Flavonoids from the Roots of *Amomum compactum* Soland Ex Maton (Zingiberaceae)

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Abstract

*Amomum compactum* Soland Ex Maton is one of the Zingiberaceae family plants which is the endemic plants from West Java, Indonesia. This study was aimed to determine the chemical structure of flavonoid compounds from n-hexane extract of *A. compactum* Sol. Ex Maton roots. Dried powder of the roots was extracted consecutively with *n*-hexane, ethyl acetate, and methanol solvents. Three flavonoids, 5-hydroxy-3,7,4'-trimethoxy kaempferol (1), 5-hydroxy-3,7,3',4'-tetra methoxy kaempferol (2) and 4'-hydroxy-3,5,7-trimethoxy kaempferol (3), have been isolated from the roots of *A. compactum* Sol. Ex Maton. The chemical structures of compounds 1-3 were identified by spectroscopy data including infrared 1D-NMR, 2D-NMR and HRTOF-MS as well as by comparison with previously reported spectral data. Compounds 1-3 were isolated from this plant for the first time and showed free radical DPPH scavenging activity.

Keywords: *Amomum compactum*, antioxidant, DPPH, flavonoid, kaempferol

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1. INTRODUCTION

*Amomum compactum* Sol. Ex Maton one of the *Amomum* genera, belonging to the family Zingiberaceae, which has 53 species widely distributed in Asia and Australia with distribution centers in Southeast Asia (A. J. Droop & Newman, 2014; J. Droop, Kaewsri, Lamxay, Poulsen, & Newman, 2013; Lamxay, 2011). *A. compactum* is known as a cardamom in Indonesia and used as a cooking spice, health drink, traditional medicine and aromatherapy (Setyawan & Bermawie, 2014). It was traditionally used in various gastrointestinal, cardiovascular and neural disorders (Aneja & Joshi, 2009). The utilization of cardamom (*A. compactum*) as cancer medicine has been reported by Deng (2012) in traditional Chinese medicine (Deng, Hu, & An, 2012). The ethyl acetate soluble fraction of cardamom seeds (*Elettaria cardamomum*) also showed antioxidant capacity with the ability to scavenge DPPH free radicals of 90% with the content of several phenolic compounds such as protocatecaldehyde and protocatechuic acid. Cardamom has also been demonstrated to decrease azoxymethane-induced colon carcinogenesis due to its antiinflammatory, antiproliferative, and proapoptotic activities (Bhagat & Chaturvedi, 2016). The Aqueous suspension of cardamom (*E. cardamomum*) together with cinnamon (*Cinnamomum burmanii*) was reported to increase detoxification enzyme activity (GST activity) and reduce lipid peroxidation that can induce colon cancer in Swiss Albino rats (Bhagat & Chaturvedi, 2016; Bhattacharjee, Rana, & Sengupta, 2007). The therapeutic potential of this spice plant is related to bioactive components such as alkaloids,
terpenoids, flavonoids, phenylpropanoids including steroids (Bhagat and Chaturvedi, 2016; Md. Uzzal Haque, 2016). Besides, the components of the fruit of this plant are also known to have antioxidant activity (Zhang, Lu, & Jiang, 2015). Studies have shown consistently that the antioxidant activity of phenolic compounds is reasonably related to their structure, namely, the substitutions on the aromatic rings and the structure of the side chain (Natella, Nardini, Di Felice, & Scaccini, 1999). Recently much attention has focused on the role and mechanism of several flavonoids as inhibitors of oxidative processes in correlation with anticancer activity (Katayama et al., 2007) and anti-diabetic (Sarian et al., 2017).

