

## Phytochemicals and Bioactivities of *Syzygium filiforme* var. *filiforme* (Fitokimia dan Bioaktiviti *Syzygium filiforme* var. *filiforme*)

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### ABSTRACT

*Syzygium filiforme* var. *filiforme* is a plant variety from dicotyledonous plant family (Myrtaceae). Phytochemical studies on *S. filiforme* var. *filiforme* stem bark have successfully isolated and characterized arjunolic acid (1), aliphatic acid (2), betulinic acid (3), ursolic acid (4), ursolic acid 3-methyl ester (5),  $\beta$ -sitosterol (6) and stigmasterol (7). The inhibitory activities against free radical, starch, and bacteria for major compounds were tested by using DPPH,  $\alpha$ -glucosidase and minimum inhibitory and bacterial concentration assays, respectively. No promising antioxidant activity was shown on tested samples except methanolic crude extract. For antidiabetic activity, methanolic and dichloromethane crude extracts displayed potent activity compared to 1-deoxyojirimycin. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) assays for antibacterial activity were evaluated on *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. All crude extracts and major compounds displayed weak and no promising activities for MIC method, respectively. Meanwhile, for MBC method, hexane crude extract and compound 1 showed inhibition against *B. subtilis*.

**Keywords:** Antibacterial; antidiabetic; antioxidant; Myrtaceae; *Syzygium filiforme* var. *filiforme*

### ABSTRAK

*Syzygium filiforme* var. *filiforme* adalah varieti tumbuhan daripada keluarga tumbuhan dicotyledonous (Myrtaceae). Kajian fitokimia pada kulit batang *S. filiforme* var. *filiforme* berjaya mengasingkan dan mencirikan asid arjunolik (1), asid alifatik (2), asid betulinik (3), asid ursolik (4), asid ursolik 3-metil ester (5),  $\beta$ -sitosterol (6) dan stigmasterol (7). Aktiviti penghambatan terhadap radikal bebas, kanji dan bakteria untuk sebatian utama diuji dengan menggunakan DPPH,  $\alpha$ -glukosidase dan uji kepekatan minimum dan kepekatan bakteria, masing-masing. Tiada aktiviti antioksidan yang menjanjikan pada sampel yang diuji kecuali ekstrak mentah metanolik. Untuk aktiviti antidiabetik, ekstrak mentah metanol dan diklorometana menunjukkan aktiviti yang kuat berbanding dengan 1-deoxyojirimycin. Kaedah kepekatan perencatan minimum (MIC) dan kepekatan bakteria minimum (MBC) untuk aktiviti antibakteria dinilai pada *Escherichia coli*, *Staphylococcus aureus* dan *Bacillus subtilis*. Semua ekstrak mentah dan sebatian utama masing-masing menunjukkan aktiviti lemah dan tidak menjanjikan untuk kaedah MIC. Sementara itu, untuk kaedah MBC, ekstrak mentah heksana dan sebatian 1 menunjukkan perencatan terhadap *B. subtilis*.

**Kata kunci:** Antibakteria; antidiabetik; antioksidan; Myrtaceae; *Syzygium filiforme* var. *filiforme*

### INTRODUCTION

*Syzygium filiforme* var. *filiforme* locally known as 'Kelat' in Malaysia is a variety of *S. filiforme* (Myrtaceae). It can be found in Australia, Southeast Asia, and South America with concentrations as a big forest tree (Frag et al. 2009; Heng et al. 2013). Since hundred years ago, the *Syzygium* species is widely used as medicinal plant for treatment of various human diseases. The *S. samarangense* flowers extract has reportedly been used for the treatments of

inflammation, asthma, and bronchitis (Minh et al. 2018; Shen et al. 2012; Swadhin et al. 2019). Meanwhile, *S. cumini* have been reported for gastropathy, stomachalgia, splenopathy, and pharyngitis treatments as well as to strengthen gums and teeth using its seeds and fruits extracts (Ajiboye et al. 2020; Chagas et al. 2015; de Jesus Soares et al. 2019; Kabra & Patel 2018). Then, the leaf of *S. cordatum* was used as antidiabetic agent in South America and Asia (Cock & Cheesman 2018; Maliehe et al. 2017; Musabayane et al. 2005).

