

Chemical components and antioxidant activities of methanol extract of *Syzygium polycephalum* Miq. stem bark (Myrtaceae)

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This study aimed to reveal chemical components and screen antioxidant activity of methanolic extract of *Syzygium polycephalum* stem bark. The chemical components were isolated by using column chromatographic techniques and determined by spectroscopic methods (UV-Vis, FTIR, MS, and NMR) and comparison with literature data, in which the antioxidant activity was performed using 2,2'-diphenyl-1-picrylhydrazyl (DPPH). The present investigation resulted in four compounds involving gallic acid, pinostrobin, 3,4,3'-tri-*O*-methyllellagic acid, and 3,3'-di-*O*-methyllellagic acid. The four compounds were all in the form of phenolic compounds found from the extract whereas 3,4,3'-tri-*O*-methyllellagic acid and 3,3'-di-*O*-methyllellagic acid were ellagic acid derivatives. The methanolic extract, the four compounds, and vitamin C (as positive control) showed antioxidant activity against DPPH with the IC₅₀ value of 99.9; 10.0; 183.2; 72.1; 63.3; and 13.9 µg/mL, respectively. The antioxidant activity of gallic acid was more active than that of vitamin C. The present study confirms that *Syzygium polycephalum* is rich in phenolic compounds and natural antioxidants.

Keywords: Antioxidant, Gallic acid, *Myrtaceae*, Pinostrobin, *Syzygium polycephalum*, 3,4,3'-tri-*O*-methyllellagic acid, 3,3'-di-*O*-methyllellagic acid.

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Introduction

The genus *Syzygium* is one of the 75 genera (3000 species) belonged to the Myrtaceae family and native to the tropics, particularly tropical America and Australia^{1,2}. The genus that is categorized as flowering plants is one of the important genus in Myrtaceae family, widely also presented in the tropical and sub-tropical region of the world, with the greatest diversity of species taking place in South East Asia such as Indonesia, Malaysia, and East India regions. On the basis of literature study, chemical component studies on *Syzygium* species have led to the identification and isolation of main compounds such as phenolic compounds as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propanes, of which are the major vegetal source is represented by *Syzygium aromaticum*³. This genus is reported to be a source of antioxidants⁴⁻⁹.

Syzygium polycephalum (Family Myrtaceae) is commonly called as "Gowok" in Indonesia. Its

synonyms include *Eugenia polycephala* Miq., *Jambosa cauliflora* DC., *Jambosa polycephala* Miq., and *S. cauliflorum* (DC) Bennet. In Indonesia, this plant grows mainly in Java and Borneo (Kalimantan). This species has rounded, purple-blackish, and acidic fruits. The tree is about 8 to 20 meters with the stem diameter up to 50 centimetres. The wood is strong and hence can be used as material for house construction¹⁰.

Some research conveyed that the ethyl acetate fraction of *S. polycephalum* wood contains 3-*O*-glucosyl-3',4',5'-trihydroxy flavonol. The compound became potential anti-fungal to inhibit the growth of *S. commune* Fr. and *Pleurotus* sp fungi¹¹. The *S. polycephalum* leaves contain non phenolic compounds namely ursolic acid, oleic acid, squalene and β-sitosterol¹². Previous research on *S. polycephalum* depicted that chloroform extract of *S. polycephalum* stem bark contained 3,3',4'-tri-*O*-methyllellagic acid¹³ and gallic acid¹⁴. To the best of the researchers' knowledge, no information had been reported in accordance with chemical components and antioxidant potentials of methanolic extract of *S. polycephalum* stem bark. Hence, the present study gives the information especially about the phenolic

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compounds and antioxidant bioactivity from methanolic extract of the *S. polycephalum* stem bark. In regard to clearer portrayals, this study also reported the finding of all compounds from the methanolic extract of the plant.

Materials and Methods

Analytical chemicals and equipment

The solvents used in this study were *n*-hexane, chloroform, methanol, ethyl acetate, and dichloromethane. The undertaken separating techniques covered Vacuum Liquid Chromatography (VLC) (using silica gel 60, 0.040-0.063 mm) and Gravitational Column Chromatography (GCC) (silica gel 60, 0.063-0.200 mm and 0.200-0.500 mm or 70-230 mesh ASTM), which were always monitored by TLC on Kieselgel gel 60 F254 (E. Merck).

