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Anti-Inflammatory and Antioxidant Activity of *Syzygium polyanthum* (Wight) Walp.

(Aktiviti Anti-Radang dan Antioksidan *Syzygium polyanthum* (Wight) Walp.)

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ABSTRACT

Syzygium polyanthum has been used as folk medicine to treat ailments related to oxidative stress and inflammation. In this study, the anti-inflammatory and antioxidant activity of stem bark and root bark of the plant were investigated. The experiments that have been carried out were estimation of total phenolic and total flavonoid in the methanol extracts and fractions of both parts, isolation and structure elucidation of chemical compounds, anti-inflammatory activity evaluation based on inhibition of prostaglandin E_2 production in the LPS-induced human whole blood using radioimmunoassay technique as well as antioxidant activity based on assays by using free radical scavenging DPPH and FRAP. Results showed high amounts of phenolics and flavonoids in both parts of *S. polyanthum*. Seven compounds were succesfully isolated from the stem bark and identified as stigmasterol (**1**), 8-hydroxy-6-methoxy-3-pentylisocoumarin (**2**), 3,3'-di-O-methylellagic acid (**3**), methylgallate (**4**), asiatic acid (**5**), arjunolic acid (**6**), and daucosterol (**7**). The ethyl acetate fraction of the root bark showed potent inhibitory activity on the production of PGE_2 (IC_{50} 3.03 ± 0.83 µg/mL). The methanol extract of the stem bark displaying promising DPPH scavenging activity (SC_{50} = 2.82 ± 0.1 µg/mL) and FRAP activity (7.02 ± 0.1 µg/µg of equivalent trolox amount). Compounds **1, 5, 6** and **7** showed pronounced inhibitory activity on the PGE_2 production with IC_{50} ranging from 0.052 to 1.25 µM. Meanwhile, compound **4** exhibited antioxidant activity toward DPPH (SC_{50} 10.60 µM) and FRAP (20.5 ± 1.0 µg/µg). The study concluded that *S. polyanthum* as a potential source for therapeutic agents with anti-inflammatory and antioxidant activities.

Keywords: Arjunolic acid; asiatic acid; myrtaceae; prostaglandin E_2; *Syzygium polyanthum*

ABSTRAK

Syzygium polyanthum telah digunakan dalam perubatan tradisi untuk merawat penyakit yang berkaitan dengan tekanan oksidatif dan radang. Penyelidikan ini dijalankan untuk mengkaji aktiviti anti-radang dan antioksidan kulit batang dan kulit akar *S. polyanthum*. Uji kaji yang dijalankan adalah untuk menentukan jumlah sebatian fenol dan flavonoid di dalam ekstrak dan fraksi metanol kedua-dua bahagian tumbuhan, pemisahan dan pengenalpastian struktur sebatian kimia, penilaian aktiviti anti-radang melalui perencatan penghasilan prostaglandin E_2 di dalam darah manusia diaruh-lipopolisakarida (LPS) dengan menggunakan teknik radioimunoasai serta aktiviti antioksidan menggunakan asai DPPH dan FRAP. Hasil kajian menunjukkan kandungan sebatian fenol dan flavonoid yang tinggi di dalam kedua-dua bahagian tumbuhan *S. polyanthum*. Tujuh sebatian telah berjaya dipencilkan daripada kulit batang *S. polyanthum* iaitu stigmasterol (**1**), 8-hidroksi-6-metoksi-3-pentilisokoumarin (**2**), 3,3'-di-O-asid metilelagik (**3**), metilgalat (**4**), asid asiatik (**5**), asid arjunolik (**6**) dan daukosterol (**7**). Fraksi etil asetat kulit akar *S. polyanthum* menunjukkan aktiviti perencatan yang poten terhadap penghasilan PGE_2 (IC_{50} 3.03 ± 0.83 µg/mL). Ekstrak metanol kulit batang menunjukkan aktiviti hapus-sisa DPPH (SC_{50} = 2.82 ± 0.1 µg/mL) dan aktiviti FRAP (7.02 ± 0.1 µg/µg bersamaan dengan jumlah

trolox). Sebatian **1**, **5**, **6** dan **7** menunjukkan aktiviti perencatan terhadap penghasilan PGE_2 dengan nilai IC_{50} antara 0.052 ke 1.25 µM. Selain itu, sebatian **4** menunjukkan aktiviti antioksidan terhadap DPPH (SC_{50} 10.60 µM) dan aktiviti FRAP (20.5 ± 1.0 µg/µg). Kajian ini menyimpulkan bahawa tumbuhan *S. polyanthum* berpotensi sebagai sumber bagi agen terapeutik untuk aktiviti anti-radang dan antioksidan.