The study on chemical constituent from this species provided useful information as sources of bioactive compounds. Triterpenoids are the most common terpenoid compounds contained in *Syzygium* species and flavonoids are also reported in some species (Babu et al. 2017; Hu et al. 2018; Ito et al. 2004; Saleem et al. 2016). Several types of flavonoids were discovered in this species such as phenols, flavanones, anthocyanins, chalcones, and anthoxanthins (Faria et al. 2011; Gaspar et al. 2020; Manaharan et al. 2012; Nguyen et al. 2016). However, there is no study regarding the biological activity and phytochemical constituents of *S. filiforme* var. *filiforme*. Thus, further investigation on the Malaysian *S. filiforme* var. *filiforme* is recommended to explore their potential as medicinal agent. In this study, we focused on chemical constituents from *S. filiforme* var. *filiforme* stem bark with their biological properties.

## MATERIALS AND METHODS

### SPECTROSCOPIC TECHNIQUES

The Bruker 500 Ultrashield NMR spectrometer was used to measure  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR at 500 and 125 MHz, respectively, in deuterated-pyridine, deuterated-methanol or deuterated-chloroform. Chemical shifts and coupling constants were recorded using  $\delta$  scale in ppm and Hz, respectively. The hot stage Gallen Kamp melting point apparatus with microscope were used to identify the melting points. Then, the Varian 640-IR spectrum one FT-IR spectrometer (KBr) were used to identify the infrared (IR) spectra. The ultraviolet (UV) and mass spectra were recorded in ethanol on Shimadzu UV-Vis 160i and on TOF LC/MS spectrometer 70 eV (Agilent Technologies 6224), respectively.

### PLANT COLLECTION

The *S. filiforme* var. *filiforme* was identified by Dr. Shamsul Khamis from Universiti Putra Malaysia (UPM). The plant stem bark (UiTM 14/2009) was collected from Pasir Raja, Terengganu and deposited at Herbarium Faculty of Applied Sciences, Universiti Teknologi MARA Malaysia, Shah Alam. It was air dried and grounded into fine powder.

### FRACTIONATION AND ISOLATION OF COMPOUNDS

The step gradient polarity solvents (hexane, dichloromethane and methanol) were used to extract successively the chemical constituents from 3 kg of stem bark plant sample (72 h, three times, 10 L). Then,

the crude extracts were evaporated until dryness under reduced pressure using rotary evaporator. About 7 fractions were yielded using vacuum liquid chromatography (VLC) from methanolic crude extract (60.3 g). From methanol extract, three compounds were obtained **1** (58.7 mg), **2** (18.7 mg) and **3** (22.6 mg). Meanwhile, **4** (8.1 mg), **5** (1.5 mg), **6** (4.2 mg) and **7** (3.9 mg) were obtained from dichloromethane extract. The structure of isolated compounds was characterized using infrared spectroscopy (IR), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR).

### SPECTRAL DATA

#### Compound 1

Weight: 58.7 mg, white amorphous solid, melting point: 292-295 °C. UV (MeOH)  $\lambda_{\text{max}}$  = 218 nm. IR  $\text{cm}^{-1}$ : 3401, 2944, 2857, 1694, 1462, 1390. MS  $m/z$ : 487.3 [M-H]<sup>-</sup>,  $\text{C}_{30}\text{H}_{48}\text{O}_5$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR were similar with published data (Djoukeng et al. 2005).

#### Compound 2

Weight: 18.7 mg, white amorphous solid, melting point: 274-277 °C. UV (MeOH)  $\lambda_{\text{max}}$  = 207 nm. IR  $\text{cm}^{-1}$ : 3435, 2942, 2868, 1692, 1454, 1376. MS  $m/z$ : 471.3 [M-H]<sup>-</sup>,  $\text{C}_{30}\text{H}_{48}\text{O}_4$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR were similar with published data (Bai et al. 2015).

#### Compound 3

Weight: 22.6 mg, white amorphous solid, melting point: 314-317 °C. UV (MeOH)  $\lambda_{\text{max}}$  = 197 nm. IR  $\text{cm}^{-1}$ : 3467, 2943, 2862, 1687, 1452, 1376. MS  $m/z$ : 455.2 [M-H]<sup>-</sup>,  $\text{C}_{30}\text{H}_{48}\text{O}_3$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR were similar with published data (Kuiate et al. 2007).

#### Compound 4

Weight: 8.1 mg, white amorphous solid, melting point: 283-286 °C. UV (MeOH)  $\lambda_{\text{max}}$  = 212 nm. IR  $\text{cm}^{-1}$ : 3429, 2924, 2851, 1688, 1463. MS  $m/z$ : 455.2 [M-H]<sup>-</sup>,  $\text{C}_{30}\text{H}_{48}\text{O}_3$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR were similar with published data (Ismail et al. 2010).