The equipment used to do extraction and fractionation (isolation) were filter paper, Buchner funnel, Hirsch funnel, Erlenmeyer flask, pipette, spatula, measuring glass, vials, containers, separating funnel, and vacuum rotary evaporator type Buchi Rotavapor R-215. The equipment used to measure the melting point of isolates was Fisher Scientific. Moreover, chromatographic techniques used to isolate chemical components from methanolic extract included VLC (using silica gel 60, 0.040-0.063 mm) and GCC (using silica gel 60, 0.063–0.200 mm and 0.200–0.500 mm or 70–230 mesh ASTM). The TLC analyses were carried out on silica gel 60 F254 chroma plates with the developing solvent systems. Checking the homogeneity of the compounds were conducted using TLC on Kieselgel gel 60 F254 pre-coated sheets (E. Merck) and the spots were detected by exposure to UV-lamp at 254 or 366 nm. Several instruments were needed to identify and characterize the isolates including UV-1800 SHIMADZU and FTIR-8400S SHIMADZU spectrophotometers. The ¹H-NMR spectra were recorded with a Bruker DRX-600 NMR Spectrometer (600 MHz, DMSO-d₆) instrument and the ¹³C-NMR spectra were obtained with the same instrument at 150 MHz in DMSO-d₆. Chemical shifts were given in δ (ppm) values relative to those of the solvent signal (DMSO-d₆) on the tetramethylsilane as an internal standard in NMR spectroscopy. The constituents were identified by ion-trap mass spectrometry in negative ion modes with EI/FAB JEOL MS Station JMS spectrometers and LC-ESI-MS with mariner Biospectrometry Hitachi L 6200.

Plant materials

The stem bark of *S. polycephalum* was collected in December 2014 from Ngawi village, East Java,

Indonesia. The identification of the plant was performed by the staff of Herbarium-LIPI, Purwodadi, East Java, Indonesia. A voucher sample is kept in the Herbarium-LIPI with the identification number of 0117/IPH.06/HM/I/2015.

Methanolic extraction and its fractionation

The stem bark of *S. polycephalum* was dried at room temperature, ground to a powder and extracted with MeOH for one day. After filtration, the solvent was removed under vacuum rotary evaporator (45 °C). Extraction of 8.3 kg of dried plant materials yielded 349 g (4.2%) of crude extract. The extract was then suspended in MeOH and defatted with hexane yielded two layers in which the upper layer (hexane soluble parts) was then separated to gain hexane fraction (22.38 g). By using the same manner, the residue was then suspended in methanol and defatted with chloroform yielded chloroform fraction (5.66 g) and methanolic extract that will be used to be materials in this study.

Isolation and characterization of pure isolates of methanolic extract

As much as 10 g of methanolic extract was isolated using VLC with eluents (dichloromethane-ethyl acetate-methanol for increasing the polarity) to yield 20 fractions. Based on TLC analysis, all fractions that gave the same value of R_f could be grouped into three major fractions namely fraction A (1-4), B (5-15), and C (16-20). Afterwards, in connection with TLC analysis, fraction A seemed to give a simple chromatogram profile because it displayed one spot only. The fraction A was then allowed to evaporate at room temperature and yielded a pure isolate as colourless needle crystal (10 mg) with m.p. 257-258 °C. The isolate was then characterized by UV-Vis and FTIR spectroscopies and by comparing with literature data. The molecular structure of isolate A was gallic acid (**1**).

Fraction B was consequently purified through recrystallization in hot methanol and followed by filtering to yield colourless filtrate. The filtrate was allowed to evaporate at room temperature and yielded a white crystal (35 mg) with m.p. 95-96 °C. The crystal was then characterized by UV-Vis, FTIR, MS, and NMR spectroscopies and by comparison with literature data and determined its structure (isolate B) to be 7-hydroxy-5-methoxyflavanone (pinostrobin) (**2**).

On the other hand, fraction C was separated by using GCC with eluents dichloromethane-methanol and resulted in 55 fractions. In regard to TLC analysis, the fractions could be combined with to give three fractions comprising C1 (1-6), C2 (7-11), and C3 (15-21). The fractions were allowed to evaporate at room temperature and yielded pure enough isolates. The isolates C2 and C3 were separately recrystallized in hot methanol and followed by filtering to yield colourless filtrate. The filtrate was allowed to evaporate at room temperature and obtained a white amorphous powder (isolate C2) with m.p. 267-269 °C and an off-white amorphous powder (isolate C3) with m.p. 293-295°C, respectively. Both isolates were then characterized by UV-Vis, FTIR, MS, and NMR spectroscopies and by comparing with literature data. The structures of isolated C2 and C3 were 3,3',4-tri-*O*-methyllellagic acid (**3**) and 3,3'-di-*O*-methyllellagic acid (**4**), respectively.