Kata kunci: Asid arjunolik; asid asiatik; myrtaceae; prostaglandin E_2; *Syzygium polyanthum*

INTRODUCTION

Inflammation is a complex reaction of vascularized tissue to local injury as results of immune response to endogenous and exogenous invades. The aim of inflammation is for limiting tissue damage and infection as well as initiating repair (Vane & Botting 1996). It is a general immune reaction, however, in the condition with aberrant inflammatory response, the reaction resulting in even more damage. Two classes of inflammation have been classified based on the duration of the reaction that are acute inflammation and chronic inflammation. The latter have been proven to exist in the development of a variety of diseases such as bone and joint inflammatory diseases, cardiovascular diseases, diabetic complications, cancers, neurological disorders, pulmonary diseases, autoimmune disorders and metabolic, and endocrine disorders (Arulselvan et al. 2016). Researches have also shown the correlation between oxidative stress and inflammation to activate and accelerate the diseases process (Willcox et al. 2016). Oxidative stress is caused by a diminished antioxidant defence and/or increased production of reactive species (Halliwell 2007). The search for active agents for treatment of diseases and the process related to inflammation and oxidative stress has increased dramatically on natural sources including plants (Azab et al. 2016; López-Alarcón & Denicola 2013). The attention has been made due to many evidences of unwanted side effects by modern medicines (Attiq et al. 2018). Studies have also suggested synergistic effects of natural products such as in herbal preparations and neutraceuticals to support better health for mankind (Alok et al. 2014; Shahidi & Zhong 2015).

Plants from the family Myrtaceae have long been used in traditional medicines to treat diseases related to inflammation and oxidative stress (Quattrocchi 2012). The family is comprised of about 145 plant genera with 5,970 accepted species names (The Plant List 2019). They are woody trees and shrubs of disjunct distribution in the wet tropics, particularly South America, tropical Asia, and Australia. The unique of Myrtaceae species is in their bark that smooth and shed annually. Another feature of the family is the presence of oil glands that produce essential oils dominated by monoterpenes and sesquiterpenes. Thus, the family is economically important for industries related to spices and pharmaceuticals (Govaerts et al. 2008). Some species of this family have also been scientifically investigated. One of the Myrtaceae species is *Syzygium polyanthum* (Wight) Walp. (synonym: *Eugenia polyantha* Wight), which is a tropical tree native to Southeast Asia countries, including Indonesia, Malaysia, Thailand, Vietnam, and the Philippines (Quattrocchi 2012; Wilson 2011). *S. polyanthum* known as 'salam' has edible flowers and fruits, and its dried leaf is used as an important flavoring condiment in Indonesian cuisine (Dalimartha 2005; Renfrew & Sanderson 2005) and several Southeast Asia countries (Parnell et al. 2007). The aromatic fragrance of the leaves is unique, hence the substitution for its fragrance rather difficult to find even among *Syzygium* species (Hutton 2003). The ripe fruits are edible although slightly astringent and the timber is used for house building and furniture (Quattrocchi 2012). The stem bark of the plant is used as dying agent for wattle and bamboo handcraft in Indonesia (Dalimartha 2005). Besides the use in culinary, 'salam' has long been used as folk medicine in Indonesia and Malaysia to treat diarrhea, hypercholesterol, gastritis, hypertension, diabetes mellitus, scabies, itches, gout, cough, cancer servix, kidney stone, arthritis, swollen, and migrain (Chooi 2008; Dalimartha 2008; Hembing 2004; Kurniawati 2010; Patria 2016). Previous studies reported the occurrence of flavonoids, phenolics, phenylpropanoids, phloroglucinols, monoterpene, and phytosterols, mainly from the leaf of the plant (Carroll 2016; Har et al. 2012; Kato et al. 2013; Lelono & Tachibana 2013a, 2013b; Saifudin et al. 2012).

In the present study, the phytochemicals of stem bark and root of the plant were investigated, employing methanol extract and organic fractions. Their anti-inflammatory activity was based on inhibitory effects on the production of prostaglandin E_2 (PGE_2) in lipopolysaccharide (LPS)-induced human whole blood and antioxidant activity was evaluated using DPPH and FRAP assays.

Materials and Methods

General

Chemicals used in this study were analytical grades of solvents, Folin-Ciocalteu's reagent, DPPH, TPTZ, PGE_2, trolox, ascorbic acid, quercetin, gallic acid, and indomethacin purchased from Merck (Darmstadt, Germany) and SigmaAldrich (St. Louis, USA). The radiolabelled PGE_2 (50 μCi/mmol) was purchased from PerkinElmer (Boston, USA). Silica gels (catalog numbers 1.05554, 1.07747, and 1.09385), Sephadex LH-20, and octadecylsilane used to perform chromatography techniques were purchased from Merck (Darmstadt, Germany).

Plant Materials

The stem bark and root bark of *S. polyanthum* were collected from the Bende district, Southeast Sulawesi, Indonesia, in April 2011. The collected samples were separately cleaned from mold and soil by washing with distilled water and drained. These parts were cut into small pieces and dried at 40 °C using an oven. Coarse powders were obtained by grinding the dried samples. Plant powders were stored separately in an airtight container at room temperature. A specimen of this plant was authenticated by the Herbarium Bogoriense, Bogor, Indonesia with a voucher number of BO-1639378.