#### Compound 5

Weight: 1.5 mg, white amorphous solid, melting point: 170-174 °C. UV (MeOH)  $\lambda_{\text{max}}$  = 218 nm. MS  $m/z$ : 471.3 [M+H]<sup>+</sup>,  $\text{C}_{31}\text{H}_{49}\text{O}_3$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR were similar with published data (Kim et al. 2005).

#### Compound 6

Weight: 4.2 mg, white amorphous solid, melting point: 133-136 °C. MS  $m/z$ : 413.1 [M-H]<sup>-</sup>,  $\text{C}_{29}\text{H}_{50}\text{O}$ .  $^1\text{H}$  and  $^{13}\text{C}$

NMR were similar with published data (Güvenalp et al. 2006).

#### Compound 7

Weight: 3.9 mg, white amorphous solid, melting point: 176-179 °C. MS  $m/z$ : 413.1  $[M+H]^+$ ,  $C_{29}H_{48}O$ .  $^1H$  and  $^{13}C$  NMR were similar with published data (De-Eknamkul & Potduang 2003).

#### DPPH ASSAY

Detailed procedure explained below as reported by Teoh et al. (2013). The 50  $\mu$ L of samples were dissolved and diluted in different concentration from 5, 10, 20, 40, 60, 80, and 100  $\mu$ g/mL. Then, ascorbic acid was used as positive control. The following equation was used to calculate the percentage of DPPH scavenging effect of antioxidant activity:

$$\text{DPPH scavenging effect} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\%$$

A is denoted as the absorbance of tested mixture samples measured at 517 nm. The experiments were conducted in triplicate.

#### ANTIDIABETIC ASSAY

The antidiabetic properties of tested samples were conducted using *in vitro*  $\alpha$ -glucosidase assay protocol reported by Lee et al. (2008). About 1 mg/mL concentration of samples and standard 1-deoxynojirimycin were prepared in 5% of DMSO. The concentration of 0.69 to 1 mg/mL was prepared by two foil serial dilutions. The following equation was used to identify the inhibition rate (%) of antidiabetic activity:

$$\% \text{ Inhibition} = \frac{(A_{30 \text{ minutes}} - A_{0 \text{ minutes}})_{\text{control}} - (A_{30 \text{ minutes}} - A_{0 \text{ minutes}})_{\text{experiment}}}{(A_{30 \text{ minutes}} - A_{0 \text{ minutes}})_{\text{control}}} \times 100\%$$

A is denoted as the absorbance of tested mixture samples measured at 405 nm.

#### ANTIBACTERIAL ASSAY

The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of samples were carried out based on previous protocol (Zakaria et al. 2010) on *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Around 100  $\mu$ L of samples were mixed

with 50  $\mu$ L of broth and gentamycin was used as positive control. The different concentration of samples (28.13 to 1800  $\mu$ g/mL) were mixed with microorganism previously inoculated and cultured for 24 h in microtiter plate by serial dilutions and were incubated for 24 h at temperature 37 °C. Then, the samples mixture in well plate was mixed with 20  $\mu$ L of MTT solution after 24 h and was incubated again for 20 min. The results were measured at 620 nm using multi-well scanning spectrophotometer (ELISA reader).

#### RESULTS AND DISCUSSION

In this study, we have successfully isolated seven compounds from *S. filiforme* var. *filiforme* stem bark (Myrtaceae) as shown in Figure 1. Arjunolic acid (**1**) was classified as oleanane type. Then, alphitolic acid (**2**) and betulinic acid (**3**) were identified as lupane type. The ursane type is ursolic acid (**4**) and ursolic acid 3-methyl ester (**5**). Meanwhile,  $\beta$ -sitosterol (**6**) and stigmasterol (**7**) were classified as phytosterol type of triterpenoids. The IR, LCMS, 1D NMR ( $^1H$ ,  $^{13}C$ , DEPTQ NMR) and 2D NMR (HMQC, HMBC, COSY, NOESY) techniques were used to characterize the structure of isolated compounds.

