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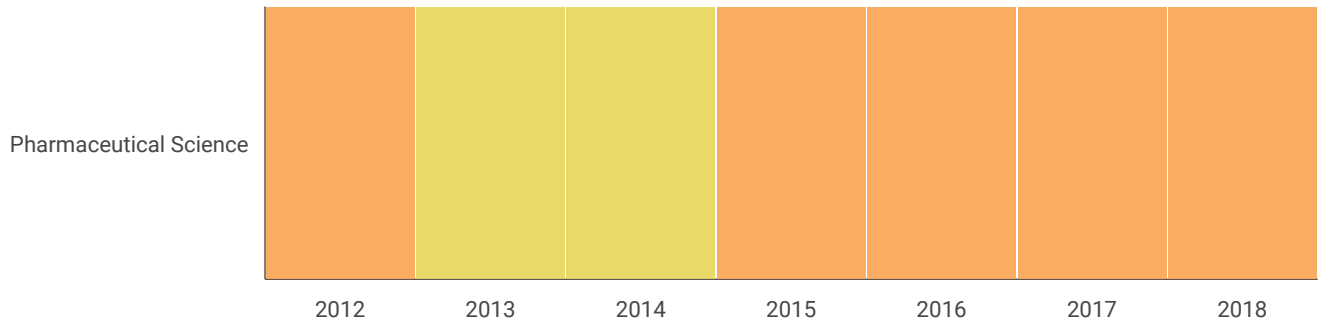

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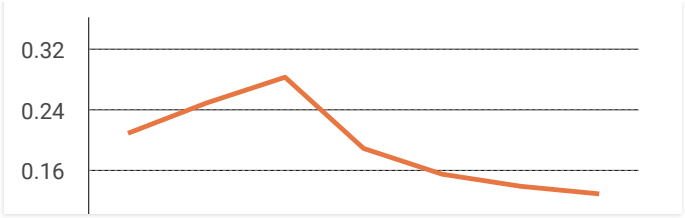

