Phytochemistry 203 (2022) 113367

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Flavonoids and phenylethanoids from the flowers and leaves of *Aeschynanthus* species and cultivars (Gesneriaceae)

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ARTICLE INFO

Keywords:
Aeschynanthus
Gesneriaceae
Hispidulin
Nepetin
Pectolinarigenin
Flavone glucuronides
Flavone methylglucuronides

ABSTRACT

Forty-one flavones, each one of flavonol, chalcone and dihydroflavonol, two flavanones, and four phenylethanoids were isolated from corollas, calyces and leaves of two *Aeschynanthus* species, *A. fulgens* and *A. pulcher*, and six cultivars, 'Mahligai', 'Mona Lisa', SoeKa', 'Redona', 'Freshya' and 'Bravera'. Flavonoids were mainly the glucuronides and/or methylglucuronides based on hispidulin, nepetin, pectolinarigenin, 6-hydroxyluteolin, scutellarein, apigenin and luteolin, and identified by UV spectra, HR-MS, LC-MS, acid hydrolysis, NMR, and/or HPLC and TLC comparisons with authentic samples. Of these flavonoids, twelve, i.e. hispidulin 7,4′-di-*O*-glucuronide, 7,4′-di-*O*-methylglucuronide, 7-*O*-methylglucuronide-4′-*O*-glucuronide, 7-*O*-glucuronide-4′-*O*-methylglucuronide, 7-*O*-glucosyl-(1 → 2)-glucuronide and 8-*C*-glucoside, nepetin 7,4′-di-*O*-glucuronide, 7-*O*-glucuronide-4′-*O*-methylglucuronide and 7-*O*-methylglucuronide-4′-*O*-glucuronide, pectolinarigenin 7-*O*-glucosyl-(1 → 2)-glucuronide and 7-*O*-xylosyl-(1 → 2)-(6″-malonylglucoside), and 6-hydroxyluteolin 7,4′-di-*O*-glucuronide, were previously undescribed.

1. Introduction

The genus *Aeschynanthus* (Gesneriaceae) consists of ca. 150 species and is mainly native to subtropical and tropical zones in China and Malesia (Mabberley, 2017). Flower colors of their species are scarlet to reddish orange in almost species and cultivars. The common name for some species is "lipstick plant" which comes from the appearance of the developing buds and their flower colors. Some cultivars are bred for ornamentals. As the flower pigments of *Aeschynanthus* species, an anthocyanin, pelargonidin 3-*O*-sambubioside has been reported from the corollas of *A. obconicus* C.B. Clarke, *A. parviflorus* (D. Don) Spreng, *A. tricolor* Hook. f. and *A. longicalyx* Ridl. (Harborne, 1966a, 1967; Lowry, 1972). Cyanidin 3-*O*-sambubioside has been found in the calyces and leaves of *A. parviflorus*, *A. ellipticus* K. Shum. & Lauterb., *A. longicaulis* Wall. ex R. Br. (as *A. marmoratus* T. Moore) (Harborne, 1966a, 1967). A chalcone, chalcononaringenin 2′-*O*-glucoside, was reported from the flowers of *A. parviflorus*, *A. ellipticus*, *A. tricolor* and *A. obconicus* (Harborne, 1966a, 1966b, 1967). Four flavanones, naringenin and its 7-*O*-glucoside, 7-*O*-apiosyl-(1 → 6)-glucoside and 6-*C*-glucoside, were found in the aerial parts of *A. bracteatus* Wall. ex A. DC., together with *C*-glycosylflavone, vicenin-2, and biflavonoid glycoside, ormocarpin (Li et al., 2008). Hispidulin and apigenin were isolated from the aerial parts of *A. superbus* C.B. Clarke (Tian and Kang, 2013). Recently, thirteen pelargonidin and cyanidin glycosides including six unreported anthocyanins were isolated and identified from the corollas and calyces of *A. fulgens* and *A. pulcher*, and six cultivars by us (Iwashina et al., 2021). In this survey, we isolated 41 flavones, each one of flavonol, chalcone and dihydroflavonol, two flavanones and four phenylethanoids from the corollas, calyces and leaves of two *Aeschynanthus* species and six cultivars, and identified by UV, HR-MS, LC-MS, acid hydrolysis, NMR, and/or HPLC and TLC comparisons with authentic samples.

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https://doi.org/10.1016/j.phytochem.2022.113367
Received 13 April 2022; Received in revised form 1 August 2022; Accepted 2 August 2022
Available online 21 August 2022

2. Results and discussion

2.1. Identification of hispidulin glycosides

Seven hispidulin glycosides (**1**–**5**, **7**, **8**) were isolated from the corollas, calyces and leaves of two *Aeschynanthus* species and six cultivars. They liberated hispidulin as aglycone by acid hydrolysis.

Flavonoid **1** was obtained as pale yellow powder, and showed a molecular ion peak at *m/z* 651.1172 $[M-H]^-$ for $C_{28}H_{27}O_{18}$ by HR-MS. Since molecular ion peak, *m/z* 653 $[M+H]^+$, and fragment ion peaks, *m/z* 477 $[M-176+H]^+$ and *m/z* 301 $[M-176-176+H]^+$, occurred on LC-MS, the attachment of 2 mol glucuronic acid to hispidulin was suggested. Practically, glucuronic acid was produced by acid hydrolysis, together with hispidulin. The attachment of glucuronic acid to 7- and 4′-positions of hispidulin was shown by UV spectral analysis according to Mabry et al. (1970). In ^{1}H and ^{13}C NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC and HMBC (Table 1, Fig. 1-1∼6S). The ^{1}H NMR spectrum of **1** showed four aromatic proton signals, δ_H 8.11 (H-2′,6′), 7.26 (H-3′,5′), 7.13 (H-8) and 7.02 (H-3), together with a methoxyl proton signal (δ_H 3.81). Anomeric proton signals of two glucuronic acid were observed at δ_H 5.32 (*d*, $J = 7.2$ Hz) and 5.26 (*d*, $J = 7.2$ Hz). The attachment of a methoxyl group to 6-position of the aglycone was confirmed by HMBC correlation between a methoxyl proton signal and C-6 carbon signal of hispidulin at δ_C 133.5. Moreover, the attachment of glucuronic acid to 7- and 4′-position of hispidulin was confirmed by HMBC correlation between two anomeric proton signals and C-7 and C-4′ carbon signals at δ_C 157.1 and 160.9, respectively. Thus, **1** was identified as hispidulin 7, 4′-di-*O*-β-D-glucuronopyranoside (Fig. 1A). The compound was found in nature for the first time (Harborne and Baxter, 1999; Williams, 2006; Buckingham et al., 2015).

Flavonoid **2** was obtained as pale yellow powder from the corollas of *Aeschynanthus* cultivar, and showed a molecular ion peak at *m/z* 703.1486 $[M+H+Na]^+$ for $C_{30}H_{32}O_{18}Na$ by HR-MS. In LC-MS, molecular ion peak, *m/z* 681 $[M+H]^+$, and fragment ion peaks, *m/z* 491 $[M-190+H]^+$, 489 $[M-190-H]^-$ occurred, showing the attachment of 2 mol methyl-glucuronic acid to hispidulin. ^{1}H NMR data was essentially the same as those of **1** except for the appearance of the additional methoxyl proton signal, δ_H 3.70 (6H, *s*) (Table 1, Figs. 2–1∼6S). On the other hand, in ^{13}C NMR, additional methoxyl carbon signals appeared at δ_C 52.9. It was shown by HMBC correlation between the methoxyl proton signal at δ_H 3.70 and 6″- and 6‴-COOH carbon signal at δ_C 170.3 that these methoxyl groups are attached to 6-position of glucuronic acid. The attachment of another methoxyl group to 6-position of the aglycone was shown by HMBC correlation between another methoxyl proton signal at δ_H 3.79 and C-6 carbon signal of the aglycone at δ_C 130.6. Although the carbon signals of C-7 and C-4′ were unclear, the attachment of methyl-glucuronic acid to 7- and 4′-positions of hispidulin was determined by UV spectral properties (Mabry et al., 1970). From the results described above, **2** was identified as hispidulin

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (DMSO-d_6) of hispidulin glycosides from Aeschynanthus species and cultivars.

position	1		2		3		4		5		6	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Hispidulin												
2		164.5		—		164.4		164.7		165.2		164.9
3	7.02 s	104.7	7.00 s	104.5	7.02 s	104.6	7.01 s	104.7	6.86 s	103.5	6.83 s	103.0
4		183.4		183.1		183.4		183.4		183.2		183.4
5		153.5		151.1		153.3		153.6		153.3		152.6
6		133.5		130.6		133.5		133.5		133.3		131.5
7		157.1		—		157.6		157.0		157.0		156.7
8	7.13 s	95.0	7.13 s	—	7.08 s	95.2	7.14 s	95.0	7.00 s	94.8		104.9
9		153.1		151.0		153.1		153.1		153.0		152.4
10		106.9		106.9		106.7		106.9		106.5		105.8
1’		125.0		—		125.1		124.9		125.0		122.5
2’	8.11 d (8.4)	129.2	8.02 d (9.0)	129.7	8.11 d (9.0)	129.2	8.10 d (9.0)	129.2	7.95 d (8.4)	129.4	8.06 d (7.2)	130.0
3’	7.26 d (9.0)	117.4	7.26 d (8.4)	117.6	7.25 d (9.0)	117.4	7.26 d (8.4)	117.5	6.95 d (7.8)	117.0	6.93 d (7.8)	116.8
4’		160.9		—		160.6		161.1		162.4		162.1
5’	7.26 d (9.0)	117.4	7.26 d (8.4)	117.6	7.25 d (9.0)	117.4	7.26 d (8.4)	117.5	6.95 d (7.8)	117.0	6.93 d (7.8)	116.8
6’	8.11 d (8.4)	129.2	8.02 d (9.0)	129.7	8.11 d (9.0)	129.2	8.10 d (9.0)	129.2	7.95 d (8.4)	129.4	8.06 d (7.2)	130.0
6-OCH_3	3.81 s	61.2	3.79 s	60.1	3.81 s	61.2	3.80 s	61.3	3.82 s	61.4	3.82 s	61.1
	7-*O*-glucuronic acid		**7-*O*-methylglucuronic acid**		**7-*O*-methylglucuronic acid**		**7-*O*-glucuronic acid**		**7-*O*-glucuronic acid**		**8-C-glucose**	
1	5.32 d (7.2)	100.4	5.33 d (7.2)	100.2	5.16 d (6.6)	100.8	5.39 d (7.2)	100.3	5.35 d (7.8)	98.6	4.76 d (10.2)	74.7
2	3.40 m	73.8	4.30-3.10	73.8	3.37 m	73.7	3.33 m	73.9	3.71 t (7.8)	81.7	3.42 m	71.5
3	3.39 m	76.8	4.30-3.10	76.5	3.40 m	76.5	3.36 m	77.1	3.59 t (9.0)	77.1	3.30 m	79.6
4	3.45 m	72.3	4.30-3.10	72.4	3.44 t (9.0)	72.3	3.36 m	72.6	3.14 t (9.6)	72.4	3.88 t (8.4)	71.9
5	4.04 d (9.6)	76.2	4.30-3.10	76.1	3.69 m	75.0	4.25 d (9.6)	76.2	3.21 t (7.8)	77.1	3.29 m	82.8
6a											3.56 t (7.8)	62.2
6b											3.80 m	
COOH		171.0		170.3		170.1		170.1		173.7		
6’’-OCH_3			3.70 s	52.9	3.70 s	52.9						
	4’-*O*-glucuronic acid		**4’-*O*-methylglucuronic acid**		**4’-*O*-glucuronic acid**		**4’-*O*-methylglucuronic acid**		**2’’-*O*-glucose**			
1	5.26 d (7.2)	100.2	5.30 m	100.3	5.33 d (7.2)	100.1	5.18 d (7.2)	100.4	4.62 d (7.8)	104.6		
2	3.36 m	73.8	4.30-3.10	73.8	3.35 m	73.9	3.42 m	73.7	3.77 m	74.5		
3	3.36 m	76.9	4.30-3.10	76.5	3.34 m	77.7	3.40 m	76.6	3.00 m	75.7		
4	3.44 m	72.2	4.30-3.10	72.4	3.25 t (9.6)	72.8	3.47 m	72.3	3.34 m	70.4		
5	3.99 d (9.6)	76.1	4.30-3.10	76.1	4.20 d (9.6)	76.0	3.83 m	75.6	3.09 m	77.7		
6a									3.41 m	61.3		
6b									3.44 m			
COOH		171.0		170.3		170.1		170.1				
6’’’-OCH_3			3.70 s	52.9			3.70 s	53.0				

