PHENOLIC COMPOUNDS FROM INDONESIAN WHITE TURMERIC (CURCUMA ZEDOARIA) RHIZOMES

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ABSTRACT

Objective: The aim of the present study is to isolate phenolic compounds from Curcuma zedoaria rhizomes grown in Bogor, West Java, Indonesia, which will enrich phytochemical information from this plant.

Methods: C. zedoaria rhizomes were macerated in methanol then followed by increasing polarity partitions with n-hexane, ethyl acetate (EtOAc), and methanol, respectively. EtOAc fraction was further fractionated using various chromatography techniques to yield two isolated fractions, Z1 and Z2. These two isolated fractions were then characterized to determine their compound structures.

Results: Fourier Transform-InfraRed (FTIR), Ultraviolet-Visible (UV-Vis), and Liquid Chromatography Mass Spectrometry tandem Mass Spectrometry LC-MS/MS spectral data, Z1 fraction was elucidated as curcuminoid derivative, that is, dimethoxycurcumin (DiMC, 1), while Z2 fraction was yielded as a mixture consisted of flavonoid and coumarin derivatives, 3,5,7-trihydroxy-4’-methoxyflavone (kaempferide, 2) and 7-methoxy coumarin (herniarin, 3).

Conclusion: This study reveals useful information regarding phenolic constituents of Indonesian C. zedoaria rhizomes. Further research needs to be carried out to purify other compounds contained and to conduct bioactivity assays.

Keywords: Curcuma zedoaria, Dimethoxycurcumin (DiMC, 1), Phenolic, 7-Methoxy coumarin (herniarin, 3), 3,5,7-Trihydroxy-4’-methoxyflavone (kaempferide, 2), White turmeric.
and de-solvation temperature of 350°C. Chemicals used for isolation were in both technical (CV. Satya Darmawan) and pro analysis (Merck) grades, such as, methanol (MeOH), n-hexane, ethyl acetate (EtOAc), dichloromethane (DCM), chloroform (CHCl₃) and acetic acid.

Collection of plant material

*C. zedoaria*, Rosc. rhizomes were collected and identified from Biopharma Innovation Centre, Institut Pertanian Bogor, West Java, Indonesia.

Isolation of phenolic compounds from *C. zedoaria* rhizomes

Fresh-harvested *C. zedoaria* rhizomes were washed, sliced into small pieces, dried, and ground into a fine powder using a powdering mill. The air-dried powdered rhizomes (2.0 kg) were then extracted 3 times with MeOH at room temperature. MeOH crude extract (65 g) was then partitioned with increasing polarity using n-hexane, EtOAc, and MeOH, respectively afforded 7.17 g n-hexane, 30.59 g EtOAc and 4.31 g MeOH extracts.

EtOAc extract (20 g) was further fractionated using VLC with gradient solvent of n-hexane:EtOAc (9:1 to 5:5, v/v), EtOAc, and MeOH, respectively, to obtain four fractions (A1-A4). A1 fraction (700 mg) was loaded on a silica gel CC and eluted with n-hexane:EtOAc (9:1, v/v) to afford four fractions (B1-B4). B2 fraction then was further fractionated using LC with gradient solvent of n-hexane:EtOAc (17:3, v/v) to yield two fractions (C1-C2). Furthermore, A2 fraction (1.0 g) was eluted with gradient solvent of n-hexane:EtOAc (17:3, v/v) in CC to obtain 16 fractions (D1-D16) while A4 fraction (1.0 g) was fractionated under the similar condition with A2 fraction to yield three fractions (E1-E3). Fractions D1 and E1 showed similar Rf with C1 fraction; therefore, these fractions were mixed and further purified using p-TLC (n-hexane:CHCl₃, 2:8, v/v) to afford Z1 fraction (10.2 mg). E3 fraction then was further purified with p-TLC (n-hexane:CHCl₃, 2:8, v/v) to afford Z2 fraction (14.4 mg). Both Z1 and Z2 fractions were subjected to characterize using FTIR, UV-Vis, and LC-MS/MS. According to spectroscopic data, Z1 fraction was identified as dimethoxycoumarin (DiMC, 1) while Z2 fraction was recognized as a mixture consistent of 3,5,7-trihydroxy-4′-methoxylavone (kaempferide, 2) and 7-methoxy coumarin (herniarin, 3).

