Pharmacogn. Res.
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ORIGINAL ARTICLE

Isolation of 5,7-Dihydroxy, 6,8-Dimethyl Flavanone from *Syzygium aqueum* with Its Antioxidant and Xanthine Oxidase Inhibitor Activities

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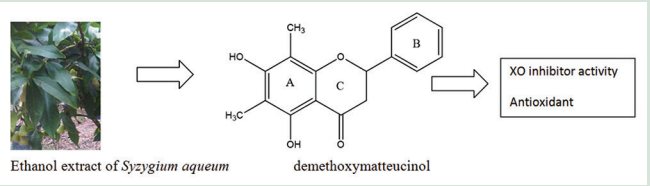
ABSTRACT

Background: *Syzygium aqueum* Burm.f. Alston (water apple) belonging to Myrtaceae family was originated from tropical areas. It was traditionally used as a medicinal plant. **Objective:** The objective of the study was to isolate the active compound from the methanolic extract of *S. aqueum* leaves. **Methods:** Extraction was done using continuous extraction with methanol as a solvent. The extract was then fractionated using liquid–liquid extraction, vacuum liquid chromatography, and radial chromatography. Recrystallization was done for purification. The structure of the compound was determined by Ultraviolet-Visible and (1D and 2D) nuclear magnetic resonance (NMR) spectrometer. **Results:** The isolate showed maximum wavelengths at 347 (band I) and 296 (band II) nm. After addition of NaOH and CH_3COONa, the maximum wavelengths of band II moved to 340 and 339 nm, respectively. There was no change in wavelengths after addition CH_3COONa/H_3BO_3 and $AlCl_3$. The ^{1}H-NMR spectrum showed 16 protons, whereas ^{13}C-NMR spectrum showed 15 carbons. Based on those data, the isolate was determined as 5,7-dihydroxy-6,8-dimethyl flavanone (demethoxymatteucinol). At a concentration of 100 and 50 µg/mL, it could inhibit 25.13% of xanthine oxidase (XO) activity and scavenge 11.87% of diphenyl-picrylhydrazyl, respectively. **Conclusion:** Demethoxymatteucinol was isolated for the first time from *S. aqueum* and it had mild antioxidant and XO inhibitory activities.

Key words: Antioxidant, flavonoid, Myrtaceae, *Syzygium aqueum*

SUMMARY

- One flavonoid compound, which 5,7-dihydroxy 6,8-dimethyl flavanone (demethoxymatteucinol), was isolated from the methanol extract of *Syzygium aqueum*. It had mild antioxidant and xanthine oxidase inhibitory activities.



Abbreviations Used: CH_3COONa/H_3BO_3: Natrium acetate/Boric acid; DPPH: Diphenyl-picrylhydrazyl, NMR: Nuclear Magnetic Resonance; ABTS: 2,2′-azinobis (3-ethyl-benzothiazline-6-sulfonic acid); AEAC: Ascorbic Acid Equivalent Antioxidant Capacity; UV-Vis: Ultraviolet-Visible; XO: Xanthine Oxidase; HSQC: Heteronuclear Single Quantum Coherence; HMBC: (Heteronuclear Multiple Bond Correlation)

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DOI: 10.4103/pr.pr_59_17

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INTRODUCTION

Myrtaceae is a huge family of plant, and it consists of 155 genera and 4000 species. Pantropical in occurrence, the family has typical Gondwanan distribution, with the center of concentrations are in South America, Southeast Asia, and Australia, but few occurrence is found in Africa.[1] Some of them possessed interesting activities such as cytotoxic, anticholinesterase, and antibacterial.[2-4] In Indonesia, Myrtaceae families are spread in certain regions, especially in Java Island, one of the members is *Syzygium aqueum* Burm.f. Alston. It is commonly known as water apple. Various parts of water apple have been used in traditional medicine.[5] The fruits and leaves contained ascorbic acid, alkaloid, tannin, glycoside, formic acid, tartaric acid, steroid, and flavonoid.[6-9] The phenolic compounds, especially flavonoid, are well known as an antioxidant. The previous studies reported that extract of fresh and dried leaves of *S. aqueum* had antioxidant activity between 58%–73% using β-carotene bleaching and 2,2'-azinobis (3-ethyl-benzothiazline-6-sulfonic acid) radical cation assay. The fresh samples had higher antioxidant activity than the dried ones.[7] The half maximal inhibitory concentration (IC_{50}) values of antioxidant and ascorbic acid equivalent antioxidant capacity of *S. aqueum* fruits were 12.0 ± 3.8 mg/ml and 31 ± 10 mg/100 g, respectively.[6]

