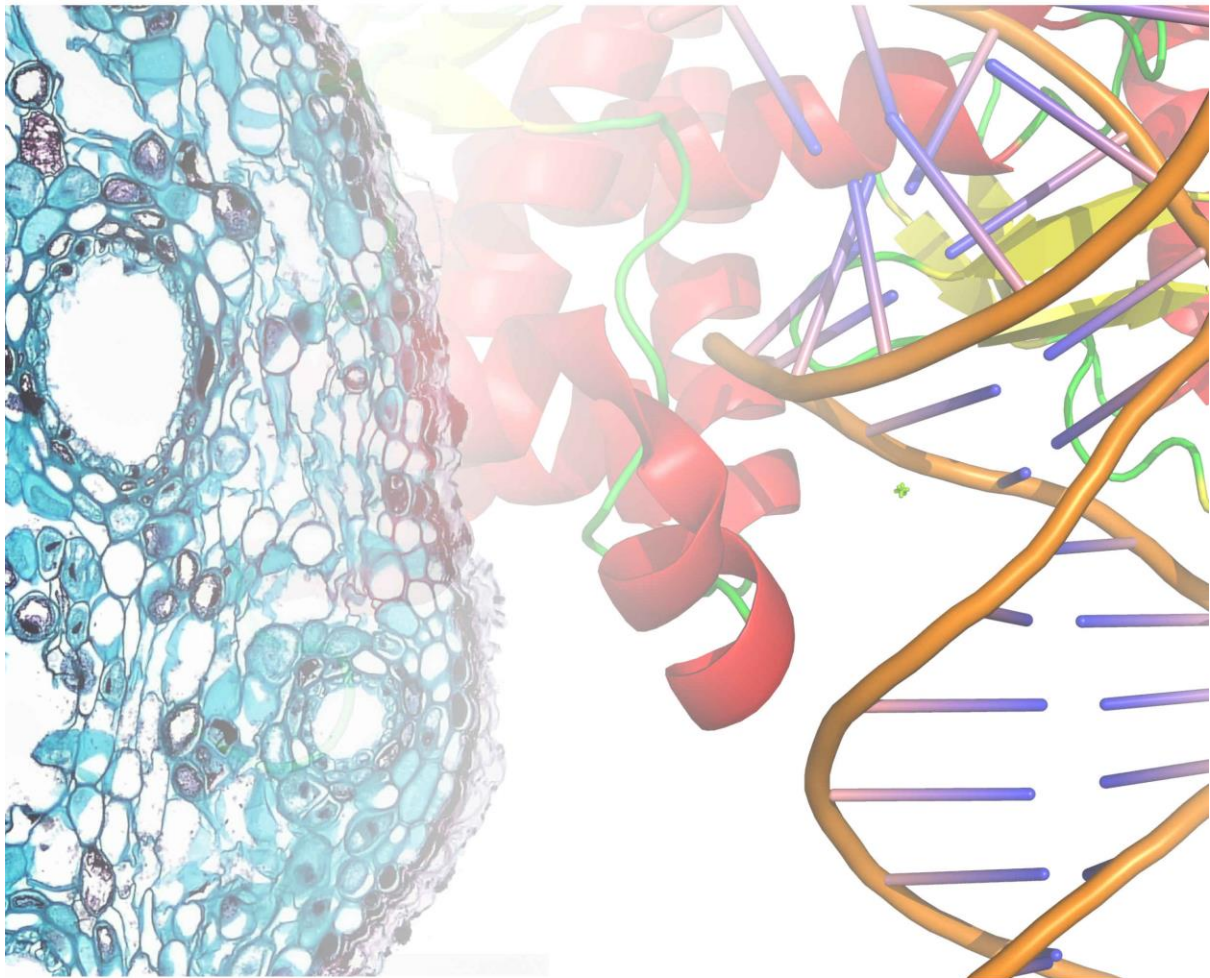


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## Luteolin, a flavonoid from *Syzygium myrtifolium* Walp.

