Phenolic compounds from the stem bark of *Saccopetalum horsfieldii* Benn

Alfinda Novi Kristanti*, Nanik Siti Aminah and Mulyadi Tanjung

*Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia*

**ABSTRACT**

Column chromatographic separation of the methanol extract from the *Saccopetalum horsfieldii* Benn's stem bark yielded four phenolic components including three flavonoids, kaempferol-3,4'-dimethylether (1), quercetin-3,7-dimethylether (2), quercetin-3,7,4'-trimethylether (3), and one alkaloid, liriodenine (4). The structures of these compounds were determined based on UV, IR, HRESIMS, 1D and 2D NMR data.

**Keywords**: flavonoid, alkaloid, *Saccopetalum horsfieldii* Benn., Anonaceae.

**INTRODUCTION**

Annonaceae is a family of plants which grows in tropical and subtropical regions. This family consists of 130 genus and more than 2000 species. In Indonesia, there are more than 20 genus. Genus which have been researched are *Annona*, *Guatteria*, *Artabotrys*, *Goniothalamus*, *Polyalthia*, *Uvaria*, *Asimia* and *Xylopia*. [1]. *Saccopetalum* is one genus that has not been much studied. There was only a small amount of research investigated the species belonged to *Saccopetalum* genus, especially *Saccopetalum horsfieldii* Benn., a plant with a synonym name *Miliusahorsfieldii* [2].

As a result of our research for phenolic compounds in this Indonesian plant, we report the isolation of phenolic compounds, kaempferol 3,4'-dimethylether (1), quercetin 3,7-dimethylether (2), quercetin 3,7,4'-trimethylether (3), and liriodenine (4), from the methanol extract of the stem bark of *Saccopetalum horsfieldii* Benn. The phytochemical data of this species has not been yet reported.

**MATERIALS AND METHODS**

**General**

UV and IR spectrum were measured with a Beckman DU-7500 and Perkin Elmer Spectrum FTIR Shimadzu 5300 spectrometer, respectively. $^1$H and $^{13}$C NMR spectrum were recorded with a JEOL 400 spectrometer operating at 400 ($^1$H) and 100 ($^{13}$C) MHz in DMSO-d$_6$ using TMS as the internal standard. Mass spectrum was obtained with a Waters LCT Premier XE. Vacuum liquid chromatography (VLC) and column chromatography were carried out using Si gel 60 GF$_{254}$ and Si gel 60. For TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF$_{254}$, 0.25 mm thickness) were used.

**Plant material**

The stem bark of *Saccopetalum horsfieldii* Benn was collected from Purwodadi Botanical Garden, Center of Biological Research and Development, National Institute of Science, Pasuruan District, East Java, Indonesia.
Extraction and isolation
Milled drystem bark of *Saccoplatamhorsfieldii* Benn (3.0kg) were macerated with methanol three times at room temperature, and then concentrated under reduced pressure. The residue was suspended in water and partitioned with n-hexane. The methanol extract was concentrated and shaken repeatedly with 5% aqueous citric acid (pH 3-4) and partitioned with dichloromethane. The dichloromethane extract (28.4 g) was fractionated on silica gel by column chromatography eluting with mixtures of n-hexane-acetone (9:1, 4:1, and 7:3) to give three major fractions A-C. Fraction B (3.6 g) was fractionated on silica gel by column chromatography eluting with mixtures of n-hexane-acetone (9:1, 4:1 and 7:3) to give compounds 2 (28 mg) and 3 (80 mg). Furthermore, fraction C (5.6 g) was fractionated on silica gel by column chromatography eluting with mixtures of n-hexane-chloroform (4:1 and 7:3), chloroform, and mixtures of chloroform-methanol (9:1, 4:1 and 4:1) to give four major fractions A-D. Fraction D (800 mg), purified using column chromatography eluted with mixtures of n-hexane-ethylacetate (9:1, 4:1, and 7:3) to give compounds 4 (26 mg).