Extraction and Fractionation

The dried powdered stem bark (5 kg) and root bark (1 kg) of *S. polyanthum* were macerated thrice with methanol (8L, 24 h each time) to yield dried methanol extracts of stem bark (600 g) and root bark (300 g). Both extracts obtained in the form of brown reddish gum. Fractionation of these extracts was carried out for HPTLC profiling and bioassay analyses, including total phenolic and total flavonoid assays. A 10 g of extract was dissolved in methanol and partitioned successively with petroleum ether and ethyl acetate, yielding residues of petroleum ether, ethyl acetate, and remaining methanol under reduced pressure. All samples were stored in amber bottles and kept in a refrigerator at 4 °C.

Phytochemical Screening

Screening of phytochemicals in methanol extracts of stem bark and root bark of *S. polyanthum* was carried out according to previous studies (Ayoola et al. 2008; Nobakht et al. 2010). Screening was carried out towards alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins.

Determination of Total Phenolic and Total Flavonoid Contents

Total phenolic dan flavonoid contents in extracts and fractions of stem bark and root bark of *S. polyanthum* were determined based on procedures in our previous study (Sabandar et al. 2019). The total phenolic content in samples was calculated using a linear gallic acid calibration curve and expressed as mg of gallic acid equivalent for each gram of sample (mg GAE/g). Meanwhile, a linear quercetin calibration curve was used to calculate the total flavonoid content expressed as mg of quercetin equivalent for each gram of sample (mg QE/g).

Isolation of Chemical Compounds and Structures Elucidation

A half portion of methanol extract of the stem bark (300 g) was dissolved in MeOH and partitioned with petroleum ether, to yield the petroleum ether soluble fraction (4.9 g). This fraction was then subjected to a vacuum liquid chromatography (VLC, silica gel 60 HF_{254}, 5-40 μm, 3 × 5 cm), eluting with mixtures of *n*-hexane–EtOAc (from 9:1 to 5:5), followed by pure EtOAc and MeOH, and yielded 28 fractions (F1-28). Based on TLC analysis, three main fractions of F4-9 (0.93 g), F15-17 (0.38 g), and F23-25 (1.70 g) were continued for further purifications. The chlorophylls content in the main fraction F4-9 (0.93 g) was removed using a Sephadex LH-20 open column chromatography (CC, 3 × 30 cm), eluted with $CHCl_3$–MeOH (99:1), and yielded 29 subfractions. Combined subfractions 4-9 (0.08 g) were applied to a silica gel CC (Si-gel 60 GF_{254}, 40-60 μm, 1 × 30 cm), eluting with gradient elution system of *n*-hexane–EtOAc (from 9:1 to 0:10), to afford compound **2** (1.2 mg). Meanwhile, re-crystallization of subfractions 10-25 (0.11 g) in methanol yielded compound **1** (8.0 mg). Furthermore, the main fraction F15-17 (0.38 g) was separated using a Sephadex LH-20 CC (3 × 30 cm) with successive elutions of pure $CHCl_3$, $CHCl_3$–MeOH (1:1), and pure MeOH, to yield 31 subfractions. The precipitation of subfractions 7-8 (0.02 g) yielded compound **3** (1.5 mg). Meanwhile, combined subfractions 15-18 (0.13 g) were chromatographed on silica gel CC (1.5 × 20 cm) using an eluent system of $CHCl_3$–MeOH (9:1), to afford compound **4** (6.0 mg). Moreover, the separation of the main fraction F23-25 (1.70 g) on a silica gel CC (5 × 30 cm) using $CHCl_3$–MeOH (9:1) as a mobile phase yielded 69 subfractions. The recrystallization of subfractions 20-21 (0.02 g) using ethanol yielded a mix of compounds **5** and **6** (8.8 mg). Meanwhile, combined subfractions 24-26 (0.19 g)

were separated using an octadecylsilane CC (ODS, 2 × 30 cm), eluted with MeOH–$CHCl_3$ (9:0.1), and yielded a mix of **5** and **6** (6.5 mg) and compound **7** (19.5 mg). The detection of these compounds was performed on TLC plates using the anisaldehyde-sulfuric acid reagent followed by heating the plate at 120 °C.

The isolated compounds were structurally elucidated using measured spectroscopic spectra of UV, IR, LC-MS/MS, HRESI-MS, 1D and 2D NMR which were obtained using a UV1800 spectrophotometer (Shimadzu, Kyoto, Japan), a Spectrum 100 FTIR spectrophotometer with ATR technique (PerkinElmer Inc., Waltham, MA, USA), a Technologie 1200 series ion trap mass spectrometer (Agilent, Santa Clara, California, USA), an Ultimate 3000 MicroTOF-Q II mass spectrometer (Bruker Daltonics, Bremen, Germany), and an Avance III 600 MHz spectrometer (Bruker BioSpin, Karlsruhe, Germany), respectively. Melting points were measured using a SMP10 apparatus (Stuart, Staffordshire, UK). All values obtained were compared to those reported in the literature.