Characterization data

**Z1 fraction** dimethoxycoumarin (DiMC, 1); yellow to orange solid; TLC spot Rf, 0.25 (n-hexane:CHCl₃, 3:7, v/v), 0.6 (n-hexane:EtOAc, 8:2, v/v), and 0.92 (DCM:MeOH 19:1, v/v); FTIR (KBr) v (cm⁻¹): 3394–3200 (O-H), 2959 (C-H), 1750 (C=O), 1616 (C=C aromatic), 1550 (aromatic C=C), and 1262 (C-O-C ether); UV-Vis (MeOH) λ_max (nm): 215, 275 (benzoyl chromophore), and 323 (cinnamoyl chromophore), addition of shift reagents see Table 1; and LC-MS/MS: LC rt 9.35 min (kaempferide, 2), MS (70 eV, m/z): 301.061 [M+H]+, 287.126, 285.080, 229.123 (base peak), 201.128, 121.102, 105.071, and 91.055, while LC rt 6.77 min (herniarin, 3), MS (70 eV, m/z): 177.065 [M+H]+ (base peak), 148.052, 135.020, 121.065, 116.986, 103.055, 91.055, and 77.039.

**RESULTS AND DISCUSSION**

*C. zedoaria*, belonging to Zingiberaceae family, was selected for the present study by recent literature showed that this species is usually used as traditional medicine and recognized to be a rich source of terpenoids [18,19]. However, relatively little was explored regarding extraction and separation processes of phenolic compounds from *C. zedoaria* rhizomes. Phenolics investigation of MeOH crude extract of *C. zedoaria* rhizomes using successive various chromatography techniques resulted in the isolation and characterization of two fractions, that is, Z1 fraction which was identified as dimethoxycoumarin (DiMC, 1) and Z2 fraction which was obtained in a mixture of 3,5,7-trihydroxy-4′-methoxylavone (kaempferide, 2) and 7-methoxy coumarin (herniarin, 3). Fig. 1 showed the chemical structures of isolated phenolics from *C. zedoaria* rhizomes which were elucidated using FTIR, UV-Vis, and LC-MS/MS.

FTIR spectra of 1 (Z1 fraction) showed characteristic peaks of phenolics at a wavenumber of 3598–3394, 2955, 1510, and 1267 cm⁻¹ indicated O-H, C=H sp3, C=C aromatic, and C-O-C ether, respectively. In addition, this compound also showed peaks for C-H sp3, C=O, and C=C alkene at a wavenumber of 2928–2860, 1733, and 1604, respectively. Phenolics have an aromatic ring with at least one hydroxyl group [20]. The presence of hydroxyl group (O-H) in the FTIR spectra of 1 is due to keto-enol tautomerization. Moreover, maximum absorbance in UV-Vis spectra of 1 appeared at the wavelength of 263 and 402 nm. A peak at 263 nm indicated benzoyl chromophore, while a peak in the visible region (402 nm) specified as a curcuminoïd chromophore proven by the yellow appearance of 1 [21]. To support FTIR and UV-Vis analysis, LC-MS/MS characterization of 1 was recorded. LC chromatogram resulted from the positive ion method showed one dominant peak at the retention time of 12.77 min (75.72%). MS spectra showed the

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**Table 1: Wavelength shifting of the mixture of 2 and 3 in various shifting reagents**

| Reagents         | Cinnamoyl (nm) | Benzoylethyl (nm) |
|------------------|----------------|------------------|
|                  | Initial | Shift | Initial | Shift |
| MeOH             | 323    | -     | 275     | -     |
| MeOH/NaOH        | 369    | +6    | 273     | -2    |
| MeOH/AlCl₂       | 323    | 0     | 275     | 0     |
| MeOH/AlCl₂/HCl   | 383    | +60   | 322     | 0     |
| MeOH/NaOAc/H₂BO₃ | 323    | 0     | 281     | +6    |
| MeOH/NaOAc/H₂BO₃ | 323    | 0     | 275     | 0     |

Flavonoids from *C. zedoaria* rhizomes which were elucidated using FTIR, UV-Vis, and LC-MS/MS.
molecular ion of 397.157 m/z [M+H]− which corresponds to C_{23}H_{24}O_{6} and fragmented peaks at 366.138, 335.167, 249.260, 205.085, 199.134 (base peak), 163.075, and 149.024 which belongs to the fragmentation scheme of 1 (Fig. 2).

