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Luteolin, a flavonoid from *Syzygium myrtifolium* Walp.

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ABSTRACT

A flavonoid, luteolin, was isolated from the leaves of *Syzygium myrtifolium*. The compound was purified using vacuum liquid chromatography with the help of a specific guidance for the flavonoid isolation. The chemical structure of this compound was determined based on measurements of UV, IR and NMR spectroscopic data. The occurrence of luteolin in *S. myrtifolium* is firstly reported from this study.

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1. Introduction

The genus *Syzygium* belongs to Myrtaceae family and consist of more than one thousand species of flowering plants. These plants are widely distributed along tropical and subtropical regions (Ahmad et al., 2016). One of the important plant from this genus is *Syzygium aromaticum* which is known as clove and commonly used as spice. Its oil has been studied for many applications (Cortés-Rojas et al., 2014). Another species also found in Indonesia is *Syzygium myrtifolium* Walp. (syn. *Syzygium campanulatum*). However, unlike clove, *S. myrtifolium* is usually grown as an ornamental plant and frequently found along roads or parks as a hedge. This plant has been used in traditional medicine to treat stomach aches (Memon et al., 2014). The plant has been reported for a potent anti-cancer activity (Aisha et al., 2013; Memon et al., 2014; Lingga et al., 2018). Current reports on phytochemical screening of the plant showed the occurrence of two flavonoids (dimethyl cardamonin and anthocyanin) (Santoni et al., 2013; Memon et al., 2014) and one triterpenoid (betulinic acid) (Aisha et al., 2013). Accordingly, the present work was aimed to investigate the phytochemical properties of the plant.

2. Materials and methods

2.1. General procedures

UV spectrum was measured using a Hewlett Packard UV-Vis spectrophotometer (Agilent Technology, Waldbronn, Germany). The IR spectrum was recorded using a One PerkinElmer instrument (Waltham, MA, USA). NMR spectra were measured using an Agilent series DD2 console spectrometer 500 (^{1}H) and 125 (^{13}C) MHz with CD_3OD as the solvent. Vacuum liquid column chromatography was performed using silica gel 60 (catalogue number: 107734, Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 PF254 plates (catalogue number: 105554, Merck, Darmstadt, Germany). A specific spray reagent, citroborate (5 g citric acid, 5 g boric acid, and 100 ml ethanol) was used for identifying flavonoids. All solvents used for the extraction and separation were technical grades, which were previously distilled before use.

2.2. Plant material

Leaves of *S. myrtifolium* were obtained from the local garden at Bandung city, West Java Province, Indonesia. The leaves were identified and confirmed by the Herbarium Jatinangor, Department of Biology, Padjadjaran University, Indonesia. Preparation of samples was performed by washing, cutting the leaves into small pieces, and drying at 40°C in an oven until a constant weight obtained. The dried leaves were powdered and stored at room temperature in an airtight container until use.

2.3. Phytochemical screening

Phytochemical screening was performed for alkaloid, flavonoids, quinone, saponin, tannin, and triterpenoid according to the method described in Harbone (1986).

2.4. Extraction and isolation

The dried leaves of *S. myrtifolium* (500 g) were extracted 3x24 hours with 2 L of ethanol 96%. The extract was concentrated with rotary evaporator to yield 62.65 g of a

dried ethanol extract. During the fractionation and chromatography, the TLC was used to monitor the profile of metabolites with the help of citroborate spray reagent. The reagent gave a yellow fluorescence to metabolites under UV 366 nm after heating, which was used as the guidance to isolate flavonoids contained in the fractions.

Fractionation was started by re-dissolving the methanol extract in methanol-water (8:2, v/v), following by liquid-liquid extraction with hexane and ethyl acetate. The evaporation resulted in 5.25 g of ethyl acetate fraction which was then subjected to a vacuum liquid column chromatography containing silica 60 H and eluted using gradient hexane, ethyl acetate and methanol. Of the 20 fractions collected, the 13rd fraction (0.92 g) was positively confirmed to contain flavonoids. This fraction was then subjected to a column chromatography over silica gel and eluted with chloroform-methanol (9:2, v/v), resulting in 38 fractions. Of them, the 5th fraction yielded yellow crystals. Final purification has obtained about 10 mg of the pure compound.

2.5. Identification and characterisation

Spectroscopy analysis was done by UV, IR and NMR. Spectral data were analysed and compared with the literature.

3. Results and discussion

3.1. Phytochemical content

The phytochemical screening of the ethanol extract of *S. myrtifolium* leaves showed the presence of flavonoid, quinone, saponin, tannin, and steroid/triterpenoid. Alkaloid was not found to be present in this plant (Table 1). The presence of flavonoids and terpenoid correspond to the previous reports (Santoni et al., 2013; Memon et al., 2014; Aisha et al., 2013).

Table 1. Results of phytochemical screening on extract of *S. myrtifolium*.

