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Isolation of 2,4-Di-tert-butylphenol and Butyrospermol 3-β-*O*-palmitate from *Syzygium aqueum* stem bark

Abstract

Syzygium genus in Myrtaceae family is a rich source of phytochemical constituents that possess various bioactivities. One of well-known species of this genus, *Syzygium aqueum*, has been already utilized as traditional medicine. This plant is cultivated in countries of tropical regions of the world such as Malaysia and Indonesia. The aim of this study was intended to isolate phytochemical constituents from the stem bark of *S. aqueum*. To date, there have been no reports of chemical substances isolated from the stem bark of this plant. The powder of the stem bark was extracted followed by partitioned in order to obtain n-hexane extract which then was separated using silica gel column chromatographic several times until the pure compounds were resulted. The isolated compounds were identified by spectroscopie method including Fouirer Transform Infrared (FTIR) and nuclear magnetic resonance (NMR) and were known as 2,4-Di-tert-butylphenol & butyrospermol 3-β-*O*-palmitate, respectively. The spectoscopie data of those compounds were compared with references.

Keywords: Myrtaceae, *Syzygium aqueum*, phytochemical constituents, phenolic, ester of fatty acid, 2,4-di-tert-butylphenol and butyrospermol 3-β-*O*-palmitate

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Introduction

Myrtaceae is an enormous family of wood flowering plants with 155 genera and around about 4000 species. The species are cultivated in South America, Southeast Asia, and Australia, but few species are found in Africa.[1,2] Tropical regions, such as Malaysia and Indonesia, are native regions for one species of this family which is *Syzygium aqueum*.[3,4] *S. aqueum* has the local name water jambu or water apple. The whole parts of the tree have been applied as traditional medicine such as for relieving child birth pains (bark), treating a cracked tongue (leaves), relieving itching and reducing swelling (roots)[5–7] Moreover, the previous researchers have been tested leaves and fruits for antioxidant, anti-inflammatory, and anticancer (MCF-7 and MDA-MB-231 cell lines) activities.[8–11] In recent, there are some reports about the phytochemicals constituents isolated from leaves such as flavonoid and tannin and some of these flavonoids have been determined the antidiabetic and antioxidant activities.[1,6,12] The phytochemical compounds of stem bark of *S. aqueum* have never been reported by any researchers. Therefore, the present study was carried out to collect the phytochemicals compounds isolated from stem bark of this plant.

Material and method

General experimental procedure

FT-IR spectra (Fouirer Transform Infrared spectroscopy) were determined on Tracer-100 spectrophotometer (Shimadzu) and the spectra showed in cm^{-1}.^{1}H NMR,^{13}C NMR (Nuclear Magnetic Resonance) and 2D NMR (HSQC, HMBC and COSY) were recorded on BRUKER 600 Hz using Tetramethylsilane (TMS). Chemical shifts of carbon and proton NMR were given in δ (ppm).

Plant material

The stem bark of *S. aquem* was collected form Wage, Taman, Sidoarjo, East Java, Indonesia. This our sample had brown color and cracked. Department of Biology, Faculty of Science and Technology, Universitas Airlangga was identified and determined the scientific name. The stem bark obtained was crushed to form a powder and then was extracted using a general protocol.[13]

Extraction and isolation

The powdered of stem bark of *S. aqueum* (1kg) was macerated with 40L of methanol at room temperature for 3 days. The methanol extract (450g) was partitioned with n-hexane. n-hexane fraction obtained (50g) was then separated using silica gel 60 (700-200 mesh ASTM) in gravity column chromatographic method (GCC) with n-hexane: ethyl acetate (9:1) as eluent.[13] The similar fractions were combined to yield 19 fractions (SA- 1 to SA-19). Fraction-1 (SA-1) was re-chromatographed by eluting with n-hexane: ethyl acetate (95:5), yielding 5 fractions (SA-1-1 to SA-1-5). One spot was found in SA-1-1 fraction and was named as **Compound-1**. Moreover, fraction-2 (SA-2) was also separated more through GCC using hexane: ethyl acetate (9:1) eluent to give 6 fractions (SA-2-1 to SA-

2-6). One spot was discovered form fraction-1 (SA-2-1) and was marked as **Compound-2**. Hereinafter, Compound-1 and Compound-2 were elucidated by spectroscopie method in an attempt to determine their stucture.