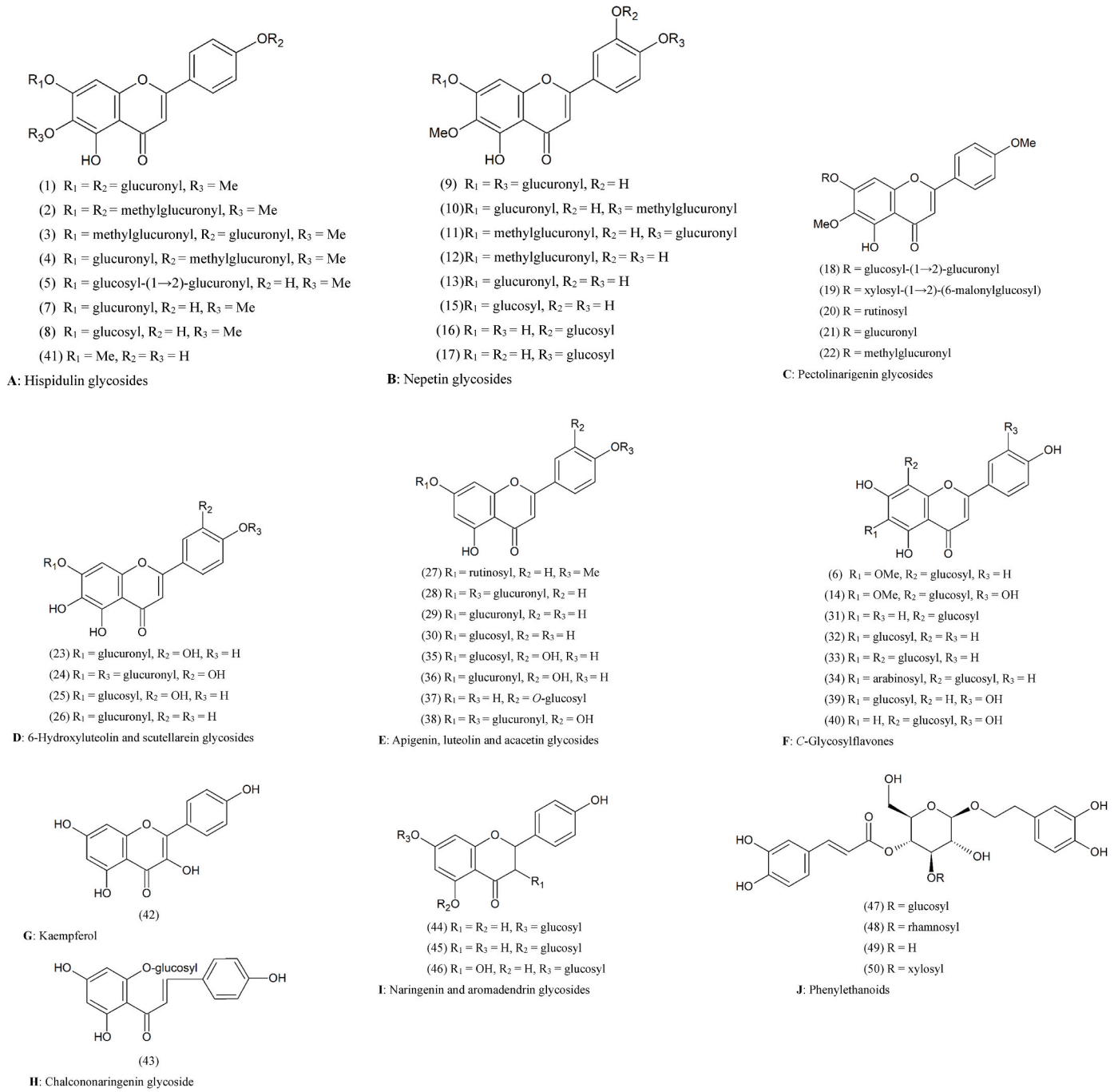

Fig. 1. Chemical structures of compounds isolated from *Aeschynanthus* species and cultivars.

7-*O*--β-D-(6″-methylglucuronopyranoside)-4′-*O*-β-D-(6‴-methylglucuronopyranoside) (Fig. 1A).

Flavonoid **3** was obtained as pale yellow powder from the corollas of *Aeschynanthus* species and cultivars, and showed a molecular ion peak at *m/z* 665.1354 $[M-H]^-$ for $C_{29}H_{29}O_{18}$ by HR-MS. LC-MS of **3** was occurred molecular ion peaks, *m/z* 667 $[M+H]^+$ and 665 $[M-H]^-$ and a fragment ion peak, *m/z* 491 $[M-176+H]^+$, showing the attachment of each 1 mol of glucuronic acid and methyl-glucuronic acid to hispidulin. In ^1H and ^{13}C NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC and HMBC (Table 1, Figs. 3–1∼6S). The ^1H NMR spectrum of **3** showed four aromatic proton signals, δ_H 8.11 (H-2′,6′), 7.25 (H-3′,5′), 7.08 (H-8) and 7.02 (H-3), together with two anomeric proton signals, δ_H 5.33 (*d*, $J = 7.2$ Hz) and 5.16 (*d*, $J = 6.6$ Hz). Two methoxyl proton signals, δ_H 3.81 and 3.70 also occurred on ^1H NMR. Of these proton signals, that of δ_H 3.81 was correlated with C-6 carbon signal of the aglycone at δ_C 133.5 by HMBC, showing a methoxyl group is attached to 6-position of the aglycone. Since another methoxyl proton signal at δ_H 3.70 was correlated with 6-carboxyl glucuronyl carbon signal at δ_C 170.1 by HMBC, it was shown that it is attached to 6-carboxyl group of glucuronic acid. Moreover, a methoxyl proton signal at δ_H 3.70 was correlated with 7-*O*-glucuronyl anomeric proton signal at δ_H 5.16 by NOESY, and also anomeric proton signal at δ_H 5.16 was correlated with C-7 carbon signal of hispidulin at δ_C 157.6 by HMBC, showing methyl-glucuronic acid is attached to 7-position of hispidulin. On the other hand, another anomeric proton signal at δ_H 5.33 correlated with C-4′ carbon signal of hispidulin at δ_C 160.6, showing the attachment of glucuronic acid to 4′-position of hispidulin. Thus, **3** was identified as

hispidulin 7-*O*-β-D-(6″-methylglucuronopyranoside)-4′-*O*-β-D-glucuronopyranoside (Fig. 1A).

Flavonoid **4** was obtained as pale yellow powder, and showed a molecular ion peak at *m/z* 665.1331 [M-H]$^-$ for $C_{29}H_{29}O_{18}$ by HR-MS. ^{1}H and ^{13}C NMR and LC-MS data of **4** were essentially the same as those of **3** (Table 1, Figs. 4–1∼6 S). However, a methoxyl proton signal at δ_H 3.70, was correlated with 4′-*O*-glucuronyl anomeric proton signal at δ_H 5.18 by NOESY and anomeric proton signal was correlated with C-4′ carbon signal at δ_C 161.1 by HMBC. On the other hand, another anomeric proton signal at δ_H 5.39 was correlated with C-7 carbon signal at δ_C 157.0 by HMBC. From the results described above, **4** was identified as hispidulin 7-*O*-β-D-glucuronopyranoside-4′-*O*-β-D-(6‴-methylglucuronopyranoside) (Fig. 1A).

Flavonoid **5** was obtained as pale yellow powder from the corollas of *Aeschynanthus* cultivars, and showed a molecular ion peak at *m/z* 661.1430 [M+H+Na]$^+$ for $C_{28}H_{30}O_{17}Na$ by HR-MS. Glucose and glucuronic acid were liberated by acid hydrolysis, together with hispidulin. ^{1}H NMR spectrum of **5** showed four aromatic proton signals, δ_H 7.95 (H-

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (DMSO-d_6) of nepetin, pectolinarigenin and 6-hydroxyluteolin glycosides from *Aeschynanthus* species and cultivars.

position	9		10		11		18		19		24	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
	Nepetin		Nepetin		Nepetin		Pectolinarigenin		Pectolinarigenin		6-Hydroxyluteolin	
2		164.7		164.9		164.6		164.8		165.0		164.5
3	6.92 s	104.3	6.94 s	104.8	6.92 s	104.7	6.98 s	104.3	6.98 s	104.4	6.89 s	104.6
4		183.3		183.3		183.3		183.3		178.3		183.3
5		153.4		153.5		153.2		153.2		154.3		147.7
6		133.5		133.5		133.5		133.4		132.9		131.5
7		157.4		157.0		157.7		157.1		157.0		152.2
8	7.09 s	95.2	7.14 s	94.9	7.05 s	95.3	7.06 s	95.0	7.06 s	94.9	7.05 s	94.8
9		153.1		153.1		153.1		153.1		153.7		150.0
10		106.8		106.9		106.7		106.5		106.9		106.9
1’		125.7		125.8		125.7		123.7		123.5		125.9
2’	7.57 brs	114.8	7.57 brs	114.8	7.57 brs	114.8	8.09 d (9.0)	129.3	8.09 d (9.0)	129.3	7.55 d (1.8)	114.8
3’		148.2		147.0		149.1	7.17 d (8.4)	115.6	7.17 d (9.0)	115.6		149.0
4’		149.5		149.8		148.1		163.4		163.4		148.0
5’	7.26 d (8.4)	117.2	7.31 d (8.4)	117.9	7.22 d (8.4)	116.7	7.17 d (8.4)	115.6	7.17 t (9.0)	115.6	7.25 d (8.4)	117.7
6’	7.57 m	119.5	7.58 brd (8.7)	119.7	7.58 brd (7.8)	119.4	8.09 d (9.0)	129.3	8.09 d (9.0)	129.3	7.57 dd (1.8, 8.6)	119.4
6-OCH$_3$	3.81 s	61.3	3.80 s	61.3	3.81 s	61.2	3.82 s	61.3	3.81 s	61.2		
4’-OCH$_3$							3.90 s	56.5	3.90 s	56.5		
	7-*O*-glucuronic acid		7-*O*-glucuronic acid		7-*O*-methylglucuronic acid		7-*O*-glucuronic acid		7-*O*-glucose		7-*O*-glucuronic acid	
1	5.25 m	100.6	5.41 d (7.2)	100.3	5.14 d (6.6)	100.9	5.35 d (7.2)	98.8	5.35 d (7.2)	104.6	5.18 d (7.8)	101.2
2	3.41 m	73.9	3.36 m	74.1	3.42 m	73.8	3.71 t (8.4)	81.7	3.66 brd (9.0)	82.0	3.42 m	73.9
3	3.38 m	76.5	3.32 m	76.8	3.42 m	76.1	3.59 t (9.0)	77.1	3.59 t (9.0)	77.3	3.38 m	76.4
4	3.39 m	72.7	3.27 t (9.6)	73.1	3.47 m	72.4	3.32 t (9.6)	72.5	3.50 m	70.4	3.41 m	72.5
5	3.90 d (7.8)	75.7	4.26 d (9.6)	76.2	3.63 brd (10.2)	74.7	3.21 t (9.0)	77.2	3.27 t (9.6)	72.5	3.97 d (9.0)	76.0
6a									3.91 m	64.1		
6b									3.97 dd (4.8, 11.7)			
COOH		172.2		170.2		172.6		172.4				171.3
6’’-OCH$_3$					3.71 s	52.9						
	4’-*O*-glucuronic acid		4’-*O*-methylglucuronic acid		4’-*O*-glucuronic acid		2’’-*O*-glucose				4’-*O*-glucuronic acid	
1	5.02 d (6.6)	102.0	4.90 d (7.2)	102.5	5.20 d (7.2)	101.2	4.62 d (7.8)	105.0			5.07 d (7.2)	101.7
2	3.39 m	73.9	3.40 m	73.8	3.36 m	73.9	3.74 brd (10.2)	74.6			3.42 m	73.8
3	3.38 m	77.3	3.40 m	76.6	3.35 m	77.8	3.00 t (8.4)	75.7			3.38 m	76.3
4	3.37 m	72.6	3.46 m	72.3	3.22 t (9.6)	72.9	3.14 t (8.4)	70.4			3.41 m	72.5
5	3.81 m	75.6	3.56 brd (9.6)	75.0	4.16 d (9.6)	76.0	3.10 m	77.7			3.89 d (9.0)	76.0
6a							3.40 m	61.5				
6b							3.45 m					
COOH		172.3		170.2		170.1						171.3
6’’’-OCH$_3$			3.70 s	53.0								
									2’’-*O*-xylose			
1									4.65 d (7.8)	98.9		
2									3.02 t (9.0)	75.6		
3									3.22 t (9.0)	76.8		
4									3.11 t (9.0)	70.4		
5a									3.15 m	67.9		
5b									3.51 m			
									6’’-malonic acid			
C=O										168.5		
CH$_2$									3.33 s	40.9		
COOH										178.3		

