Phenolic Compounds Isolated from The Fern *Chingia sakayensis* (Zeiller) Holtt

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ABSTRACT

Four known phenolic compounds namely kaemferol, matteucinol, farrerol, and matteucinol-7-O-β-D-glucoside were isolated for the first time from the fern *Chingia sakayensis* (Zeiller) Holtt's. All substances were separated by chromatographic techniques, purified by recrystalization, elucidated on the basis of spectroscopic evidence and by comparation with those reported data in literature. These results were very important to study the phytochemicals of the other fern in Chingia genus based on the chemotaxonomic approach.

Keywords: Fern, *Chingia sakayensis*, kaemferol, matteucinol, farrerol, matteucinol-7-O-β-D-glucoside

INTRODUCTION

C*hingia sakayensis* was one of the ferns belonging the Thelypteridaceae family distributed in Thailand, Malaysia, Serawak, Sumatra, and Java. It usually grew in the forest, often near streams, at altitude 150-1200 m. Because of the difference of environment condition, the specimens from Java and Sumatra were much ticker in texture, with very strongly raised veins and sinus membrane on the lower (Steenish & Holttum 1982). The young fronds of the plant can be eaten cooked or raw, an extract of mature fronds in water some times sprinkled on fever, and a decoction was used as tonic after childbirth (Piggott 1988).

In the course of our studies, several secondary metabolites had been isolated from the leaves and stem of *C. sakayensis* including wax ester, steroid, and flavonoid (Sutoyo *et al*. 2007). In this paper, we reported the isolation and structure determination of the four flavonoids, kaemferol (1), matteucinol (2), farrerol (3), and matteucinol-7-O-β-D-glucoside (4), isolated from *C. sakayensis* (Figure 1).

METHODS

General experimental procedures

Melting point was measured by Fisher John melting point apparatus and was uncorrected. Optical rotation was determined on polarimeter Perkin-Elmer 341**.** UV spectra were recorded on Shimadzu Pharmaspec UV-1700 spectrophotometer. IR spectrum in KBr film was determined by JASCO FT/IR-5300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured by JEOL JNM-ECP 400 spectrometer [operating at 400 MHz (^{1}H) and 100.5 MHz (^{13}C)] and JEOL JNM-AL300/AL 400 spectrometer [operating at 400 MHz (^{1}H) and 100.4 MHz (^{13}C)] using tetra methyl silane (TMS) as the internal standart. Mass spectrum (MS) was recorded on JEOL JMS-LX 1000 spectrometer using ion mode EI, CI^{+}, and FAB^{+} [3-nitro benzyl alcohol (m-NBA) as matrix]. Kieselgel 60 GF-254 (Merck) and silica gel G 60 63-200 μm (Merck) were used for vacuum liquid chromatography (VLC) and flash chromatography (FC), respectively. Precoated silica gel 60 F-254 (Merck) 0.25 mm, 20 x 20 cm was used for thin layer chromatography (TLC) and spots were detected by spraying with the sulphuric acid solution 5% (v/v) in ethanol followed by heating.

Plant material

The leaves and stem of *C. sakayensis* was collected from Kletak forest, Nongkojajar, Pasuruan, East Java, Indonesia in January 2002. A voucher spesimen was deposited at the herbarium of the Purwodadi Botanical Garden, Indonesia.

Isolation

(1). The leaves of *C. sakayensis*

The dried powdered leaves of *C. sakayensis* (1.5 kg) was exhaustively extracted successively with n-hexane (6 L x 3), dichloromethane (6 L x 3), and methanol (6 L x 3) at room temperature. The methanol extract was evaporated in vacum to obtain the concentrate methanol extract (104 g).