Water Apple was also used traditionally for various diseases, and it was proved for its antioxidant, antihyperglycemic agent, and antibacterial effects.[7-11] Until now, the study of phytochemical compounds in *S. aqueum* is limited. Phytochemical constituent and pharmacological studies are still promising to be explored. Based on these facts, the aim of this research was to isolate the active compound from the methanolic extract of *S. aqueum* leaves.

MATERIALS AND METHODS

The fresh leaves of *S. aqueum* (water apple) were collected from Bandung, West Java, Indonesia. The determination was done in Herbarium School

Cite this article as: Insanu M, Ramadhania ZM, Halim EN, Hartati R, Wirasutisna KR. Isolation of 5,7-Dihydroxy, 6,8-Dimethyl Flavanone from *Syzygium aqueum* with Its Antioxidant and Xanthine Oxidase Inhibitor Activities. Phcog Res 2018;10:60-3.

of Life Sciences, Institut Teknologi Bandung and deposited with serial number 1070/I1.CO2.2/PL/2014. The leaves were sorted, cleaned, dried, and milled to obtain a powder.

Ultraviolet-Visible (UV-Vis) spectrophotometer (Hewlett Packard®8453), ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) (Agilent® series) were used.

Extraction and isolation

Three hundred grams of powdered dried leaves of *S. aqueum* were extracted by Soxhlet apparatus using gradient polarity solvents (*n*-hexane, ethyl acetate, and methanol) to give *n*-hexane extract (6.99 g), ethyl acetate extract (25.97 g), and methanolic extract (75.92 g).

The ethyl acetate extract was fractionated by vacuum liquid chromatography with gradient elution using *n*-hexane, ethyl acetate, and methanol. It produced nine fractions. Fraction 4 (4.2 g) was rechromatographed using the same system and produced 15 subfractions. Subfraction 5 (315 mg) was further processed using radial thin layer chromatography with gradient elution followed by recrystallization to obtain 24.5 mg compound X. It was characterized using specific spray reagents (H_2SO_4, $AlCl_3$, $FeCl_3$, and citroboric acid), UV-Vis, and NMR (H-NMR, C-NMR, HSQC, HMBC).

Compound X, Yellow amorphous powder, UV (MeOH; MeOH-NaOH; MeOH-CH_3COONa) λ_{max} (nm): (347, 296; 340; 339) nm [Figure 1].

BIOLOGICAL ASSAY

Diphenyl-picrylhydrazyl scavenging activity

The diphenyl-picrylhydrazyl (DPPH) radicals scavenging assay was adopted from Blois (1958) and modified. The free radical scavenging activity of the sample was measured by the decrease absorbance of the methanolic DPPH solution at 517 nm. Each extract (50 µg/mL) was mixed with DPPH solution at concentration 50 µg/mL (1:1). After 30 min incubation, the absorbance was observed by spectrophotometer.[12,13] Antioxidant activity of the sample was determined by calculating the percentage of radical scavenging activity. Ascorbic acid was used as a standard. All samples were analyzed in triplicate.

Assay of inhibitor xanthine oxidase activity

The xanthine oxidase (XO) inhibitor activity was observed by UV spectrophotometer.[14] The mixture was consisted of 1 mL sample (100 µg/mL); 2.9 mL potassium phosphate buffer (50 mM; pH 7.5 at 25°C) initiated by adding to 2 mL of the substrate solution (xanthine 0.15 mM). The mixture was incubated at 25°C for 15 min. It was added to 0.1 mL (0.1 U/mL in phosphate buffer, pH 7.5 at 25°C) XO (from bovine milk, Sigma X4875) and incubated at 25°C for 30 min. The reaction was stopped by adding 1 mL HCl 1 N after 30 min. The absorbance was recorded at 284 nm. Allopurinol (100 µg/mL) was used as positive control.[15-17] One unit will convert 1.0 µmol of xanthine to uric acid per minute.