2′,6′), 7.00 (H-8), 6.95 (H-3′,5′) and 6.86 (H-3) and a methoxyl proton signal, δ_H 3.82 (Table 1, Figs. 5–1~6S). In LC-MS, **5** exhibited the molecular ion peaks at *m/z* 639 $[M+H]^+$ and 637 $[M-H]^-$, showing the attachment of each 1 mol of glucose and glucuronic acid to hispidulin. Two anomeric proton signals corresponding to glucose and glucuronic acid occurred at δ_H 4.62 (*d*, *J* = 7.8 Hz) and 5.35 (*d*, *J* = 7.8 Hz), respectively. Of these signals, the latter was correlated with C-7 carbon signal of hispidulin at δ_C 157.0 by HMBC. On the other hand, the former correlated with C-2 carbon signal of glucuronic acid at δ_C 81.7. The attachment of methoxyl group to 6-position of the aglycone was confirmed by HMBC correlation between a methoxyl proton signal at δ_H 3.82 and C-6 carbon signal of the aglycone at δ_C 133.3. Thus, **5** was identified as hispidulin 7-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranoside (Fig. 1A).

Flavonoids **7** and **8** were characterized as hispidulin 7-*O*-glucuronide and hispidulin 7-*O*-glucoside (Fig. 1A) by UV, LC-MS, acid hydrolysis, and HPLC and TLC comparison with authentic samples.

2.2. Identification of nepetin glycosides

Eight nepetin glycosides (**9**–**13**, **15**–**17**) were isolated from the calyces and leaves of two *Aeschynanthus* species and six cultivars.

Flavonoid **9** was obtained as pale yellow powder from the calyces of *Aeschynanthus* cultivars, and showed a molecular ion peak at *m/z* 691.1148 $[M+H+Na]^+$ for $C_{28}H_{28}O_{19}Na$ by HR-MS. Acid hydrolysis of **9** liberated nepetin and glucuronic acid. Since the molecular ion peaks, *m/z* 669 $[M+H]^+$ and 667 $[M-H]^-$, and the fragment ion peaks, *m/z* 493 $[M-176+H]^+$ and 491 $[M-176-H]^-$, occurred on LC-MS, the attachment of 2 mol glucuronic acid to nepetin was shown. In ^1H and ^{13}C NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC and HMBC (Table 2, Figs. 7–1~6S). The ^1H NMR spectrum of **9** showed five aromatic proton signals, δ_H 7.57, 7.57, 7.26, 7.09 and 6.92 corresponding to H-2′, H-6′, H-5′, H-8 and H-3. Two glucuronyl anomeric proton signals at δ_H 5.25 (*m*) and 5.02 (*d*, *J* = 6.6 Hz) occurred, together with a methoxyl proton signal at δ_H 3.81. The attachment of a methoxyl group to 6-position of the aglycone was revealed by HMBC correlation between a methoxyl proton signal and C-6 carbon signal of the aglycone at δ_C 133.5. On the other hand, since two anomeric proton signals at δ_H 5.25 and 5.02 correlated with C-7 and C-4′ carbon signals of nepetin at δ_C 157.4 and 149.5, respectively, it was shown that glucuronic acids are attached to 7- and 4′-positions of nepetin, respectively. Thus, **9** was identified as nepetin 7,4′-di-*O*-β-D-glucuronopyranoside (Fig. 1B).

Flavonoid **10** was obtained as pale yellow powder from the calyces of *Aeschynanthus* cultivars, and showed a molecular ion peak at *m/z* 705.1297 $[M+H+Na]^+$ for $C_{29}H_{30}O_{19}Na$ by HR-MS. Nepetin was produced by acid hydrolysis of **10**. ^1H and ^{13}C NMR data were essentially the same as those of **9** except for the occurrence of additional methoxyl proton and carbon signals at δ_H 3.70 and δ_C 53.0 (Table 2, Figs. 8–1~6S). Practically, the molecular ion peaks, *m/z* 683 $[M+H]^+$ and 681 $[M-H]^-$, and fragment ion peaks, *m/z* 493 $[M-190+H]^+$, 505 $[M-176-H]^-$ and 317 $[M-176-190+H]^+$ occurred on LC-MS, showing the attachment of each 1 mol of glucuronic acid and methyl-glucuronic acid to nepetin. Two anomeric proton signals, δ_H 5.41 (*d*, *J* = 7.2 Hz) and 4.90 (*d*, *J* = 7.2 Hz) occurred on ^1H NMR, and was correlated with C-7 and C-4′ carbon signals at δ_C 157.0 and 149.8 by HMBC, showing that the sugars were attached to 7- and 4′-positions of nepetin. Moreover, a methoxyl proton signal at δ_H 3.80 correlated with C-6 carbon signal at δ_C 133.5. On the other hand, another methoxyl proton signal at δ_H 3.70 correlated with carboxyl carbon signal of glucuronic acid at δ_C 170.2, showing that the methoxyl group is attached to carboxyl group of glucuronic acid. A methoxyl proton signal at δ_H 3.70 was correlated with 4′-*O*-glucuronyl anomeric proton signal at δ_H 4.90 by NOESY, exhibiting that methyl-glucuronic acid is attached to 4′-position of nepetin. Thus, **10** was identified as nepetin 7-*O*-β-D-glucuronopyranoside-4′-*O*-β-D-(6‴-methylglucuronopyranoside) (Fig. 1B).

Flavonoid **11** was obtained as pale yellow powder from the calyces of *Aeschynanthus* cultivars, and showed a molecular ion peak at *m/z* 705.1309 $[M+H+Na]^+$ for $C_{29}H_{30}O_{19}Na$ by HR-MS. Acid hydrolysis of **11** liberated nepetin. LC-MS and NMR data were the same as those of **10** (Table 2, Figs. 9–1~6S), showing that **11** is nepetin glycoside which attached glucuronic acid and methyl-glucuronic acid to 4′- and 7-positions. Since a methoxyl proton signal at δ_H 3.71 correlated with 7-*O*-glucuronyl anomeric proton signal at δ_H 5.14 by NOESY, showing that methyl-glucuronic acid is attached to 7-position of nepetin. From the results described above, **11** was identified as nepetin 7-*O*-β-D-(6″-methylglucuronopyranoside)-4′-*O*-β-D-glucuronopyranoside (Fig. 1B).

Flavonoids **12** and **13** were identified as nepetin 7-*O*-β-D-(6″-methylglucuronopyranoside) and nepetin 7-*O*-β-D-glucuronopyranoside (Fig. 13S) by UV, LC-MS, acid hydrolysis and NMR (Tables 1S and 2S). Flavonoids **15** and **16** were characterized as nepetin 7-*O*-glucoside and nepetin 3′-*O*-glucoside (Fig. 1B) by UV, LC-MS and acid hydrolysis, respectively. Flavonoid **17** was identified as nepetin 4′-*O*-glucoside (Fig. 1B) by UV, LC-MS, acid hydrolysis, and HPLC and TLC comparison with authentic sample.

2.3. Identification of pectolinarigenin glycosides

Five pectolinarigenin glycosides (**18**–**22**) were isolated from the corollas of *Aeschynanthus* species and cultivars. Flavonoid **18** was obtained as pale yellow powder, and showed a molecular ion peak at *m/z* 651.1558 $[M-H]^-$ for $C_{29}H_{31}O_{17}$ by HR-MS. Pectolinarigenin, glucose and glucuronic acid were produced by acid hydrolysis. The attachment of each 1 mol of glucose and glucuronic acid to pectolinarigenin was determined by LC-MS, i.e. the occurrence of the molecular ion peaks, *m/z* 653 $[M+H]^+$ and 651 $[M-H]^-$. In ^1H and ^{13}C NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC and HMBC. The ^1H NMR spectrum of **18** exhibited four aromatic proton signals at δ_H 8.09, 7.17, 7.06 and 6.98 corresponding to H-2′,6′, H-3′,5′, H-8 and H-3, together with two methoxyl proton signals at δ_H 3.90 and 3.82 (Table 2, Figs. 13–1~6S). Two anomeric proton signals corresponding to glucuronic acid and glucose occurred at δ_H 5.35 (*d*, *J* = 7.2 Hz) and 4.62 (*d*, *J* = 7.8 Hz). Since two methoxyl proton signals at δ_H 3.90 and 3.82 were correlated with C-4′and C-6 carbon signals of the aglycone at δ_C 163.4 and 133.4 by HMBC, it was confirmed that they are attached to 4′- and 6-positions of the aglycone. Glucuronyl anomeric proton signal at δ_H 5.35 correlated with C-7 carbon signal of pectolinarigenin at δ_C 157.1, showing the attachment of glucuronic acid to 7-position of pectolinarigenin. It was confirmed by the occurrence of a fragment ion peak, *m/z* 491 $[M-162+H]^+$ on LC-MS. Moreover, since glucosyl anomeric proton signal at δ_H 4.62 was correlated with C-2 carbon signal of glucuronic acid at δ_C 81.7 by HMBC, the attachment of glucose to 2-position of glucuronic acid was shown. Thus, **18** was identified as pectolinarigenin 7-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranoside (Fig. 1C).