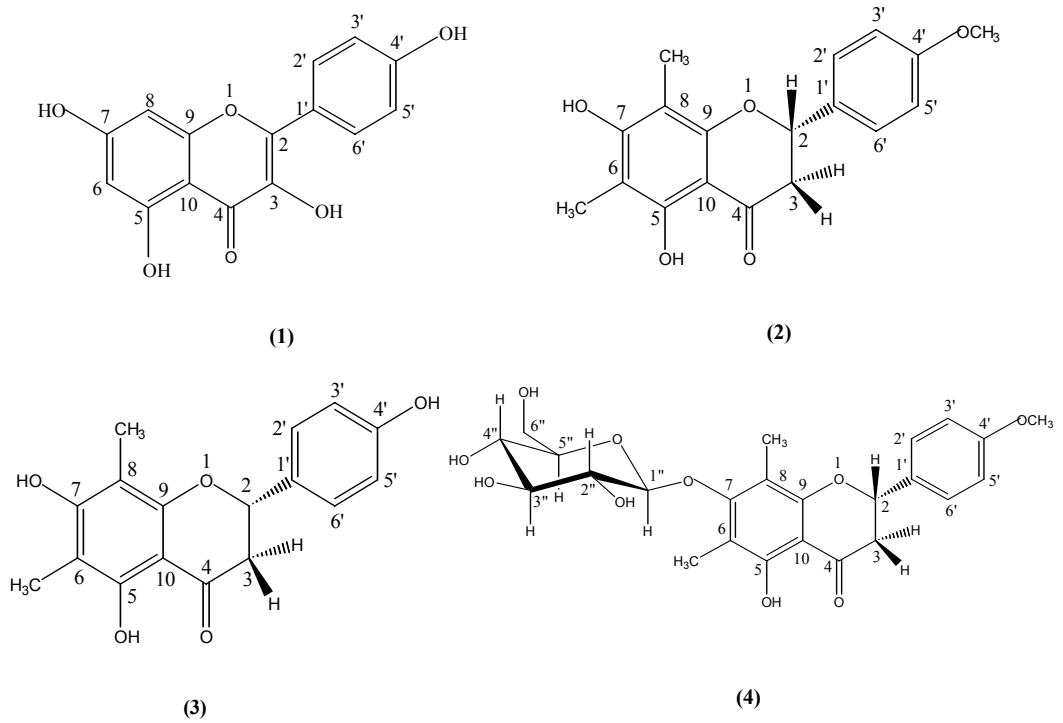

Figure 1. Structure of the four flavonoids isolated from *C. sakayensis*: kaemferol (1), matteucinol (2), farrerol (3), and matteucinol-7-O-β-D-glucoside (4).

Futhermore it was extracted with ethyl acetate-water mixture (1 : 1) (400 mL x 3). Removal of the solvent under reduced pressure of the ethyl acetate soluble fraction afforded a greenish brown residue (28 g). A portion of it (8 g) was chromatographed by VLC and eluted with solvents of increasing polarity (n-hexane, n-hexane-$CHCl_3$, $CHCl_3$, $CHCl_3$-MeOH) yielded 200 fractions (15 mL each). Removal of the solvent under reduced pressure of the combined fractions of 125-180 gave the brownish yellow solid (271 mg). A part of it (203 mg) was rechromatographed by FC with $CHCl_3$-acetone (4:1) as eluen, obtained 25 fractions (10 mL each). The fractions 6-9 were collected, recrystalized in $CHCl_3$-acetone yielded compound 1 (28 mg).

(2). The stem of *C. sakayensis.*

The dried powdered stem of *C. sakayensis* (677 g) was exhaustively extracted successively with n-hexane (4 L x 3), dichloromethane (4 L x 3), and methanol (4 L x 3) at room temperature. The methanol extract was evaporated in vacuo to afford the concentrate methanol extract (65 g). Futhermore it was extracted with ethyl acetate-water mixture (1 : 1) (400 mL x 3). Removal of the solvent under reduced pressure of the ethyl acetate soluble fraction yielded a brown residue (10 g). A portion of it (5 g) was chromatographed by VLC and eluted with solvents of increasing polarity (n-hexane, n-hexane-$CHCl_3$, $CHCl_3$, $CHCl_3$-MeOH) yielded 225 fractions (15 mL each).

The combined fractions of 34-50 (216 mg) was purified by FC with n-hexane-EtOAc (3:2) as eluen yielded compound 2 (30 mg). While purification of the combined fractions of 60-67 (160 mg) by FC with n-hexane-EtOAc (3:2) as eluen afforded compound 3 (18 mg). Recrystalization of the combined fractions of 128-135 (360 mg) in $CHCl_3$-MeOH afforded compound 4 (58 mg).