HPTLC PROFILING

HPTLC profiling of extracts, fractions, and isolated compounds of *S. polyanthum* was carried out according to the HPTLC Association guidelines with some adaptations (HPTLC Association 2012). Samples (10 mg for extracts and fractions and 1 mg for isolated compounds) were dissolved in 1 mL methanol. Sample (10 µL) was applied onto HPTLC glass plates silica gel 60 F_{254} (20 × 10 cm, 0.2 mm thickness) by using a semi-automatic applicator Linomat 5 (CAMAG, Basel, Switzerland) at room temperature with nitrogen as spray gas. Sample applications include 14 tracks, band length 8 mm, track distance 13 mm, and distance from lower edge 15 mm. Samples bands were developed in a twin trough chamber (20 × 10 cm). Chloroform-ethyl acetate-formic acid (5:4:1) and chloroform-methanol (9:1) were used as mobile phase systems. The derivatization was carried out using a dipping method with anisaldehyde-sulfuric acid reagent, followed by heating the plate at 120 °C for 5 min in an oven. Images (clean, developed, and derivatized plate) were captured using a CAMAG visualizer completed with a digital camera DXA252 (12 mm, f4.0) under daylight and UV lights at 254 and 366 nm. The analysis was managed using a WinCATS Planar Chromatography software (CAMAG, Basel, Switzerland).

PROSTAGLANDIN E_2 (PGE_2) ASSAY

The production of prostaglandin E_2 in human whole blood induced by LPS was analyzed using a radioimmunoassay method as described previously (Jalil et al. 2015). The use of human blood was in the consent of the Human Ethics Committee of Universiti Kebangsaan Malaysia (UKM) with the number of UKM.15.3.5/244/NF-016-2013. A semi-logarithmic calibration curve of PGE_2 was prepared within concentrations of 2.45 to 400 pg/0.1 mL. The percentage inhibition of PGE_2 production (%I) by extract and isolated compounds was calculated as: [(1 – Concentration of PGE_2 in samples or standard)/ Concentration of PGE_2 in control] × 100. Active samples and indomethacin as a positive control were determined for their IC_{50} values with serial dilutions ranging from 1.25 to 10 µg/mL.

DPPH AND FRAP ASSAYS

DPPH and FRAP assays of plant extracts, fractions, and isolated compounds were performed according to methods in our previous study (Sabandar et al. 2019). The free radical scavenging activity was calculated as: $[(A_{blank} - A_{sample})/A_{blank}] \times 100$ where A was the absorbances at 540 nm. Samples with activity more than 50% proceeded to the SC_{50} determination, prepared within concentrations of 1.56 to 100 µg/mL. Meanwhile, the FRAP value expressed as the equivalent trolox amount (µg/µg) was calculated using a trolox linear calibration curve, prepared within concentrations of 0.08 to 10 µg/mL. Ascorbic acid, trolox, gallic acid, and quercetin were used as positive controls.

STATISTICAL ANALYSIS

All bioactivity data were presented as mean ± standard deviation which was obtained from three repetitions. Data were analyzed statistically through one-way ANOVA with Tukey's test ($p<0.05$ was significantly different) and IC_{50} values were determined using GraphPad Prism 5 (La Jolla, CA, USA). Pearson correlation was used to measure the significance between parameters.

RESULTS AND DISCUSSION

The present study demonstrated the anti-inflammatory and antioxidant activity of methanol extracts, fractions, dan chemical compounds of stem bark and root bark of *S. polyanthum*. Qualitative screening of methanol extracts of stem bark and root bark of *S. polyanthum* showed the occurrence of flavonoids, tannins, and terpenoids including steroids and saponins, while alkaloids were absent in both extracts. Previous studies have reported flavonoids, phenolics, and steroids from the leaf part of the plant (Lelono & Tachibana 2013b; Saifudin et al.

2012). Only three phenolics, including flavonoids, have been reported from the stem bark thus far, that are gallic acid, (+)-catechin, and rutin (Lelono & Tachibana 2013a). To the best of our knowledge, the study on the root bark has not been investigated previously.