Flavonoid **19** was obtained as pale yellow powder from the corollas of *Aeschynanthus fulgens*, and a molecular ion peak at *m/z* 693.1635 $[M-H]^-$ for $C_{31}H_{33}O_{18}$ by HR-MS. Pectolinarigenin, glucose and xylose were liberated by acid hydrolysis of **19**. However, the molecular ion peaks, *m/z* 695 $[M+H]^+$ and 693 $[M-H]^-$ occurred on LC-MS, together with the fragment ion peaks, *m/z* 609 $[M-86+H]^+$, 477 $[M-86-132+H]^+$ and 315 $[M-86-132-162+H]^+$, showing the attachment of 1 mol malonic acid to pectolinarigenin with xylose and glucose. The ^1H NMR spectrum of **19** showed four aromatic proton signals at δ_H 8.09 (H-2′,6′), 7.17 (H-3′,5′), 7.06 (H-8) and 6.98 (H-3), together with two methoxyl proton signals at δ_H 3.90 (4′-OCH_3) and 3.81 (6-OCH_3) (Table 2, Figs. 14–1~6S). Glucosyl anomeric proton signal at δ_H 5.35 (*d*, *J* = 7.2 Hz) occurred in ^1H NMR and was correlated with C-7 carbon signal at δ_C 157.0 by HMBC. On the other hand, xylosyl anomeric proton signal at δ_H 4.65 (*d*, *J* = 7.8 Hz) correlated with C-2 carbon signal of glucose at δ_C 82.0. Moreover, malonyl CH_2 proton signal at δ_H 3.33, and carbon signals at δ_C 178.3 (COOH), 40.9 (CH_2) and 168.5 (C═O) occurred in ^1H and ^{13}C NMR. Of these malonyl carbon signals, malonyl C═O carbon signal at δ_C 168.5 was correlated with glucosyl H-6a and H-6b proton signals at δ_H 3.91

and 3.97 by HMBC. Thus, **19** was identified as pectolinarigenin 7-*O*-β-D-[xylopyranosyl-(1 → 2)-(6″-malonylglucopyranoside)] (Fig. 1C).

Flavonoids **27** and **20** were isolated as a mixture, and characterized as acacetin 7-*O*-rutinoside (Fig. 1E) and pectolinarigenin 7-*O*-rutinoside (Fig. 1C) by UV, LC-MS and acid hydrolysis, and HPLC comparisons with authentic samples. Flavonoids **21** and **22** were identified as pectolinarigenin 7-*O*-glucuronide and pectolinarigenin 7-*O*-methyglucuronide (Fig. 1C) by UV, LC-MS and acid hydrolysis, respectively.

2.4. Identification of 6-hydroxyluteolin and scutellarein glycosides

Three 6-hydroxyluteolin glycosides **23**, **24** and **25**, and one scutellarein glycoside **26** were isolated from the corollas (**26**) and calyces (**23**–**25**) of *Aeschynanthus* cultivars as pale yellow powders.

HR-MS of **24** showed a molecular ion peak at *m/z* 653.1006 $[M-H]^-$ for $C_{27}H_{25}O_{19}$. In LC-MS, **24** showed the molecular ion peaks, *m/z* 655 $[M+H]^+$ and 653 $[M-H]^-$, and fragment ion peaks, *m/z* 479 $[M-176+H]^+$, 303 $[M-176-176+H]^+$ and 301 $[M-176-176-H]^-$, suggesting the attachment of 2 mol glucuronic acid to pentahydroxyflavone. Five aromatic proton signals corresponding to H-3, H-8, H-2′, H-5′ and H-6′ appeared on ^{1}H NMR, together with two glucuronyl anomeric proton signals, δ_H 5.18 (*d*, *J* = 7.8 Hz) and 5.07 (*d*, *J* = 7.2 Hz) (Table 2). Since these anomeric proton signals were correlated with C-7 and C-4′ carbon signals of 6-hydroxyluteolin at δ_C 152.2 and 148.0 by HMBC, it was shown that glucuronic acids are attached to 7- and 4′-positions of 6-hydroxyluteolin. Thus, **24** was identified as 6-hydroxyluteolin 7,4′-di-*O*-β-D-glucuronopyranoside (Fig. 1D).

Flavonoids **23** and **26** were identified as 6-hydroxyluteolin 7-*O*-β-D-glucuronopyranoside and scutellarein 7-*O*-β-D-glucuronopyranoside (Fig. 1D) by UV, LC-MS, acid hydrolysis and NMR (Tables 4S and 5S). Flavonoid **25** was characterized as 6-hydroxyluteolin 7-*O*-glucoside (Fig. 1D) by UV, LC-MS and acid hydrolysis.

2.5. Identification of C-glycosylflavones

Flavonoid **6** was obtained as pale yellow powder from the corollas of *Aeschynanthus* cultivars, and showed a molecular ion peak at *m/z* 461.1065 $[M-H]^-$ for $C_{22}H_{21}O_{11}$ by HR-MS. In LC-MS, **6** showed the molecular ion peaks, *m/z* 463 $[M+H]^+$ and 461 $[M-H]^-$, showing the attachment of 1 mol hexose to trihydroxy-monomethoxyflavone. However, **6** was unhydrolyzable by acid hydrolysis and presumed to be *C*-glycosylflavone. In ^{1}H and ^{13}C NMR, the proton and carbon signals were assigned by COSY, NOESY, HSQC and HMBC (Table 1, Figs. 6–1∼6S). The ^{1}H NMR spectrum of **6** showed three aromatic proton signals, δ_H 8.06, 6.93 and 6.83 corresponding to H-2′,6′, H-3′,5′ and H-3, together with a methoxyl proton signal, δ_H 3.82 and anomeric proton signal, δ_H 4.76 (*d*, *J* = 10.2 Hz). The attachment of a methoxyl group to 6-position of the aglycone was shown by HMBC correlation between a methoxyl proton signal and C-6 carbon signal at δ_C 131.5. From the results described above, aglycone was determined as hispidulin. It was shown by COSY, NOESY and HMBC that the hexose is glucose. Moreover, the attachment of glucose to 8-position of hispidulin was shown by HMBC correlation between anomeric proton signal at δ_H 4.76 and C-8 carbon signal of hispidulin at δ_C 104.9. Thus, **6** was identified as hispidulin 8-*C*-β-D-glucopyranoside (Fig. 1F).

Flavonoid **14** was identified as nepetin 8-*C*-β-D-glucopyranoside (Fig. 1F) by UV, LC-MS, acid hydrolysis and NMR (Table 3S). Other *C*-glycosylflavones **31**–**34**, **39** and **40** were identified as isovitexin (**31**), vitexin (**32**), vicenin-2 (**33**), isoschaftoside (**34**), isoorientin (**39**) and orientin (**40**) (Fig. 1F) by UV, LC-MS, and HPLC comparisons with authentic samples.

2.6. Identification of other flavonoids and phenylethanoids

Three apigenin *O*-glycosides, **28**–**30**, and four luteolin *O*-glycosides, **35**–**38** were isolated from the corollas, calyces and leaves of *Aeschynanthus* species and cultivars. Of these compounds, **28** and **38** were identified as apigenin 7,4′-di-*O*-β-D-glucuronopyranoside and luteolin 7,4′-di-*O*-β-D-glucuronopyranoside (Fig. 1E) by UV, LC-MS, acid hydrolysis and NMR (Tables 6S and 7S). Other flavone *O*-glycosides **29** and **30** were characterized as apigenin 7-*O*-glucuronide and apigenin 7-*O*-glucoside (Fig. 1E) by UV spectral properties, LC-MS, acid hydrolysis, and HPLC and TLC comparisons with authentic samples. Flavonoids **35** and **36** were identified as luteolin 7-*O*-glucoside and luteolin 7-*O*-glucuronide (Fig. 1E) by UV, LC-MS, acid hydrolysis, and HPLC comparisons with authentic samples. Flavonoid **37** was characterized as luteolin 3′-*O*-glucoside (Fig. 1E) by UV, LC-MS and acid hydrolysis.

Each one of flavone aglycone (**41**), flavonol (**42**), chalcone (**43**) and dihydroflavonol (**46**), two flavanones (**44**, **45**), and four phenylethanoids (**47**–**50**) were isolated from the corollas, calyces and leaves of *Aeschynanthus* species and cultivars. Of these compounds, **41** and **42** were agreed with authentic pedalitin (Fig. 1A) and kaempferol (Fig. 1G) by HPLC comparisons. Flavonoid **43** is chalcone and was identified as chalcononaringenin 2′-*O*-glucoside (Fig. 1H) by HPLC and TLC comparison with authentic sample. Flavonoids **44**–**46** were flavanones (**44**, **45**) and dihydroflavonol (**46**), and **45** and **46** were identified as naringenin 5-*O*-β-D-glucopyranoside and aromadendrin 7-*O*-β-D-glucopyranoside (Fig. 1I) by UV, LC-MS, acid hydrolysis and NMR (Tables 8S and 9S), respectively. On the other hand, **44** was characterized as naringenin 7-*O*-glucoside (Fig. 1I) by HPLC and TLC comparison with authentic sample. Although naringenin and its 7-*O*-glucoside have been found in the aerial parts of *Aeschynanthus bracteatus* (Li et al., 2008), dihydroflavonol was reported from the genus for the first time.

Compounds **47**–**50** were those of typical caffeoyl derivatives. Of these compounds, **47** and **48** were identified as plantamajoside and acteoside (Fig. 1J) by UV, LC-MS, and HPLC and TLC comparisons with authentic samples. On the other hand, **49** and **50** were identified as 2-(3′,4′-dihydroxyphenyl)ethyl-4-*E*-caffeoyl-β-D-glucopyranose and 2-(3′,4′-dihydroxyphenyl)ethyl-4-*E*-caffeoyl-3-*O*-β-xylofuranosyl-β-D-glucopyranose (Fig. 1J) by NMR (Tables 10S and 11S). Of these phenylethanoids, although **48** has been found in *A. bracteatus* (Li et al., 2008), other compounds were reported from the genus for the first time.

2.7. Occurrence of flavonoids and phenylethanoids from Aeschynanthus species and cultivars

Of forty-six flavonoids and four phenylethanoids which were isolated

Table 3
Isolation of flavonoids and phenylethanoids from *Aeschynanthus* species and cultivars.

Species and Cultivars	Flavonoids and Phenylethanoids
corollas	
A. fulgens	**5, 18, 19, 20, 21, 27, 30, 34, 43, 45, 47, 48, 49, 50**
A. pulcher	**1, 8, 43, 44, 45, 46, 47, 48, 49, 50**
'Mahligai'	**1, 6, 7, 8, 18, 20, 26, 34, 43, 44, 45, 46, 47, 48, 49, 50**
'Mona Lisa'	**1, 3, 4, 6, 8, 18, 20, 28, 32, 33, 34, 43, 44, 45, 46, 47, 48, 49, 50**
'SoeKa'	**1, 8, 26, 28, 29, 33, 34, 38, 43, 45, 47, 48, 49, 50**
'Redona'	**1, 2, 3, 4, 7, 8, 17, 34, 42, 43, 44, 45, 46, 47, 48, 49, 50**
'Freshya'	**1, 8, 43, 44, 45, 46, 47, 48, 49, 50**
'Bravera'	**1, 8, 22, 43, 44, 45, 46, 47, 48, 49, 50**
calyces	
A. pulcher	**1**
'Mahligai'	**1, 36**
'Mona Lisa'	**1, 9, 13, 15, 31, 48**
'SoeKa'	**1, 23, 24, 25**
'Redona'	**1, 10, 11**
'Freshya'	**1**
'Bravera'	**1**
leaves	
'Mahligai'	**1, 12, 14, 16, 17, 35, 37, 40, 41**
'Mona Lisa'	**14, 37, 39, 41**

from *Aeschynanthus* species and cultivars, chalcone (**43**), flavanones (**44** and **45**), and dihydroflavonol (**46**) were found only the corollas (Table 3). Phenylethanoids (**47**–**50**) were also isolated from the corollas except for **48** which was found in the calyces of 'Mona Lisa'. Major flavonoid **1** was isolated from almost corollas, calyces and leaves of *Aeschynanthus* species and cultivars. B-ring monohydroxyl flavone glycosides such as hispidulin, pectolinarigenin, apigenin and scutellarein occurred in the corollas. On the other hand, B-ring dihydroxy flavone glycosides and *C*-glycosylflavones were isolated from the calyces and leaves, except for **38** from cultivar 'SoeKa', and **6** from 'Mona Lisa' and 'Mahligai', and **33** from 'Mona Lisa' and 'SoeKa'. Flavonol aglycone (**42**) was isolated from the corollas of 'Redona' and free flavone (**41**) was found in the leaves of 'Mahligai' and 'Mona Lisa'. The correct flavonoid composition of each *Aeschynanthus* species and cultivars could not be cought by HPLC for the occurrence of numerous flavonoid and related compounds. However, the flavonoid diversity of the genus *Aeschynanthus* was determined in this survey.