RESULTS AND DISCUSSION

Compound 1

Compound 1 was obtained as yellow crystal ($CHCl_3$-acetone), mp. 271-273^oC, which gave positive test (green colour) with $FeCl_3$ and Shinoda test (Mg-HCl). UV (MeOH) λ_{max} (log ε) : 273 (2.87), 324 (sh) (2.63) and 375 (2.85) nm; (MeOH + NaOH): 285 (3.91) and 410 (3.69) nm; (MeOH+$AlCl_3$): 276 (3.00), 312 (sh) (2.49), 355 (sh) (2.53) and 432 (2.93) nm; (MeOH+$AlCl_3$+HCl): 276 (2.97), 311 (sh) (2.51), 355 (sh) (2.55) and 432 (2.89) nm; (MeOH+NaOAc): 282 (2.97) and 391 (2.81) nm; (MeOH+NaOAc+H_3BO_3): 274 (2.92) and 375 (2.87) nm. IR (KBr) ν_{max} : 3333 (OH),

1659 (chelated C=O), 1617, 1570, 1509 (aromatic C=C) cm^{-1}. ^{1}H-NMR (400 MHz,CD_3OH) δ (ppm) : 6.18 (1H, *d*, *J* = 2 Hz, H-6); 6.39 (1H, *d*, *J* = 2 Hz, H-8); 6.90 (2H, *d*, *J* = 9 Hz, H-3' and H-5') and 8.08 (2H, *d*, *J* = 9 Hz, H-2' and H-6'). ^{13}C-NMR (100.5 MHz, CD_3OH) δ (ppm) : 94.5 (C-8); 99.3 (C-6); 104.5 (C-10); 116.3 (C-3' and C-5'); 123.7 (C-1'); 130.7 (C-2' and C-6'); 137.1 (C-3); 148.1 (C-2); 158.3 (C-5); 160.5 (C-4'); 162.5 (C-9); 165.6 (C-7) and 177.4 (C-4). FABMS, m/z (rel.int.): 287 ($M+H^+$)(44), 176 (m-NBA + Na^+)(34), 154 (m-NBA + H^+)(100), 136 (m-NBA-OH)(84).

The FABMS spectrum of 1 showed a quasi molecular ion peak at m/z 287 [$M+H^+$], corresponding to a molecular formula $C_{15}H_{10}O_6$. The absorbtion maxima at 273 (band II) and 375 nm (band I) in the UV spectrum supported that 1 was a flavonol with a free 3-hydroxyl group (Markham 1982). The batochromic shift of band I on adding NaOH reagent (35 nm) and $AlCl_3$ + HCl reagent (57 nm) indicated the presence of a hydroxyl group at C-4' and C-5, respectively. The presence of a hydroxyl group at C-7 was exhibited by batochromic shift of band II (9 nm) on adding NaOAc reagent.

No batochromic shift on adding NaOAc + H_3BO_3 reagent supported that 1 didn't have ortho-di hydroxyl group at B-ring. The IR spectrum of 1 clearly disclosed absorbtion bands for OH group (3333 cm^{-1}), chelated carbonyl group (1659 cm^{-1}), and aromatic C=C (1617, 1570, 1509 cm^{-1}). The ^{1}H-NMR spectrum of 1 exhibited four doublet proton signals at δ_H 6.18, 6.39, 6.90, and 8.08 (Table 1). Two doublet proton signals at δ_H 6.18 (*J* =1.8 Hz) and 6.39 (*J* = 2.2 Hz) due to a pair of meta coupled protons H-6 and H-8 in the A-ring, respectively, supported the presence of a hydroxyl group at C-5 and C-7.

While two doublet proton signals at δH 6.90 (J = 8.8 Hz, H-3',5') and 8.08 (J = 8.8, H-2',6') due to two pairs of ortho-cop;ed protons in the B-ring, confirmed the presence of a hydroxyl group at C-4'. The 13C-NMR spectrum exhibited 15 carbon signals which corresponded to 1, containing five oxy aryl carbons [δC 148.1 (C-2), 158.3 (C-5), 160.5 (C-4'), 162.5 (C-9), and 165.6 (C-7)], one oxyolefine carbon [δC 137.1 (C-3)], and one carbonyl carbon [δC 177.4 (C-4)] (Table 1). The correlation spectroscopy (1H-1H COSY, HMQC, and HMBC) spectral data supported complete assignment of all proton-bearing carbon signals of 1.

Futher supporting evidence of structure 1 for kaemferol came from comparison of the 1H-NMR and 13C-NMR spectral data with those of reported data in literature (Markham & Geiger 1994, Bin & Yongming 2003). From the above results, 1 was proposed for the structure of kaemferol (3,5,7,4'-tetrahydroxy flavone).

Table 1. ^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR data of **1** in CD_3OH.