Compound **1** appeared as white amorphous solid with a melting point 292-295 °C and was isolated from methanolic crude extract. Its spectral data has similarity with arjunolic acid isolated from *S. guineense* (Myrtaceae) (Djoukeng et al. 2005). Two lupane type triterpenoids, compounds **2** and **3** were also isolated from methanolic crude extract. Compound **2** was isolated as white amorphous solid with a melting point 274-277 °C and its spectral data has compared with literature value (Bai et al. 2015) and confirmed that the compound **2** is alphitolic acid. Meanwhile, compound **3** was appeared as white amorphous solid with a melting point 314-317 °C and identified as betulinic acid previously isolated from the stem bark of *S. jambos* (L.) Alston (Myrtaceae) (Kuiate et al. 2007).

Two ursane type triterpenoids, compounds **4** and **5** were isolated as white amorphous solid with melting point 283-286 °C and 170-174 °C, respectively, from dichloromethane extract. It was reported that compound **4** was previously contained in *S. malaccense* leaves extract (Myrtaceae) (Ismail et al. 2010) while compound **5** from *Campsis grandiflora* flower extract (Bignoniaceae) (Kim et al. 2005). Furthermore, compounds **6** and **7** were obtained as white amorphous solid with melting point 133-136 °C and 176-179 °C, respectively, from dichloromethane extract. They are classified as well-known phytosterols and easily found in most of the medicinal plants.