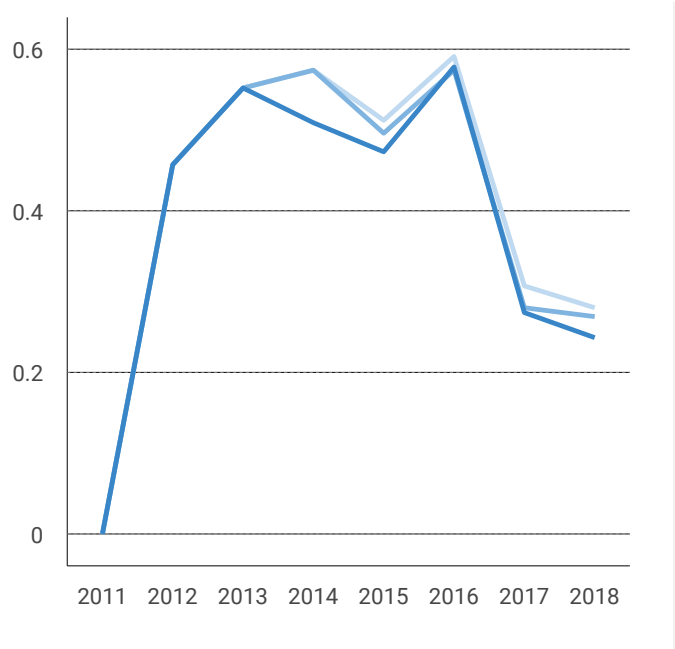

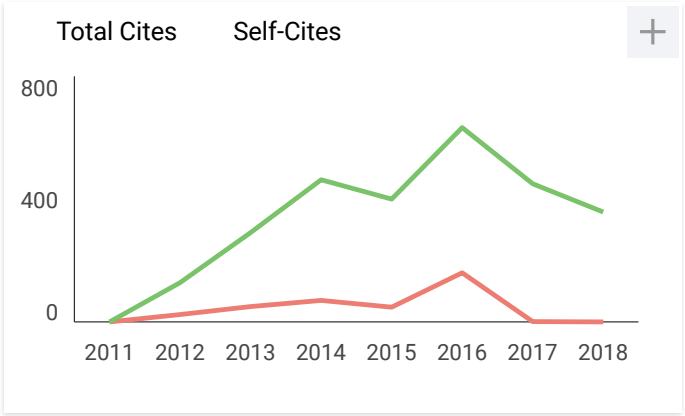

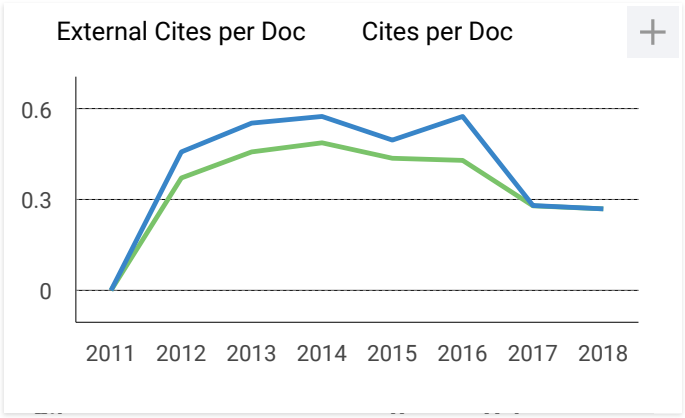

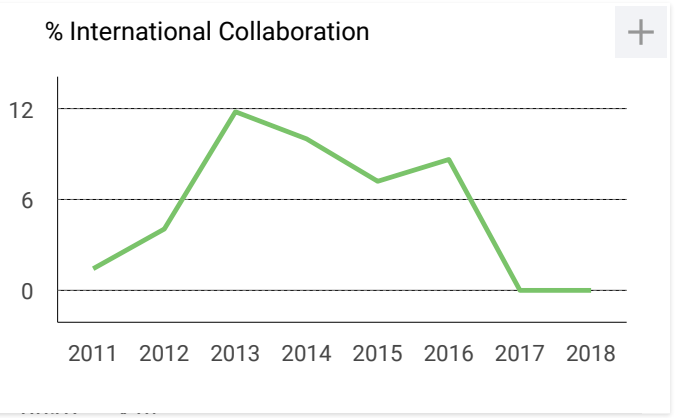

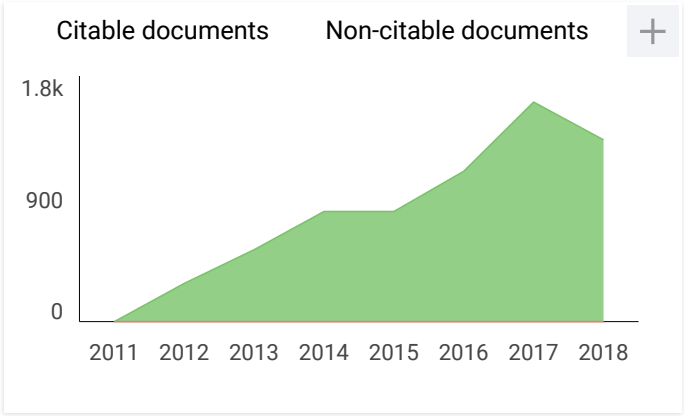

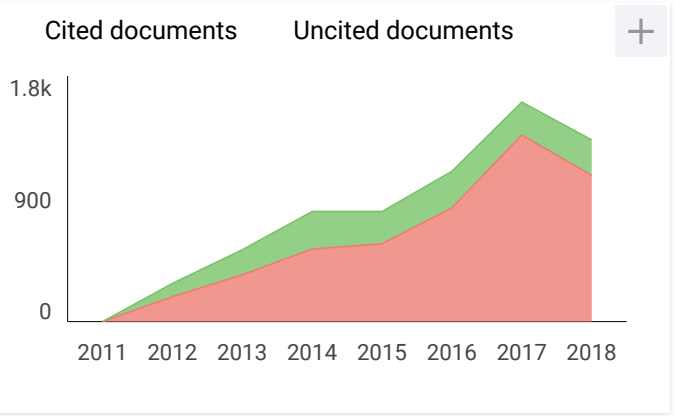

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Phenolic compounds from the stem bark of *Saccopetalumhors fieldii* Benn

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ABSTRACK

*Column chromatographic separation of the methanol extract from the Saccopatlumhorsfieldii Benn'sstem 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, 1Dand2DNMR data.*

Keywords: flavonoid, alkaloid,*Saccopatlumhorsfieldii* Benn, Annonaceae.