Position of Atom C	δ_H (mult.,*J* in Hz)	δ_C	^{1}H-^{13}C HMBC
1	-	-	-
2	-	148.1	-
3	-	137.1	-
4	-	177.3	-
5	-	158.3	-
6	6.18 (*d,* 1.8)	99.3	C-7,C-8,C-9, C-10
7	-	165.6	-
8	6.39 (*d*, 2.2)	94.5	C-4,C-5,C-6,C-7,C-10
9	-	162.5	-
10	-	104.5	-
1'	-	123.7	-
2'	8.08 (*d,*8.8)	130.7	C-2,C-3',C-4',C-5',C-6'
3'	6.90 (*d,*8.8)	116.3	C-1',C-4',C-5'
4'	-	160.5	-
5'	6.90 (*d,*8.8)	116.3	C-1', C-3', C-4'
6'	8.08 (*d,*8.8)	130.7	C-2,C-2',C-3',C-4',C-5'

Compound 2

Compound 2 was obtained as pale yellow crystal (benzene), mp. 167-168oC, [α]D20 = -26o (MeOH, c.0.1), gave positive test with FeCl3 (green) and shinoda test (Mg-HCl)(red). It showed a single spot by TLC on silica gel with Rf = 0.25 (n-hexane : CH_2Cl_2 = 1 : 4), Rf = 0.70 (CH_2Cl_2 : EtOAc = 95 :5) and Rf = 0.80 (CH_2Cl_2: EtOAc = 9:1). UV (MeOH) λ maks (log ε) : 298 (4.13) dan 349 (sh) (3.40) nm; (MeOH + NaOH): 341 (4.37)nm; (MeOH+$AlCl_3$): 299 (4.10), 354 (sh)(3.32) nm; (MeOH+$AlCl_3$+HCl): 309 (4.05) nm; (MeOH+NaOAc): 341 (4.26) nm; (MeOH+NaOAc+H_3BO_3): 299 (4.07) dan 346 (sh)(3.72) nm. IR (KBr) νmaks : 3453 (OH), 3005 (aromatic C-H), 2922, 2840 (alkyl C-H), 1630 (chelated C=O), 1520 (aromatic C=C), 1454, 1397 cm-1. 1H-NMR (300 MHz,$CDCl_3$) δ (ppm) : 2.03(3H, s, 6-CH_3), 2.05 (3H, s, 8-CH_3), 2.78 (1H, dd, J = 17 Hz, 3 Hz, H-3β), 3.03 (1H, dd, J = 17 Hz, 13 Hz, H-3α), 3.83 (3H, s, OCH_3), 5.32 (1H, dd, J = 13 Hz, 3 Hz, H-2), 6.95 (2H, d, J = 9 Hz, H-3',5'), 7.39 (2H, d, J = 9 Hz, H-2',6'), 12.29 (1H, s, chelated 5-OH). 13C-NMR (100.5 MHz,$CDCl_3$) δ (ppm) : 6.9 (6-CH_3), 7.6 (8-CH_3), 43.1 (3α,3β), 55.2 (O CH_3), 78.2 (C-1), 102.3 (C-8), 102.7 (C-10), 103.5 (C-6), 114.0 (C-3',5'), 127.4 (C-2',6'), 131.0 (C-1'), 157.7 (C-9), 158.8 (C-5), 159.6 (C-4'), 162.1 (C-7), 196.5 (C-4). EIMS, m/z (rel.int.): 314 (M+)(100), 207 (M-C_7H_7O)+(9), 206 (M-C_7H_7O-H)+(6), 180 (M-C_9H10O)+(89), 152 (M-C_9H10O-CO)+(70), 134 (M-$C_9H_8O_4$)+(36), 121 (24), 91 (10), 77 (5), 69 (5), 55 (4).

The EIMS spectrum of 2 showed a molecular ion peak at m/z 314, corresponding a molecular formula $C_{18}H_{18}O_5$. The UV spectrum of 2 indicated absorbtion characteristic of flavanone-type compounds at 297 nm (band II) and 343 nm (sh) (band I) (Markham 1982). The absorbtion bands of alkyl C-H (2922, 2840 cm-1), chelated carbonyl group (1630 cm-1), and aromatic C=C (1520 cm-1) in the IR spectrum, together with the existence of the ABX-type proton signals at δH 2.78 (dd, H-3β), 3.05 (dd, H-3α), and 5.35 (dd, H-2) in the 1H-NMR spectrum (Table 2) also supported that 2 was a flavanone (Tanaka *et al.* 1985). The batochromic shift of band II (42 nm) on adding NaOH and NaOAc reagent showed the presence of a hydroxyl group at C-7. The presence of OH group at C-5 was supported by the batochromic shift of band II (11 nm) on adding AlCl3 + HCl reagent. No batochromic shift on adding NaOAc + H_3BO_3 reagent supported that 2 didn't have ortho-dihydroxyl group at the A-ring. The chelated proton signal at δH 12.29 (s) indicated the presence of a hydroxyl group at C-5.