2.8. Effect to flower color of isolated flavonoids

Thirteen anthocyanins have been isolated and identified from the corollas and calyces of *Aeschynanthus* species and cultivars (Iwashina et al., 2021), and 46 flavonoids were identified in this survey. Almost flower colors of *Aeschynanthus* species and cultivars are scarlet to reddish orange, and other flower colors are hardly present. Flavonoids are known as copigment substances affecting the flower color (Asen et al., 1972; Iwashina, 2015). We performed in vitro examination using two major corolla anthocyanins of Aeschynanthus, pelargonidin 3-*O*-samububioside (**A1**), pelargonidin 3-*O*-(6″-malonylsambubioside) (**A2**), and one calyx anthocyanin, cyanidin 3-*O*-(6″-malonylsambubioside) (**A3**) and major flavonoids **1**, **6**, **7**, **21** and **29**. Copigmentation effect of each anthocyanin and flavonoid is shown in Supplementary data (Table 12S). The bathochromic shift of 7.0–0.5 nm of visible absorbance maxima were recognized except for the mixtures of **A1** and **6**, and **A3** and **7**. These results showed that the presence of these flavonoids is effective to change of the flower color. However, these bathochromic shifts are comparatively slight. We presumed that blue *Aeschynanthus* flower do not need in nature. The flowers of *Aeschynanthus* have been estimated as to be specialized for bird pollination (Chen et al., 2019). The absence of flavonoids which is effective to bluing may be related to the selection pressure by the pollinator. As the results, although the flower color of almost *Aeschynanthus* species and cultivars are scarlet to reddish orange, the change to other flower color such as red purple and purple may be bred by the change of pelargonidin to other anthocyanidins such as cyanidin and delphinidin rather than the formation of copigmentation.

3. Conclusions

Forty-one flavones, each one of flavonol, chalcone and dihydroflavonol, two flavanones, and four phenylethanoids were isolated from *Aeschynanthus* species and cultivars. Flavonoids were mainly the glucuronides and/or methylglucuronides based on hispidulin, nepetin, pectolinarigenin, 6-hydroxyluteolin, scutellarein, apigenin and luteolin. Of these flavonoids, twelve, i.e. hispidulin 7,4′-di-*O*-glucuronide (**1**), 7,4′-di-*O*-methylglucuronide (**2**), 7-*O*-methylglucuronide-4′-*O*-glucuronide (**3**), 7-*O*-glucuronide-4′-*O*-methylglucuronide (**4**), 7-*O*-glucosyl-(1 → 2)-glucuronide (**5**) and 8-*C*-glucoside (**6**), nepetin 7,4′-di-*O*-glucuronide (**9**), 7-*O*-glucuronide-4′-*O*-methylglucuronide (**10**) and 7-*O*-methylglucuronide-4′-*O*-glucuronide (**11**), pectolinarigenin 7-*O*-glucosyl-(1 → 2)-glucuronide (**18**) and 7-*O*-xylosyl-(1 → 2)-(6″-malonylglucoside) (**19**), and 6-hydroxyluteolin 7,4′-di-*O*-glucuronide (**24**), were not previously described.

4. Materials and methods

4.1. General

Analytical high performance liquid chromatography (HPLC) was performed with Shimadzu HPLC systems using Inertsil ODS-4 column (I. D. 6.0 × 150 mm, GL Science Inc., Tokyo) at flow-rate of 1.0 ml min^{-1}. Detection wavelength was 350 nm (flavones, flavonol, chalcone and phenylethanoids) and 270 nm (flavanones and dihydroflavonol). Eluent was acetonitrile (MeCN)/H_2O/H_3PO_4 [20:80:0.2 (solv. I) and 25:70:0.2 (solv. II) for glycosides and 40:60:0.2 (solv. III) for aglycones]. Liquid chromatograph-mass spectra (LC-MS) was performed with Shimadzu LC-MS systems using Inertsil ODS-4 column (I.D. 2.1 × 100 mm) at a flow rate of 0.2 ml min^{-1}, electrospray ionization (ESI^+) 4.5 kV, ESI^- 3.5 kV, 250 °C. Eluent was MeCN/H_2O/HCOOH (15:80:5 for glycosides and 30:65:5 for aglycones). Nuclear magnetic resonance (NMR) spectra were measured by Bruker AV-600 spectrometer in dimethylsulfoxide-d_6 (DMSO-d_6). UV–visible absorption spectra were measured with Shimadzu MPS-2000 multipurpose recording spectrophotometer. Acid hydrolysis was performed in 12% aq. HCl, 100 °C, 30 min. After shaking with diethyl ether, aglycones were migrated to organic layer. On the other hand, sugars and *C*-glycosylflavones were left in aqueous layer. Preparative HPLC was performed with Shimadzu HPLC systems using Inertsil ODS-4 column (I.D. 10 × 250 mm) at a flow rate of 1.5 ml min^{-1}, detection wavelength of 250, 270 or 350 nm, and eluents were MeCN/H_2O/HCOOH (17:78:5, 20:75:5 or 30:65:5). Preparative paper chromatography (prep. PC) was performed with solvent systems, BAW (n-BuOH/HOAc/H_2O = 4:1:5, upper phase) and 15% HOAc.

4.2. Plant materials

Aeschynanthus fulgens Wall. ex R. Br., *A. pulcher* (Blume) G. Don and cultivar 'Freshya' are cultivated in the Bogor Botanic Gardens, Indonesia. *Aeschynanthus* cultivars, 'Mahligai', 'Mona Lisa' and 'Redona' are growing in Sunbur Makmur, Lembang-Bandung, Indonesia. *Aeschynanthus* cultivars, 'SoeKa' and 'Bravera' are cultivated in the Experiment Gardens, Ministry of Agriculture, Manoko-Lembang, Indonesia. Of these cultivars, 'Mahligai', 'Mona Lisa', 'Redona' and 'Freshya' were the new varieties of the Bogor Botanic Gardens as Dr. Rahayu's research results. On the other hand, 'SoeKa' and 'Bravera' were the interspecific cross-hybrid between *A. radicans* and *A. tricolor*, and between *A. pulcher* and *A. longiflorus*, respectively. 'Mahligai', 'Redona' and 'Freshya' were the mutant from irradiation treatment of *A. pulcher*. Voucher specimens and live specimens are deposited in Bogor Botanic Gardens (BBG), Indonesia, and these specimen numbers are as follow; *A. pulcher* (B200408570), *A. fulgens* (SRY-A1701), *A.* cultivar "SoeKa" (PR-22007), *A.* cultivar "Mahligai" (LR-01), *A.* cultivar "Bravera" (PL-62009), *A.* cultivar "Mona Lisa" (SRY-A1003), *A.* cultivar "Redona" (LR-07) and *A.* cultivar "Freshya" (LR-09).

4.3. Extraction and isolation

Fresh corollas of *Aeschynanthus fulgens* (6.4 g), *A. pulcher* (8.17 g), cultivars 'Mahligai' (92.35 g), 'Mona Lisa' (41.66 g), 'SoeKa' (35 g), 'Redona' (29 g), 'Freshya' (0.76 g) and 'Bravera' (7.55 g) were extracted with methanol (MeOH)/HCOOH (9:1) in the Bogor Botanic Garden, Indonesia in 20 and 21 Feb., 2019. Fresh calyces of *A. pulcher* (5.52 g), 'Mahligai' (116.8 g), 'Mona Lisa' (28.18 g), 'SoeKa' (32.2 g), 'Redona' (17.61 g), 'Freshya' (0.33 g) and 'Bravera' (4.28 g) were also extracted with MeOH/HCOOH (9:1). Fresh leaves of 'Mona Lisa' (49.79 g) and 'Mahligai' (35.61 g) were extracted with MeOH. After check of flavonoid and phenylethanoid composition by HPLC, corolla, calyx and leaf extracts were gathered and applied to prep. PC (see, General), respectively. Compounds **1**, **3**, **7**, **28**, **43**, **45** and **47**–**49** were purified by Sephadex LH-20 column chromatography using solvent system, 70% MeOH. Other compounds **2**, **4**–**6**, **8**–**27**, **29**–**42**, **44**, **46** and **50** were obtained as the

mixtures and further separated with prep. HPLC (see, General). The flavonoids and phenylethanoids were dissolved in water, freeze dried (Eyela EDU-1200, Tokyo Rika-kiki, Ltd., Tokyo) and obtained as pale yellow or white powders, i.e. **1** (69.5 mg), **2** (1.1 mg), **3** (14.0 mg), **4** (11.7 mg), **5** (1.5 mg), **6** (3.2 mg), **7** (21.0 mg), **9** (3.8 mg), **10** (1.3 mg), **11** (1.6 mg), **12** (1.8 mg), **13** (3.9 mg), **14** (4.4 mg), **15** (1.0 mg), **17** (0.7 mg), **18** (2.0 mg), **19** (1.3 mg), **20** (1.3 mg), **21** (1.1 mg), **22** (0.4 mg), **23** (1.0 mg), **24** (1.4 mg), **25** (0.9 mg), **26** (2.0 mg), **28** (9.1 mg), **29** (1.9 mg), **33** (3.8 mg), **38** (0.3 mg), **43** (78.9 mg), **44** (1.3 mg), **45** (14.2 mg), **46** (5.3 mg), **47** (105.4 mg), **48** (106.0 mg), **49** (190.9 mg) and **50** (1.5 mg). Other compounds **8**, **16**, **30**–**32**, **34**–**37**, and **40**–**42** were obtained as pure MeOH solutions.

4.4. Identification of flavonoids and phenylethanoids

Flavonoids and phenylethanoids were identified by UV–vis. spectral survey, HR-MS, LC-MS, characterization of hydrolysates, NMR, and HPLC and TLC comparisons with authentic samples. NMR spectra and the signal assignment for flavonoids and phenylethanoids were shown in Tables 1 and 2, and supplementary data (Tables 1S–11S, Figs. 1 S–12 S). The origins of the authentic samples used in this survey were as follows: hispidulin 7-*O*-glucuronide from the leaves of *Uncarina grandidieri* (Bail.) Stepf. (Yamazaki et al., 2007); hispidulin 7-*O*-glucoside, pedalitin, acteoside and plantamajoside from the leaves of *Plantago asiatica* L. (Aritomi, 1967; Ravn et al., 1990; Murai et al., 2009); nepetin 4′-*O*-glucoside from the leaves of *Cirsium oligophyllum* (Franch. et Savat.) Matsum. (Iwashina et al., 1999); acacetin 7-*O*-rutinoside and pectolinarigenin 7-*O*-rutinoside from the leaves of *Cirsium* spp. (Iwashina et al., 1995); vitexin and isovitexin from the flowers of *Iris ensata* Thunb. (Iwashina et al., 1996); vicenin-2 from the fronds of *Asplenium normale* D. Don (Iwashina et al., 2010b); isoschaftoside from the aerial parts of *Osyris alba* L. (Iwashina et al., 2008); isoorientin and orientin from the fronds of *Cyrtomium* spp. (Iwashina et al., 2006); apigenin 7-*O*-glucuronide from *Aeginetia indica* L. (Iwashina, 2010); apigenin 7-*O*-glucoside and naringenin 7-*O*-glucoside from Extrasynthese (Genay, France); luteolin 7-*O*-glucoside from the leaves of *Schmalhausenia nidulans* (Regel) Petr. (Iwashina and Kadota, 1999); luteolin 7-*O*-glucuronide from the leaves of *Saussurea daurica* Adams (Iwashina et al., 2010a); kaempferol from hydrolysate of kaempferol 3-*O*-alloside from *Glaucidium palmatum* Sieb. et Zucc. (Iwashina and Ootani, 1990); and chalcononaringenin 2′-*O*-glucoside from the flowers of yellow carnation (Yoshida et al., 2004).