Due to its potential biological properties of flavonoids, the isolation of flavonoids from the Amomum compactum plant is of great interest. The first reported flavonoids isolated from the genus Amomum, namely fruits of A. tsao-ko, were catechin and epicatechin. Both flavonoids reported to have strong antioxidant activity. The radical scavenging activity of the isolated compounds was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and colorimetric and electron spin resonance (ESR) analyses (Martin, Kikuzaki, Hisamoto, & Nakatani, 2000). Epicatechin has also been reported to have a neuroprotective activity (Zhang, Lu, & Jiang, 2014). Meanwhile, quercetin, quercetin 3-O-glycoside and quercetin-7-O-glycoside, were also isolated from the genus and reported as neuroprotective agents similar to epicatechin (Zhang et al., 2014). However, all these studies at present on A. compactum plant have not been reported to detail chemical constitution analysis, and bioactivity studies were restricted to its crude extracts. In our continuous effort to discover interesting molecules from the Amomum plant, this study revealed the presence of flavonoid compounds from A.compactum Sol. Ex Maton. Although the compounds have been previously reported by Cornelius et al. (2010) (Cornelius et al., 2010) these secondary metabolites in A.compactum Sol. Ex Maton. has not been reported yet.

2. MATERIALS AND METHODS

The experimental section including general experimental procedure, plant material, plant extraction and isolation, and antioxidant activity assays (DPPH radicals scavenging activity).

General Experimental Procedures

The Fourier transform infrared (FTIR) spectra were recorded on a Thermo Scientific. The mass spectra were recorded with a Waters Xevo High Resolution Top of Flight Mass Spectrometry (HRTOF-MS). Nuclear magnetic resonance (NMR) Data were recorded on the Bruker Top Spin spectrometer at 500 MHz and JEOL ECZR 600 MHz for 1D and 2D NMR using TMS as an internal standard. Column chromatography was conducted on silica gel 60, 200-400 Mesh (Merck, and Kanto Chemical). Thin layer chromatography (TLC) plates were precoated with silica gel GF254 (Merck, 0.25 mm) and detection was achieved by spraying with 10% H2SO4 in ethanol and also using AlCl3 in 10% ethanol, following by observing under ultraviolet light at wavelength 254 nm and 367 nm, and also by heating respectively.

Plant Material

The roots of A. compactum Soland Ex Maton was collected from community cultivation in Garut Regency, West Java, Indonesia. The plant was determined at the Laboratory of Taxonomy of Biology, Universitas Padjadjaran, Indonesia The sample (No.466 HB) has been deposited at the herbarium.

Plant Extraction and Isolations

Cardamom dried root (A. compactum Soland Ex Maton) (5.4 kg) was macerated with 20 L 96% ethanol for 2x24 hours repeatedly at room temperature, then the combined macerate was concentrated with a rotary evaporator. The ethanolic extracts was obtained for 142.5 g. The extracts was dissolved with distilled water and partitioned sequentially with n-hexane, ethyl acetate and n-butanol then each organic fraction was concentrated to obtain crude extracts of n-hexane (38.85 g), ethyl acetate (14.40 g), n-butanol (44.35), and water (26.32 g). Each extract was tested for its free radical DPPH scavenging activity. The n-hexane extract was then fractionated by vacuum liquid chromatography (silica gel G30) with the solvent of a mixture of n-hexane, ethyl acetate and methanol with a polarity increase of 20% to obtain 10 fractions (A-J) that were combined according to TLC analysis. Fraction D (3.2 g) was then separated by column chromatography on silica gel using a gradient of n-hexane–ethyl acetate-methanol with a 5% stepwise polarity.
increase to produce 15 subfractions (D₁-D₁₅). D₁₂ fraction (155.2 mg) was subjected to column chromatography on silica gel using n-hexane-ethyl acetate and methanol, with a 5% stepwise polarity increase to afford 7 subfractions (D₁₂A-D₁₂F). Subfraction D₁₂-B (120.6 mg) was column chromatographed on ODS, eluted with methanol: H₂O (9:1) to give 1 (71.3 mg). Fraction E (1.9 g) was subjected to column chromatography on silica gel with 5% gradient of n-hexane–ethyl acetate-methanol to result in 13 subfractions (E₁₁–E₁₃). Subfraction E₄ (45.8 mg) was then separated by column chromatography on silica gel using n-hexane-dichloromethane-ethyl acetate (7.5:4.5:3) to give combined subfraction 5 (E₄A–E₄E). Subfraction E₄-D (14.9 mg), further column chromatographed on ODS using methanol-water (8:2) and obtained 2 (2.2 mg). Fraction G (1.0 g) was separated by column chromatography on silica gel using a gradient mixture of n-hexane–ethyl acetate-methanol to result in 13 subfractions. Fraction G₅ (35 mg) was subjected on a column chromatography on silica gel using CHCl₃–ethyl acetate–methanol (71.3 mg). Fraction E (1.9 g) was subjected to column chromatography to afford 13 subfractions (E₁–E₁₃). Fraction E₄ (1.5 mg) further column chromatography on ODS, eluted with methanol: H₂O then tested samples. Ascorbic acid was used as a reference compound (Kassim et al., 2013; Molyneux P, 2004).