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 *Saccopetalumhorsfieldii*Benn., a plant with a synonym name *Miliusahorsfieldii* [2].

As a result of our research for phenolic compound in this Indonesian plant, we report the isolation of phenolic compounds, kaempferol 3,4'-dimethylether(**1**), quercetin3,7-dimethylether(**2**), quercetin3,7,4'-trimethylether(**3**), and liriodenine (**4**). from the methanol extract of the stem bark of *Saccopatlumhorsfieldii* 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-7500and Perkin Elmer SpectrumFTIR Shimadzu 5300 spectrometer, respectively. ^{1}H and ^{13}C NMR spectrum were recorded with a JEOL400 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 coloumn 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 *Saccopatlumhorsfieldii* Bennwas collected from Purwodadi Botanical Garden, Center of Biological Research and Development, National Institute of Science, Pasuruan District, EastJava, Indonesia.

Extraction and isolation

Milled drystem bark of *Saccopatlumhorsfieldii* 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 repeatly with 5% aqueous citric acid (pH 3-4)and partitioned with dichloromethane. The dichloromethane extract (28.4 g) was fractionated on silica gel by VLC eluting with mixtures *n*-hexane-acetone (19:1, 8:1, 4:1, and 7:3) to give three major fractions A-C. Fraction B (3.6 g), purified using coloumn chromatography eluted with mixture *n*-hexane-ethylacetate (9:1, and 4:1) to give compounds **2**(28 mg) and **3**(80 mg). Furthermore, fraction C (5.6 g) eluted withmixture *n*-hexane-acetone (9:1, 4:1and 7:3) yieldedcompounds **2** (18 mg). The acid fractionwasbasifiedwith 28% ammoniasolution (pH 8-9) and partitioned with ethylacetate to yield of crude alkaloids.The crude alkaloids (5.0 g) was fractionated on silica gel by coloumn chromatography eluting with mixture *n*-hexane-chloroform (4:1 and 7:3), chloroform, and mixtures of chloroform-methanol (9:1, and 4:1)to give four major fractions A-D.Fraction D (800mg), purified using coloumn chromatography eluted with *n*-hexane-acetone (9:1, 4:1, and 7:3), to give compounds **4** (26 mg).

Kaempferol3,4'-dimethyl ether(1): Pale yellow solid; m.p. 237^{o}C;UV (MeOH) λ_{max} (nm) (log ε): 203 (4.68), 264 (4.28), 346 (3.80); LC-ESI-MS *m/z* 314[M]$^{+}$; ^{1}H NMR (400 MHz, acetone-d$_6$) δ_H (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'),3.84 (3H, s, 3-OCH$_3$), 3.87 (3H, s, 4'-OCH$_3$), 12.75 (1H, s, 5-OH); ^{13}C NMR (100 MHz, acetone-d$_6$) δ_H (ppm): 156.5 (C-2), 139.4 (C-3), 179.5 (C-4), 106.3 (C-4a), 169.0 (C-5), 97.0 (C-6), 164.8 (C-7), 94.6 (C-8), 157.8 (C-8a), 126.0 (C-1'), 131.1 (C-2'/6'), 115.3 (C-3'/5), 162.7 (C-4'), 60.4 (3-OCH$_3$), 55.8 (4'-OCH$_3$).