**Kaempferol 3,4'-dimethyl ether (1):** Pale yellow solid; m.p. 237°C; UV (MeOH) λ_max (nm) (log ε): 203 (4.68), 264 (4.28), 346 (3.80); LC-ESI-MS m/z 314 [M]^+; ^1H NMR (400 MHz, acetone-d₆) δH (ppm): 6.23 (1H, d, J = 2.4Hz, H-6), 6.47 (1H, d, J = 2.4Hz, H-8), 8.05 (2H, d, J = 9.2Hz, H-2'/6'), 7.05 (2H, d, J = 9.2Hz, H-3'/5'), 8.34 (3H, s, 3-OCH₃), 8.37 (3H, s, 4'-OCH₃), 12.75 (1H, s, 5-OH); ^13C NMR (100 MHz, DMSO-d₆) δC (ppm): 146.1 (C-2), 137.7 (C-3), 177.8 (C-6), 105.0 (C-4a), 169.0 (C-5), 97.0 (C-6), 164.8 (C-7), 94.6 (C-8), 157.8 (C-8a), 59.5 (4'-OCH₃).

**Quercetin 3,7-dimethylether (2):** Pale yellow solid; m.p. 224-226°C; UV (MeOH) λ_max (nm) (log ε): 203 (4.62), 257 (4.25), 359 (3.78); IR (KBr) ν_max (cm⁻¹): 3204 (OH), 2928, 2921 (CH alkyl), 1643 (conj. C=O), and 1545, 1390 C=C aromatic. ^1H NMR (400 MHz, acetone-d₆) δH (ppm): 6.35 (1H, d, J = 2.4Hz, H-6), 6.67 (1H, d, J = 2.4Hz, H-8), 7.47 (1H, dd, J = 8.4Hz, H-5'), 7.47 (1H, dd, J = 2.2Hz, H-6'), 3.80 (3H, s, 3-OCH₃), 3.86 (3H, s, 7-OCH₃), 12.61 (1H, s, 5-OH); ^13C NMR (100 MHz, DMSO-d₆) δC (ppm): 150.5 (C-2), 137.7 (C-3), 177.7 (C-4), 105.0 (C-4a), 160.7 (C-5), 95.5 (C-6), 164.8 (C-7), 92.0 (C-8), 156.0 (C-8a), 120.5 (C-1'), 115.5 (C-2'), 148.6 (C-3'), 155.7 (C-4'), 115.4 (C-5'), 60.4 (3-OCH₃), 55.8 (4'-OCH₃).

**Quercetin 3,4'-trimethylether (3):** Pale yellow solid; m.p. 173-175°C; UV (MeOH) λ_max (nm) (log ε): 204 (4.62), 255 (4.25), 348 (3.78); IR (KBr) ν_max (cm⁻¹): 3443 (OH), 1641 (conj. C=O), and 1580, 1421 C=C aromatic. ^1H NMR (400 MHz, DMSO-d₆) δH (ppm): 6.33 (1H, d, J = 2.2Hz, H-6), 6.67 (1H, d, J = 2.2Hz, H-8), 7.54 (1H, d, J = 2.0Hz, H-2'), 7.09 (1H, d, J = 8.2Hz, H-5'), 7.55 (1H, dd, J = 8.2, 2.0Hz, H-6'), 3.88 (3H, s, 3-OCH₃), 3.86 (3H, s, 7-OCH₃), 3.81 (3H, s, 4'-OCH₃), 12.61 (1H, s, 5-OH); ^13C NMR (100 MHz, DMSO-d₆) δC (ppm): 146.1 (C-2), 137.7 (C-3), 177.8 (C-4), 105.0 (C-4a), 160.7 (C-5), 97.5 (C-6), 164.8 (C-7), 92.0 (C-8), 156.0 (C-8a), 122.0 (C-1'), 115.5 (C-2'), 150.1 (C-3'), 155.3 (C-4'), 111.7 (C-5'), 120.2 (C-6'), 59.6 (3-OCH₃), 55.9 (7-OCH₃), 55.6 (4'-OCH₃).