3. RESULTS AND DISCUSSION
At the initial stage, the dried root of *Amomum compactum* Sol. Ex Maton was macerated with ethanol and the combined macerates were concentrated to a crude extract. The ethanol extract was partitioned with n-hexane, ethyl acetate, n-butanol and water then tested for its free radical scavenging activity using the DPPH radical method to give of IC₅₀ values of 147.93, 1185.98, 60.04, 58.48 and 1999.66 mg/mL, respectively. Referring to Blois (1958) in (Molyneux P, 2004), extracts with an IC₅₀ value < 50 µg/mL are considered as very strong antioxidant activity, whereas an IC₅₀ value >200 µg/mL is considered as very weak. As a continuing study in the search of flavonoid compounds, from the n-hexane fraction was chromatographed over vacuum-liquid chromatography (VLC) and silica gel column chromatography to afford compounds 1, 2, and 3. The three compounds showed similarities from the TLC analysis, which were yellow sprayed with 10% H₂SO₄ reagent and also AlCl₃ in ethanol, each of which was followed by heating indicated that compounds 1-3 have conjugated double bonds. The all three ultraviolet spectrums showed the presence of two absorption bands of the cinnamoyl and benzoyl rings, which are typical for flavonoid compounds with flavone skeletons (Markham, 2007). The all three infrared spectrums showed the presence of an absorption band that confirmed the presence of aliphatic CH stretch, the double bond of the aromatic ring assisted by absorption in the fingerprint region, then the presence of conjugated carbonyl and ether. The structure of the compounds was analysed using spectroscopic data including FTIR, 1D and 2D NMR as well as HRTOF-MS (Figure 1).
The DPPH assay has been widely used to measure radical scavengers because it is stable, and the reaction system covers only direct reactions between radicals and antioxidants (Kassim et al., 2013). The DPPH radical exhibits a strong maximum absorption at 517 nm, resulting in a purple color (Bellik et al., 2013). When a solution of DPPH (radical form) is mixed with a substance that can donate a hydrogen atom, then this gives the reduced form (unradical) with the disappearance of this purple color (although there will be expected to be a pale yellow remnant of extant picryl groups (Molyneux P, 2004).

5-hydroxy-3,7,4'-trimethoxy kaempferol (1). Yellow amorphous crystalline; melting point 145.5 °C; UV \( \lambda_{\text{max}} \) 254 and 365 nm; IR (FTIR) \( \nu_{\text{max}} \) (cm\(^{-1}\)) 2847, 1655, 1582, 1000, 742; \( ^1H \) NMR (CDCl\(_3\), 500 MHz); \(^{13}C \) NMR (CDCl\(_3\), 125 MHz) see Table 1; HRTOF-MS (m/z) 329.1027 [M+H]+, calculation for C\(_{16}\)H\(_{10}\)O\(_6\) (m/z) 328.1025.