Raden Herni Kusriani<sup>a,\*</sup>, Shinta Maulida Rosandhy<sup>a</sup>, and Elfahmi<sup>b</sup>

<sup>a</sup>Bandung School of Pharmacy, Bandung, Indonesia

<sup>b</sup>School of Pharmacy, Institut Teknologi Bandung, Bandung, Indonesia 40132

### 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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\*Corresponding author:

herni.kusriani@stfb.ac.id

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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 (<sup>1</sup>H) and 125 (<sup>13</sup>C) MHz with CD<sub>3</sub>OD as the

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

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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 5<sup>th</sup> fraction yielded yellow crystals. Final purification has obtained about 10 mg of the pure compound.

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

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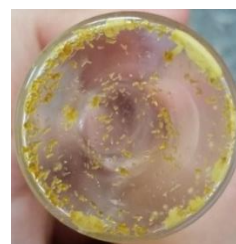
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**Table 1.** Results of phytochemical screening on extract of *S. myrtifolium*.

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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 ( $\nu_{\max}$  3475, 3371, and 3211  $\text{cm}^{-1}$ ), carbonyl group ( $\nu_{\max}$  1606  $\text{cm}^{-1}$ ), alkene group ( $\nu_{\max}$  1562  $\text{cm}^{-1}$ ), aromatic rings ( $\nu_{\max}$  1458  $\text{cm}^{-1}$ ), and phenols including OH bending ( $\nu_{\max}$  1355, 1294) and C–O stretching ( $\nu_{\max}$  1197, 1117, 1068, and 1023  $\text{cm}^{-1}$ ).

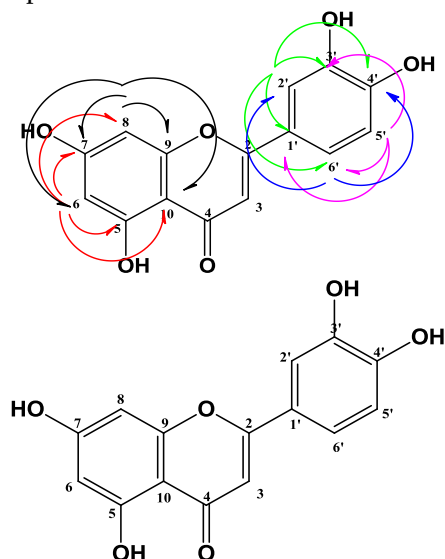


**Fig. 1.** Yellow crystal of isolated flavonoid from *S. myrtifolium*.

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

Carbon number	NMR data of the isolated compound			NMR data from references	
	$^{13}\text{C}$ ( $\text{CD}_3\text{OD}$ )	$^1\text{H}$ ( $\text{CD}_3\text{OD}$ )	HMBC	$^{13}\text{C}$ (DMSO) (Liu et al., 2010)	$^{13}\text{C}$ ( $\text{C}_5\text{D}_5\text{N}$ ) (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  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were analysed (Table 2). The  $^1\text{H}$ -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 ( $^1\text{H}\leftrightarrow^{13}\text{C}$ ) (left) and the structure of the isolate (right).

The  $^{13}\text{C}$ -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  $\alpha$ -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.