Futher the 1H-NMR spectrum of 2 showed the existence of two aromatic methyl groups [δH 2.03 (s), 2.05 (s)] and a methoxyphenyl group [δH 3.85 (s)] in the flavanone skeleton (Table 2). In the HMBC spectrum of 2, the proton signal of the first aromatic methyl group (δH 2.03) showed correlation with carbon signals of C-5 (δC 158.8), C-6 (δC 103.5), C-7 (δC 162.1), while the proton signal of the second aromatic methyl group (δH 2.05) correlated with carbon signals of C-7 (δC 162.1), C-8 (δC 102.3), C-9 (δC 157.7) (Table 2). These results indicated that the first and the second aromatic methyl groups should be located at C-6 and C-8, respectively. The correlation between proton signal of methoxyphenyl group (δH 3.85) with carbon signal of C-4' (δC 159.6) in the HMBC spectrum, together with the appearance of two aromatic proton signals at δH 6.95 (d, J = 9.0 Hz, H-3',5') and 7.39 (d, J = 8.7 Hz, H-2',6') due to two pairs of ortho-copled aromatic protons in the B-ring indicated the presence of a methoxy group at C-4'. The other significant correlations of 2 can be seen in Table 2. Futher supporting evidence of structure 2 for matteucinol came from comparison of the 1H-NMR, 13C-NMR, and EIMS spectral data with those of reported data in literature (Tanaka *et al.* 1985, Miraglia *et al.* 1985). From the above results, compound 2 was identified as matteucinol (5,7-dihydroxy-4'-methoxy-6,8-dimethyl flavanone).

Compound 3

Compound 3 was obtained as pale yellow crystal ($CHCl_3$-MeOH), mp. 224-226°C, $[\alpha]_D^{20}$ = -26° (MeOH, *c*.0.1), gave positive test with $FeCl_3$ (green) and shinoda test (Mg-HCl)(red). It showed a single spot by TLC on silica gel with Rf = 0.38 ($CHCl_3$: EtOAc = 5 : 1), Rf = 0,36 (*n*-hexane : EtOAc = 3 : 2), and Rf = 0.07 (*n*-hexane : EtOAc = 4:1). UV (MeOH) λ_{maks} (log ε) : 297 (3.76) dan 348 (sh) (3.08) nm; (MeOH + NaOH): 339 (3.96)nm; (MeOH+$AlCl_3$): 297 (3.70), 357 (sh)(3.00) nm; (MeOH+$AlCl_3$+HCl): 299 (3.67), 358 (sh) (2.97) nm; (MeOH+NaOAc):

339 (3.83) nm; (MeOH+NaOAc+H_3BO_3): 297 (3.75), 350 (sh)(3.37) nm. IR (KBr) ν_{maks} : 3425 (OH), 2924 (alkyl C-H), 1636 (chelated C=O), 1520 (aromatic C=C), 1458, 1367, 1119, 833 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm) : 1.98(3H, *s*, 6-CH_3), 1.99 (3H, *s*, 8-CH_3), 2.70 (1H, *dd*, *J* = 16.8 Hz, 2.8 Hz, H-3β), 3.05 (1H, *dd*, *J* = 16.8 Hz, 12.8 Hz, H-3α), 5.29 (1H, *dd*, *J* = 12.8 Hz, 2.8 Hz, H-2), 6.82 (2H, *d*, *J* = 8.4 Hz, H-3',5'), 7.32 (2H, *d*, *J* = 8.4 Hz, H-2',6'). ^{13}C-NMR (100.6 MHz,CD_3OD) δ (ppm) : 7.4 (6-CH_3), 8.2 (8-CH_3), 44.1 (3α,3β), 80.1 (C-1), 103.2 (C-8), 104.1 (C-10), 104.8 (C-6), 116.3 (C-3',5'), 128.8 (C-2',6'), 131.5 (C-1'), 158.9 (C-9), 159.3 (C-5), 160.3 (C-4'), 164.2 (C-7), 198.4 (C-4). EIMS, m/z (rel.int.): 300 (M^+)(82), 282 (3), 271 (1), 257 (3), 207 (M-C_6H_5O)$^+$(6), 194 (12), 180 (M-C_8H_8O)$^+$(65), 152 (M-C_8H_8O-CO)$^+$(100), 154 (6), 120 (24), 107 (6), 91 (18), 77 (9), 65 (12), 55 (12).