5-hydroxy-3,7,3',4'-tetramethoxy kaempferol (2). Yellow amorphous crystals; melting point 140\(^\circ\)C; UV \( \lambda_{\text{max}} \) 243 nm and 350 nm; IR (FTIR) \( \nu_{\text{max}} \) (cm\(^{-1}\)) 2922, 2851, 1734, 1648, 1125 and 935; \(^1H \) NMR (CDCl\(_3\), 500 MHz) \( \delta_H \) (ppm); \(^{13}C \) NMR (CDCl\(_3\), 125 MHz) see Table 1; HRTOF-MS (m/z) 359.1137 [M+H]+, calculation for C\(_{19}\)H\(_{10}\)O\(_7\), (m/z) 359,1131.

4'-hydroxy-3,5,7-trimethoxy kaempferol (3). Yellow amorphous crystalline; melting point 143\(^\circ\)C; \( \lambda_{\text{max}} \) 283 and 348 nm; IR (FTIR) \( \nu_{\text{max}} \) (cm\(^{-1}\)) 2920, 1655, 1582, 1216, 768; \(^1H \) NMR (CDCl\(_3\), 600 MHz); \(^{13}C \) NMR (CDCl\(_3\), 150 MHz) see Table 1; HRTOF-MS (m/z) 329.1039 [M+Na]+, calculation for C\(_{19}\)H\(_{10}\)O\(_6\) (m/z) 329.1025.

Compound (1) was obtained as a yellow amorphous crystalline. HRTOF-MS spectrum of 1 showed ([M+H]+ m/z 329.1027 (calcd. m/z 328.1025), which corresponds to the molecular formula of C\(_{16}\)H\(_{10}\)O\(_6\) thus providing eleven degrees of unsaturation. UV spectra in methanol showed \( \lambda_{\text{max}} \) 283 and 348 nm indicated the presence of flavon skeleton (Marby, TJ, Markam, K.R. and Thomas, 1970; Markham, 2007). The IR spectra showed the peaks at \( \nu_{\text{max}} \) (cm\(^{-1}\)) 2847, 1655, 1582, 1000, 742 indicating the presence of hydroxyl groups, C=C aromatics, symmetric and asymmetric of C-O-C, C=O, and substituted benzene ring, respectively. The \(^1H \)NMR spectrum of compound 1 showed the presence of six olefinic methine protons consisted of two protons resonating at \( \delta_H \) 6.33 and 6.42 ppm (each 1H, \( d, J=2.2 \) Hz) that were assigned for H-6 and H-8 in A ring. Two pairs of methine protons resonating at \( \delta_H \) 8.05 (each 1H, \( dd, J=9.1, 2.9 \) Hz, H-2' and H-6') and the others at \( \delta_H \) 7.00 (each 1H, \( dd, J=9.0, 2.9 \) Hz, H-3' and H-5') were assigned to be ABC proton type in B ring. The spectrum also showed the presence of 3 oxygenated methyl protons at \( \delta_H \) 3.83 (3H, s, 3-OCH\(_3\)), 3.85 (3H, s, 7-OCH\(_3\)), and 3.87 (3H, s, 4'-OCH\(_3\)). Compound 1 also showed the presence of a hydroxyl proton resonating at \( \delta_H \) 12.63 (1H, s), which was attached to C-5. Two meta-protons at ring A evidenced by the \( J \) coupling constant of H-6 and H-8 as 2.2 Hz and by HMBC correlation between H-6 to C-5, C-7, and H-8 to C-7 and C-9 (Figure 2). A \( p \)-substituted benzene of C ring observed at \( \delta_H \) 8.05 (each 1H, \( dd, J=9.1,2.9 \) Hz, H-2' and H-6') and the others at \( \delta_H \) 7.00 (each 1H, \( dd, J=9.0, 2.9 \) Hz, H-3' and H-5') were supported by \(^1H \)H-COSY cross peak H-2'/H-3' and H-5'/H-6' (Figure 2). The flavonol skeleton in ring C was evidenced by the presence of quaternary carbon at \( \delta_C \) 139.0 ppm (C-3) and 178.9 (C-4) and through HMBC correlation between \( \delta_H \) 3.83 (3-OCH\(_3\)) to \( \delta_C \) 139.0 (C-3). The \(^{13}C \) NMR and DEPT
135° spectra of the compound showed the presence of six olefinic methines and six quaternary olefinic carbon (12 sp² carbons), three methoxy carbons, C=O aromatic ring and one ketone carbon. These functionalities accounted for eight of the total eleven degrees of unsaturation, and the remaining three degrees of unsaturation were consistent with the flavonol structure. Detailed comparison of NMR spectra of 1 to those of reported 3,7,4’-trimethoxy kaempferol (Cornelius et al., 2010; Rossi, Yoshida, & Maia, 1997), revealed that the structure was very similar, consequently, compound 1 was identified as 5-hydroxy-3,7,4’-trimethoxy kaempferol.

