192 [M\(^{+}\)- CH\(_3\)=C-O], 150 [M\(^{+}\)- CH\(_2\)=C-O], 149, 132 [M\(^{+}\)- C-O], 104 and 77 [M\(^{+}\)- CH\(_2\)=CH\(_2\)]. The presence of this phenylpropanoid skeleton was disclosed by analysis of \(^1\)H NMR, \(^{13}\)C NMR, COSY and HMBC spectral data (Table 3 and Fig. 4). The \(^1\)H NMR spectrum showed two methyl at \(\delta_H\) 2.08 (3H, s) and \(\delta_H\) 2.27 (3H, s) corresponding to the methyl groups attached directly with the ester carbonyl groups, C-5' and C-2" respectively. The olefin proton H-2' and H-3' appeared as a multiplet and doublet of doublet at \(\delta_H\) 5.98 (1H, \(m\)) and \(\delta_H\) 5.26 (2H, \(dd\), \(J = 10.5, J' = 14.6\)). A set of doublets corresponding to four proton signals of H-2/H-6 and H-3/H-5 were appeared at \(\delta_H\) 7.33 (2H, \(d\), \(J = 8.8\)) and \(\delta_H\) 7.03 (2H, \(d\), \(J = 8.8\)) respectively indicating that the aromatic ring is 1,4-disubstituted. The COSY \(^1\)H-\(^1\)H spectrum nicely displays the connectives within the allylic moiety H-1', H-2' and H-3', demonstrating that in a COSY spectrum not only vicinal and geminal but also allylic spin coupling constants yield cross peaks (Fig. 4). The \(^{13}\)C NMR spectrum reveals a typical pattern of \textit{para}-substituted aromatic compound. The assignment for two ester carbonyl carbon atoms C-1" and C-4' at \(\delta_C\) 169.4 and \(\delta_C\) 169.7 are obvious. The peak at \(\delta_C\) 75.6 could be assigned to methane C-1' attached to the terminal alkene. The peak at \(\delta_C\) 136.1 might be attributed to C-2', while at \(\delta_C\) 117.2 to C-3' of the terminal vinyl group. Furthermore, the peak at \(\delta_C\) 136.5 and \(\delta_C\) 150.5 might be attributed to the quaternary carbons, C-1 and C-4 respectively. In addition, the \(sp^2\) carbon at position C-2/C-6 and C-3/C-5 resonated at \(\delta_C\) 128.5 and \(\delta_C\) 121.7. Comparison of the observed data with the literature value (Watanabe \textit{et al.} 1995) confirmed that compound 1 was \(1'S-1'\)-acetoxychavicol acetate.

Compound 2 was obtained as yellowish oil. The IR spectrum of compound 2 showed absorption bands indicative of an ester functionality at 1705 cm\(^{-1}\) (C=O ester) and 1229 cm\(^{-1}\) (C-O ester). The molecular formula of C\(_{13}\)H\(_{14}\)O\(_4\) was determined by GC-EIMS at m/z 234 [M\(^{+}\)] (calc'd 234.2479). Analysis of the \(^1\)H NMR, \(^{13}\)C NMR and HMBC data of compound 2 revealed the characteristic of phenylpropanoid, including 1,4-disubstituted benzene ring (Table 3 and Fig. 4). The \(^1\)H NMR spectrum of compound 2 displayed two set of doublets \(\delta_H\) 7.03 (2H, \(d\), \(J = 8.6\)) and 7.38 (2H, \(d\), \(J = 8.6\)) corresponding to two protons each, attributable to H-3/H-5 and H-2/H-6 respectively. Two methyl singlets at \(\delta_H\) 2.29 (3H, s) and \(\delta_H\) 2.10 (3H, s) corresponding to the methyl groups attached directly with the ester carbonyl groups, C-5' and C-2" respectively. In aromatic region, an AX spin system at \(\delta_H\) 6.22 and 6.60 with a spin coupling of 15.9 Hz, which can easily be assigned to the protons H-2' and H-1' of the \textit{trans} olefinic bond. The \(^{13}\)C NMR spectrum reveals a typical pattern of \textit{para}-substituted aromatic compound. The long-range correlations between H-3' and the carbonyl carbon at \(\delta_C\) 170.7 (C-4'), H-1' and C-3' (\(\delta_C\) 64.8), H-2 and C-1', C-4 in HMBC spectrum (Fig. 4) confirmed the connection of a 1,4-disubstituted benzene ring with C-1'. The presence of two acetyl groups in these phenylpropanoid were deduced from the proton signals at \(\delta_H\) 2.10 (3H, s) and
Table 3: $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data of compounds 1–3 (CDCl$_3$).