The EIMS spectrum of 2 showed a molecular ion peak at m/z 300, suggesting a molecular formula $C_{17}H_{16}O_5$. The UV spectrum of 2 indicated absorbtion characteristic of flavanone-type compounds at 297 nm (band II) and 348 nm (sh) (band I) (Markham 1982).

The absorbtion bands of alkyl C-H (2924 cm-1), chelated carbonyl group (1636 cm-1), and aromatic C=C (1520 cm-1) in the IR spectrum, together with the existence of the ABX-type proton signals at δH 2.70 (dd, H-3β), 3.05 (dd, H-3α), and 5.29 (dd, H-2) in the 1H-NMR spectrum (Table 3) also supported that 2 was a flavanone (Tanaka *et al.* 1985). The batochromic shift of band II (42 nm) on adding NaOH and NaOAc reagent showed the presence of a hydroxyl group at C-7. The presence of OH group at C-5 was supported by the batochromic shift of band II (2 nm) on adding $AlCl_3$ + HCl reagent. No batochromic shift on adding NaOAc + H_3BO_3 reagent supported that 3 didn't have ortho-dihydroxyl group at the A-ring. Futher the 1H-NMR spectrum of 3 showed the existence of two aromatic methyl groups [δH 1.98 (s), 1.99 (s)] in the flavanone skeleton (Table 3). The DEPT spectrum of 3 showed that it had five methine carbons, one methylene carbon, two methyl carbons, and nine quartenary carbons. The 1H-NMR and 13C-NMR spectral data of 3 were similar to those of related compound, matteucinol, except a methoxy signal at δH 3.83 (s) and δC 55.2, respectively.

Table 2. ^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR data of **2** in $CDCl_3$.

Position of Atom C	^{1}H-NMR (δ ppm, mult.,*J*)	^{13}C-NMR (δ ppm)	^{1}H-^{13}C HMBC
1	-	-	-
2	5.32 (*dd*,*J*=13 Hz,3 Hz)	78.2	C-1',C-2',C-6'
3α	3.03 (*dd*,*J*=17 Hz,13 Hz)	43.1	C-4, C-10
3β	2.78 (*dd*,*J* = 17 Hz,3 Hz)	43.1	C-2, C-4, C-1'
4		196.5	-
5	-	158.8	-
6	-	103.5	-
7	-	162.1	-
8	-	102.3	-
9	-	157.7	-
10	-	102.7	-
1'	-	131.0	-
2'	7.39 (*d*,*J*=9 Hz)	127.4	C-2, C-3', C-4', C-6'
3'	6.95 (*d*,*J*= 9 Hz)	114.0	C-1',C-4', C-5'
4'	-	159.6	-
5'	6.95 (*d*,*J*= 9 Hz)	114.0	C-1',C-4', C-5'
6'	7.39 (*d*,*J*=9 Hz)	127.4	C-2, C-2', C-4', C-5'
6-CH_3	2.03 (*s*)	6.9	C-5, C-6, C-7
8-CH_3	2.05 (*s*)	7.6	C-7, C-9
4'-OCH_3	3.83 (*s*)	55.2	C-4'
5-OH	12.29 (s)	-	-

Table 3. ^{1}H-NMR, ^{13}C-NMR, and DEPT spectral data of farrerol in CD_3OD.

Position	^{1}H-NMR (δ ppm, mult.,*J*)	^{13}C-NMR (δ ppm)	DEPT 90	DEPT 135
1	-	-	-	-
2	5.29 (*dd*,*J*=12.8 Hz, 2.8 Hz)	80.1	CH	CH
3α	3.05 (*dd*,*J*=16.8 Hz, 12.8 Hz)	44.1	-	CH_2
3β	2.7 (*dd*,*J* = 16.8 Hz, 2.8 Hz)	44.1	-	CH_2
4		198.4	-	-
5	-	159.3	-	-
6	-	104.8	-	-
7	-	164.2	-	-
8	-	103.2	-	-
9	-	158.9	-	-
10	-	104.1	-	-
1’	-	131.5	-	-
2’	7.32 (*d*,*J*=8.4 Hz)	128.8	CH	CH
3’	6.82 (*d*,*J*=8.4 Hz)	116.3	CH	CH
4’	-	160.3	-	-
5’	6.82 (*d*,*J*=8.4 Hz)	116.3	CH	CH
6’	7.32 (*d*,*J*=8.4 Hz)	128.8	CH	CH
6-CH_3	1.98 (*s*)	7.4	-	CH_3
8-CH_3	1.99 (*s*)	8.2	-	CH_3

Further supporting evidence of structure 3 for matteucinol came from comparison of the EIMS data with those of the base data at the GCMS instrument. From the above results, compound 3 was identified as farrerol (5,7, 4’-trihydroxy-6,8-dimethyl flavanone).