4.4.1. Hispidulin 7,4′-di-O-glucuronide (1)

HPLC (retention time, *t*R): 9.03 min (solv. I). UV: λ_{max} (nm) MeOH 277, 321; +NaOMe 293, 380 (dec.); +$AlCl_3$ 286sh, 297, 344, 386sh; +$AlCl_3$/HCl 285sh, 296, 338, 386sh; +NaOAc 282, 307sh; +NaOAc/H_3BO_3 277, 326. HR-MS (ESI) $[M+H]^+$ Calcd. for $C_{28}H_{27}O_{18}$: 651.1197, Found: 651.1172. LC-MS: *m/z* 653 $[M+H]^+$ (hispidulin +2 mol glucuronic acid), *m/z* 477 $[M-176+H]^+$ (hispidulin +1 mol glucuronic acid) and *m/z* 301 $[M-176-176+H]^+$ (hispidulin). ^1H and ^{13}C NMR, see, Table 1.

4.4.2. Hispidulin 7,4′-di-O-methylglucuronide (2)

HPLC (*t*R): 11.64 min (solv. II). UV: λ_{max} (nm) MeOH 278, 322; +NaOMe 294, 377 (dec.); +$AlCl_3$ 281, 297sh, 336; +$AlCl_3$/HCl 285, 296, 336; +NaOAc 280, 307sh; +NaOAc/H_3BO_3 277, 321. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{30}H_{32}O_{18}Na$: 703.1486, Found 703.1486. LC-MS: *m/z* 681 $[M+H]^+$ (hispidulin +2 mol methyl-glucuronic acid), *m/z* 491 $[M-190+H]^+$, 489 $[M-190-H]^-$ (hispidulin +1 mol methyl-glucuronic acid). ^1H and ^{13}C NMR, see, Table 1.

4.4.3. Hispidulin 7-O-methylglucuronide-4′-O-glucuronide (3)

HPLC (*t*R): 18.88 min (solv. I). UV: λ_{max} (nm) MeOH 277, 322; +NaOMe 293, 379 (dec.); +$AlCl_3$ 286sh, 297, 348, 387sh; +$AlCl_3$/HCl 285sh, 296, 339, 386sh; +NaOAc 284, 386sh; +NaOAc/H_3BO_3 277, 323. HR-MS (ESI) $[M-H]^-$ Calcd. for $C_{29}H_{29}O_{18}$: 665.1354, Found 665.1354. LC-MS: *m/z* 667 $[M+H]^+$, 665 $[M-H]^-$ (hispidulin + each 1 mol of glucuronic acid and methyl-glucuronic acid), *m/z* 491 $[M-176+H]^+$ (hispidulin +1 mol methyl-glucuronic acid). ^1H and ^{13}C NMR, see, Table 1.

4.4.4. Hispidulin 7-O-glucuronide-4′-O-methylglucuronide (4)

HPLC (*t*R): 18.37 min (solv. I). UV: λ_{max} (nm) MeOH 277, 321; +NaOMe 293, 380 (dec.); +$AlCl_3$ 287sh, 297, 346, 386sh; +$AlCl_3$/HCl 286sh, 296, 339, 386sh; +NaOAc 282, 313sh; +NaOAc/H_3BO_3 276, 323. HR-MS (ESI) $[M-H]^-$ Calcd. for $C_{29}H_{29}O_{18}$: 665.1354, Found 665.1331. LC-MS: *m/z* 667 $[M+H]^+$, 665 $[M-H]^-$ (hispidulin + each 1 mol of glucuronic acid and methyl-glucuronic acid), *m/z* 491 $[M-176+H]^+$, 489 $[M-176-H]^-$ (hispidulin +1 mol methyl-glucuronic acid), *m/z* 477 $[M-190+H]^+$ (hispidulin +1 mol glucuronic acid). ^1H and ^{13}C NMR, see, Table 1.

4.4.5. Hispidulin 7-O-glucosyl-(1 → 2)-glucuronide (5)

HPLC (*t*R): 15.26 min (solv. I). UV: λ_{max} (nm) MeOH 274, 334; +NaOMe 276, 358, 377sh (inc.); +$AlCl_3$ 281, 300, 351, 391sh; +$AlCl_3$/HCl 283, 297, 347, 389sh; +NaOAc 272, 346sh, 390; +NaOAc/H_3BO_3 272, 338. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{28}H_{30}O_{17}Na$: 661.1381, Found: 661.1430. LC-MS: *m/z* 639 $[M+H]^+$, 637 $[M-H]^-$ (hispidulin + each 1 mol of glucose and glucuronic acid). ^1H and ^{13}C NMR, see, Table 1.

4.4.6. Hispidulin 8-C-glucoside (6)

HPLC (*t*R): 13.43 min (solv. I). UV: λ_{max} (nm) MeOH 277, 334; +NaOMe 279, 327, 395 (inc.); +$AlCl_3$ 282, 304, 360, 390sh; +$AlCl_3$/HCl 285sh, 301, 351, 390sh; +NaOAc 279, 316, 334, 393; +NaOAc/H_3BO_3 282, 324, 344. HR-MS (ESI) $[M-H]^-$ Calcd. for $C_{22}H_{21}O_{11}$: 461.1084, Found 461.1065. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (hispidulin +1 mol glucose). ^1H and ^{13}C NMR, see, Table 1.

4.4.7. Hispidulin 7-O-glucuronide (7)

HPLC (*t*R): 31.09 min (solv. I). UV: λ_{max} (nm) MeOH 275, 331; +NaOMe 275, 359, 376sh (inc.); +$AlCl_3$ 280, 297sh, 341, 387sh; +$AlCl_3$/HCl 282, 297, 346, 387sh; +NaOAc 274, 338, 387; +NaOAc/H_3BO_3 275, 336. LC-MS: *m/z* 477 $[M+H]^+$, 475 $[M-H]^-$ (hispidulin +1 mol glucuronic acid), *m/z* 301 $[M-176+H]^+$ (hispidulin).

4.4.8. Hispidulin 7-O-glucoside (8)

HPLC (*t*R): 27.07 min (solv. I). UV: λ_{max} (nm) MeOH 276, 331; +NaOMe 277, 358, 377sh (inc.); +$AlCl_3$ 287, 300, 359, 387sh; +$AlCl_3$/HCl 285sh, 298, 348, 387sh; +NaOAc 274, 389; +NaOAc/H_3BO_3 274, 337. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (hispidulin +1 mol glucose), *m/z* 301 $[M-162+H]^+$ (hispidulin).

4.4.9. Nepetin 7,4′-di-O-glucuronide (9)

HPLC (*t*R): 7.28 min (solv. I). UV: λ_{max} (nm) MeOH 276, 331; +NaOMe 273, 365 (dec.); +$AlCl_3$ 280, 342, 386sh; +$AlCl_3$/HCl 286, 344, 391sh; +NaOAc 273, 323; +NaOAc/H_3BO_3 275, 333. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{28}H_{28}O_{19}Na$: 691.1123, Found: 691.1148. LC-MS: *m/z* 669 $[M+H]^+$, 667 $[M-H]^-$ (nepetin +2 mol glucuronic acid), *m/z* 493 $[M-176+H]^+$, 491 $[M-176-H]^-$ (nepetin +1 mol glucuronic acid). ^1H and ^{13}C NMR, see, Table 2.

4.4.10. Nepetin 7-O-glucuronide-4′-O-methylglucuronide (10)

HPLC (*t*R): 14.66 min (solv. I). UV: λ_{max} (nm) MeOH 276, 332; +NaOMe 269, 364 (dec.); +$AlCl_3$ 285sh, 291, 357, 389sh; +$AlCl_3$/HCl 286sh, 289, 349, 387sh; +NaOAc 270, 315sh, 366sh; +NaOAc/H_3BO_3 276, 335. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{29}H_{30}O_{19}Na$: 705.1279, Found: 705.1297. LC-MS: *m/z* 683 $[M+H]^+$, 681 $[M-H]^-$ (nepetin + each 1 mol of glucuronic acid and methyl-glucuronic acid), *m/z* 493 $[M-190+H]^+$ (nepetin +1 mol glucuronic acid), *m/z* 505 $[M-176-H]^-$ (nepetin +1 mol methyl-glucuronic acid), *m/z* 317 $[M-176-190+H]^+$

(nepetin). ^{1}H and ^{13}C NMR, see, Table 2.

4.4.11. Nepetin 7-O-methylglucuronide-4′-O-glucuronide (11)

HPLC (*t*R): 14.92 min (solv. I). UV: λ_{max} (nm) MeOH 276, 332; +NaOMe 269, 364 (dec.); +$AlCl_3$ 285sh, 291, 357, 389sh; +$AlCl_3$/HCl 286sh, 289, 349, 387sh; +NaOAc 270, 315sh, 366sh; +NaOAc/H_3BO_3 276, 335. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{29}H_{30}O_{19}Na$: 705.1279, Found: 705.1309. LC-MS: *m/z* 683 $[M+H]^+$, 681 $[M-H]^-$ (nepetin + each 1 mol of glucuronic acid and methyl-glucuronic acid), *m/z* 493 $[M-190+H]^+$ (nepetin +1 mol glucuronic acid), m/z 505 $[M-176-H]^-$ (nepetin +1 mol methyl-glucuronic acid), *m/z* 317 $[M-176-190+H]^+$ (nepetin). ^{1}H and ^{13}C NMR, see, Table 2.

4.4.12. Nepetin 7-O-methylglucuronide (12)

HPLC (*t*R): 44.89 min (solv. I). UV: λ_{max} (nm) MeOH 256, 271, 346; +NaOMe 273, 397 (inc.); +$AlCl_3$ 275, 428; +$AlCl_3$/HCl 261sh, 281, 293sh, 364, 386sh; +NaOAc 265, 404; +NaOAc/H_3BO_3 262, 375. LC-MS: *m/z* 507 $[M+H]^+$, 505 $[M-H]^-$ (nepetin +1 mol methyl-glucuronic acid), *m/z* 317 $[M-190+H]^+$ (nepetin). ^{1}H and ^{13}C NMR, see, Table 1S.

4.4.13. Nepetin 7-O-glucuronide (13)

HPLC (*t*R): 16.62 min (solv. I). UV: λ_{max} (nm) MeOH 255sh, 273, 347; +NaOMe 275, 407 (inc.); +$AlCl_3$ 274, 296sh, 365, 394sh; +$AlCl_3$/HCl 260sh, 279, 293sh, 362; +NaOAc 273, 358, 408sh; +NaOAc/H_3BO_3 263, 375. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{22}H_{20}O_{13}Na$: 515.0802, Found: 515.0815. LC-MS: *m/z* 493 $[M+H]^+$, 491 $[M-H]^-$ (nepetin +1 mol glucuronic acid). ^{1}H and ^{13}C NMR, see, Table 2S.

4.4.14. Nepetin 8-C-glucoside (14)

HPLC (*t*R): 10.46 min (solv. I). UV: λ_{max} (nm) MeOH 255, 273, 347; +NaOMe 270, 344sh, 411 (inc.); +$AlCl_3$ 276, 425; +$AlCl_3$/HCl 262, 283, 295sh, 364, 386sh; +NaOAc 278, 398; +NaOAc/H_3BO_3 268, 383, 424sh. HR-MS (ESI) $[M+H+Na]^+$ Calcd. for $C_{22}H_{22}O_{12}Na$: 501.1009, Found: 501.1006. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (nepetin +1 mol glucose). ^{1}H and ^{13}C NMR, see, Table 3S.

4.4.15. Nepetin 7-O-glucoside (15)

HPLC (*t*R): 16.01 min (solv. I). UV: λ_{max} (nm) MeOH 271, 349. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (nepetin +1 mol glucose).