| Position | 1 | 2 | 3 |
|----------|---|---|---|
|          | $\delta_\text{H}$ ($J$ in Hz) | $\delta_\text{C}$ | $\delta_\text{H}$ ($J$ in Hz) | $\delta_\text{C}$ | $\delta_\text{H}$ ($J$ in Hz) | $\delta_\text{C}$ |
| 1        | - | 136.5 | - | 133.9 | - | 150.1 |
| 2        | 7.33 (2H, d, $J = 8.8$) | 128.5 | 7.38 (2H, d, $J = 8.6$) | 127.5 | 7.38 (2H, d, $J = 8.8$) | 127.4 |
| 3        | 7.03 (2H, d, $J = 8.8$) | 121.7 | 7.03 (2H, d, $J = 8.6$) | 121.5 | 7.03 (2H, d, $J = 8.5$) | 121.7 |
| 4        | - | 150.5 | - | 150.3 | - | 134.5 |
| 5        | 7.03 (2H, d, $J = 8.8$) | 121.7 | 7.03 (2H, d, $J = 8.6$) | 121.5 | 7.03 (2H, d, $J = 8.5$) | 121.7 |
| 6        | 7.33 (2H, d, $J = 8.8$) | 128.5 | 7.38 (2H, d, $J = 8.6$) | 127.5 | 7.38 (2H, d, $J = 8.8$) | 127.4 |
| 1\'      | 6.23 (1H, d, $J = 5.8$) | 75.6 | 6.60 (1H, d, $J = 15.9$) | 133.0 | 6.58 (1H, d, $J = 15.9$) | 130.1 |
| 2\'      | 5.98 (1H, m) | 136.1 | 6.22 (1H, m) | 121.6 | 6.32 (1H, m) | 128.7 |
| 3\'      | 5.22 (2H, dd) ($J = 10.5$, $J = 14.6$) | 117.2 | 4.70 (2H, d, $J = 5.1$) | 64.8 | 4.31 (2H, d, $J = 5.6$) | 63.6 |
| 4\'      | - | 169.7 | - | 170.7 | - | 169.5 |
| 5\'      | 2.08 (3H, s) | 21.2 | 2.29 (3H, s) | 21.0 | 2.30 (3H, s) | 21.1 |
| 1\"      | - | 169.4 | - | 169.3 |
| 2\"      | 2.27 (3H, s) | 21.3 | 2.10 (3H, s) | 20.9 |
$\delta_H$ 2.29 (3H, s) and the carbon signals at $\delta_C$ 169.3 (C-1"), 20.9 (C-2"), 170.7 (C-4’) and 21.0 (C-5’). The locations of the acetyl group at C-3’ was determined by the $^3J$ (C-H) correlation between H-3’ and C-4’. Thorough analysis of the observed data and comparison with the literatures (Matsuda et al. 2003a,b) led to the deduction that compound 2 is trans-p-coumaryl diacetate.