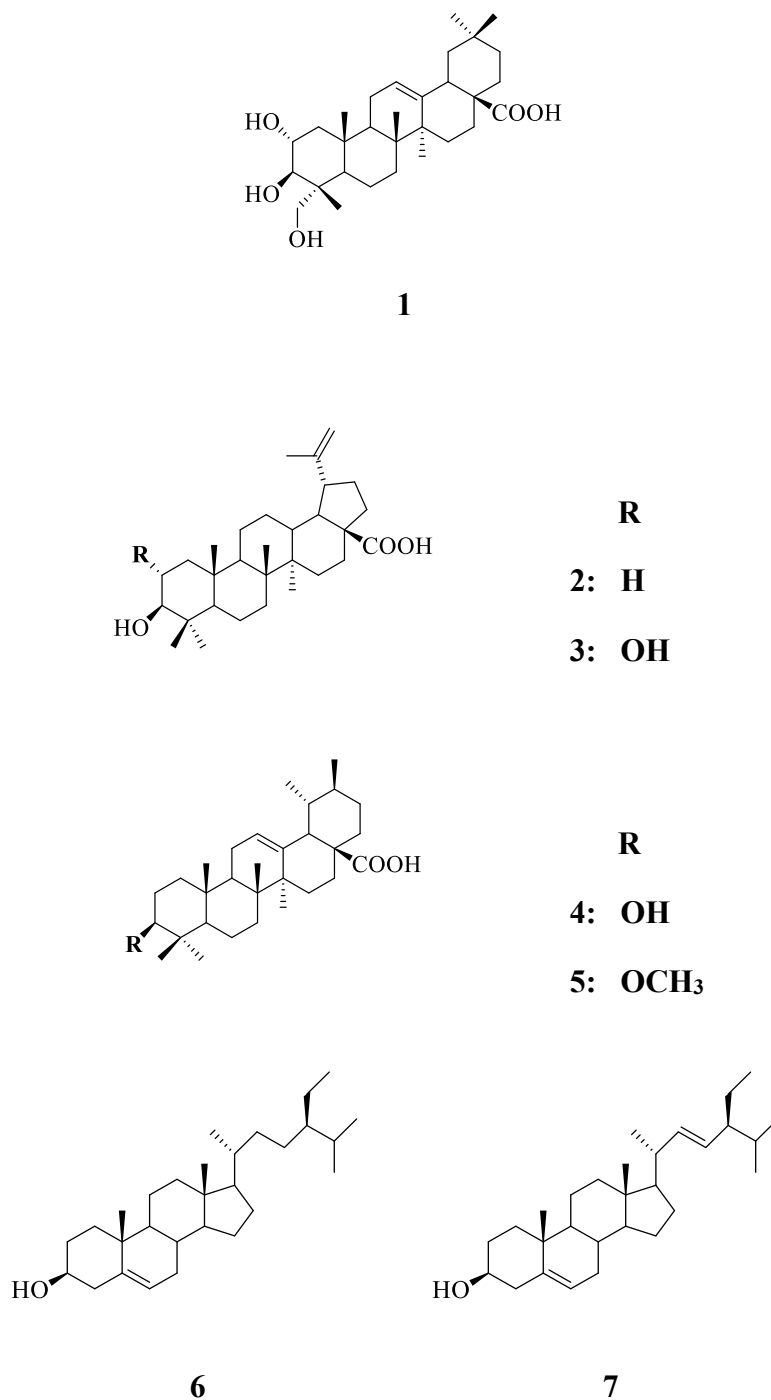


FIGURE 1. Isolated compounds from *Syzygium filiforme* var. *filiforme*. 2 $\alpha$ , 3 $\beta$ , 23-trihydroxyolean-12-en-28-oic acid (arjunolic acid, **1**), 2 $\alpha$ , 3 $\beta$ -dihydroxylup-20(29)-en-28-oic acid (alphitolic acid, **2**) and 3 $\beta$ -hydroxylup-20(29)-en-28-oic acid (betulinic acid, **3**), 3 $\beta$ -hydroxyurs-12-en-28-oic acid (ursolic acid, **4**) and ursolic acid 3-methyl ester (**5**),  $\beta$ -sitosterol (**6**) and stigmasterol (**7**)

The radical scavenging activity for tested samples was identified by the different in percentage inhibition towards free radicals. The potential of each samples was observe using the established protocol for DPPH assay. Moreover, the greater activity was observed with the lowest IC<sub>50</sub> values (Banerjee et al. 2005). The sample concentration required to inhibit 50% of free radicals is known as IC<sub>50</sub> value. The tested samples with final concentrations of 5, 10, 20, 40, 60, 80, and 100 µg/mL were used to evaluate the antioxidant activity of tested samples.

Table 1 shows the radical scavenging activity of tested samples with their IC<sub>50</sub> values compared with standard ascorbic acid. Among all tested samples, methanolic

crude extract demonstrated the good potential as radical scavenger with IC<sub>50</sub> value of 44.7±6.42 µg/mL. Similar result was obtained with study on methanolic crude extract from leaf of *S. malaccense* (L.) Merr and Perr which has antiradical effect with IC<sub>50</sub> value of 25.74±0.0 µg/mL (Savitha et al. 2011). It is also similar with methanolic crude extract of *S. jambos*, *S. javanicum*, *S. samarangense*, and *S. curranii* which has promising antioxidant activity with IC<sub>50</sub> values 92.0±8.24, 81.4±6.24, 77.5±4.19, and 33.4±2.52 µg/mL, respectively (Reynertson et al. 2008). Finally, dichloromethane and hexane crude extracts as well as major compounds showed no promising antioxidant properties towards DPPH radicals.

TABLE 1. The IC<sub>50</sub> values of *S. filiforme* var. *filiforme* crude

extracts, major compounds and ascorbic acid

Sample	IC <sub>50</sub> (µg/mL)
MeOH crude extract	44.7±6.42
DCM crude extract	NA
Hexane crude extract	NA
Arjunolic acid	NA
Betulinic acid	NA
Alphitolic acid	NA
Ursolic acid	NA
Ascorbic acid	7.9±1.20

The α-glucosidase activity of tested samples is displayed in Table 2 with their IC<sub>50</sub> values compared with standard 1-deoxyojirimycin. The samples stock solution (1 mg/

mL) of tested samples was diluted to final concentrations 69.3 to 1000 µg/mL for evaluation of their antidiabetic properties.

TABLE 2. The IC<sub>50</sub> values of *S. filiforme* var. *filiforme* crude extracts, major compounds and 1-deoxyojirimycin

Sample	IC <sub>50</sub> (µg/mL)
MeOH extract	6.31 ± 0.90
DCM extract	100 ± 9.90
Hexane extract	NA
Arjunolic acid	562.34 ± 11.80
Betulinic acid	501.19 ± 8.20
Alphitolic acid	NA
Ursolic acid	NA
1-deoxyojirimycin	103.79 ± 6.36

Methanolic ( $IC_{50}=6.31\pm0.90$   $\mu\text{g/mL}$ ) and dichloromethane ( $IC_{50}=100\pm0.90$   $\mu\text{g/mL}$ ) crude extracts displayed promising antidiabetic effect compared to 1-deoxynojirimycin. It can be noted that most of crude extracts showed great potential to convert carbohydrates into monosaccharide. This is similar with butanolic crude extract of *S. cumini* seed kernels which showed good antidiabetic activity with  $IC_{50}$  value of  $8.3\pm0.2$   $\mu\text{g/mL}$  compared to standard 1-deoxynojirimycin (Shinde et al. 2008). Radical scavenging effect of compounds **1** and **3** indicated promising antidiabetic activity with  $IC_{50}$  values of  $562.34\pm11.80$  and  $501.19\pm8.20$   $\mu\text{g/mL}$ , respectively. Then, no promising activity was identified for the other samples.