DPPH radical scavenging activity

The 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants⁵. The antioxidant activity (free radical scavenging activity) of the test samples (methanolic extract of *S. polycephalum* stem bark, compounds **1**, **2**, **3** and **4** as well as vitamin C) on the stable radical 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) was determined according to previously described methods¹⁵. The following concentrations of methanolic extract were prepared in methanol: 10, 25, 50, 75, 100 µg/mL and 0.3 mL of each concentration was mixed with 3 mL containing DPPH radicals (0.004%, w/w). The mixture was shaken vigorously and left to stand for 30 minutes in the dark before measuring the absorbance at 515 nm against a blank¹⁶. Lower absorbance reading of the reaction mixture indicated higher free radical scavenging activity.

The same manner, compounds **1**, **2**, **3** and **4** as well as vitamin C (used as standard to compare) were prepared in methanol with the following concentrations: 1, 5, 10, 15 and 20 µg/mL and 0.3 mL of each concentration were added at an equal volume (3 mL) to methanolic solution of DPPH (400 µg/mL). Blank solutions were prepared by mixing 2 mL of methanol with 4 mL of DPPH (400 µg/mL) solutions in methanol. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capacity to scavenge the DPPH radical

was calculated by using the following equation:

$$\% \text{ inhibition} = 100 \times (\text{Abs blank} - \text{Abs sample}) / \text{Abs control}$$

where Abs blank is the absorbance value of the control and Abs sample is the absorbance value of the test samples¹⁷. The equation was used to obtain the IC₅₀ value that was defined as the amount of antioxidant substance required to scavenge 50% of free-radicals (DPPH) present in the assay system. In other words, IC₅₀ (50% inhibitory concentration) values were obtained through extrapolation from the concentration of test samples necessary to scavenge 50% of free-radicals (DPPH). A lower IC₅₀ value indicated greater radical scavenging activity. IC₅₀ < 50 µg/mL is very active; 50 µg/mL < IC₅₀ < 100 µg/mL is active; 100 µg/mL < IC₅₀ < 200 µg/mL is moderately active; and IC₅₀ > 200 µg/mL is not active¹⁸.

Results and Discussion

Structural determination of the isolated compounds

Methanolic extract of *S. polycephalum* stem bark that had been partitioned by hexane and chloroform was separated using various chromatographic techniques and followed by purification methods including recrystallization. The results yielded compounds **1**, **2**, **3**, and **4**. The data of all compounds were presented as follows.

Fig. 1 depicts the structure of compound **1** namely gallic acid, which was a colourless crystal (10 mg) with m.p. 257-258 °C. The maximum wavelengths of UV-Vis (MeOH, λ_{max}) of compound **1** lied at 216.90 and 272.80 nm indicated the absorption of the phenolic group. The IR spectrum (KBr, ν_{max}) showed the absorption band at 3489, 3368, 3277, 3080, 3005, 1701, 1612, 1535, 1448, 1246, 1026, and 702 cm⁻¹. IR absorption bands at 3489, 3368, 3277, 3080, and 3005 cm⁻¹ indicated the presence of hydroxyl groups and at 1701 cm⁻¹ indicated the carbonyl group. The absorption bands at 1613, 1535, and 1449 cm⁻¹ represented a benzene ring system. For a while,

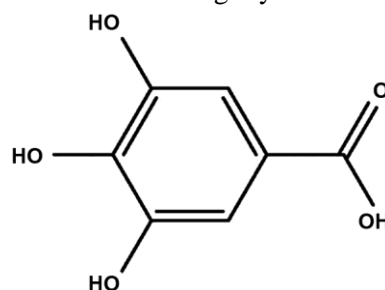


Fig 1 — Structure of gallic acid (**1**)

absorption bands at 1246, and 1026 cm^{-1} indicated the presence of three $-\text{O}-\text{aryl}$ groups directly attached at the benzene ring in which two of their groups are similar, namely *meta*-position towards carbonyl group. The last absorption band at 702 cm^{-1} showed substituted benzene. By comparing the IR data of compound **1** with that of an authentic sample (gallic acid) and gallic acid isolated from *S. littorale*⁹, it was confirmed that the compound **1** was gallic acid (see Table 1). The finding of compound **1** was the first report from *S. polycephalum*, although it had previously been found in other *Syzygium* species such as *S. cumini*¹⁹, *S. polyanthum*⁷, and *S. littorale*⁹.