The fractionation of total extract with different polarity index of organic solvents such as petroleum ether, ethyl acetate, and methanol can provide selective non-polar and medium to polar compounds, following the principle of 'like dissolves like' (Seidel 2006). Yields of fractionation of methanol extracts of both parts of *S. polyanthum* were in a solvent-dependent manner and found to increase in the order of increasing polarity, indicating the high content of polar compounds. The estimation of total phenolic content in methanol extracts of stem bark and root bark showed comparably high amounts of phenolics, while the total flavonoid in the root bark was higher than the stem bark. The high content of phenolics has been reported in a hydromethanol extract of the stem bark of this plant with values of 856 mg gallic acid equivalent/g and 161 mg catechin equivalent/g (Lelono & Tachibana 2009). Moreover, total phenolic and flavonoid in fractions of the stem bark were in a solvent-dependent manner, increasing in the order of methanol > ethyl acetate > petroleum ether. However, this trend was not observed in the root bark fractions, considering that the ethyl acetate fraction contained the least amount of both total phenolic and flavonoid compared to others. Yields of fractionation, total phenolic, and flavonoid contents in methanol extracts and fractions of both parts of the plant are displayed in Table 1.

TABLE 1. Yield, total phenolic content (TPC), and total flavonoid content (TFC) of extracts and fractions of *S. polyanthum*

Extract/fraction	Sample code	Yield (g)	TPC (mg GAE/g)	TFC (mg QE/g)
Stem bark				
Methanol extract	SPS-Extract	600	187.50 ± 1.3^{a}	114.82 ± 2.3^{a}
Petroleum ether fraction	SPS-Pe	0.07	5.23 ± 0.1^{b}	1.75 ± 1.3^{b}
Ethyl acetate fraction	SPS-Ea	0.98	105.29 ± 0.7^{c}	17.18 ± 0.5^{c}
Methanol fraction	SPS-M	8.9	134.85 ± 3.3^{d}	35.85 ± 0.7^{d}
Root bark				
Methanol extract	SPR-Extract	300	189.80 ± 2.0^{a}	134.85 ± 0.5^{e}
Petroleum ether fraction	SPR-PEF	0.07	119.20 ± 0.9^{e}	47.95 ± 1.6^{f}
Ethyl acetate fraction	SPR-EAF	1.2	33.50 ± 0.6^{f}	18.54 ± 0.6^{c}
Methanol fraction	SPR-MF	8.7	179.83 ± 1.3^{g}	27.29 ± 2.0^{g}

Note: Values with different superscript letters on the same column are significantly different ($p<0.05$)

Chemical purification of methanol extract of the stem bark of *S. polyanthum* using chromatography techniques yielded stigmasterol (**1**), 8-hydroxy-6-methoxy-3-pentylisocoumarin (**2**), 3,3'-di-*O*-methylellagic acid (**3**), methylgallate (**4**), a mixture of asiatic acid (**5**) and arjunolic acid (**6**), and daucosterol (**7**). Structures of these compounds are displayed in Figure 1.

To the best of our knowledge, the occurrence of phenolics (**1**-**3**) and terpenoids (**1**, **4**-**6**) in *S. polyanthum* is reported for the first time in the present study. Compound **2** classified as an isocoumarin, has been previously reported from the genus *Knema*, *Pongamia*, and *Tessmania* (Kihampa et al. 2009; Kijjoa et al. 1991; Yin et al. 2006). However, the compound has not yet reported previously from the family Myrtaceae according to literature study. The occurrences of **3** and **4** have been found in other *Syzygium* species, including *S. cumini* seeds and *S. jambolana* leaves (Bhatia & Bajaj 1975; Mahmoud 2001). Moreover, the occurrence of **5** has been found in