To the best of our knowledge, this is the first report on isolating compound 1 from MeOH crude extract of Indonesian *C. zedoaria* rhizomes. This compound belonging to curcuminoids was obtained previously from Indian turmeric species (*C. longa* Linn.) [22,23]. This compound is an analog of curcumin which is commonly known as synthetic curcumin derivative displayed a wide range of bioactivities such as antiproliferative, antioxidant, anti-inflammatory, and anticancer [24-27]. Moreover, curcuminoid derivatives are found to be the major compounds in several turmeric species. Bisdemethoxy curcumin had previously reported from Chinese *C. zedoaria* rhizomes [28]. Other curcuminoids, such as curcumin, demethoxy curcumin, and bisdemethoxy curcumin had been reported to be isolated from Vietnamese, Indonesian, and Indian *C. longa* rhizomes [29-31].

A mixture of 2 and 3 (Z2 fraction) exhibited typical absorption peaks of phenolics, that is, O-H, C-H sp\(^2\), C-O-C ether, and C=C aromatic at the wavenumber of 3314-3176, 2959, 1550, and 1262 cm\(^{-1}\), respectively. Besides, this mixture also showed peaks at the wavenumber of 2927-2857, 1750, and 1616 cm\(^{-1}\) indicated C-H sp\(^3\), C=O, and C=C alkene, respectively. Furthermore, UV-Vis spectra of the mixture showed the maximum wavelength of 275 and 323 nm belongs to benzoyl and

**Fig. 2:** Fragmentation scheme of 1

**Fig. 3:** Fragmentation scheme of 2
cinnamoyl chromophores, respectively, indicated the characteristic peaks for flavonoid, especially flavonol [32]. Shift reagents were then used to elucidate the substituent in flavonoids [33] and summarized in Table 1.

According to Table 1, the mixture consisted of predominant flavonol having three free hydroxyl groups at positions of C3, C5, and C7. Hydroxyl group, which usually appears at C4' position, appeared as methoxy (−OCH3) substituent. Furthermore, there were no o-dihydroxy groups in both ring A and B in flavonol. Since the fraction was still in a mixture proven by the appearance of a λmax of 215 nm, LC-MS/MS was carried out to analyze the minor compound. LC chromatogram of the mixture revealed two peaks at the retention time of 6.77 and 9.35 min with the average area of 24.29% and 68.16%, respectively. A peak at 9.35 min was identified as 2 showing the dominant compound in the mixture, while a peak at 6.77 min belonged to 3 based on MS spectra analysis. Compound 3 also showed the cinnamoyl chromophore analyzed using UV-vis. Therefore, this peak in UV-Vis appeared in a simultaneous way with a similar chromophore of compound 2. Moreover, the coumarin derivative 3 was reported to have a strong absorption peak at 200–250 and 300–350 nm [34,35]. MS spectra of a peak at 9.35 min exhibited the molecular ion of 301.061 [M+H]+, which corresponds to the molecular ion of 287.126, 285.080, 229.123 (base peak), 201.128, 121.102, 105.071, and 91.055, which belongs to the fragmentation scheme of 2. Furthermore, MS spectra at 6.77 min of retention time showed the typical MS peak for 3 ([C10H8O3]+) as base peak and fragmented peaks of 287.126, 285.080, 229.123 (base peak), 201.128, 121.102, 105.071, and 91.055 which belongs to the fragmentation scheme of 2 and 3 are presented in Figs 3 and 4, respectively.

Based on the literature study, this is the first finding of compounds 2 and 3 from Indonesian C. zedoaria rhizomes. Compound 2 had been isolated previously from Alpinia galanga (Zingiberaceae), Tecomaria capensis var. aurea (Bignoniaceae), and Tamarix gallica (Tamaricaceae) [36-38]. Furthermore, compound 3 had been reported previously from Matricaria chamomilla, Zanthoxylum zanthoxyloides, and Alpinia calcarata (Zingiberaceae) [39-41].

CONCLUSIONS
From MeOH crude extract of C. zedoaria rhizomes, three phenolic compounds were successfully isolated through various chromatography techniques, and identified as, dimethoxycurcumin (DiMC, 1) and a mixture of 3,5,7-trihydroxy-4-methoxyflavone (kaempferide, 2) and 7-methoxy coumarin (herniarin, 3). Further research needs to be carried out to purify other compounds contained and to conduct bioactivity assays.

AUTHORS’ CONTRIBUTIONS
Conceptualization, DUCR; formal analysis and data acquisition, DAS, DUCR, HD; investigation, DAS; writing – original draft preparation, DUCR; writing – review and editing, DUCR, HD, PS; supervision, DUCR, HD, PS; project administration, DUCR; funding acquisition, DUCR. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest related to this work. The funders had no role in the design of the study; in the data collection, analyses, or interpretation in the writing of the manuscript; or in the decision to publish the results.

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