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), alfitolic acid (2), betulinic acid (3), ursolic acid (4), ursolic acid 3-methyl ester (5), β-sitosterol (6) and stigmasterol (7). The inhibitory activities against free radical, starch, and bacteria for major compounds were tested by using DPPH, α-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-deoxynojirimycin. 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

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 (Farag 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, stomachalia, spleenopathy, 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 ¹H-NMR and ¹³C-NMR at 500 and 125 MHz, respectively, in deuterated-pyridine, deuterated-methanol or deuterated-chloroform. Chemical shifts and coupling constants were recorded using δ 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 MARA 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) λmax = 218 nm. IR cm⁻¹: 3401, 2944, 2857, 1694, 1462, 1390. MS m/z: 487.3 [M+H]⁺, C₆₀H₄₈O₄. ¹H and ¹³C 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) λmax = 207 nm. IR cm⁻¹: 3435, 2942, 2868, 1692, 1454, 1376. MS m/z: 471.3 [M+H]⁺, C₅₀H₄₆O₄. ¹H and ¹³C 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) λmax = 197 nm. IR cm⁻¹: 3467, 2943, 2862, 1687, 1452, 1376. MS m/z: 455.2 [M+H]⁺, C₄₀H₄₄O₄. ¹H and ¹³C 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) λmax = 212 nm. IR cm⁻¹: 3429, 2924, 2851, 1688, 1463. MS m/z: 455.2 [M+H]⁺, C₆₀H₄₈O₄. ¹H and ¹³C 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) λmax = 218 nm. MS m/z: 471.3 [M+H]⁺, C₃₀H₂₄O₄. ¹H and ¹³C 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]⁺, C₂₉H₂₀O₄. ¹H and ¹³C
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]+, C29H40O. 1H and 13C 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 µL of samples were dissolved and diluted in different concentration from 5, 10, 20, 40, 60, 80, and 100 µg/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 α-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:

$$\% \text{ Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})_{\text{control}} - (A_{\text{blank}} - A_{\text{sample}})_{\text{sample}}}{(A_{\text{blank}} - A_{\text{sample}})_{\text{control}}} \times 100\%$$

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 µL of samples were mixed with 50 µL of broth and gentamycin was used as positive control. The different concentration of samples (28.13 to 1800 µg/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 µL 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, β-sitosterol (6) and stigmasteryl (7) were classified as phytosterol type of triterpenoids. The IR, LCMS, 1D NMR (1H, 13C, 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*. 1, 3β, 23-trihydroxyolean-12-en-28-oic acid (arjunolic acid, 1), 2α, 3β-dihydroxyup-20(29)-en-28-oic acid (alphitolic acid, 2) and 3β-hydroxyup-20(29)-en-28-oic acid (betulinic acid, 3), 3β-hydroxyurs-12-en-28-oic acid (ursolic acid, 4) and ursolic acid 3-methyl ester (5), β-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$_{50}$ values (Banerjee et al. 2005). The sample concentration required to inhibit 50% of free radicals is known as IC$_{50}$ 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$_{50}$ values compared with standard ascorbic acid. Among all tested samples, methanolic crude extract demonstrated the good potential as radical scavenger with IC$_{50}$ 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$_{50}$ 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$_{50}$ 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$_{50}$ values of *S. filiforme* var. *filiforme* crude extracts, major compounds and ascorbic acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH crude extract</td>
<td>44.7±6.42</td>
</tr>
<tr>
<td>DCM crude extract</td>
<td>NA</td>
</tr>
<tr>
<td>Hexane crude extract</td>
<td>NA</td>
</tr>
<tr>
<td>Arjunolic acid</td>
<td>NA</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>NA</td>
</tr>
<tr>
<td>Alphitolic acid</td>
<td>NA</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>NA</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>7.9±1.20</td>
</tr>
</tbody>
</table>

The α-glucosidase activity of tested samples is displayed in Table 2 with their IC$_{50}$ values compared with standard 1-deoxynojirimycin. 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$_{50}$ values of *S. filiforme* var. *filiforme* crude extracts, major compounds and 1-deoxynojirimycin

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>6.31±0.90</td>
</tr>
<tr>
<td>DCM extract</td>
<td>100±9.90</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>NA</td>
</tr>
<tr>
<td>Arjunolic acid</td>
<td>562.34±11.80</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>501.19±8.20</td>
</tr>
<tr>
<td>Alphitolic acid</td>
<td>NA</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>NA</td>
</tr>
<tr>
<td>1-deoxynojirimycin</td>
<td>103.79±6.36</td>
</tr>
</tbody>
</table>
Methanolic ($IC_{50}=6.31\pm0.90$ µg/mL) and dichloromethane ($IC_{50}=100\pm0.90$ µg/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±0.2 µg/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±11.80 and 501.19±8.20 µg/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 µg/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 µg/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>
<thead>
<tr>
<th>Sample</th>
<th><em>E. coli</em> (µg/mL)</th>
<th><em>S. aureus</em> (µg/mL)</th>
<th><em>B. subtilis</em> (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>NA</td>
<td>900</td>
<td>NA</td>
</tr>
<tr>
<td>DCM extract</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>1800</td>
<td>900</td>
<td>1800</td>
</tr>
<tr>
<td>Arjunolic acid</td>
<td>1800</td>
<td>450</td>
<td>900</td>
</tr>
<tr>
<td>Alphitolic acid</td>
<td>NA</td>
<td>900</td>
<td>NA</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>1800</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Two major compounds, compounds 1 and 2 showed inhibition against *S. aureus* with MIC values of 450 and 900 µg/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 µg/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 µg/mL, respectively. On the other hand, the MBC values of compounds 1 (560 µg/mL) and 2 (1000 µg/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 µg/mL, respectively.
TABLE 4. The minimum bactericidal concentration (MBC) of *S. filiforme* var. *filiforme* crude extracts, major compounds and gentamycin

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>E. coli</em> (µg/mL)</th>
<th><em>S. aureus</em> (µg/mL)</th>
<th><em>B. subtilis</em> (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>NA</td>
<td>1000</td>
<td>NA</td>
</tr>
<tr>
<td>DCM extract</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>1800</td>
<td>1100</td>
<td>1800</td>
</tr>
<tr>
<td>Arjunolic acid</td>
<td>1800</td>
<td>560</td>
<td>1100</td>
</tr>
<tr>
<td>Alphitolic acid</td>
<td>NA</td>
<td>1000</td>
<td>NA</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>1800</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Methanol and dichloromethane extracts showed potent antidiabetic activity compared to 1-deoxynojirimycin using α-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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