Figure 1. Structures of phenolic compounds

**Scholar Research Library**

150
Lirioidenine(4): Pale yellow solid: UV (MeOH) \( \lambda_{max} \): 272, 317 nm; FAB-MS m/z: 276[M+H]⁺; ¹H NMR (400 MHz, DMSO-d₆) \( \delta_{H} \) (ppm): 7.58 (1H, s, H-3), 8.05 (1H, d, J = 5.2 Hz, H-2'), 8.83 (1H, d, J = 5.2 Hz, H-5), 8.38 (1H, dd, J = 8.0, 2.0 Hz, H-8), 7.66 (1H, t, J = 8.0 Hz, H-9), 7.90 (1H, t, J = 8.0 Hz, H-10), 8.67 (1H, d, J = 8.0 Hz, H-11), 6.51 (2H, s, -O-CH₂-O-). ¹³C NMR (100 MHz, DMSO-d₆) \( \delta_{C} \) (ppm): 148.3 (C-1), 151.4 (C-2), 103.1 (C-3), 144.3(C-3a), 124.3 (C-4), 144.2 (C-5), 135.2 (C-6a), 180.9 (C-7), 132.3 (C-7a), 126.8 (C-8), 127.6 (C-9), 133.9 (C-10), 128.3 (C-11), 130.6 (C-11a), 106.0 (C-11β), 122.4 (C-11c), 103.0 (O-CH₂-O).

RESULTS AND DISCUSSION

Four phenolic compounds, namely kaempferol 3,4′-dimethyl ether(1), quercetin 3,7-dimethyl ether(2), quercetin 3,7,4′-trimethyl ether (3) and lirioidenine(4) have been isolated from the stem bark of *Saccopatuum horsfieldii* Benn.

Kaempferol 3,4′-dimethyl ether(1) was isolated as a pale yellow solid. The UV spectrum exhibited maximum absorption on 203, 257, and 359 nm typical for a flavonol compound and showed bathochromic shifts on addition of AlCl₃ and NaOAc [3]. In the ¹³C NMR spectrum, 15 carbon signals representing 17 carbon atoms were observed. Two of them, namely the signals at 156.5 and 179.5, are characteristic for C-3 and C-4 of a flavonol structure [4]. The presence of five oxyaryl signals (δC 156.5, 157.8, 162.7, 164.8, and 169.0) indicated that the flavonol is a derivative of kaempferol. The ¹H NMR spectrum showed the presence of proton signals of a pair of doublets (J = 2.4 Hz) in the aromatic region at 6.23 and 6.47 ppm, characteristic for H-6 and H-8 proton signals of the ring A. Furthermore, in the ¹H NMR spectrum, a pair of doublets (J = 9.2 Hz) was appeared in the aromatic region at 6.805 and 7.05 ppm (each 2H) characteristic for a hydroxyl phenyl group of the ring B. The ¹H NMR spectrum of 1 also showed twomethoxy groups at δH 3.84 and 3.87 and a proton singlet signal at δH 12.75 that is consistent with the presence of an OH-phenolic at C-5. The placement of methoxy group in kaempferol structure shown in HMQC and HMBC spectrum. By analysis of HMQC and HMBC spectrum of 1, the methoxy signal (δH 3.87) exhibited ¹H-¹³C long range correlation with an oxyaryl carbon signal (δC 162.7), meanwhile correlation of the signal at δH 8.05 in the ring B correspond to the methoxyl group at C-4′. Furthermore, correlation methoxy signal δH 3.84 with δC 139.4 suggested that the methoxy was unambiguously located at C-3. From these NMR data analysis, the flavonol isolated was assigned as kaempferol 3,4′-dimethyl ether[5]. Other HMQC and HMBC correlations, as well as ¹³C NMR data assignment, that are consistent with the structure 1 are shown in Fig. 2.