Compound 3 was isolated as yellowish oil. The GC-EIMS of compound 3 revealed a molecular ion peak corresponding to [M+] at m/z 192 indicating the molecular formula C$_{11}$H$_{12}$O$_{3}$. The IR spectrum showed absorption band at 3400 cm$^{-1}$ (OH) and 1737 cm$^{-1}$ (C=O ester). The $^1$H NMR and $^{13}$C NMR spectroscopic data of compound 3 (Table 3) indicated phenylpropanoid skeleton, which is similar to that of compound 2; both have acetyl moiety at C-3’ ($\delta_C$ 64.8 in 2; 63.6 in 3) and trans double bond ($\delta_C$ 133.0, 121.6 in 2; 130.1, 128.7 in 3) directly attached to 1,4-disubstituted benzene ring. The principal difference in 1D NMR spectra of compound 3 was that with the replacement of acetyl moiety with hydroxy bearing quaternary carbon at C-4 ($\delta_C$ 134.5). The structure was further confirmed by HMBC (Fig. 4) correlation between (H-5/C-4’; H-3’/C-2’; H-2’/C-3’, C-1’; H-1’/C-3’, C-2, C-6; H-3, H-5/C-4, C-1; H-2, H-6/C-3, C-5, C-1’, C-1) respectively. Similarly, the doublet attributed to H-1’ showed $J = 15.9$ Hz, which is an indicative of trans configuration at C-1’/C-2’. Thus, upon comparison of the obtained data and the literature values (Matsuda et al. 2003a,b; 2005), one may conclude that compound 3 is p-hydroxycinnamyl acetate.

Figure 4: HMBC and $^1$H-$^1$H COSY correlation of compounds 1–5.
Compound 4 was isolated as yellow oil. The GC-EIMS of compound 4 revealed a molecular ion peak corresponding to [M+] at m/z 192 indicating the molecular formula C_{11}H_{12}O_{3}. The ^1H NMR of compound 4 showed all features of chavicol acetate. The IR spectrum displayed absorption bands at 3080 cm^{-1} (OH), 1756 cm^{-1} (aliphatic ester C=O) and 1201 cm^{-1} (ester moiety C-O). The ^1H NMR spectrum displayed the typical AA'XX' pattern of a para-substituted benzene ring (Table 4). A strong singlet at δ_H 2.32 (3H, s) indicated a methyl group attached directly with the ester carbonyl group. Existence of a doublet at δ_H 5.18 (1H, d, J = 9.3) is belong to the methine group, H-1' which directly attached to the benzene ring, the hydroxyl group and the ethylene group. Furthermore, the doublet at δ_H 5.32 might be attributable to ethylene proton H-3'a (1H, d, J = 17.1) while H-2' emerged as a multiplet at 6.01 (1H, m). A set of doublets corresponding to four proton signals of H-2/H-6 and H-3/H-5 were appeared at δ_H 7.36 (2H, d, J = 8.6) and δ_H 7.05 (2H, d, J = 8.5) respectively. The ^13C NMR spectrum of compound 4 showed the presence of 11 carbon atoms. The DEPT experiment indicated the presence of one ethyl, one CH₂, six CH groups and three quaternary carbons. The ^13C NMR spectrum of compound 4 also showed all the features of chavicol acetate (Table 4). The ^13C NMR spectrum indicates the presence of ethylene carbon (δ_C 115.4 and 140.1), carbonyl group (δ_C 169.5) and methyl signal at δ_C 21.1. Further it also displayed signal at δ_C 140.0 and δ_C 150.1 due to quaternary carbon corresponding to para-disubstituted benzene ring. Meanwhile, the δ_C 140.1 is assignable to methine carbon C-2'. The complete assignment of protons and carbons was assisted by HMBC, COSY and HSQC experiments. The carbon skeletons suggested by several diagnostic correlations (H-2"/C-4, C-1"; H-1'/C-3', C-2, C-6, C-2'; H-3'/C-1', C-2'; H-2'/C-1'; H-3, H-5/C-1, C-4; H-2, H-6/C-1', C-4) and ^1H-1H-COSY (H-2/H-3; H-5/H-6; H-2'/H-3'). All the key HMBC correlations are depicted in Fig. 4. Based on these data and comparison with literature values (Jansen & Scheffer 1985), compound 4 was identified as (S)-4-(1-hydroxyally) phenyl acetate, a known phenylpropanoid and trivially named as 1'S-1'-hydroxychavicol acetate.