Result and discussion

Compound-1 (2mg) was collected as yellow oil. ^{1}H NMR δ_H: 1.29 (9H, s), 1.34 (9H, s), 7.13 (1H, dd, *J*= 2.5 and 8.7Hz), 7.36 (1H, d, *J*= 2.5Hz), 7.54 (1H, d, *J*= 8.7Hz). Three protons (δ_H: 7.13, 7.36, 7.54) showed in aromatic protons region. The remaining 18 protons (δ_H: 1.29 & 1.34) supposed to be six methyls. And then, COSY (^{1}H-^{1}H) and HMBC correlations (^{1}H-^{13}C) were joined for confirmation of the structure. The NMR spectra were also compared to previous literature data.[14] From these data **Compound-1** was determined as **2,4-di-tert-butylphenol** and the molecular formula was $C_{14}H_{22}O$.

Compound-2 was obtained as pale yellow oil. FTIR spectra showed at 2926-2854 cm^{-1} (sp^3 C-H stretching), 1708 cm^{-1} (C=O). ^{1}H NMR δ_H: 0.77 (3H, s), 0.81 (3H, s), 0.86 (3H, s), 0.89 (6H, m), 0.94 (3H, s), 0.98 (3H,s), 1.19 (1H, m), 1.26 (24H, m), 1.42 (3H, m), 1.50 (4H, m), 1.61 (3H,s), 1.65 (3H, m), 1.67 (3H, m), 1.69 (3H, s), 1.78 (1H, m), 1.93 (2H, m), 1.98 (3H, m), 2.04 (2H, m), 2.13 (1H, m), 2.23 (1H, m), 2.30 (2H, t, *J*=7.5 Hz), 4.53 (1H, dd, *J*=3.9, 11.5Hz), 5.12 (1H, t, *J*=7.1Hz), 5.25 (1H, m). The proton signal at δ_H: 4.53ppm (C-3) belonged to the proton attached to the carbon that binds the ester group (C-3). The proton signal of 5.25 (1H, m) and 5.12 (1H, t, *J*= 7.1 Hz) linked to sp^2 hybridized carbons (C-7 and C-24 respectively). COSY (^{1}H-^{1}H) and HMBC correlations (^{1}H-^{13}C) were joined for confirmation of structure. NMR spectra also compared to previous literature data.[15,16] From these data **Compound 2** was identified as Butyrospermol 3-β-*O*-palmitate and molecular formula was $C_{46}H_{80}O_2$ (Figure 1) (Figure 2) (Table 1) (Table 2).