Quercetin 3,7-dimethyl ether(2) was isolated as a pale yellow solid, and its UV spectrum exhibited maximum absorption on 203, 257, and 359 nm typical for a flavonol. The IR spectrum indicated absorptions for hydroxyl (3204 cm⁻¹), conjugated carbonyl (1643 cm⁻¹), and aromatic (1545, 1390 cm⁻¹) groups. The ¹H NMR spectrum of 2 showed ABX system at δH 7.58 (d, J = 2.2 Hz, H-2’), 6.91 (d, J = 8.4 Hz, H-5’), 7.47 (dd, J = 8.4, 2.2 Hz, H-6’) characteristic for aromatic in the ring B. The presence of proton signals of a pair of doublets (J = 2.2 Hz) in the aromatic region at δH 6.33 and 6.68 ppm, characteristic for H-6 and H-8 in the ring A. The ¹H NMR spectrum of 2 also showed two methoxyl signals (δH 3.80; 3.86) and a proton singlet signal at δH 12.67 that is consistent with an OH-phenolic at C-5. The ¹³C NMR spectrum of 2 showed 17 carbon signals were observed. Two of them, namely the signals at δC 145.0, 148.6, 155.7, 156.0, 160.7, and 164.8) indicated that the flavonol is a derivative of quercetin. Further support for the structure 2 was also obtained from the comparison of the NMR data with those reported for quercetin 3,7-dimethyl ether from *Ericameria diffusa*[6].

Quercetin 3,7,4′-trimethyl ether(3) was isolated as a pale yellow solid. The UV and IR spectrum very similar with compound 2. The ¹H NMR spectrum of 3 showed ABX system at δH 7.54 (d, J = 2.0 Hz, H-2’), 7.09 (d, J = 8.2 Hz, H-5’), 7.55 (dd, J = 8.2, 2.0 Hz, H-6’) and a pair of doublets (J = 2.2 Hz) in the aromatic region at δH 6.33 and 6.67 ppm, three methoxyl signals (δH 3.88; 3.86; 3.81) and an OH-phenolic at C-5a; δH 12.61. The ¹³C NMR spectrum of 3 showed 18 carbon signals were observed. Two of them, namely the signals at δC 137.7 and 177.8 are characteristic.
flavonol structure and six oxyaryl signals (δ_C 146.1, 150.1, 155.3, 156.0, 160.7, and 164.8) indicated that the flavonol is a derivative of quercetin. The structure of 3 agreed with those recorded by Urbatsch[6].

Liriodenine(4) was obtained as an pale yellow solid. Its UV spectrum (λ_{max} 272, 317 nm) indicated characteristic of oxoaporphine alkaloid. The FABMS spectrum showed a molecular ion [M+H]^+ at m/z 276 consistent to the molecular formula C_{17}H_{10}NO_{3}. The ^1H NMR spectrum of 4 showed the presence of one methylenedioxy group and seven aromatic protons. In the ^1H-NMR spectrum of 4 showed a proton singlet signal of methylenedioxy signal at δ_H 6.51, a pair of doublets (J = 5.2 Hz) in the aromatic region at δ_H 8.05 and 8.38 are characteristic for H-4 and H-5 of an oxoaporphine structure, a proton singlet signal at δ_H 7.66, a pair of doublets (J = 5.2 Hz) in the aromatic region at δ_H 8.05 and 8.83 are characteristic for H-4 and H-5 of a oxoaporphine structure, a proton singlet signal at δ_H 7.90, a pair of doublets (J = 5.2 Hz) in the aromatic region at δ_H 8.05 and 8.83 were assigned to H-8, H-9, H-10 and H-11, respectively. In the ^13C NMR spectrum, 17 carbon signals were observed. Two of them, the signals at δ_C 148.3 and 151.4 are characteristic for ortho oxygenated and one carbonyl group at δ_C 180.9. Based on ^1H and ^13C NMR data were similar to those of the known compound liriodenine [7].

CONCLUSION

Three flavonoids, kaempferol 3,4’-dimethyl ether(1), quercetin 3,7-dimethyl ether(2), quercetin 3,7,4’-trimethyl ether (3), and alkaloid, liriodenine(4) have been isolated from the stem bark of Saccopatulumhorsfieldii Benn. Their structures were elucidated on the basis of spectroscopic data.

Acknowledgements

We would like to thank Purwodadi Botanical Garden, Pasuruan, Indonesia for the availability and identification the species.

REFERENCES