Antibacterial activity for tested samples were carried out using minimum inhibitory concentration (MIC) technique with final concentrations of 28.13 to 1800  $\mu\text{g/mL}$  against *E. coli*, *S. aureus*, and *B. subtilis* as shown in Table 3. All crude extracts showed antibacterial activity against tested bacteria except dichloromethane extract. Hexane crude extract at highest concentration demonstrated more potential while methanolic crude extract displayed antibacterial activity against *S. aureus* with  $IC_{50}$  value of 900  $\mu\text{g/mL}$ . Similar results were observed with study on leaves crude extracts of *S. cumini* which showed antibacterial effect against *E. coli*, *S. aureus*, and *B. subtilis* (Ugbabe et al. 2010).

TABLE 3. The minimum inhibitory concentration (MIC) of *S. filiforme* var. *filiforme* crude extracts, major compounds and gentamycin

Sample	<i>E. coli</i> ( $\mu\text{g/mL}$ )	<i>S. aureus</i> ( $\mu\text{g/mL}$ )	<i>B. subtilis</i> ( $\mu\text{g/mL}$ )
MeOH extract	NA	900	NA
DCM extract	NA	NA	NA
Hexane extract	1800	900	1800
Arjunolic acid	1800	450	900
Alphitolic acid	NA	900	NA
Betulinic acid	NA	NA	NA
Ursolic acid	1800	NA	NA
Gentamycin	28.13	28.13	28.13

Two major compounds, compounds **1** and **2** showed inhibition against *S. aureus* with MIC values of 450 and 900  $\mu\text{g/mL}$ , respectively. Interestingly, compound **1** is also showed antibacterial effect against *E. coli* and *B. subtilis* at highest concentration. Meanwhile, compound **4** at highest concentration demonstrated antibacterial activity against *E. coli* but compound **3** showed no inhibition against tested bacteria. Based on literature, compounds **3** and **4** at highest concentration displayed mild antibacterial effect against *E. coli* and *B. subtilis* (Chandramu et al. 2003).

The minimum bacterial concentration (MBC) of crude extracts and major compounds compared to

standard gentamycin is displayed in Table 4. Hexane crude extract, compounds **1** and **4** showed promising antibacterial effect against *E. coli* with MBC values of 1800  $\mu\text{g/mL}$  equivalent to their MIC values. Meanwhile, the MBC values of methanolic and hexane crude extracts against *S. aureus* were determined at concentration 1000 and 1100  $\mu\text{g/mL}$ , respectively. On the other hand, the MBC values of compounds **1** (560  $\mu\text{g/mL}$ ) and **2** (1000  $\mu\text{g/mL}$ ) on the same bacteria showed higher than their MIC values. Finally, all tested samples displayed no inhibition against *B. subtilis* except hexane crude extract and compound **1** with MBC values 1800 and 1100  $\mu\text{g/mL}$ , respectively.

TABLE 4. The minimum bactericidal concentration (MBC) of *S. filiforme* var. *filiforme* crude extracts, major compounds and gentamycin

Sample	<i>E. coli</i> (µg/mL)	<i>S. aureus</i> (µg/mL)	<i>B. subtilis</i> (µg/mL)
MeOH extract	NA	1000	NA
DCM extract	NA	NA	NA
Hexane extract	1800	1100	1800
Arjunolic acid	1800	560	1100
Alphitolic acid	NA	1000	NA
Betulinic acid	NA	NA	NA
Ursolic acid	1800	NA	NA
Gentamycin	28.13	28.13	28.13

#### CONCLUSION

Methanol and dichloromethane extracts showed potent antidiabetic activity compared to 1-deoxynojirimycin using  $\alpha$ -glucosidase assay. This might be due to the traditional uses of *Syzygium* species as antidiabetic for old folks. For antibacterial activity, to the MBC values of hexane extract, compounds **1** and **4** against *E. coli* were equivalent to MIC values 1800 µg/mL. Methanol extract, hexane extract, compounds **1** and **2** showed activity on *S. aureus* with MBC values of 1000, 1100, 560, and 1000 µg/mL, respectively, higher than MIC values against same bacteria. Then, all samples have no promising activity against *B. subtilis* except hexane extract and compound **1** with MBC values 1800 and 1100 µg/mL, respectively. Finally, the *S. filiforme* var. *filiforme* species could become potential as antidiabetic and medicinal agent for drug discovery.

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