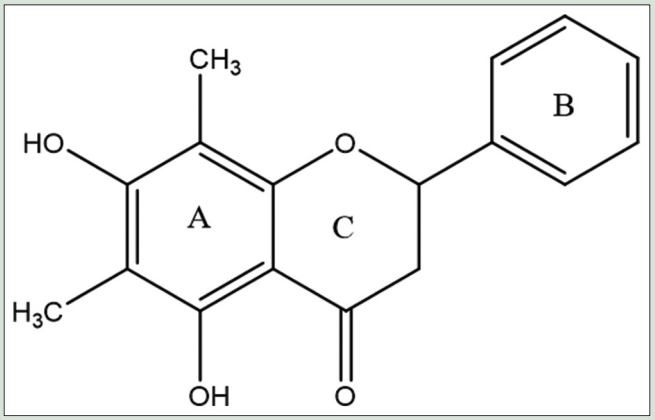

Figure 1: Structure of demethoxymatteucinol from *Syzygium aqueum*

RESULTS

Our preliminary study revealed antioxidant, and XO inhibitor activities from the leaves of several plants from Myrtaceae family, which were *Psidium guajava* (guava), *Syzygium aromaticum* (clove), *Syzygium polyanthum* (bay), *S. aqueum* (water apple), and *Melaleuca leucadendra* (eucalyptus) leaves.[18] The results showed the methanol extract of *S. aqueum* leaves had the best antioxidant activity with an IC_{50} value of 20.24 µg/mL, whereas its XO inhibitory activity was 47.22%. It was the best among all plants. Because it had the best activity, the extract was continued to further process.

The UV-vis spectra of the isolated compound showed maximum wavelengths at 347 (band I) and 296 (band II) nm. These findings showed that the compound was identified as flavonoid with flavanone backbone. The presence of C-3 hydroxyl group in flavonoid and the absence of C2-C3 double bond gave particular characteristic in UV spectra. The flavanones had their primary absorption peak (band II) in the range of 270–295 nm with only a shoulder or low-intensity band I peak.[11]

Flavonoid structure can be determined using shifting reagent. After addition of NaOH and CH_3COONa, band II moved to 340 and 339 nm, respectively. The difference of 35 and 34–37 nm showed that compound X was 5,7-dihydroxyflavanones, whereas there was no change in the wavelengths after addition of CH3COONa/H_3BO_3 and $AlCl_3$. Those revealed that the B-ring had no conjugation with the dominant chromophore.[11]

Compound X was obtained as a yellow-pale crystal. The ^{1}H-NMR showed 16 protons, which were multiplet of one proton signal at δ 7.38 ppm, multiplet of two protons at δ 7.45 ppm, and multiplet signal of two protons at δ 7.58 ppm. Those signal represented the presence of the B-ring which had five different protons and were not hydroxylated. Singlet signal at δ 12.4 ppm was related to the presence of 5-OH whereas singlet signal at δ 5.5 ppm showed 7-OH. From those spectra, the possibility of substituted was at any C atom on Ring A. The ^{13}C-NMR showed 15 carbons which were δ 7.0 and 7.7 ppm of methyl groups (CH_3), those indicated the substitution of CH_3 at the C6 and C8 in the ring A. Signal at δ 196.49 ppm showed the presence of carbonyl atom on C4 whereas signals at δ 42.77 ppm and 78.61 ppm indicated CH_2 and CH. Signals between δ 100 and 165 ppm showed aromatic C and double bonds of flavanones. By comparison with previous data, compound X can be predicted as demethoxymatteucinol [Table 1].[19] It was also proved by 2D-NMR spectra (HSQC and HMBC) [Figure 2]. This was the first time demethoxymatteucinol was isolated from the leaves of *S. aqueum*. Demethoxymatteucinol was tested for its antioxidant and inhibitor XO activities. It could inhibit 11.87% of DPPH activity at concentration of 50 µg/mL, whereas at 100 µg/mL could inhibit 25.15% XO activity [Table 2].