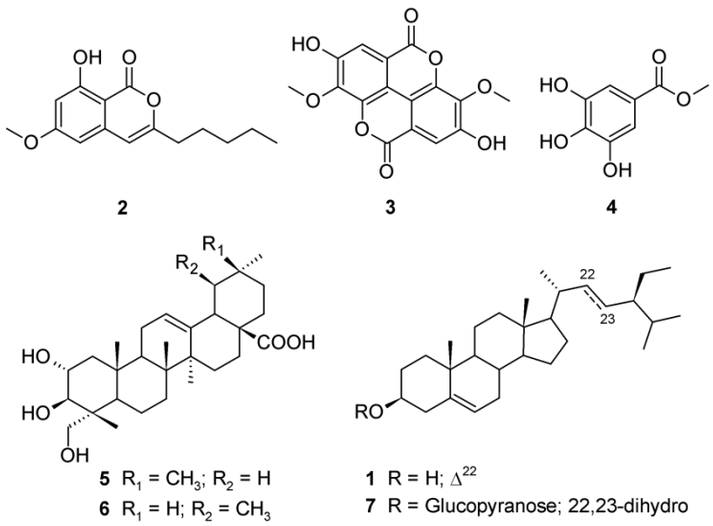

FIGURE 1. Structure of isolated compounds **1-7**

the leaves of *S. claviflorum* and *S. guineense* (Djoukeng et al. 2005; Kashiwada et al. 1998). Meanwhile, compound **6** has been isolated from four *Syzygium* species, including *S. claviflorum* leaves, *S. cordatum* bark and sapwood, *S. guineense* leaves, and *S. samarangense* aerial parts (Candy et al. 1968; Djoukeng et al. 2005; Kashiwada et al. 1998; Srivastava et al. 1995). In this study, compounds **5** and **6** were isolated as a mixture with a ratio of 1:0.5 based on the peaks integration of ^{1}H NMR spectrum. Furthermore, compound **7** has been reported from the stem bark of *S. jambolana* (Bhargava et al. 1974). The HPTLC profiling of these compounds in methanol extracts and fractions of stem bark and root bark is displayed in Figure 2. HPTLC profiles of methanol extracts (tracks 1 and 5) were considered analogs with varied intensity. Some separated bands of compounds at R_f 0.15–0.60 absorbed UV light at 254 nm, indicating the presence of other phenolics beside **2**–**4** (Figure 2(A)). Several bands only appeared after derivatization using the anisaldehyde-sulfuric acid reagent, showing typical of terpenoids such as triterpenoids and steroids (Figure 2(C) & 2(D)). The mixture of **5** and **6** was present in all samples with varied intensity, similar to compound **7**. R_f values of these compounds are displayed in Table 2.

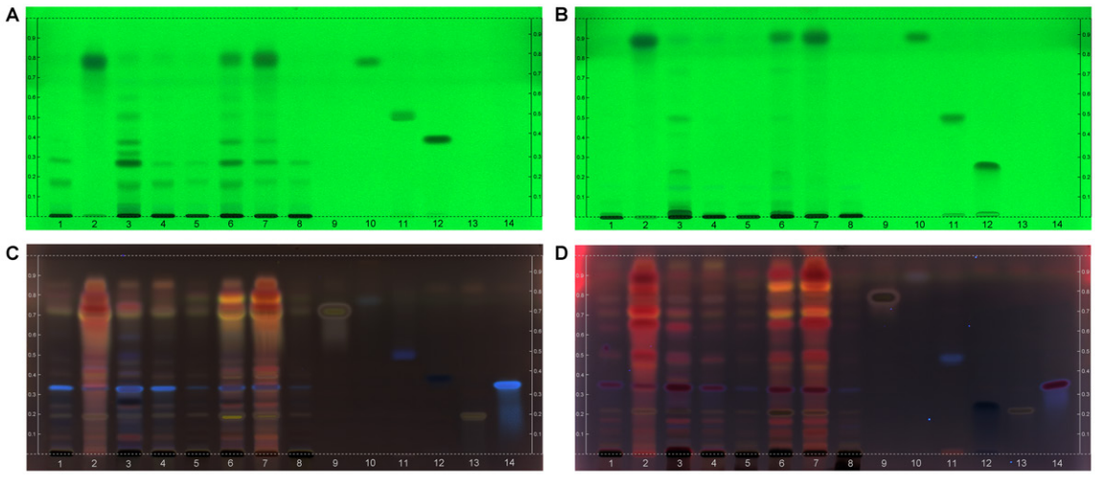

FIGURE 2. HPTLC chromatograms of extracts, fractions, and compounds **1-7** of *S. polyanthum*

(A, C) Developed using chloroform-ethyl acetate-formic acid (5:4:1), (B, D) developed using chloroform-methanol (9:1), (A, B) bands were observed under UV 254 nm before derivatization, (C, D) bands were observed under UV 366 nm after derivatization; tracks: 1 (SPS-Extract), 2 (SPS-Pe), 3 (SPS-Ea), 4 (SPS-Me), 5 (SPR-Extract), 6 (SPR-Pe), 7 (SPR-Ea), 8 (SPR-Me), 9 (compound **1**), 10 (compound **2**), 11 (compound **3**), 12 (compound **4**), 13 (compound **7**), 14 (mixture **5** and **6**).

TABLE 2. Retention factor (R_f) values of the isolated compounds **1-7**

Sample	Mobile phase system	
	$CHCl_3$-EtOAc-FA (5:4:1)	$CHCl_3$-MeOH (9:1)
Compound **1**	0.73	0.76
Compound **2**	0.77	0.89
Compound **3**	0.50	0.48
Compound **4**	0.39	0.24
Mixture of **5** and **6**	0.35	0.33
Compound **7**	0.20	0.21