## References

- Aisha AF, Ismail Z, Abu-Salah KM, Siddiqui JM, Ghafar G, Majid AM. 2013. *Syzygium campanulatum* korth methanolic extract inhibits angiogenesis and tumor growth in nude mice. *BMC Complement Altern Med* 13:168. DOI: 10.1186/1472-6882-13-168.
- Ahmad B, Baider C, Bernardini B, Biffin E, Brambach F, Burslem D, Byng JW, Christenhusz M, Florens FV, Lucas E, Ray A. 2016. *Syzygium* (Myrtaceae): Monographing a taxonomic giant via 22 coordinated regional revisions. *PeerJ Preprints* 4:e1930v1. DOI: 10.7287/peerj.preprints.1930v1.
- Chaturvedula VSP, Prakash I. 2013. Flavonoids from *Astragalus propinquus*. *J Chem Pharm Res* 5(1): 261-5.
- Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (*Syzygium aromaticum*): a precious spice. *Asian Pac J Trop Biomed* 4(2): 90-6. DOI: 10.1016/S2221-1691(14)60215-X.
- Cavatão de Freitas T, Oliveira RJ, Mendonça RJ, Candido PA, Pereira S, Lopes L, Devienne KF, Carolina da Silva A, Pereira CA. 2019. Identification of bioactive compounds and analysis of inhibitory potential of the digestive enzymes from *Syzygium* sp. extracts. *J Chem*. DOI:10.1155/2019/3410953.
- Fransworth NR. 1966. Biological and phytochemical studies on *Ruta chalapensis* Lamarck. *Nat Prod Res* 19(3):203-10.
- Harbone J. *Phytochemical Method* (second ed.) New York: Chapman Hall. 1986.
- Lin Y, Shi R, Wang X, Shen HM. 2008. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Cur Cancer Drug Tar* 8(7):634-46. DOI: 10.2174/156800908786241050.
- Lingga RS, Harahap U, Yuandani Y. 2018. Antimutagenic effects of ethanol extract of *Syzygium myrtifolium* Walp. in cyclophosphamide-induced mice. *Asian J Pharm Clin Res* 11(5):210-3. DOI: 10.22159/ajpcr.2018.v11i6.24350.
- Liu H, Mou Y, Zhao J, Wang J, Zhou L, Wang M, Wang D, Han J, Yu Z, Yang F. 2010. Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Molecules* 15(11):7933-45. DOI: 10.3390/molecules15117933.
- Mabry T, Markham KR, Thomas MB. 1970. *The Systematic Identification of Flavonoids*. New York: Springer-Verlag Berlin Heidelberg. DOI: 10.1007/978-3-642-88458-0.
- Mahmoud I, Marzouk M, Moharram M, El-Gindi M, Hassan A. 2001. Acylated flavonol glycosides from *Eugenia jambolana* leaves. *Phytochem* 58:1239-44.
- Memon AH, Ismail Z, Aisha AF, Al-Suede FS, Hamil MS, Hashim S, Saeed MA, Laghari M, Majid A, Shah AM. 2014. Isolation, characterization, crystal structure elucidation, and anticancer study of dimethyl cardamonin, isolated from *Syzygium campanulatum* Korth. *Evidence-Based Compl Altern Med*. DOI: 10.1155/2014/470179.
- Santoni A, Darwis D, Syahri S. 2013. Isolation of antosianin from red shoot (*Syzygium campanulatum* Korth.) as well as antosianin testing and applications as natural dyes. Lampung: *Prosiding Semirata Unila*, pp. 1-10.



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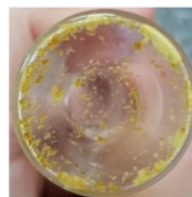
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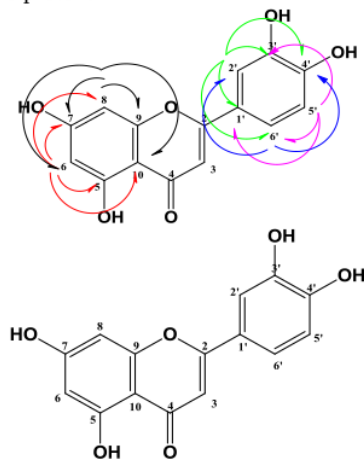


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8	94.7	6.39 (1H, d, J=2Hz)	C-7, C-9, C-10, C-6	94.3	94.7
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10	105.6	-		102.3	103.8
1'	145.9	-		119.8	119.0
2'	117.4	7.74 (1H, d, J=2.2 Hz)	C-1', C-3', C-6', C-4'	112.2	113.2
3'	149.9	-		146.4	146.0
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  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were analysed (Table 2). The  $^1\text{H}$ -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 ( $^1\text{H}\leftrightarrow^{13}\text{C}$ ) (left) and the structure of the isolate (right).