Fig. 2 shows the structure of compound **2** namely 7-hydroxy-5-methoxyflavanone (pinostrobin). The compound was characterized as a white crystals with m.p. 95-96°C; UV (MeOH, λ_{max}): 212.4 and 287.7 nm; IR (KBr, ν_{max}): = 3223, 3034, 1643, 1622, 1579, 1207, and 1157 cm^{-1} ; ¹H NMR (600 MHz, DMSO-*d*₆, ppm): δ (ppm) = 5.64 (1H, H-2), 2.84 (1H, H-3a), 3.29 (1H, H-3b), 6.10 (1H, H-6), 6.15 (1H, H-8), 7.43 (2H, H-2' and H-6'), 7.40 (2H, H-3' and H-5'), 7.52 (1H, H-

4'), 3.79 (3H, 7-OMe), and 12.09 (1H, 5-OH); ¹³C NMR (150 MHz, DMSO-*d*₆, ppm): δ (ppm) = 78.46 (1C, C-2), 42.04 (1C, C-3), 196.45 (1C, C-4), 163.11 (1C, C-5), 167.39 (1C, C-7), 162.56 (1C, C-9), 55.83 (1C, C-7 OMe), 94.68 (1C, C-6), 93.80 (1C, C-8), 102.55 (1C, C-10), 138.44 (1C, C-1'), 128.45 (2C, C-2' and C-6'), 128.51 (2C, C-3' and C-5') and 126.55 (1C, C-4); EI-MS (*m/z* 270 for C₁₆H₁₄O₄).

Compound **2** was obtained as a white crystal (35 mg) with m.p. 95-96 °C. The UV-Vis (MeOH, λ_{max}) spectrum of compound **2** showed maximum absorption at 212.4 and 287.7 nm. The IR spectrum of the compound displayed characteristic absorption bands for hydroxyl group (3223 cm^{-1}), carbonyl group (1643 cm^{-1}), ring benzene system (1622, 1579, and 1442 cm^{-1}) and C-O-C bond (1157 cm^{-1}). This is the first report of compound 7-hydroxy-5-methoxyflavanone (pinostrobin) from *S. polycephalum*, although it has previously been found in other *Syzygium* species such as *S. samarangense* (Amor *et al.*, 2005). The ¹³C-NMR spectrum (150 MHz, DMSO-*d*₆) of compound **2** showed one signal for methine group located at δ_{C} 78.46 (C-2), one aliphatic carbon at δ_{C} 42.04 (C-3), one carbon carbonyl at δ_{C} 196.45 (C-4), three oxyaryl carbon at δ_{C} 163.11 (C-5), 167.39 (C-7), and 162.56 (C-9). Additionally, the ¹³C-NMR also displayed a methoxy group at δ_{C} 55.83 (7-OCH₃) and nine aromatic carbon at δ_{C} 94.68 (C-6), 93.80 (C-8), 102.55 (C-10), 138.44 (C-1'), 128.45 (C-2' and C-6'), 128.51 (C-3' and C-5') and 126.55 (C-4). The ¹H-NMR spectrum (600 MHz, DMSO-*d*₆) of compound **2** showed the presence of an oxyalkyl proton signal located at δ_{H} 5.64 (1H, H-2), aliphatic proton at δ_{H} 2.84 (1H, H-3a) and 3.29 (1H, H-3b), one of *meta*-coupled aromatic protons at δ_{H} 6.10 (1H, H-6) and 6.15 (1H, H-8), monosubstituted phenyl ring at δ_{H} 7.43 (2H, H-2' and H-6'), 7.40 (2H, H-3' and H-5'), and 7.52 (1H, H-4') ppm. In addition, the ¹H-NMR displayed a methoxy group at δ_{H} 3.79 (3H, 5-OCH₃) and hydroxyl proton at δ_{H} 12.09 (1H, 7-OH) ppm. When ¹H-NMR and ¹³C-NMR spectra of compound **2** were compared with those of 7-hydroxy-5-methoxyflavanone (pinostrobin) as reported in literature data^{20,21}, the compounds were the same (see Table 2).