The assessment of anti-inflammatory activity of methanol extracts and fractions of stem bark and root bark of *S. polyanthum* was carried out using the prostaglandin E_2 (PGE_2) production in LPS-induced human whole blood. PGE_2 is known as a mediator of inflammation. The use of whole blood is believed as the most resemble *in vivo* situations that use intact human cells, which are target cells for performing the anti-inflammatory activities. The production of PGE_2 induced by lipopolysaccharide (LPS) in whole blood has been measured as a reflection of cyclooxygenase-2 (COX-2) activity of blood cells such as monocytes (Pairet et al. 1998; Patrignani et al. 1994). Hence, the inhibition of PGE_2 production in whole blood can be expressed as an inhibition of the enzymatic activity of COX-2 and/or inhibition of the gene expression of COX-2, such as suppression of protein and mRNA (Perera et al. 2001). Results showed that the inhibitory effect of methanol extracts and fractions of *S. polyanthum* stem bark and root bark toward the PGE_2 production with percentage inhibitions ranging from 15.92 to 54.60% at a concentration of 10 µg/mL (Table 3). The ethyl acetate fraction of the root bark exhibited potent inhibitory activity, achieving the lowest IC_{50} value of 3.03 µg/mL. The inhibition exhibited by extracts and fractions were found not to correlate significantly to their phenolic and flavonoid contents based on the correlation analysis (Table 4). It is also noted that compounds **2** and **3** (phenolics) were considered weakly active to inhibit the PGE_2 production, while compound **4** showed moderate inhibition with an IC_{50} of 5.86 µg/mL. On the other hand, triterpenoids and steroids isolated from the plant seemed to play an important role to support the activity of extracts and fractions, indicating by their low IC_{50} values. Indeed, the mixture of **5** and **6** and compound **7** gave IC_{50} values lower than indomethacin, suggesting their potential as cyclooxygenase inhibitors, in agreement with previous studies (Huang et al. 2010; Mavar-Manga et al. 2008). However, triterpenoids and related groups in the family Myrtaceae occurred in minor amounts (Wilson 2011), which might affect the weak inhibitory activity of PGE_2 production by extracts and fractions. Hence, the quantification of these compounds in extracts and fractions of *S. polyanthum* needs further investigation. The previous study has reported the anti-inflammatory of compound **5** toward nitric oxide (NO) and PGE_2 production in RAW 264.7 macrophages induced by LPS. The compound also suppressed iNOS and COX-2 expressions at protein and mRNA levels (Yun et al. 2008). Meanwhile, compound **6** inhibited the arachidonic acid-induced ear edema by 55.5% inhibition, similar action with positive control indomethacin (Ghosh & Sil 2013). In contrast to this study, compound **7** was reported to exhibit weak COX-2 inhibition via prostaglandin D_2 (PGD_2) production in mouse bone marrow-derived mast cells, which caused only 2.2% inhibition at 12.5 µg/mL (Kim et al. 2006). However, this compound has been attributed as PGH_2 and TXA_2 receptor agonist as well as platelet aggregation inhibitor (Zhou et al. 2011). Based on these studies, the active compounds are suggested to play a significant role in promoting anti-inflammatory activity, which might be involving multiple pathways and signaling.

The evaluation of antioxidant activity of methanol extracts and fractions of *S. polyanthum* stem bark and root bark showed scavenging activity toward DPPH radical (Table 3). The activity was in a dose-dependent manner with SC_{50} values in the range of 2.82–9.28 µg/mL, indicating their potent antiradical activity at low concentrations. The antiradical activity was also comparable to ascorbic acid, trolox, quercetin, and gallic acid as positive controls of the assay. Furthermore, FRAP values of the methanol extracts and fractions were found to vary ranging from 0.11 to 7.02 µg/µg equivalent trolox amount. The highest FRAP value was demonstrated by methanol extract of the stem bark, followed by methanol fraction and extract of the root bark (Table 3). However, this activity was considered weakly active when compared with quercetin and gallic acid as positive controls. Pearson analysis showed a strong positive correlation between phenolic content in methanol extracts and fractions and their antioxidant

TABLE 3. DPPH scavenging activity, FRAP values, and PGE_2 inhibition of extract, fractions, and isolated compounds of *S. polyanthum*