Compound 4

Compound 4 was obtained as pale yellow crystal (MeOH-$CHCl_3$), mp. 135-136 ^{o}C, $[\alpha]_D^{20}$ = +7^{o} (MeOH, *c*.0.1), gave positive test with $FeCl_3$ (green) and shinoda test (Mg-HCl)(pale red). It showed a single spot by TLC on silica gel with Rf = 0.14 ($CHCl_3$ - EtOAc = 1 : 4), Rf = 0.28 ($CHCl_3$ -MeOH = 9 :1) and Rf = 0.38 ($CHCl_3$-MeOH = 5:1). UV (MeOH) λ_{maks} (log ε) : 282 (3.41), 361 (sh) (2.77) nm; (MeOH + NaOH): 284 (3.33), 372 (sh) (2.88)nm; (MeOH+$AlCl_3$): 281 (3.37), 362 (sh)(2.73) nm; (MeOH+$AlCl_3$+HCl): 283 (3.36), 363 (sh) (2.76) nm; (MeOH+NaOAc): 282 (3.41), 362 (sh) (2.77) nm; (MeOH+NaOAc+H_3BO_3): 282 (3.41), 363 (sh) (2.74) nm. IR (KBr) ν_{maks} : 3432 (OH), 2928 (alkyl C-H), 1636 (chelated C=O), 1516 (aromatic C=C), 1456, 1356, 1125, 1069, 835 cm^{-1}. ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm) : 2.05(3H, *s*, 6-CH_3), 2.07 (3H, *s*, 8-CH_3), 2.84 (1H, *dd*, *J* = 17.2 Hz, 2.8 Hz, H-3β), 3.05 (1H, *dd*, *J* = 18 Hz, 13.6 Hz, H-3α), 3.10-3.62 (5H, *m*, H-2”-6”), 3.76 (3H, *s*, 4’-OCH_3), 4.58 (1H, *d, J* = 7.2 Hz, H-1”), 5.54 (1H, *m*, H-2), 6.98 (2H, *d*, *J* = 8.4 Hz, H-3’,5’), 7.45 (2H, *d*, *J* = 8.8 Hz, H-2’,6’), 12.10 (1H, *s*, chelated 5-OH). ^{13}C-NMR (100.4 MHz, DMSO-d_6) δ (ppm) : 8.70 (6-CH_3), 9.27 (8-CH_3), 42.19 (3α,3β), 55.16 (OCH_3), 61.05 (C-6”), 69.86 (C-4”), 74.06 (C-2”), 76.34 (C-3”), 77.03 (C-5”), 77.84 (C-2), 104.19 (C-1”), 109.96 (C-10), 110.13 (C-8), 111.21 (C-6), 113.94 (C-3’,5’), 127.99 (C-2’,6’), 130.82 (C-1’), 157.27 (C-9), 157.85 (C-5), 159.36 (C-4’), 161.40 (C-7), 198.46 (C-4). FABMS, m/z (rel.int.): 515 ($M+K^+$)(2), 477 ($M+H^+$) (1), 345 (2 m-NBA+K^+)(22), 315 (aglycon+H^+), 314 (aglycon) (3), 307 (2 m-NBA+H^+)(14), 192 (m-NBA+H^+)(100), 136 (m-NBA-OH) (86).

The FABMS spectrum of 4 a quasi molecular ion peak at m/z 477 [M+H+], suggesting a molecular formula $C_{24}H_{28}O_{10}$. The absorption maxima at 282 nm (band II) and 361 nm (sh) (band I) in the UV spectrum supported that compound supported that compound 4 was a flavanone (Markham 1982). The presence of absorbtion bands for alkyl C-H (2928 cm-1), chelated carbonyl group (1636 cm-1), and aromatic C=C (1516 cm-1) in the IR spectrum, together with the existence of the ABX-type signals at δH 2.84 (dd, H-3β), 3.05

(dd, H-3α), and 5.35 (dd, H-2) in the 1H-NMR spectrum (Table 3) also supported that compound 4 had the flavanone skeleton. No batochromic shift of band II on adding NaOH and NaOAc reagent showed that 4 didn't have a free hydroxyl group at C-7. No batochromic shift on adding NaOAc + H3BO3 reagent supported that 4 didn't have ortho-dihydroxy group at A-ring.