Fig. 3 depicts the structure of compound **3** namely 3,3',4-Tri-O-methylgallic acid, which was a white amorphous powder; m.p. 267-269 °C; UV (MeOH, λ_{max}): 247.0 and 371.3 nm; IR (KBr, ν_{max}): = 3433, 3082, 2955, 2853, 1753, 1726, 1609, 1578, 1493, 1360, 1302, 1115, 1092, 988 and 914 cm^{-1} ; ¹H NMR (600 MHz, DMSO-*d*₆, ppm): δ (ppm) = 7.624 (1H, s,

Table 1 — FTIR spectral data for compound **1** and Gallic acid

Position	IR (ν_{max} , cm^{-1})	
	Gallic acid ⁹	Gallic acid*
3489	3462	3466
3368	3367	3365
3277	3290	3292
3080	3065	3064
3005	3013	3008
1701	1705	1705
1613	1620	1612
1535	1541	1537
1449	1450	1450
1246	1246	1246
1026	1028	1026
702	702	702

* Gallic acid (authentic sample)

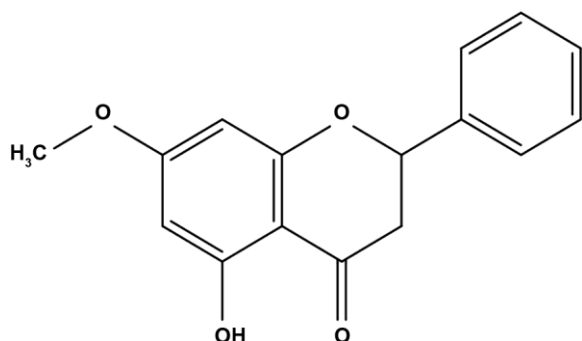
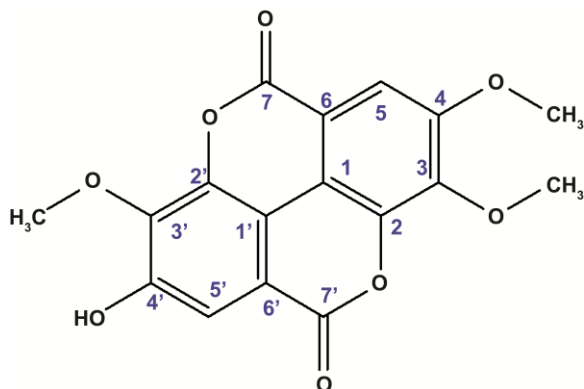


Fig 2 — Structure of 5-hydroxy-7-methoxyflavanone (pinostrobin) (**2**)

Table 2 — H- and C-NMR spectral data for the compound **2** and Pinostrobin

Position	Compound 2 (DMSO)		Pinostrobin (DMSO) ²¹		Pinostrobin (CDCl ₃) ²⁰	
	δ_C (150 MHz)	δ_H (600 MHz)	δ_C (100 MHz)	δ_H (300 MHz)	δ_C (125 MHz)	δ_H (500 MHz)
2 (CH)	78.46	5.64 (dd)	79.44	5.44 (dd)	77.45	5.43 (dd)
3 (CH ₂)	42.04	3.29 (dd) 2.84 (dd)	43.03	3.48 (dd) 2.84 (dd)	43.50	3.08 (dd) 2.84 (dd)
4 (C)	196.45	-	196.60	-	195.93	-
5 (C)	163.11	-	164.10	-	164.30	-
6 (CH)	94.68	6.10 (d)	94.80	6.10 (d)	95.30	6.04 (d)
7 (C)	167.39	-	168.36	-	168.30	-
8 (CH)	93.80	6.15 (d)	95.66	6.15 (d)	94.43	6.06 (d)
9 (C)	162.56	-	163.52	-	163.14	-
10 (C)	102.55	-	103.52	-	103.30	-
1' (C)	138.44	-	139.41	-	138.54	-
2'/6' (CH)	126.55	7.52 (m)	129.49	7.55 (m)	126.30	7.43 (m)
3'/5' (CH)	128.51	7.40 (m)	127.51	7.43 (m)	129.00	7.42 (m)
4' (CH)	128.45	7.43 (m)	129.43	7.43 (m)	126.30	7.43 (m)
5-OH	-	12.09 (s)	-	12.10 (s)	-	12.03 (s)
7-OCH ₃	55.83	3.79 (s)	56.57	3.81 (s)	55.85	3.81 (s)

Fig 3 — Structure of 3,4,3'-tri-O-methylellagic acid (**3**)

H-5) and 7.529 (1H, s, H-5'), 3.313 (1H, *br*, OH), 4.058, 4.044, and 4.000 (3H, s, -OCH₃); ¹³C NMR (150 MHz, DMSO-*d*₆): δ (ppm) = 158.45 (1C, C-7), 158.25 (C1, C-7'), 153.65 (C, C-4), 152.93 (C, C-4'), 112.44 (C, C-5), 107.39 (C, C-5'), 141.41 (C, C-3), 140.20 (C, C-3'), 140.89 (C, C-2), 140.72 (C, C-2'), 113.38 (C, C-6), 111.79 (C, C-6'), 111.68 (C, C-1), 110.86 (C, C-1'), 61.20 (-OCH₃, C-3), 60.85 (-OCH₃, C-3'), 56.63 (-OCH₃, C-4); LC-ESI-MS (*m/z* 345.39 [M+H]⁺) for C₁₇H₁₂O₈.