Compound 5 was obtained as yellowish oil. The IR spectrum exhibited absorption bands of carbonyl group at 1683 cm^{-1} (aldehyde) and hydroxyl group at 3369 cm^{-1}. The molecular formula C_{7}H_{6}O_{2} was deduced on the basis of GC-EIMS (m/z 122 [M⁺]; calcd 122.1213). The ^1H NMR data showed at δ_H 6.95 (2H, d, J = 8.8) and δ_H 7.81 (2H, d, J = 8.3) showed the presence of para-substituted benzene ring and methine signal at δ_H 9.87 (1H, s) (Table 4). The ^13C NMR spectra revealed the presence of aldehyde conjugated with aromatic ring, resonating at δ_C 191.4 and a quaternary carbon linked with OH group, confirmed by the signal at δ_C 161.8. The remaining five carbon signal are for methine unit at 1,4-disubstituted benzene ring (δ_C 116.1 x 2 and 132.6 x 2) and a quaternary carbon at δ_C 129.7 (C-1) (Table 4). Comparison of ^1H NMR and ^13C NMR data of compound 5 with those of the known compound indicated that this compound was ρ-hydroxybenzaldehyde.
Table 4: $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data of compound 4–5 (CDCl$_3$).

| Position | $\delta$$_H$ (J in Hz) | $\delta$$_C$ | $\delta$$_H$ (J in Hz) | $\delta$$_C$ |
|----------|-------------------------|--------------|-------------------------|--------------|
| 1        | -                       | 140.0        | -                       | 129.7        |
| 2        | 7.36 (2H, d, $J$ = 8.6) | 127.3        | 6.97 (2H, d, $J$ = 8.6) | 116.1        |
| 3        | 7.05 (2H, d, $J$ = 8.5) | 121.5        | 7.81 (2H, d, $J$ = 8.6) | 132.6        |
| 4        | -                       | 150.2        | -                       | 161.8        |
| 5        | 7.05 (2H, d, $J$ = 8.5) | 121.5        | 7.82 (2H, d, $J$ = 8.6) | 132.6        |
| 6        | 7.36 (2H, d, $J$ = 8.6) | 127.6        | 6.97 (2H, d, $J$ = 8.6) | 116.1        |
| 1'       | 5.18 (1H, d, $J$ = 9.3) | 74.7         | 9.82 (1H, s)            | 191.4        |
| 2'       | 6.01 (1H, m)            | 140.2        |                         |              |
| 3'a      | 5.18 (1H, d, $J$ = 9.3) | 115.2        |                         |              |
|          | 5.32 (1H, d, $J$ = 17.1)|             |                         |              |
| 1"       | -                       | 169.4        |                         |              |
| 2"       | 2.32 (3H, s)            | 21.2         |                         |              |