The chelated proton signal at δH 12.10 (s) indicated the existence of a hydroxyl group at C-5. The 1H-NMR spectrum of 4 exhibited proton signals due to a 4'-methoxyphenyl group at δH 3.76 (3H, s, 4'-OCH3), 6.98 (2H, d, J=8.4 Hz), and 7.45 (2H, d, J=8.8 Hz), two aromatic methyl group at δH 2.05 (3H, s) and 2.07 (3H, s) as well as a glycosyl group at δH 4.58 (1H, d, J = 7.2 Hz, H-1") and δH 3.10-3.62 (6-H glycosyl, m)(Table 3). The glycosyl group of **4** could be identified as a glucosyl group because its carbon signals resembled those of reported data in literature [8]. In the HMBC spectrum of **4**, proton signal of methoxyphenyl group (δ_H 3.76) showed correlation with carbon signal of C-4' (δ_C 159.36), proton signal of the first aromatic methyl group (δ_H 2.05) correlated with carbon signals of C-5 (δ_C 157.85), C-6 (δ_C 111,21), C-7 (δ_C 161.40), and the proton signal of the second aromatic methyl group (δ_H 2.07) correlated with carbon signals of C-7 (δ_C 161.40), C-8 (δ_C 110.13), C-9 (δ_C 157.27) (Table 3).

These results suggested the presence of methoxyphenyl group at C-4' and aromatic methyl group at C-6 and C-8, respectively. The correlation beetwen proton signal of anomeric proton of glucosyl group (δH 4.58) with carbon signal of C-7 (δC 161.40) in the HMBC spectrum of 4 showed the presence of glucosyl group at C-7.

Table 4. ^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR data of 4 in DMSO-d_6.

Position of Atom C	δ_H (mult.,*J* in Hz)	δ_C	^{1}H-^{13}C HMBC
1	-	-	-
2	5.54 (*m*)	77.84	C-1',C-2',C-6', C-4
3α	3.05 (*dd,*18.0,13.6)	42.19	C-4
3β	2.84 (*dd,*17.2,2.8)	42.19	C-4
4		198.46	-
5	-	157.85	-
6	-	111.21	-
7	-	161.40	-
8	-	110.13	-
9	-	157.27	-
10	-	109.96	-
1'	-	130.82	-
2'	7.45 (*d,*8.8)	127.99	C-2, C-3', C-4', C-6'
3'	6.98 (*d,*8.4)	113.94	C-1',C-2',C-4', C-5'
4'	-	159.36	-
5'	6.98 (*d,*8.4)	113.94	C-1',C-3',C-4', C-6'
6'	7.45 (*d,*8.8)	127.99	C-2, C-2', C-4', C-5'
6-CH_3	2.05 (*s*)	8.70	C-5, C-6, C-7
8-CH_3	2.07 (*s*)	9.27	C-7, C-8,C-9
4'-OCH_3	3.76 (*s*)	55.16	C-4'
5-OH	12.10 (*s)*	-	C-5,C-6,C-8
1"	4.58 (*d*, 7.2)	104.19	C-7
2"		74.06	
3"		76.34	
4"	3.10-3.62 (*m*)	69.86	
5"		77.03	
6"		61.05	

Meanwhile the coupling constant value of the anomeric proton was 7.2 Hz, indicated the presence of a β-glycosydic linkage to a aglycon (Markham & Geiger 1994). Futher supporting evidence of structure 4 for matteucinol-7-O-β-D-glucoside came from comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data with those of reported data in literature (Tanaka *et al.* 1985). From above results compound 4 was suggested to be a matteucinol-7-O-β-D-glucoside.

CONCLUSIONS

Four phenolic compounds, kaemferol, matteucinol, farrerol, and matteucinol-7-O-β-D-glucoside were separated from the fern *Chingia sakayensis* (Zeiller) Holtt's. All compounds isolated for the first time from this fern and they can be used to study phytochemicals of the other plants in *Chingia* genus based on the chemotaxonomic approach.

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