Compound **3** was obtained as a white amorphous powder (5 mg), m.p. 267-269 °C and its molecular formula C₁₇H₁₂O₈ was determined by the LC-ESI-MS (*m/z* 345.39). The UV-Vis (MeOH, λ_{max}) spectrum of compound **3** showed maximum absorption at 247.0 and 371.3 nm indicating phenolic compound with conjugated benzene ring of the carbonyl group. The IR (KBr, ν_{max}) spectrum of compound **3** exhibited the

following absorption frequencies: 3433, 3082, 2955, 2853, 1753, 1726, 1609, 1578, 1493, 1360, 1302, 1115, 1092, 988 and 914 cm⁻¹. The IR spectrum of the compound showed sharp absorption bands at 3433 cm⁻¹ indicating hydroxyl group, at 3082, 2955, and 2853 cm⁻¹ representing C-H stretching, at 1753, and 1726 cm⁻¹ revealing the presence of two carbonyl groups. The characteristic absorption bands at 1609, 1578, and 1493 cm⁻¹ indicated a benzene ring system. The presence of a methyl group is shown specifically at 1360 cm⁻¹. For a while, absorption bands at 1302, 1115, and 1092 cm⁻¹ indicated -O-aryl and -O-CH₃, respectively. The last absorption band at 988 and 914 cm⁻¹ showed substituted benzene. The ¹H-NMR spectrum (600 MHz, DMSO-*d*₆, ppm) of compound **3** revealed the presence of six significant proton signals that could be explained as follows. Two signals located at δ_H 7.62 (1H, s, H-5) and 7.53 (1H, s, H-5') indicated two aromatic protons due to the ellagic acid skeleton. The spectrum of the compound also displayed one signal at δ_H 3.31 (1H, *br*, OH) suggesting the presence of an aromatic hydroxyl group (aryl -OH). In addition, three signals located at δ_H 4.06, 4.04, and 4.00 (3H, s, -OCH₃) showed three methoxyl groups. The ¹³C-NMR spectrum (150 MHz, DMSO-*d*₆, ppm) of compound **3** displayed seventeen carbon signals that could be described as follows. The spectrum showed 17 signals, of which 14 signals were assigned to the ellagic acid portion and the rest signals were three methoxyl groups. Two carbon signals located at δ_C 158.45 (C, C-7), and 158.25 (C, C-7') confirming clearly for two carbonyl groups were

attributed to ellagic acid lactone carbonyl signals, two carbon signals at δ_C 153.65 (C, C-4), and 152.93 (C, C-4') confirmed as benzene ring attached by methoxyl and hydroxyl groups, and two carbon signals at δ_C 112.44 (C, C-5), and 107.39 (C, C-5') indicated benzene ring attached by hydrogen. Then, two carbon signals located at δ_C 141.41 (C, C-3), and 140.20 (C, C-3') with high intensity indicated as benzene ring attached by methoxyl groups. For a while, two carbon signals on the position of δ_C 140.89 (C, C-2), and 140.72 (C, C-2') represented benzene ring attached by the respect lactone groups and δ_C 113.38 (C, C-6), and 111.79 (C, C-6') revealed benzene ring attached by carboxyl groups. Two carbon signals located at δ_C 111.68 (C, C-1), and 110.86 (C, C-1') revealed benzene ring attached by other phenyl group and assigned as an ellagic acid skeleton. Finally, three carbon signals located at δ_C 61.20 (-OCH₃, C-3), 60.85 (-OCH₃, C-3'), and 56.63 (-OCH₃, C-4) revealed carbon methoxy groups. When, ¹H-NMR and ¹³C-NMR spectra of compound **3** were compared with those of 3,4,3'-tri-*O*-methyllellagic acid as reported in literature data^{13,22,23}, the compounds were the same (see Table 3).