The  $^{13}\text{C}$ -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  $\alpha$ -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.

## References

- Aisha AF, Ismail Z, Abu-Salah KM, Siddiqui JM, Ghafar G, Majid AM. 2013. *Syzygium campanulatum* korth methanolic extract inhibits angiogenesis and tumor growth in nude mice. *BMC Complement Altern Med* 13:168. DOI: 10.1186/1472-6882-13-168.
- Ahmad B, Baider C, Bernardini B, Biffin E, Brambach F, Burslem D, Byng JW, Christenhusz M, Florens FV, Lucas E, Ray A. 2016. *Syzygium* (Myrtaceae): Monographing a taxonomic giant via 22 coordinated regional revisions. *PeerJ Preprints* 4:e1930v1. DOI: 10.7287/peerj.preprints.1930v1.
- Chaturvedula VSP, Prakash I. 2013. Flavonoids from *Astragalus propinquus*. *J Chem Pharm Res* 5(1): 261-5.
- Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (*Syzygium aromaticum*): a precious spice. *Asian Pac J Trop Biomed* 4(2): 90-6. DOI: 10.1016/S2221-1691(14)60215-X.
- Cavatão de Freitas T, Oliveira RJ, Mendonça RJ, Candido PA, Pereira S, Lopes L, Devienne KF, Carolina da Silva A, Pereira CA. 2019. Identification of bioactive compounds and analysis of inhibitory potential of the digestive enzymes from *Syzygium* sp. extracts. *J Chem.* DOI:10.1155/2019/3410953.
- Fransworth NR. 1966. Biological and phytochemical studies on *Ruta chalapensis* Lamarck. *Nat Prod Res* 19(3):203-10.
- Harbone J. *Phytochemical Method* (second ed.) New York: Chapman Hall. 1986.
- Lin Y, Shi R, Wang X, Shen HM. 2008. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Cur Cancer Drug Tar* 8(7):634-46. DOI: 10.2174/156800908786241050.
- Lingga RS, Harahap U, Yuandani Y. 2018. Antimutagenic effects of ethanol extract of *Syzygium myrtifolium* Walp. in cyclophosphamide-induced mice. *Asian J Pharm Clin Res* 11(5):210-3. DOI: 10.22159/ajpcr.2018.v11i6.24350.
- Liu H, Mou Y, Zhao J, Wang J, Zhou L, Wang M, Wang D, Han J, Yu Z, Yang F. 2010. Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Molecules* 15(11):7933-45. DOI: 10.3390/molecules15117933.
- Mabry T, Markham KR, Thomas MB. 1970. *The Systematic Identification of Flavonoids*. New York: Springer-Verlag Berlin Heidelberg. DOI: 10.1007/978-3-642-88458-0.
- Mahmoud I, Marzouk M, Moharram M, El-Gindi M, Hassan A. 2001. Acylated flavonol glycosides from *Eugenia jambolana* leaves. *Phytochem* 58:1239-44.
- Memon AH, Ismail Z, Aisha AF, Al-Suede FS, Hamil MS, Hashim S, Saeed MA, Laghari M, Majid A, Shah AM. 2014. Isolation, characterization, crystal structure elucidation, and anticancer study of dimethyl cardamonin, isolated from *Syzygium campanulatum* Korth. *Evidence-Based Compl Altern Med*. DOI: 10.1155/2014/470179.
- Santoni A, Darwis D, Syahri S. 2013. Isolation of antosianin from red shoot (*Syzygium campanulatum* Korth.) as well as antosianin testing and applications as natural dyes. Lampung: *Prosiding Semirata Unila*, pp. 1-10.