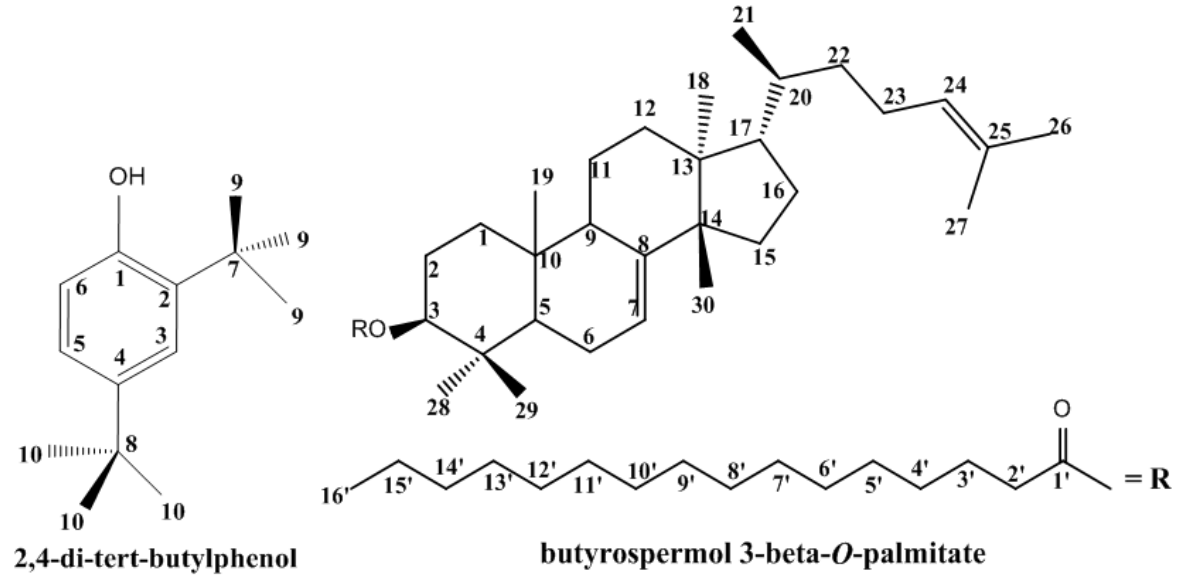

Figure 1 Chemical structure of 2,4-di-tert-butylphenol and butyrospermol 3-β-*O*-palmitate.

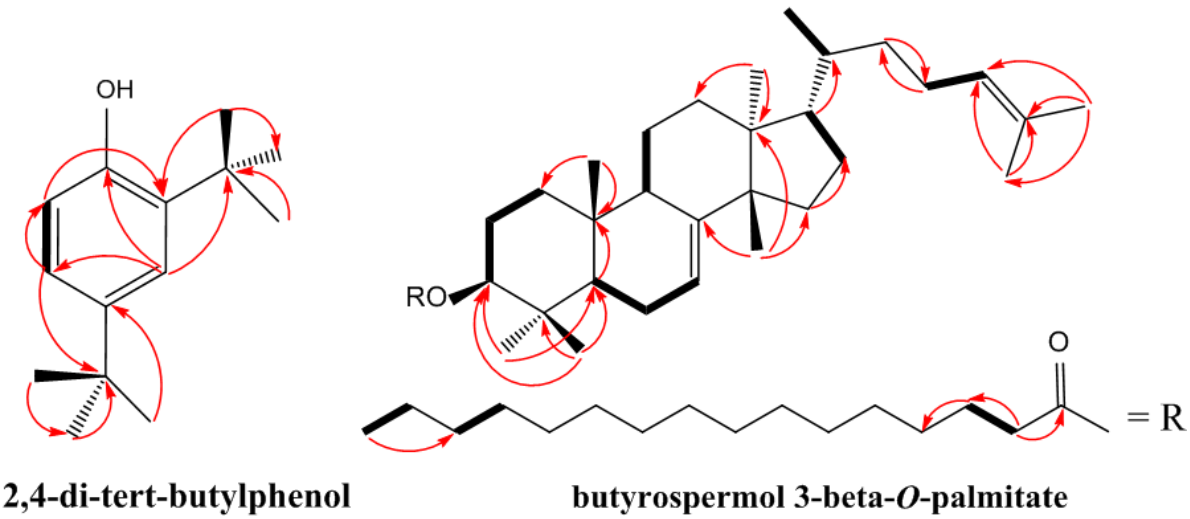

Figure 2 Selected ( ) HMBC, ( ) COSY of 2,4-di-tert-butylphenol and butyrospermol 3-β-*O*-palmitate.

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Table 1 NMR data ($CDCl_3$, 600 MHz) of 2,4-di-tert-butylphenol

Position	DEPT	HSQC (^1H-^{13}C)		HMBC	COSY
		δX	δH		
1	C	147.7	-	H-3	-
2	C	138.4	-	H-9, H-6	-
3	CH	124.4	7.36	H-5	-
			(*d*, *J*= 2.5 Hz)		
4	C	147.1	-	H-6, H-10	-
5	CH	123.9	7.13	H-3	H-6
			(*dd*, *J*= 2.5, 8.7 Hz)		
6	CH	119.1	7.54	H-5	H-5
			(*d*, *J*= 8.7 Hz)		
7	C	34.8	-	H-9, H-3	-
8	C	34.5	-	H-10, H-5	-
9	$3CH_3$	30.2	1.34 (s)	-	-
10	$3CH_3$	31.4	1.29 (s)	-	-