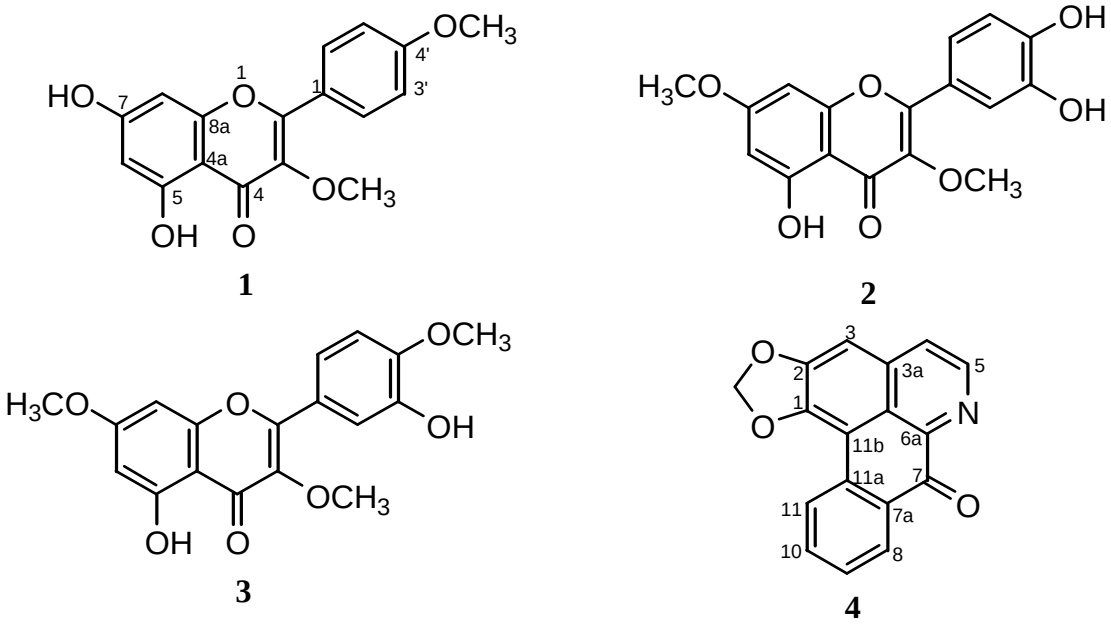

Figure 1. Structures of phenolic compounds

Quercetin 3,7-dimethylether(2):Pale yellow solid; m.p. 224-226^{o}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). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H (ppm): 6.35 (1H, d, *J* = 2.2Hz, H-6),6.68 (1H, d, *J* = 2.2Hz, H-8),7.58 (1H, d, *J* = 2.2Hz, H-2'),6.91 (1H, d, *J* = 8.4Hz, H-5'),7.47 (1H, dd, *J* = 8.4, 2.2Hz, H-6'),3.80 (3H, s, 3-OCH$_3$),3.86 (3H, s, 7-OCH$_3$), 12.67 (1H, s, 5-OH); ^{13}C NMR (100 MHz, DMSO d$_6$) δ_H (ppm): 145.0 (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'), 120.4 (C-6'), 59.5 (3-OCH$_3$), 55.9 (7-OCH$_3$).

Quercetin 3,7,4'-trimethylether(3): Pale yellow solid; m.p. 173-175^{o}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). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H (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-OCH$_3$),3.86 (3H, s, 7-OCH$_3$),3.81 (3H, s, 4'-OCH$_3$), 12.61 (1H, s, 5-OH);^{13}C NMR (100 MHz, DMSO d$_6$) δ_H (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-OCH$_3$), 55.9 (7-OCH$_3$), 55.6 (4'-OCH$_3$).

Liriodenine(4): Pale yellow solid: UV (MeOH) λ_{max} 272, 317nm; FAB-MS *m/z*276[M+H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 7.58 (1H, s, H-3),8.05 (1H, d, *J* = 5.2, H-4),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-CH_2-O-);^{13}C NMR (100 MHz, DMSO d_6) δ_H (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-11b), 122.4 (C-11c), 103.0 (O-CH_2-O).

RESULTS AND DISCUSSION

Four phenoliccompounds,namelykaempferol 3,4'-dimethyl ether(**1**), quercetin 3,7-dimethyl ether(**2**),quercetin 3,7,4'-trimethyl ether (**3**),andliriodenine(**4**) havebeen isolatedfromthe stembark of *Saccopatlumhorsfieldii* Benn.