Fig. 4 shows the structure of compound **4** namely 3,3'-Di-*O*-methyllellagic acid, which was an off-white amorphous powder; m.p. 293-295 °C; UV (MeOH, λ_{max}); 248.5 and 365.1 nm; IR (KBr, ν_{max}): = 3277, 2999, 2955, 2851, 1724, 1613, 1578, 1352, 1213, and

1177 cm⁻¹; ¹H NMR (600 MHz, DMSO-*d*₆, ppm): δ (ppm) = 7.505 (2H, s, H-5 and H-5') and 4.044 (3H, s, -OCH₃); ¹³C NMR (150 MHz, DMSO-*d*₆, ppm): δ (ppm) = 111.49 (2C, C-1 and C-1'); 141.30 (2C, C-2 and C-2'), 140.17 (2C, C-3 and C-3'), 152.22 (2C, C-4 and C-4'), 111.39 (2C, C-5 and C-5'), 111.99 (2C, C-6 and C-6'), 158.39 (2C, C-7 and C-7'), and 60.85 (2C, -OCH₃, C-3 and C-3'); EI-MS (m/z 330 for C₁₆H₁₀O₈).

Compound **4** was obtained as a white amorphous powder (12 mg) with m.p. 293-295 °C. The UV-Vis (MeOH, λ_{max}) spectrum of the isolated compound showed maximum absorption at 248.5 and 365.1 nm. The IR (KBr, ν_{max}) spectrum exhibited the following absorption frequencies: 3277, 2999, 2955, 2851, 1724, 1613, 1578, 1352, 1213, and 1177 cm⁻¹. The IR

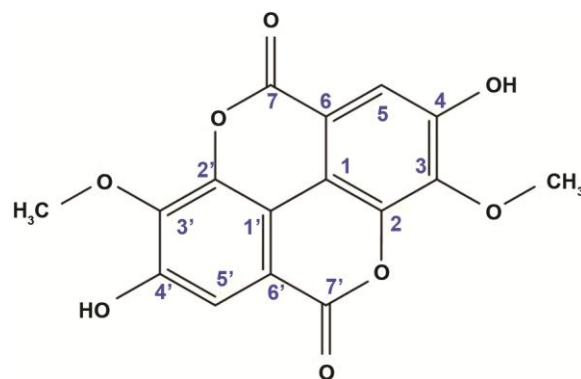


Fig 4 — Structure of 3,3'-di-*O*-methyllellagic acid (**4**)

Table 3 — H- and C-NMR spectral data for compound **3** and 3,4,3'-tri-*O*-methyllellagic acid

Position	Compound 3		3,4,3'-tri- <i>O</i> -methyllellagic acid					
	δ_C (150 MHz)	δ_H (600 MHz)	Wardana (2016) ¹³		Hiranrat (2010) ²²		Gao <i>et al.</i> (2014) ²³	
			δ_C (150 MHz)	δ_H (600 MHz)	δ_C (125 MHz)	δ_H (500 MHz)	δ_C (160 MHz)	δ_H (600 MHz)
1 (C)	111.68	-	111.76	-	112.01	-	111.62	-
2 (C)	140.89	-	140.95	-	141.38	-	141.23	-
3 (C)	141.41	-	141.48	-	141.81	-	140.26	-
4 (C)	153.65	-	153.71	-	154.04	-	152.23	-
5 (CH)	107.39	7.53 (s)	107.44	7.52 (s)	107.72	7.64 (s)	111.42	7.73 (s)
6 (C)	113.38	-	113.44	-	114.03	-	112.11	-
7 (C=O)	158.45	-	158.52	-	159.06	-	158.52	-
1'(C)	110.86	-	110.89	-	111.68	-	111.65	-
2'(C)	140.72	-	140.77	-	141.03	-	141.25	-
3'(C)	140.20	-	140.28	-	140.74	-	140.23	-
4'(C)	152.93	-	153.06	-	152.77	-	152.22	-
5'(CH)	112.44	7.62 (s)	112.49	7.61 (s)	112.77	7.68 (s)	111.45	7.61 (s)
6'(C)	111.79	-	111.84	-	112.70	-	112.12	-
7'(C=O)	158.25	-	158.32	-	158.74	-	158.63	-
3-OCH ₃	61.20	4.04 (s)	61.28	4.04 (s)	61.76	4.17 (s)	61.53	4.19 (s)
4-OCH ₃	56.63	4.00 (s)	56.70	3.99 (s)	56.77	4.04 (s)	56.51	4.04 (s)
3'-OCH ₃	60.85	4.09 (s)	60.92	4.06 (s)	61.54	4.19 (s)	61.26	4.14 (s)