DISCUSSION

Flavonoid, phenols, polyphenols and tannins, Coumarins, plant growth regulators, and folic compounds were reported to be potent XO inhibitors.[15,20] The hydroxyl groups at C-5 and C-7 and the double bond between C-2 and C-3 were essential for a high inhibitory activity on XO. The presence of a hydroxyl group at C-3, C-8, and C-2' slightly decreases the inhibitory activity. The structure of flavanones different with flavones

Table 1: ^{1}H and ^{13}C data of compound X and demethoxymatteucinol

Position	Compound X ($CDCl_3$)		Demethoxymatteucinol ($CDCl_3$)[19]	
	^{1}H mult.	^{13}C mult.	^{1}H mult.	^{13}C mult.
2	5.4 dd	78.6 CH	5.40 dd	78.7 CH
3	3.0 dd	43.6 CH_2	2.85 dd	43.5 CH_2
4	-	196.4 qC	-	196.3 qC
5	-	159.1 qC	-	159.3 qC
6	-	102.2 qC	-	102.9 qC
7	-	160.9 qC	-	160.8 qC
8	-	102.5 qC	-	102.9 qC
9	-	157.7 qC	-	157.7 qC
10	-	103.4 qC	-	103.0 qC
1'	-	139.0 qC	-	138.9 qC
2'	7.58 d	126.0 CH	7.50 m	125.9 CH
3'	7.45 t	128.5 CH	7.44 m	128.8 CH
4'	7.38 t	128.3 CH	7.40 m	128.6 CH
5'	7.45 t	128.5 CH	7.44 m	128.8 CH
6'	7.58 d	126.1 CH	7.50 m	125.9 CH
6-Me	2.08 s	7.7 CH_3	2.08 s	7.6 CH_3
8-Me	2.07 s	7.0 CH_3	2.07 s	6.8 CH_3
5-OH	12.7 s		12.27 s	
7-OH	5.4 s			

δ (ppm) 500 MHz for ^{1}H and 125 MHz for ^{13}C: (Mult.=Multiplicities)

Table 2: Xanthine oxidase inhibitory activity

Samples (100 µg/mL)	XO inhibition (%)
Methanolic extract	47.22
Ethyl acetate fraction	47.68
Compound X	25.15
Allopurinol	97.14

XO: Xanthine oxidase

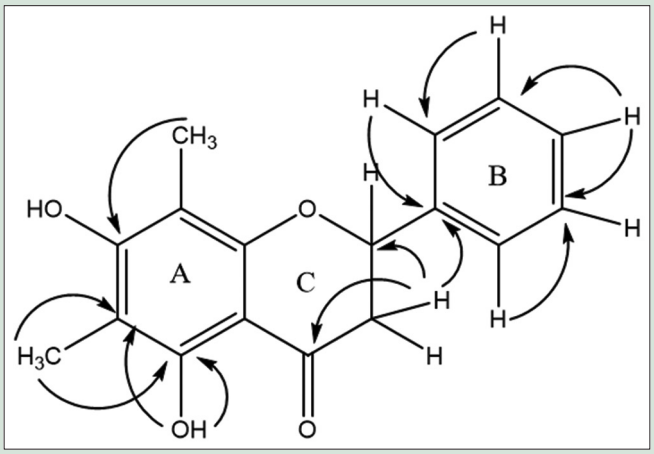

Figure 2: Selected heteronuclear multiple bond correlation for demethoxymatteucinol

and flavonols because of the presence of a single bond between C-2 and C-3. Apparently, this structural difference influenced the inhibitory effect of XO. With a double bond between C-2 and C-3, ring B will be coplanar with ring A and C due to the conjugation. Saturation of this double bond will destroy conjugation and coplanarity.[21] These facts caused demethoxymatteucinol had mild antioxidant and XO inhibitor activities.

Previous research reported that compound 5,7-dihydroxy 6,8-dimethyl flavanone (demethoxymatteucinol) was isolated from the methanolic extract of *Syzygium jambos* (Linn.) Alston flower, *Matteuccia struthiopteris* rhizomes, *Syzygium samarangense* leaves and flower buds of *Cleistocalyx operculatus*.[22-25] There was no information regarding its isolation from *S. aqueum*. We claim that our work is the first report for demethoxymatteucinol isolation from *S. aqueum* leaves.

CONCLUSION

Compound X 5,7-dihydroxy 6,8-dimethyl flavanone (demethoxymatteucinol) was the active constituent of *S. aqueum*, and it had mild antioxidant and xanthine oxidase inhibitory activities.

Acknowledgement

This work was supported by Riset Inovasi KK ITB.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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