4.4.16. Nepetin 3′-O-glucoside (16)

HPLC (*t*R): 36.10 min (solv. I). UV: λ_{max} (nm) MeOH 274, 337; +NaOMe 276, 329, 395 (inc.); +$AlCl_3$ 255, 288, 295sh, 364, 386sh; +$AlCl_3$/HCl 249sh, 289, 355, 386sh; +NaOAc 276, 333, 398; +NaOAc/H_3BO_3 276, 345. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (nepetin + 1 mol glucose), *m/z* 317 $[M-162+H]^+$ (nepetin).

4.4.17. Nepetin 4′-O-glucoside (17)

HPLC (*t*R): 26.69 min (solv. I). UV: λ_{max} (nm) MeOH 272, 334; +NaOMe 265, 294sh, 371 (dec.); +$AlCl_3$ 255, 292, 355, 385sh; +$AlCl_3$/HCl 254, 289, 351, 386sh; +NaOAc 272, 367; +NaOAc/H_3BO_3 272, 338. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (nepetin + 1 mol glucose), *m/z* 317 $[M-162+H]^+$, 315 $[M-162-H]^-$ (nepetin).

4.4.18. Pectolinarigenin 7-O-glucosyl-(1 → 2)-glucuronide (18)

HPLC (*t*R): 16.65 min (solv. II). UV: λ_{max} (nm) MeOH 276, 329; +NaOMe 295, 376 (dec.); +$AlCl_3$ 282, 300, 343, 387sh; +$AlCl_3$/HCl 285, 298, 343, 387sh; +NaOAc 278, 329; +NaOAc/H_3BO_3 276, 332. HR-MS (ESI) $[M-H]^-$ Calcd. for $C_{29}H_{31}O_{17}$: 651.1561, Found 651.1558. LC-MS: *m/z* 653 $[M+H]^+$, 651 $[M-H]^-$ (pectolinarigenin + each 1 mol of glucose and glucuronic acid), *m/z* 491 $[M-162+H]^+$ (pectolinarigenin +1 mol glucuronic acid), *m/z* 315 $[M-162-176+H]^+$ (pectolinarigenin). ^{1}H and ^{13}C NMR, see, Table 2.

4.4.19. Pectolinarigenin 7-O-[xylosyl-(1 → 2)-(6″-malonylglucoside)] (19)

HPLC (*t*R): 29.14 min (solv. II). UV: λ_{max} (nm) MeOH 280, 334; +NaOMe 297, 376 (dec.); +$AlCl_3$ 284, 301, 346, 386sh; +$AlCl_3$/HCl 285, 299, 345, 386sh; +NaOAc 280, 332; +NaOAc/H_3BO_3 276, 334. HR-MS (ESI) $[M-H]^-$ Calcd. for $C_{31}H_{33}O_{18}$: 693.1667, Found 693.1635. LC-MS: *m/z* 695 $[M+H]^+$, 693 $[M-H]^-$ (pectolinarigenin + each 1 mol of glucose, xylose and malonic acid), *m/z* 609 $[M-86+H]^+$ (pectolinarigenin + each 1 mol of glucose and xylose), *m/z* 477 $[M-86-132+H]^+$ (pectolinarigenin +1 mol glucose), *m/z* 315 $[M-86-132-162+H]^+$ (pectolinarigenin). ^{1}H and ^{13}C NMR, see, Table 2.

4.4.20. Pectolinarigenin 7-O-rutinoside (Pectolinarin, 20)

HPLC (*t*R): 23.94 min (solv. II). UV: λ_{max} (nm) MeOH 277, 331; +NaOMe 296, 378 (dec.); +$AlCl_3$ 284sh, 299, 351, 387sh; +$AlCl_3$/HCl 283sh, 298, 344, 386sh; +NaOAc 282, 323; +NaOAc/H_3BO_3 274, 333. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (pectolinarigenin + each 1 mol of glucose and rhamnose), *m/z* 477 $[M-146+H]^+$ (pectolinarigenin +1 mol glucose), *m/z* 315 $[M-146-162+H]^+$, 313 $[M-146-162-H]^-$ (pectolinarigenin).

4.4.21. Pectolinarigenin 7-O-glucuronide (21)

HPLC (*t*R): 37.26 min (solv. II). UV: λ_{max} (nm) MeOH 276, 329; +NaOMe 296, 377 (dec.); +$AlCl_3$ 281, 301, 341, 386sh; +$AlCl_3$/HCl 284, 298, 345, 386sh; +NaOAc 278, 325; +NaOAc/H_3BO_3 275, 330. LC-MS: *m/z* 491 $[M+H]^+$, 489 $[M-H]^-$ (pectolinarigenin +1 mol glucuronic acid), *m/z* 315 $[M-176+H]^+$, 313 $[M-176-H]^-$ (pectolinarigenin).

4.4.22. Pectolinarigenin 7-O-methylglucuronide (22)

HPLC (*t*R): 45.23 min (solv. II). UV: λ_{max} (nm) MeOH 275, 326; +NaOMe 292, 372 (dec.); +$AlCl_3$ 280, 297sh, 343, 388sh; +$AlCl_3$/HCl 282, 297sh, 337, 386sh; +NaOAc 274, 324; +NaOAc/H_3BO_3 272, 333. LC-MS: *m/z* 505 $[M+H]^+$ (pectolinarigenin +1 mol methyl-glucuronic acid), *m/z* 315 $[M-190+H]^+$ (pectolinarigenin).

4.4.23. 6-Hydroxyluteolin 7-O-glucuronide (23)

HPLC (*t*R): 8.03 min (solv. I). UV: λ_{max} (nm) MeOH 284, 345; +NaOMe 272sh, 304sh, 396 (inc.); +$AlCl_3$ 274, 299, 422; +$AlCl_3$/HCl 261sh, 295, 367; +NaOAc 259sh, 302, 393; +NaOAc/H_3BO_3 261, 287sh, 361. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (6-hydroxyluteolin + 1 mol glucuronic acid), *m/z* 303 $[M-176+H]^+$, 301 $[M-176-H]^-$ (6-hydroxyluteolin). ^{1}H and ^{13}C NMR, see, Table 4S.

4.4.24. 6-Hydroxyluteolin 7,4′-di-O-glucuronide (24)

HPLC (*t*R): 4.17 min (solv. I). UV: λ_{max} (nm) MeOH 282, 330; +NaOMe 267sh, 305, 364sh (dec.); +$AlCl_3$ 255sh, 295, 355; +$AlCl_3$/HCl 252sh, 295, 352; +NaOAc 268sh, 306, 374sh; +NaOAc/H_3BO_3 287, 324. HR-MS (ESI) $[M-H]^-$ Calcd. for $C_{27}H_{25}O_{19}$: 653.0990, Found 653.1006. LC-MS: *m/z* 655 $[M+H]^+$, 653 $[M-H]^-$ (6-hydroxyluteolin + 2 mol glucuronic acid), *m/z* 479 $[M-176+H]^+$ (6-hydroxyluteolin + 1 mol glucuronic acid), *m/z* 303 $[M-176-176+H]^+$, 301 $[M-176-176-H]^-$ (6-hydroxyluteolin). ^{1}H and ^{13}C NMR, see, Table 2.

4.4.25. 6-Hydroxyluteolin 7-O-glucoside (25)

HPLC (*t*R): 7.84 min (solv. I). UV: λ_{max} (nm) MeOH 280, 346. LC-MS: *m/z* 465 $[M+H]^+$, 463 $[M-H]^-$ (6-hydroxyluteolin + 1 mol glucose), *m/z* 303 $[M-162+H]^+$ (6-hydroxyluteolin).

4.4.26. Scutellarein 7-O-glucuronide (26)

HPLC (*t*R): 14.30 min (solv. I). UV: λ_{max} (nm) MeOH 284, 334; +NaOMe 313sh, 372 (inc.); +$AlCl_3$ 289sh, 302, 362; +$AlCl_3$/HCl 289sh, 300, 356; +NaOAc 313, 376; +NaOAc/H_3BO_3 293, 330. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (scutellarein +1 mol glucuronic acid), *m/z* 287 $[M-176+H]^+$ (scutellarein). ^{1}H and ^{13}C NMR, see, Table 5S.

*4.4.27. Acacetin 7-O-rutinoside (Linarin, **27**)*

HPLC (*t*R): 22.53 min (solv. II). UV: λ_{max} (nm) MeOH 277, 331; +NaOMe 296, 378 (dec.); +$AlCl_3$ 284sh, 299, 351, 387sh; +$AlCl_3$/HCl 283sh, 298, 344, 386sh; +NaOAc 282, 323; +NaOAc/H_3BO_3 274, 333. LC-MS: *m/z* 593 $[M+H]^+$ (acacetin + each 1 mol of glucose and rhamnose), *m/z* 447 $[M-146+H]^+$ (acacetin +1 mol glucose), *m/z* 285 $[M-146-162+H]^+$, 283 $[M-146-162-H]^-$ (acacetin).

*4.4.28. Apigenin 7,4′-di-O-glucuronide (**28**)*

HPLC (*t*R): 7.68 min (solv. I). UV: λ_{max} (nm) MeOH 270, 318; +NaOMe 287, 372 (dec.); +$AlCl_3$ 271, 300, 330, 387sh; +$AlCl_3$/HCl 275, 298, 329, 386sh; +NaOAc 270, 314; +NaOAc/H_3BO_3 270, 320. LC-MS: *m/z* 623 $[M+H]^+$, 621 $[M-H]^-$ (apigenin +2 mol glucuronic acid), *m/z* 445 $[M-176+H]^+$ (apigenin +1 mol glucuronic acid), *m/z* 271 $[M-176-176+H]^+$ (apigenin). ^1H and ^{13}C NMR, see, Table 6S.

*4.4.29. Apigenin 7-O-glucuronide (**29**)*

HPLC (*t*R): 25.74 min (solv. I). UV: λ_{max} (nm) MeOH 267, 335; +NaOMe 271, 379 (inc.); +$AlCl_3$ 269, 299, 344, 384sh; +$AlCl_3$/HCl 274, 297, 339, 378sh; +NaOAc 256sh, 266, 389; +NaOAc/H_3BO_3 268, 343. LC-MS: *m/z* 447 $[M+H]^+$, 445 $[M-H]^-$ (apigenin +1 mol glucuronic acid).

*4.4.30. Apigenin 7-O-glucoside (Cosmosiin, **30**)*

HPLC (*t*R): 22.16 min (solv. I). UV: λ_{max} (nm) MeOH 269, 332; +NaOMe 274, 378 (inc.); +$AlCl_3$ 277, 299, 346, 377sh; +$AlCl_3$/HCl 277, 298, 341, 380sh; +NaOAc 268, 386; +NaOAc/H_3BO_3 266, 342. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (apigenin +1 mol glucose), *m/z* 271 $[M-162+H]^+$ (apigenin).

*4.4.31. Apigenin 6-C-glucoside (Isovitexin, **31**)*

HPLC (*t*R): 11.72 min (solv. I). UV: λ_{max} (nm) MeOH 270, 331; +NaOMe 277, 320, 392 (inc.); +$AlCl_3$ 274, 304, 346, 378sh; +$AlCl_3$/HCl 273, 304, 344, 376sh; +NaOAc 277, 347; +NaOAc/H_3BO_3 272, 303, 329. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (apigenin +1 mol glucose).

*4.4.32. Apigenin 8-C-glucoside (Vitexin, **32**)*

HPLC (*t*R): 10.94 min (solv. I). UV: λ_{max} (nm) MeOH 271, 334; +NaOMe 279, 325, 392 (inc.); +$AlCl_3$ 275, 305, 344, 384sh; +$AlCl_3$/HCl 276, 302, 339, 378sh; +NaOAc 279, 380; +NaOAc/H_3BO_3 272, 344. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (apigenin +1 mol glucose).

*4.4.33. Apigenin 6,8-di-C-glucoside (Vicenin-2, **33**)*

HPLC (*t*R): 5.05 min (solv. I). UV: λ_{max} (nm) MeOH 273, 333; +NaOMe 283, 333, 399 (inc.); +$AlCl_3$ 280, 305, 349, 383sh; +$AlCl_3$/HCl 280, 304, 343, 379; +NaOAc 282, 314sh, 336, 395; +NaOAc/H_3BO_3 284, 321, 348, 405sh. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (apigenin +2 mol glucose).