Table 2 NMR data ($CDCl_3$, 600 MHz) of Butyrospermol 3-β-*O*-palmitate

Position	DEPT	HSQC (^1H-^{13}C)		HMBC	COSY
		13C	1H		
1	CH_2	36.8	1.67(m)	H-19	H-2
			1,19(m)		
2	CH_2	25.7	1.67 (m)	-	H-1, H-3
3	CH	81	4.53	H-28, H-29	H-2
			(dd, J= 3.9, 11.5 Hz)		
4	C	38	-	H-29	-
5	CH	50.7	1.42 (m)	H-28, H-29	H-6
6	CH_2	23.8	2.13 (m)	-	H-5,H-7
			1.98 (m)		
7	CH	117.6	5.25 (m)	-	H-6
8	C	146	-	H-30	-
9	CH	48.8	2.23 (m)	-	H-11
10	C	34.8	-	H-19,H-5	-
11	CH_2	18.2	1.50 (m)	-	H-9
12	CH_2	33.8	1.65 (m)	H-18	-
			1.78 (m)		
13	C	43.6	-	H-18,H-30	-
14	C	51.9	-	H-18,H-30	-
15	CH_2	34	1.42 (m)	H-30	-
			1.50 (m)		
16	CH_2	29.2	1.26 (m)	H-15	H-17

Table Continued...

Position	DEPT	HSQC (^{1}H-^{13}C)		HMBC	COSY
17	CH	53.2	1.50 (m)	-	H-16
18	CH_3	22.1	0.81 (s)	-	-
19	CH_3	13.1	0.77 (s)	-	-
20	CH	35.7	1.42 (m)	H-17	H-21
21	CH_3	18.2	0.89 (m)	-	H-20
22	CH_2	39.8	1.98 (m)	H-23	-
23	CH_2	26.8	2.04 (m)	H-22	H-24
24	CH	125.1	5.12	H-26, H-27	H-23
			(t, J= 7.1 Hz)		
25	C	130.9	-	H-26, H-27	-
26	CH_3	17.7	1.61 (s)	H-27	-
27	CH_3	25.7	1.69 (s)	H-26	-
28	CH_3	27.7	0.86 (s)	-	-
29	CH_3	16	0.94 (s)	-	-
30	CH_3	27.3	0.98 (s)	H-15	-
1'	C	173.6	-	H-2'	-
2'	CH_2	35	2.3	-	H-3'
			(t, J= 7.5 Hz)		
3'	CH_2	25.1	1.65 (m)	H-2'	H-2'
4'	CH_2	29.7	1.26 (m)	H-3'	
5	CH_2	29.7	1.26 (m)	-	-
6'	CH_2	29.6	1.26 (m)	-	-
7'	CH_2	29.6	1.26 (m)	-	-
8'	CH_2	29.6	1.26 (m)	-	-
9'	CH_2	29.5	1.26 (m)	-	-
10'	CH_2	29.4	1.26 (m)	-	-
11'	CH_2	29.3	1.26 (m)	-	-
12'	CH_2	29.2	1.26 (m)	-	-
13'	CH_2	28.4	1.93 (m)	-	H-14'
14'	CH_2	31.9	1.26 (m)	H-16'	H-13'
15'	CH_2	22.7	1.26 (m)	-	H-16'
16'	CH_3	14.1	0.89 (m)	-	H-15'

The bioactivities of 2,4-Di-tert-butylphenol has been previously reported such as moderate cytotoxicity against HeLa and MCF-7, high percentage of antioxidant activity, active antibacterial against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, prevention the fungal mycelial growth of *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium chrysogenumon*.[17–19] However, Butyrospermol 3-β-*O*-palmitate hasn't been reported any bioactivities in literature. Therefore, we would like to recommend studying the various bioactivities of this compound for the next research.

Conclusion

In this study, 2,4-di-tert-butylphenol and Butyrospermol 3-β-*O*-palmitate were isolated from stem bark of *S. aqueum*. These phytochemical constituents are the first to be reported from the stem bark of this species.

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Conflicts of interest

The authors declare there are no conflicts of interest.

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