Compounds 6 and 7 were obtained as a mixture which is a white crystalline substance. These substances give the positive test to Salkowski and Liebermann-Burchard reagent for steroidal nucleus (Jain et al. 2009; Habib et al. 2007). The mass spectrum displays the typical pattern of polycyclic compound with many CH$_2$ and CH groups. The spectrum showed a parent molecular ion [M$^+$] peak at m/z 412 and 414, which corresponded to the molecular formula C$_{29}$H$_{48}$O and C$_{29}$H$_{50}$O respectively. The fragmentation peaks for compound 6 observed at m/z 412, 369, 327, 300, 271, 199, 173, 145 and 119, while m/z 414, 382, 273, 213, 187, 159, 133, 105, 81 and 55 for compound 7. The IR spectroscopic analysis led to observation of some important bands 3430 cm$^{-1}$ (hydroxyl stretching), 1653 cm$^{-1}$ (C=C absorption), 1463 cm$^{-1}$ (cyclic (CH$_2$)$_n$) and 1378 cm$^{-1}$ (–CH$_2$(CH$_3$)$_2$γ). The absorption frequency at 1049 cm$^{-1}$ signifies cycloalkane. Compounds 6 and 7 have an identical sterol skeleton. Thus, the $^1$H and $^{13}$C NMR spectrums are similar to one another (Table 5). The $^1$H NMR spectrum of both compounds have revealed the existence of singlets at $\delta$$_H$ 0.67 and $\delta$$_H$ 1.00 corresponding to H-18/H-18' and H-19/H-19’ methyl protons. Other methyl protons (H-21/H-21’, H-26/H-26’, H-27/H-27’ and H-29/H-29’) resonated between $\delta$$_H$ 0.65 to $\delta$$_H$ 1.00. The H-3/H-3’ protons appeared as a multiplet at $\delta$$_H$ 3.50, and H-6/H-6’ olefinic proton signals appeared as a multiplet at $\delta$$_H$ 5.33. The most significant difference on the $^1$H NMR chemical shift of these two molecules was the olefinic proton signals for H-22 and H-23 of compound 6, which resonated as a doublet of doublet signals at $\delta$$_H$ 5.17 (1H, dd, $J_{22}$ = 8.7, $J'_{22}$ = 15.6) and $\delta$$_H$ 5.03 (1H, dd, $J_{23}$ = 8.7, $J'_{23}$ = 15.6) respectively. The C-22’ and C-23’ ethylene proton signals of compound 7 resonated as multiplets in the region of $\delta$$_H$ 0.90 to $\delta$$_H$ 1.30. The rest of the protons resonated as multiplets in the region of $\delta$$_H$ 0.70 to $\delta$$_H$ 3.50. Many signals of both compounds were overlapping.
and gave the same $^1$H NMR signals. Since compounds 6 and 7 have an identical sterol skeleton, the $^{13}$C NMR spectrum for both compounds showed similar profile. The main different between compounds 6 and 7 were the signals of C-22/C-22' and C-23/C-23'. For compound 6, the sp$^2$ carbons; C-22 and C-23 resonated at $\delta_C$ 138.3 and $\delta_C$ 129.2 respectively. While for compound 7, the two carbons resonated at $\delta_C$ 33.9 and $\delta_C$ 26.0 respectively. Another important signal was observed at 71.8 ppm corresponded to C-3 β-hydroxy group. The presence of the double bond also moved C-20, C-21, C-24, C-25 and C-28 further downfield at $\delta_C$ 40.5, 21.1, 51.2, 31.9 and 25.4 respectively as compared to that of compound 7, which showed the signals at $\delta_C$ 36.1, 21.1, 45.8, 29.1 and 23.0 for C-20', C-21', C-24', C-25' and C-28' respectively. From the above observations and comparison of the obtained spectral data with the literature values (Jayaprakasha et al. 2007; Forgo & Kövér 2004), compounds 6 and 7 were found to be stigmasterol and β-sitosterol, respectively.