*4.4.34. Apigenin 6-C-arabinoside-8-C-glucoside (Isoschaftoside, **34**)*

HPLC (*t*R): 5.70 min (solv. I). UV: λ_{max} (nm) MeOH 272, 329. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (apigenin + each 1 mol of glucose and arabinose).

*4.4.35. Luteolin 7-O-glucoside (**35**)*

HPLC (*t*R): 14.75 min (solv. I). UV: λ_{max} (nm) MeOH 255, 267sh, 346; +NaOMe 271, 393 (inc.); +$AlCl_3$ 273, 422; +$AlCl_3$/HCl 261, 275sh, 295sh, 356, 385sh; +NaOAc 257, 401; +NaOAc/H_3BO_3 257, 373. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin +1 mol glucose), *m/z* 287 $[M-162+H]^+$, 285 $[M-162-H]^-$ (luteolin).

*4.4.36. Luteolin 7-O-glucuronide (**36**)*

HPLC (*t*R): 14.61 min (solv. I). UV: λ_{max} (nm) MeOH 254, 266sh, 348; +NaOMe 272, 400 (inc.); +$AlCl_3$ 274, 424; +$AlCl_3$/HCl 270, 295sh, 357, 384sh; +NaOAc 260, 400; +NaOAc/H_3BO_3 259, 372. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (luteolin +1 mol glucuronic acid), *m/z* 285 $[M-176-H]^-$ (luteolin).

*4.4.37. Luteolin 3′-O-glucuronide (**37**)*

HPLC (*t*R): 16.47 min (solv. I). UV: λ_{max} (nm) MeOH 255, 267sh, 346; +NaOMe 273, 400 (inc.); +$AlCl_3$ 272, 292sh, 362, 394sh; +$AlCl_3$/HCl 272, 295sh, 355, 387sh; +NaOAc 258sh, 359, 403sh; +NaOAc/H_3BO_3 259, 371. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (luteolin +1 mol glucuronic acid).

*4.4.38. Luteolin 7,4′-di-O-glucuronide (**38**)*

HPLC (*t*R): 7.12 min (solv. I). UV: λ_{max} (nm) MeOH 270, 333; +NaOMe 267, 369 (dec.); +$AlCl_3$ 257sh, 269, 295sh, 343, 387sh; +$AlCl_3$/HCl 257sh, 270, 294sh, 337, 386sh; +NaOAc 265, 333; +NaOAc/H_3BO_3 269, 336. LC-MS: *m/z* 639 $[M+H]^+$, 637 $[M-H]^-$ (luteolin +2 mol glucuronic acid), *m/z* 287 $[M-176-176+H]^+$ (luteolin). ^1H and ^{13}C NMR, see, Table 7S.

*4.4.39. Luteolin 6-C-glucoside (Isoorientin, **39**)*

HPLC (*t*R): 7.79 min (solv. I). UV: λ_{max} (nm) MeOH 256, 269sh, 353; +NaOMe 273, 409 (inc.); +$AlCl_3$ 275, 426; +$AlCl_3$/HCl 258, 276, 297sh, 356, 385sh; +NaOAc 269, 276sh, 400; +NaOAc/H_3BO_3 263, 376, 433sh. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin +1 mol glucose).

*4.4.40. Luteolin 8-C-glucoside (Orientin, **40**)*

HPLC (*t*R): 9.26 min (solv. I). UV: λ_{max} (nm) MeOH 255, 270sh, 350; +NaOMe 274, 408 (inc.); +$AlCl_3$ 273, 387; +$AlCl_3$/HCl 260, 277sh, 296, 357, 386sh; +NaOAc 271, 388; +NaOAc/H_3BO_3 262, 372, 425sh. LC-MS: *m/z* 449 $[M+H]^+$, 447 $[M-H]^-$ (luteolin +1 mol glucose).

*4.4.41. 5,6,3′,4′-tetrahydroxy-7-methoxyflavone (Pedalitin, **41**)*

HPLC (*t*R): 5.75 min (solv. III). UV: λ_{max} (nm) MeOH 285, 344; +NaOMe 269sh, 307sh, 393 (inc.); +$AlCl_3$ 273, 292, 421; +$AlCl_3$/HCl 256sh, 288, 369; +NaOAc 263sh, 388; +NaOAc/H_3BO_3 259sh, 286, 361. LC-MS: *m/z* 317 $[M+H]^+$, 315 $[M-H]^-$ (tetrahydroxy-monomethoxyflavone).

*4.4.42. 3,5,7,4′-tetrahydroxyflavone (Kaempferol, **42**)*

HPLC (*t*R): 16.59 min (solv. III). UV: λ_{max} (nm) MeOH 265, 365; +NaOMe 282, 323sh, 426 (decomp.); +$AlCl_3$ 256sh, 269, 304, 351, 422; +$AlCl_3$/HCl 258, 268sh, 303, 354, 422; +NaOAc 275, 310sh, 394; +NaOAc/H_3BO_3 264, 369. LC-MS: m/z 287 $[M+H]^+$, 285 $[M-H]^-$ (tetrahydroxyflavone).

*4.4.43. Chalcononaringenin 2′-O-glucoside (Isosalipurposide, **43**)*

HPLC (*t*R): 33.35 min (solv. I). UV: λ_{max} (nm) MeOH 246sh, 368; +NaOMe 246sh, 319sh, 432 (inc.); +$AlCl_3$ 253sh, 341, 415; +$AlCl_3$/HCl 246sh, 323sh, 399; +NaOAc 323sh, 401; +NaOAc/H_3BO_3 353sh, 436. LC-MS: m/z 433 $[M+H]^+$ (chalcononaringenin +1 mol glucose), *m/z* 273 $[M-162+H]^+$, 271 $[M-162-H]^-$ (chalcononaringenin).

*4.4.44. Naringenin 7-O-glucoside (**44**)*

HPLC (*t*R): 19.31 min (solv. I). UV: λ_{max} (nm) MeOH 283, 327sh; +NaOMe 242, 285, 360; +$AlCl_3$ 225, 286, 304, 356sh; +$AlCl_3$/HCl 224, 289sh, 307, 351; +NaOAc 283, 329; +NaOAc/H_3BO_3 283, 329. LC-MS: *m/z* 435 $[M+H]^+$, 433 $[M-H]^-$ (naringenin +1 mol glucose), *m/z* 273 $[M-162+H]^+$, 271 $[M-162-H]^-$ (naringenin).

*4.4.45. Naringenin 5-O-glucoside (**45**)*

HPLC (*t*R): 11.82 min (solv. I). UV: λ_{max} (nm) MeOH 226, 282, 314sh; +NaOMe 248, 324, 415; +$AlCl_3$ 226, 282, 314sh; +$AlCl_3$/HCl 226, 282, 314sh; +NaOAc 249sh, 284sh, 324; +NaOAc/H_3BO_3 225, 283, 317sh. LC-MS: *m/z* 433 $[M-H]^-$ (naringenin +1 mol glucose), *m/z* 273 $[M-162+H]^+$ (naringenin).

4.4.46. Aromadendrin 7-O-glucoside (46)

HPLC (*t*R): 5.99 min (solv. I). UV: λ_{max} (nm) MeOH 226sh, 285, 326sh; +NaOMe 243, 288, 365; +$AlCl_3$ 223sh, 288, 313sh, 343sh; +$AlCl_3$/HCl 222sh, 287, 312sh, 343sh; +NaOAc 287, 328sh, 367sh; +NaOAc/H_3BO_3 286, 338. LC-MS: *m/z* 451 $[M+H]^+$, 449 $[M-H]^-$ (aromadendrin +1 mol glucose), *m/z* 289 $[M-162+H]^+$, 287 $[M-162-H]^-$ (aromadendrin). ^1H and ^{13}C NMR, see, Table 9S.

4.4.47. 2-(3′,4′-dihydroxyphenyl)ethyl-4-E-caffeoyl-3-O-glucosyl-glucose (Plantamajoside, 47)

HPLC (*t*R): 8.28 min (solv. I). UV: λ_{max} (nm) MeOH 247sh, 292, 329; +NaOMe 253, 385 (inc.); +$AlCl_3$ 252sh, 305sh, 346; +$AlCl_3$/HCl 246sh, 289sh, 329; +NaOAc 288sh, 342, 387sh; +NaOAc/H_3BO_3 257sh, 295, 351. LC-MS: *m/z* 639 $[M-H]^-$.

4.4.48. 2-(3′,4′-dihydroxyphenyl)ethyl-4-E-caffeoyl-3-O-rhamnosyl-glucose (Acteoside, 48)

HPLC (*t*R): 11.12 min (solv. I). UV: λ_{max} (nm) MeOH 245sh, 290sh, 332; +NaOMe 256sh, 391 (inc.); +$AlCl_3$ 263, 306sh, 361; +$AlCl_3$/HCl 245sh, 290sh, 331; +NaOAc 289, 347sh, 372; +NaOAc/H_3BO_3 258sh, 294, 355. LC-MS: *m/z* 623 $[M-H]^-$.

4.4.49. 2-(3′,4′-dihydroxyphenyl)ethyl-4-E-caffeoyl-glucose (49)

HPLC (*t*R): 11.06 min (solv. I). UV: λ_{max} (nm) MeOH 245sh, 292, 331; +NaOMe 257, 379 (inc.); +$AlCl_3$ 254sh, 292, 343; +$AlCl_3$/HCl 247sh, 291, 332; +NaOAc 290, 337, 384sh; +NaOAc/H_3BO_3 256sh, 295, 353. LC-MS: *m/z* 477 $[M-H]^-$. ^1H and ^{13}C NMR, see, Table 10S.

4.4.50. 2-(3′,4′-dihydroxyphenyl)ethyl-4-E-caffeoyl-3-O-xylofuranosyl-glucose (50)

HPLC (*t*R): 9.91 min (solv. I). UV: λ_{max} (nm) MeOH 245sh, 291, 331; +NaOMe 257, 387 (inc.); +$AlCl_3$ 250sh, 289sh, 344; +$AlCl_3$/HCl 245sh, 290sh, 332; +NaOAc 290, 337, 386sh; +NaOAc/H_3BO_3 257sh, 294, 356. LC-MS: *m/z* 609 $[M-H]^-$. ^1H and ^{13}C NMR, see, Table 11S.

4.5. In vitro examination of flower color

This experiment was conducted referring to our previous study (Mizuno et al., 2021). Three anthocyanins, pelargonidin 3-*O*-sambubioside (**A1**), pelargonidin 3-*O*-(6″-malonylsambubioside) (**A2**) and cyanidin 3-*O*-(6″-malonylsambubioside) (**A3**) which were isolated from *Aeschynanthus* flowers (Iwashina et al., 2021) were used for measurement of the copigment effects of five flavonoids, hispidulin 7,4′-di-*O*-glucuronide (**1**), 8-*C*-glucoside (**6**) and 7-*O*-glucuronide (**7**), pectolinarigenin 7-*O*-glucuronide (**21**), and apigenin 7-*O*-glucuronide (**29**). Each compound was dissolved in 2.5 μL DMSO and diluted to final concentration of 1 mM anthocyanin and 3 mM flavonoid using 0.1 mol L^{-1} acetate buffer solution (pH 5.0). The UV–vis absorption spectra (350–750 nm) of these solutions were measured on a UV-2600 spectrophotometer (Shimadzu, Japan) using Scinco Nano Stick (Seoul, Korea).

Declaration of competing interest

The authors declare that they no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

Authors thank to the staffs of the Bogor Botanic Garden, Sunbur Makmur Lembang Bandung, and Manoko Lembang Research Station for support and assistance of plant care, collection and pre-extraction.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2022.113367.

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