Phytochemicals	Occurrence
Alkaloid	-
Flavonoid	+
Quinone	+
Saponin	+
Tannin	+
Triterpenoid	+

The purification of flavonoids from this plant was guided with citroborate, the specific reagent for detection of flavonoids (Mabry et al., 1970). A series purification steps eventually yielded 10 mg of yellow crystal (Fig. 1). The UV spectra of the isolated compound showed strong absorbance at two different wavelengths, in which the maximum absorbance at 358 nm and the second absorbance at 284 nm. These absorbance values indicated the presence of a flavonoid compound (Mabry et al., 1970). The result was emphasised by the infrared spectra, which ascribed the presence of hydroxyls (v_{max} 3475, 3371,and 3211 cm^{-1}), carbonyl group (v_{max} 1606 cm^{-1}), alkene group (v_{max}1562 cm^{-1}), aromatic rings (v_{max} 1458 cm^{-1}), and phenols including OH bending (v_{max} 1355, 1294) and C–O stretching (v_{max} 1197, 1117, 1068, and 1023 cm^{-1}).

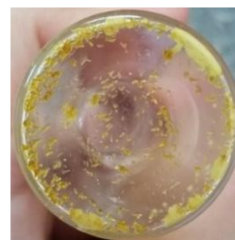

Fig. 1. Yellow crystal of isolated flavonoid from *S. myrtifolium*.

Table 2. ^{13}C and ^{1}H NMR data of the isolated compound

	NMR data of the isolated compound			NMR data from references	
Carbon number	^{13}C (CD_3OD)	^{1}H (CD_3OD)	HMBC	^{13}C (DMSO) (Liu et al., 2010)	^{13}C (C_5D_5N) (Sai et al., 2013)
2	163.0	-		167.6	164.0
3	104.6	5.18 (1H, d, J=6.5 Hz)		101.8	
4	179.4	-		181.0	181.8
5	163.0	-		161.3	157.6
6	99.9	6.20 (1H, d, J=1.5 Hz)	C-7, C-5, C-10, C-8	99.6	99.2
7	166.1	-		163.6	164.3
8	94.7	6.39 (1H, d, J=2Hz)	C-7, C-9, C-10, C-6	94.3	94.7
9	158.4	-		157.5	162.1
10	105.6	-		102.3	103.8
1'	145.9	-		119.8	119.0
2'	117.4	7.74 (1H, d, *J*=2.2 Hz)	C-1', C-3', C-6', C-4'	112.2	113.2
3'	149.9	-		146.4	146.0
4'	158.7	-		152.0	149.7
5'	116.1	6.87 (1H, d, *J*=8.5 Hz	C-1', C-3', C-6'	115.8	116.8
6'	123.0	7.57 (1H, dd, *J*=2.2, 8.4 Hz)	C-3', C-2'	118.9	120.8

To confirm the exact structure of the isolated flavonoid, data from ^{1}H and ^{13}C-NMR spectra were analysed (Table 2). The ^{1}H-NMR spectrum showed 6 methine signals. One proton of vinylic group was located downfield at 5.13 ppm. Meanwhile, five methines were further located downfield at range of 6.20 to 7.70 ppm, indicating typical of aromatic proton.

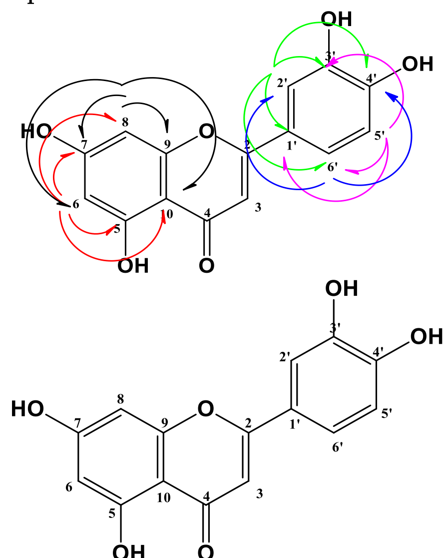

Fig. 2. Selected of the important HMBC correlations ($^{1}H\leftrightarrow^{13}C$) (left) and the structure of the isolate (right).

The ^{13}C-NMR spectra showed 15 signals, observing between 94.7 to 166.1 ppm. Fourteen carbons of the aromatic benzene located at 94.7 to 166.1 ppm and one carbonyl at 176.5 ppm. The spectrum has aromatic protons (6.20 and 6.39) on 2 shielded aromatic carbons at 94.7 (C-8) and 99.9 ppm (C-6), implying the typical skeleton of flavonoids (rings A and B).

Further evidences for the structure were obtained by multiple-bonds correlations found in the HMBC spectra as shown in Fig. 2. After comparing their physicochemical and spectrometric data with those reported in literatures (Liu et al. 2010; Sai et al. 2013), the isolate identified as a known compound and confirmed as luteolin (Fig. 2). Luteolin was reported to have multiple pharmacological effects, such as anti-oxidant, anti-inflammatory, anti-allergy, and anti-cancer (Lin et al. 2008). Although the isolated flavonoid were known compound and has been found to occur in various species of plants, to our knowledge, no report regarding its presence and isolation from *S. myrtifolium*. This might be the first report of luteolin isolated from leaves of *S. myrtifolium*. A study from de Freitas et al. (2019) has reported the presence of luteolin in *Syzygium* sp. using MALDI-TOF spectroscopy. They also found that this compound might be responsible for α-amylase inhibitory activity of *Syzygium* sp. extract. However, which species of *Syzygium* plant they used was not clearly described.

Conclusion

A flavone, luteolin was successfully purified from leaves of S. myrtifolium using a specific guidance extraction for flavonoids.

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