**Table 5: $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data of compound 6–7 (CDCl$_3$)**

| Position | 6      | 7      |
|----------|--------|--------|
|          | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ |
| 1        | 1.82 (1H, m) | 37.2 | 1.82 (1H, m) | 37.2 |
|          | 1.06 (1H, m) |     | 1.06 (1H, m) |     |
| 2        | 1.82 (1H, m) | 31.6 | 1.82 (1H, m) | 31.6 |
|          | 1.50 (1H, m) |     | 1.50 (1H, m) |     |
| 3        | 3.50 (1H, m) | 71.8 | 3.50 (1H, m) | 71.8 |
| 4        | 2.29 (1H, m) | 42.3 | 2.29 (1H, m) | 40.2 |
|          | 2.22 (1H, m) |     | 2.22 (1H, m) |     |
| 5        | -       | 140.7 | -       | 140.7 |
| 6        | 5.33 (1H, m) | 121.7 | 5.33 (1H, m) | 121.7 |
| 7        | 1.98 (1H, m) | 31.9 | 1.98 (1H, m) | 31.9 |
|          | 1.50 (1H, m) |     | 1.50 (1H, m) |     |
| 8        | 1.46 (1H, m) | 31.9 | 1.46 (1H, m) | 31.9 |
| 9        | 0.92 (1H, m) | 50.1 | 0.92 (1H, m) | 50.1 |
| 10       | -       | 36.5 | -       | 36.5 |
| 11       | 1.50 (1H, m) | 21.1 | 1.50 (1H, m) | 21.1 |
| 12       | 1.98 (1H, m) | 39.7 | 1.98 (1H, m) | 39.8 |
|          | 1.15 (1H, m) |     | 1.15 (1H, m) |     |
| 13       | -       | 42.3 | -       | 42.3 |
| 14       | 1.01 (1H, m) | 56.8 | 1.01 (1H, m) | 56.7 |

(Continued on next page)
A major compound, compound 1, was proceeded to the chemical transformation. Treatment of compound 1 with sodium acetate in acidic media afforded the rearrangement derivative, whose spectroscopic data were identical with those of compound 2. Thus, the biosynthesis and the transformation of compound 2 was confirmed via [3,3]-sigmatropic rearrangement (Fig. 5). This transformation had been done because both compounds (i.e. compounds 1 and 2) were a pair of isomers, and isolated as a major component from this plant.

Three isolated compounds 1, 2 and 5 were evaluated for their antimicrobial activity against MRSA using broth microdilution technique. The minimum inhibitory concentration (MIC) was used to evaluate the potential compounds as inhibitory agents against the strains of MRSA. Tetracycline was included as comparison for antimicrobial potential. Based on the results obtained in Table 6, the range of MIC values for all tested compounds is from 0.25 to 4.0 mg/mL. Compound 1 showed the good antimicrobial activity against the strain of MRSA with MIC value of 0.5 mg/mL. Meanwhile, compounds 2 and 5 exhibited moderate activity with MIC value between 1.0 and 2.0 mg/mL.
Figure 5: Chemical transformation of 1’S-1’-acetoxychavicol acetate (1).

Table 6: Antimicrobial activity of isolate compounds against strain of MRSA ATCC 43300.

| Chemical compounds                        | MIC (mg/mL) |
|-------------------------------------------|-------------|
| Tetracycline (control)                    | 0.0005      |
| 1’S-1’-acetoxychavicol acetate (1)        | 0.5         |
| trans-p-coumaryl diacetate (2)            | 1.0         |
| p-hydroxybenzaldehyde (5)                 | 2.0         |

*Note: MIC = minimum inhibitory concentration

CONCLUSION

The phytochemical investigation of the rhizomes of cultivate Alpinia conchigera Griff has led to the isolation of seven compounds namely 1’S-1’-acetoxychavicol acetate (1), trans-p-coumaryl diacetate (2), p-hydroxycinnamyl acetate (3), 1’S-1’-hydroxychavicol acetate (4), p-hydroxybenzaldehyde (5), stigmasterol (6) and β-sitosterol (7). Compounds 1, 2 and 5 were screened for antimicrobial activity against MRSA ATCC 43300. Compound 1 exhibited good antimicrobial activity with MIC value of 0.5 mg/mL. It was also shown that compound 1 was a major compound from the rhizomes of Alpinia conchigera Griff. and can be further evaluated as a potential candidate for antimicrobial treatment against MRSA. These results provide some evidence to support the traditional application of this herb in treatment of skin infection as reported in Kelantan, East Coast Malaysia (Ibrahim et al. 2000).
ACKNOWLEDGEMENTS

We would like to thank to Universiti Sains Malaysia, Pulau Pinang for providing grant 304/PKIMIA/6313330 which supported this research.

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