Compound 2 was obtained as colorless needle crystals. HRTOF-MS (m/z) 359.1137 [M+H]⁺, calculation for C₁₇H₁₃O₇, (m/z) 359.1131, required twelve unsaturation. IR (FTIR) νmax cm⁻¹: 2922 (C-H aliphatic), 2851 (C-H sp²), 1734 (C=O), 1648 (C=C aromatic), 1125 (asymmetric C-O-C stretch and 935 (substituted benzene ring). The NMR spectra were very similar to those of compound 1 except for the addition of methoxy at C-3’. The methoxy substituent at C-3’ increased the number of hydroxyl groups in combination with the conjugated p-electron system, allowing them to act as free radical scavengers via hydrogen atom or electron donation (Martinez-Perez et al., 2014). Generally, position and number of hydroxylation correlate reasonably to the anti-oxidation activity of flavonoids. Compound 2 with 4 methoxy groups and compounds 1 & 3 with 3 methoxy groups showed low DPPH radical scavenging activity, it’s consistent with the structure-activity relationship study of flavonoid compounds, noticed that the premise of at least two hydroxyl groups in ring B for anti-oxidant capacity is suggested based on significantly improved anti-oxidant effects (Wang, Li, & Bi, 2018).

Hydrogens and electrons are donated by ring B hydroxyl groups to hydroxyl, peroxy, and peroxy nitrite radicals, forming relatively stable flavonoid radicals. Due to reducing activities of phenolic hydroxyl groups, flavonoids are able to donate hydrogen. Along with delocalization of phenoxy radical products, flavonoids can protect against various disease damage from ROS. On the other side, flavonoids could scavenge the resulting radicals to neutralize the prior effect (Verma et al., 2012). The structure activity relationship of flavonoid compound from the roots of A. compactum Sol. Ex Maton was as follows: (a) the presence of hydroxyl in ring A (5-OH and 7-OH) increases activity, and loss of it decreases activity; (b) The loss of two Ortho 3’,4’ hydroxyl in ring B decreases activity; (c) C=O bonds at C-2 & C-3, 4-keto (C=O) and 3-OH in ring C increase activity, substitution of 3-OCH₃ decreases activity.
These flavonoid compounds, 5-hydroxy-3,7,4′-trimethoxy kaempferol (1), 5-hydroxy-3,7,3′,4′-tetramethoxy kaempferol (2) and 4′-hydroxy-3,5,7-trimethoxy kaempferol (3) were isolated in this plant for the first time and support also the presence of flavonoid compound in the *Amomum* genus beside other metabolites compounds as a chemical marker. The antioxidant capacity correlated with the position and number of hydroxylation of the flavonoid compounds.

**4. CONCLUSION**

Three flavonoid compounds, 5-hydroxy-3,7,4′-trimethoxy kaempferol (1), 5-hydroxy-3,7,3′,4′-tetramethoxy kaempferol (2) and 4′-hydroxy-3,5,7-trimethoxy kaempferol (3) have been isolated from the roots of *Amomum compactum* Soland Ex Maton. These compounds were isolated from this plant for the first time and having free radical DPPH scavenging activity. These findings support efforts to develop natural antioxidant...
compounds as lead compounds for the development of degenerative disease drugs.

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