Kaempferol 3,4'-dimethyl ether(**1**) was isolated as an pale yellow solid.The UV spectrum of **1**exhibited maximum absorption on 203,257,and 359 nm typical for a flavonolcompound and showed bathochromic shifts on addition of $AlCl_3$ and NaOAc [3].In the ^{13}C NMR spectrum, 15 carbon signals representing 17 carbon atoms were observed. Two of them, namely the signals at δ_C139.4and 179.5, are characteristic for C-3 and C-4 of a flavonol structure [4]. The presence of five oxyaryl signals (δ_C 156.5, 157.8, 162.7, 164.8, and 169.0) indicated that the flavonol is a derivative of kaempferol.The ^{1}H NMR spectrum showed the presence of the proton signals of a pair of doublets (J = 2.4 Hz) in the aromatic region at δ_H 6.23 and 6.47 ppm, characteristic for H-6 and H-8 proton signals of the ring A. Furthermore, in the ^{1}H NMR spectrum, a pair of doublets (J = 9.2 Hz) was appearedin the aromatic region at δ_H8.05 and 7.05 ppm (each 2H) characteristic for a hydroxyl phenyl group of the ring B.The ^{1}H NMR spectrum of **1** also showed twomethoxy groups at δ_H3.84 and 3.87 and a proton singlet signal at δ_H 12.75 that is consistent with the presence of an OH-phenolic at C-5.The placement of methoxy groupsin kaempferol structure shown in HMQC and HMBC spectrum. By analysis of HMQC and HMBC spectrum of **1**, the methoxy signal (δ_H3.87) exhibited ^{1}H-^{13}C long range correlation with an oxyaryl carbon signal (δ_C 162.7), meanwhilecorrelation ofthe signal at δ_H8.05in the ring Bcorrespond to the methoxygroup at C-4'. Furthermore, correlation methoxyl signal δ_H3.84 with δ_C139.4suggested that the methoxyl was unambiguously located at C-3.From these NMR data analysis, the flavonol isolated was assigned as kaempferol3,4'-dimethyl ether[5]. Other HMQC and HMBC correlations, as well as ^{13}C NMR data assignment, that are consistent with the structure **1** are shown in Fig. 2.

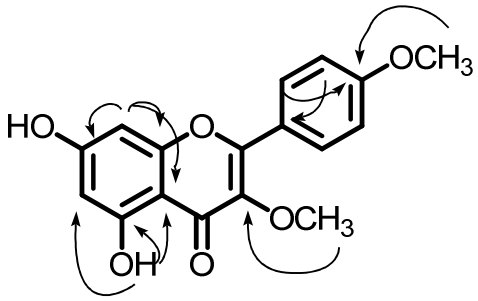

Figure 2. Significant HMBC correlation for 1

Quercetin 3,7-dimethyl ether(**2**) was isolated as an pale yellow solid,and its UV spectrumexhibited 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 ^{1}H NMR spectrum of **2** showedABX system at δ_H7.58 (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 the proton signals of a pair of doublets (J = 2.2 Hz) in the aromatic region at δ_H6.35and 6.68ppm, characteristic for H-6 and H-8 in the ring A. The ^{1}H NMR spectrum of **2** also showed twomethoxyl signals(δ_H3.80; 3.86) and a proton singlet signal at δ_H 12.67 that is consistent with an OH-phenolic at C-5.The ^{13}C NMR spectrum of **2** showed 17 carbon signals were observed. Two of them, namely the signals at δ_C137.7and 177.7 are characteristic for C-3 and C-4 of a flavonol structure [4]. The presence of six oxyaryl signals (δ_C 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 an pale yellow solid.The UV and IR spectrumvery similar with compound **2**.The ^{1}H NMR spectrum of **3** showedABX system at at δ_H7.54 (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 δ_H6.33and 6.67ppm,threemethoxyl signals(δ_H3.88; 3.86; 3.81) and a OH-phenolic at C-5at δ_H 12.61.The ^{13}C NMR spectrum of **3**showed 18 carbon signals were observed. Two of them, namely the signals at δ_C137.7and 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* 276consistent to the molecular formula $C_{17}H_{10}NO_3$. The ^{1}H NMR spectrum of **4** showed the presence of one methylenedioxy groupand seven aromatic protons.In the ^{1}H-NMR spectrum of **4** showed a proton singlet signal of methylenedioxy signal at δ_H6.51, a pair of doublets (J = 5.2 Hz) in the aromatic region at δ_H8.05and 8.83are characteristic for H-4 and H-5 of a oxoaporphine structure, a proton singlet signal at δ_H7.58 characteristic for H-3. In the aromatic region, the four aromatic region at δ_H 8.38 (dd, *J* = 8.0, 2,0 Hz),7.66 (t, *J* = 8.0 Hz), 7.90 (t, *J* = 8.0 Hz),8.67 (d, *J* = 8.0 Hz) were assigned to H-8, H-9, H-10 and H-11, respectively. In the ^{13}C NMR spectrum, 17 carbon signals were observed. Two of them, the signals at δ_C148.3and 151.4 are characteristic for ortho oxygenated and one carbonyl group at δ_C 180.9. Based on^{1}H and ^{13}C 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**),andalkaloid, liriodenine(**4**) havebeen isolated from the stembark of *Saccopatlumhorsfieldii* Benn. Their structures were elucidated on the basis of spectroscopic data.