Pinostrobin, one of dihydroflavanones (flavonoid types) isolated from *S. polycephalum*, has never been found previously from the genus *Syzygium* plants. The compound had been just found in another genus of Myrtaceae namely *Eucalyptus nitida*³¹. The flavonoid reported from the genus *Syzygium* could be conveniently grouped into the following types: flavones and flavone glycosides, flavonols and flavonol glycosides, dihydroflavanones, dihydroflavonols, chalcones, dihydrochalcones, anthocyanidins and anthocyanins. The following flavonoids commonly exist in *Syzygium* plants such as anthocyanins, quercetin, isoquercetin, kaempferol, myricetin, myricitrin, flavonol glycosides myricetin 3-O-(4''-acetyl)- α -L-rhamnopyranosides, and acylated flavonol glycosides that had been isolated from *S. cumini*³². The flavonoids of eugenin, kaempferol, rhamnetin, and eugentin have been also isolated from *S. aromaticum*³³, C-methylated chalcones: 2'-hydroxy-4',6'-dimethoxy-3'-methylchalcone, 2',4'-dihydroxy-6' methoxy-3',5'-dimethylchalcone, 2',4'-dihydroxy-6'-methoxy-3'-methylchalcone (stercuren-sin), 7-hydroxy-5-methoxy-6,8-dimethylflavanone, 2',4'-dihydroxy-6'-methoxychalcone (cardamonin) found in *S. samarangense*^{34,29}, and dimethylcardamonin (called as 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone) isolated from *S. campanulatum* Korth leaf extract³⁵, etc.

Further, 3,4,3'-tri-*O*-methyllellagic acid (3) and 3,3'-di-*O*-methyllellagic acid (4) were known as ellagic acid derivatives³⁶. Based on literature data, ellagic acid had been mostly isolated from the genus *Syzygium* such as *S. cumini*^{37,38}, *S. samarangense*²⁹, and *S. aromaticum*³⁹. Besides, compound 3 had been also isolated from *S. aromaticum*²⁸ and *S. jambos*³⁶, and compound 4 had been also found from *S. cumini*⁴⁰. It seems that both compounds were ellagic acid derivatives rarely found in *Syzygium* plants.

On the other hand, the free radical scavenging activities of the tested samples including methanolic extract, compounds isolated from *S. polycephalum* (compounds 1, 2, 3, and 4), and vitamin C towards DPPH radical had been investigated. DPPH was a stable radical that had been used to evaluate the antioxidant activity of plant and microbial extracts⁴¹. The antioxidant activity of methanolic extract of *S. polycephalum* steam bark and compounds 1, 2, 3, and 4 was tested using the DPPH method with vitamin C as the positive control. The testing principle using DPPH method was carried out based on the ability of antioxidant compounds to donate protons on free radical compounds. The free radical retention

Table 5 — Antioxidant Activity (IC₅₀) of Methanolic Extract of *S. polycephalum*, Compounds 1, 2, 3, and 4 as well as Vitamin C against DPPH

Tested Samples	IC ₅₀ (µg/mL)
Methanolic extract	99.9
Gallic acid (1)	10.0
Pinostrobin (2)	183.2
3,4,3'-tri- <i>O</i> -methyllellagic acid (3)	72.1
3,3'-di- <i>O</i> -methyllellagic acid (4)	63.3
Vitamin C	13.9

activity was measured at a 515 nm wavelength obtained from the optimum wavelength optimization of DPPH. The value of IC₅₀ was the concentration of an antioxidant substance to absorb 50% of DPPH radicals. The IC₅₀ value of methanolic extract, gallic acid (1), pinostrobin (2), 3,4,3'-tri-*O*-methyllellagic acid (3), and 3,3'-di-*O*-methyllellagic acid (4) are given in Table 5.

In accordance with Table 5, it seemed that the antioxidant activity of compound 1 was very active because of the IC₅₀ value of 10.0 µg/mL. The IC₅₀ value of compound 1 was more active than that of Vitamin C (IC₅₀ value of 13.9 µg/mL). The antioxidant activity of methanolic extract of compounds 3 and 4 was active while that of compound 2 was moderately active. The previous studies on plants of Myrtaceae family revealed that phenolic compounds such as gallic acid and ellagic acid (their derivatives) contributed to antioxidant activity⁴².

Conclusion

The analysis of the methanolic extract of *S. polycephalum* stem bark reveals four chemical components namely gallic acid (1), pinostrobin (2), 3,4,3'-tri-*O*-methyllellagic acid (3), and 3,3'-di-*O*-methyllellagic acid (4). The antioxidant activity against DPPH for methanolic extract, compound 1, 2, 3 and 4, and vitamin C result in the IC₅₀ value of 99.9, 10.0, 183.2, 72.1, 63.3, and 13.9 µg/mL, respectively. The antioxidant activity of gallic acid is more active than that of vitamin C and pinostrobin, which show moderately active.

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