Sample	DPPH		FRAP	PGE_2 inhibition	
	%SA	SC_{50}		%I	IC_{50}
Stem bark					
SPS-Extract	77.38 ± 1.5[a]	2.82 ± 0.1[a,b]	7.02 ± 0.1[a]	47.71 ± 1.9[a,b]	-
SPS-Pe	49.14 ± 0.3[b]	-	0.11 ± 0.02[b]	15.92 ± 0.2[c]	-
SPS-Ea	77.28 ± 1.7[a]	4.16 ± 0.2[a,b]	3.92 ± 0.02[c,d]	37.43 ± 2.9[a,d]	-
SPS-M	78.02 ± 2.5[a]	4.47 ± 0.6[a,b]	4.96 ± 0.03[d]	48.24 ± 3.2[a,b]	-
Root bark					
SPR-Extract	82.20 ± 0.6[c]	4.43 ± 0.1[a,b]	6.07 ± 0.2[a]	54.60 ± 3.2[b]	27.69 ± 9.3[a]
SPR-Pe	78.41 ± 0.7[a]	3.93 ± 0.3[a,b]	4.13 ± 0.1[c,d]	17.12 ± 3.4[c]	-
SPR-Ea	61.99 ± 1.7[d]	9.28 ± 1.1[b]	1.00 ± 0.04[b]	53.26 ± 4.0[b]	3.03 ± 0.83[b]
SPR-M	79.67 ± 0.8[a,c]	3.40 ± 0.1[a,b]	6.90 ± 0.2[a]	34.19 ± 9.5[d]	-
Isolated compounds					
Compound **1**	52.30 ± 0.6[b]	175.55 ± 10.2 (430.23)[c]	0.13 ± 0.02[b]	78.20 ± 1.1[e]	0.51 ± 0.1 (1.25)[c]
Compound **2**	nd	-	nd	48.81 ± 1.4[a,b]	-
Compound **3**	nd	-	nd	13.30 ± 2.0[c]	-
Compound **4**	89.39 ± 0.2[e]	1.95 ± 0.02 (10.60)[a]	20.5 ± 1.0[e]	53.47 ± 5.7[b]	5.86 ± 1.0 (33.62)[d]
Mixture of **5** and **6**	49.37 ± 0.9[b,f]	-	-0.03 ± 0.03[b]	79.02 ± 2.8[e]	0.025 ± 0.02 (0.052)[c]
Compound **7**	44.83 ± 0.8[f]	-	-0.01 ± 0.02[b]	72.50 ± 1.6[e]	0.041 ± 0.03 (0.078)[c]
Positive controls					
Ascorbic acid	92.78 ± 0.9[g]	1.41 ± 0.4 (8.01)[a]	3.37 ± 0.3[c]		
Trolox	90.64 ± 0.6[e]	2.53 ± 0.02 (10.11)[a]	-		
Quercetin	66.35 ± 1.9[h]	3.79 ± 0.9 (12.54)[a,b]	25.80 ± 0.8[f]		
Gallic acid	91.42 ± 0.6[e,g]	1.43 ± 0.03 (8.41)[a]	32.35 ± 0.4[g]		
Indomethacin				83.9 ± 0.3[e]	0.12 ± 0.03 (0.34)[c]

Note: Percentage of radical scavenging activity (%SA) at 100 µg/mL; SC_{50} (50% radical scavenging concentration) and IC_{50} (half-maximal inhibitory concentration) are expressed in µg/mL; FRAP values are expressed in µg/µg equivalent trolox amount; Percentage inhibition of PGE_2 (%I) at 10 µg/mL; Values in brackets are expressed in µM; Values with different superscript letters on the same column are statistically different ($p<0.05$); nd: not determined due to the low amount

activities as presented in Table 4. This result was supported by a previous study which reported the antioxidant activity and high concentration of phenolics in the bark of *S. polyanthum* from Java, Indonesia (Lelono et al. 2009). In contrast, the analysis showed a weak correlation of flavonoid content to support the antioxidant activity of extracts and fractions of both parts of the plant. In line with antioxidant activity by the active extracts and fractions, the isolated compound **4** exhibited a significant DPPH scavenging activity and FRAP activity. In contrast, compounds **1** and **5**–**6** were weakly active to scavenge DPPH radical and considered inactive as reducing agents, indicating that the presence of these compounds and the similar group may interfere the antioxidant activity of extracts and fractions of *S. polyanthum* stem bark and root bark. However, previous studies have reported promising antioxidant activity of compounds **5** and **6** in their pure form when assayed using *in vitro* and *in vivo* assays (Lv et al. 2017; Manna et al. 2007). Hence, further research on *in vivo* antioxidant assays of extracts and fractions is needed to explore the synergistic effects among prominent chemical compounds accumulated in the plant.

TABLE 4. Pearson correlation of TPC and TFC toward DPPH, FRAP, and PGE_2

Parameters	Pearson correlation (r)	Linear curve (r^2)
TPC × DPPH	+0.9031*	0.8156
TPC × FRAP	+0.9900*	0.9801
TPC × PGE_2	+0.3981	0.1585
TFC × DPPH	+0.5639	0.3180
TFC × FRAP	+0.6594	0.4348
TFC × PGE_2	+0.5043	0.2543

Note: *Significant correlation ($p<0.05$, two-tailed)

CONCLUSION

The use of *S. polyanthum* in culinary and traditional medicine has shown the importance of this plant to support human life and health. Investigation on chemistry and biological activity of this plant would then facilitate its future direction in the development of new drug agents from nature. Our study concluded a potential of methanol extracts and fractions of stem bark and root bark the plant as sources of therapeutic agents with antioxidant and anti-inflammatory activities. Both parts of this plant contained a high content of phenolics and flavonoids. Seven compounds, including a mixture, are reported for the first time from this plant. The phenolic showed potent antiradical and ferric reduction activity, while the triterpenoids and steroids exhibited remarkable inhibitory activity on prostaglandin E_2 production in LPS-induced human whole blood. The occurrence of these compounds may provide synergistic effects, which may support the traditional and herbal preparations of *S. polyanthum* for treatment of diseases related to oxidative stress and inflammation. Further investigations are also required to provide more evidence on the efficacy and safety of this plant for clinical applications.

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