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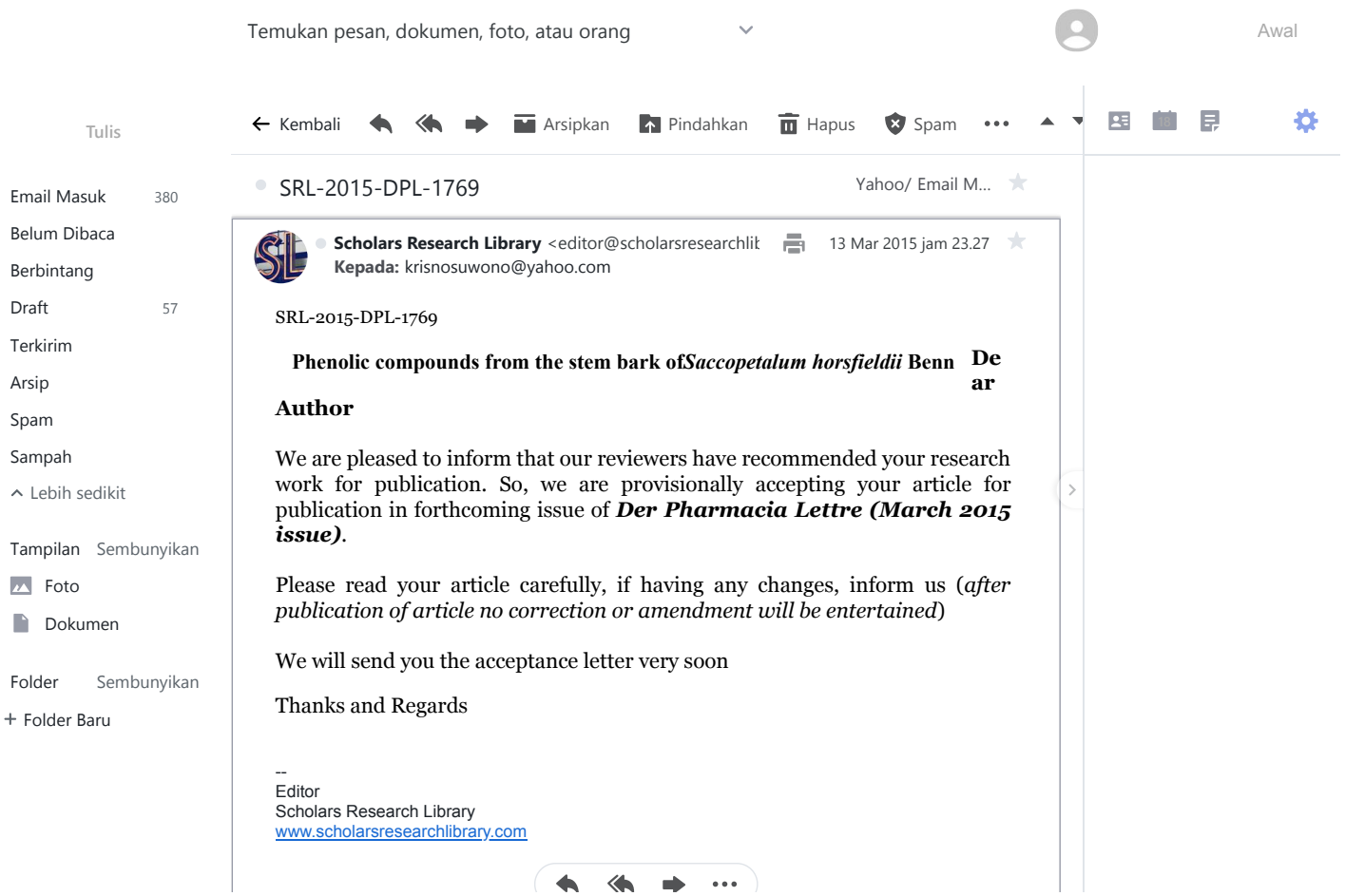
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