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Research Article

PHENOLIC COMPOUNDS FROM INDONESIAN WHITE TURMERIC (*CURCUMA ZEDOARIA*) RHIZOMES

DYAH UTAMI CAHYANING RAHAYU[1]*, DWI AYU SETYANI[1], HANHAN DIANHAR[2], PURWANTININGSIH SUGITA[3]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia. [2]Chemistry Study Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta 13220, Indonesia. [3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Dramaga Bogor 16680, Indonesia. Email: dyahutamicr@sci.ui.ac.id

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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.

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INTRODUCTION

In recent years, natural products derived from terrestrial plants, animals, microorganisms, and marine organisms play an important role in traditional medicines [1]. *Curcuma zedoaria*, also known as temu putih, white turmeric, *zedoaria*, or *gajutsu*, is belonging to Zingiberaceae family and close relative to *Curcuma longa*. This plant has dark orange-fleshed tubers similar to *C. longa*. This plant is indigenous to Bangladesh, Sri Lanka, India and is also widely cultivated in China, Japan, Brazil, Nepal, and Thailand [2]. Its rhizomes are commonly consumed traditionally as medicine in Asia for curing stomach diseases, toothache, blood stagnation, leucoderma, tuberculosis, enlargement of spleen, and for promoting menstruation, while the roots are usually used in the treatment of flatulence, dyspepsia, cold, cough, fever, and infections [3]. Phytochemical investigation of this plant showed that *C. zedoaria* is a rich source of essential oils, terpenoids, and curcuminoids [4,5]. *C. zedoaria* was also reported to have wide range of pharmacological activities such as antimicrobial and antifungal, anti-amoebic, larvicidal effect, antinociceptive, analgesic, antiallergic, antiulcer, anti-inflammatory, hemagglutinating, antimutagenic, anticancer, and hepatoprotective [5,6].

Having predominant of terpenoids, *C. zedoaria* rhizomes capture scholars' attention to investigate novel terpenoids or essential oils from this plant. Two diterpenes, curcuzedoalide and curcuminol D, were obtained from *C. zedoaria* cultivated in South Korea [7]. Curdione and curcumol belonging to sesquiterpenes had been isolated previously from essential oils of Chinese *C. zedoaria* rhizomes [8]. From methanol extract of *C. zedoaria* rhizomes, purchased from Kyoungdong Herbal Market in Seoul, five sesquiterpenes had been reported such as isoprocurcumenol, germacrone, curzerenone, curcumenol, and curcuzedoalide [9]. Moreover, since known as terpenoids-rich plant, this plant, together with other species *Curcuma*, had been profiled for distinguishing Indian *Curcuma* species based on its non-polar terpenes contains [10]. Nevertheless, there are still limited studies on phenolic compounds contained from *C. zedoaria* rhizomes. Align with our interest in a phytochemical investigation of Indonesian *Curcuma* [11-17], in the present study, we conduct phenolic isolation and characterization from methanol crude extract of *C. zedoaria* rhizomes grown in West Java, Indonesia.

METHODS

Chemicals and instrument

Fractions were monitored using thin-layer chromatography (TLC) and performed on precoated 0.25 mm thickness of silica gel 60 GF_{254} plates (Merck). TLC spots were visualized under ultraviolet (UV) light (254 and 366 nm) and stained using $Ce(SO_4)_2.4H_2O$ 1.5% in H_2SO_4 2N. Moreover, all silica gel for various chromatography techniques were also purchased from Merck, such as, Si 60 G (column pack) and Si 60 0.2–0.5 mm (sample adsorbed) from vacuum liquid chromatography (VLC), Si 60 (0.063–0.200 mm) for column chromatography (CC), and Si 60 PF_{254} containing gypsum for radial chromatography (RC) and preparative-TLC (p-TLC). The isolated fractions were elucidate using Fourier Transform-InfraRed (FTIR) Shimadzu IR Prestige 21, Ultraviolet-Visible (UV-Vis) Shimadzu UV-2450, and Liquid Chromatography Mass Spectrometry tandem Mass Scpectrometry (LC-MS/MS) with LC system ACQUITY UPLC® H-Class System (Waters, USA) and MS Xevo G2-S QTof (Waters, USA). LC-MS/MS with MS detection at 50 eV was performed under the following conditions: Column: C18 (1.8 µm 2.1 mm×100 mm) HSS, temperature: 50°C (column) and 25°C (room), mobile phase: Water + 5 mM ammonium formic and acetonitrile + 0.05% formic acid, flow rate: 0.2 mL/min (gradient-step) running 23 min, and injection volume: 5 µL with MS system of ES (electrospray ionization) in ion positive mode

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 ($CHCl_3$), and acetone.

Collection of plant material

C. zedoaria, Rosc. rhizomes were collected and identified from Biopharmaca Research 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 RC 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 R_f with C1 fraction; therefore, these fractions were mixed and further purified using p-TLC (*n*-hexane:$CHCl_3$ 2:8, v/v) afforded Z1 fraction (10.2 mg). E3 fraction then was further purified with p-TLC (*n*-hexane:$CHCl_3$ 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 dimethoxycurcumin (DiMC, **1**), while Z2 fraction was recognized as a mixture consisted of 3,5,7-trihydroxy-4'-methoxyflavone (kaempferide, **2**) and 7-methoxy coumarin (herniarin, **3**).

Characterization data

Z1 fraction dimethoxycurcumin (DiMC, **1**); yellow to orange solid; TLC spot R_f 0.25 (*n*-hexane:$CHCl_3$ 3:7, v/v), 0.6 (*n*-hexane:EtOAc 8:2, v/v), and 0.92 (DCM:MeOH 19:1, v/v); FTIR (KBr) υ (cm^{-1}): 3598–3394 (O-H), 2955 (C-H sp^2), 2928–2860 (C-H sp^3), 1733 (C=O), 1604 (C=C alkene), 1510 (C=C aromatic), and 1267 (C-O-C ether); UV-Vis (MeOH) λ_{max} (nm): 263 (benzoyl chromophore) and 402 (curcuminoid chromophore), no shift wavelength observed in addition of shift reagents; and LC-MS/MS: LC rt 12.77 min, MS (70 eV, m/z): 397.157 $[M+H]^+$, 366.138, 335.167, 249.260, 205.085, 199.134 (base peak), 163.075, and 149.024.

Z2 fraction mixture of 3,5,7-trihydroxy-4'-methoxyflavone (kaempferide, **2**) and 7-methoxy coumarin (herniarin, **3**); light-yellowish oil; FTIR (KBr) υ (cm^{-1}): 3314–3176 (O-H), 2959 (C-H sp^2), 2927–2857 (C-H sp^3), 1750 (C=O), 1616 (C=C), 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): 177.055 $[M+H]^+$ (base peak), 148.052, 135.020, 121.065, 116.986, 103.055, 91.055, and 77.039.

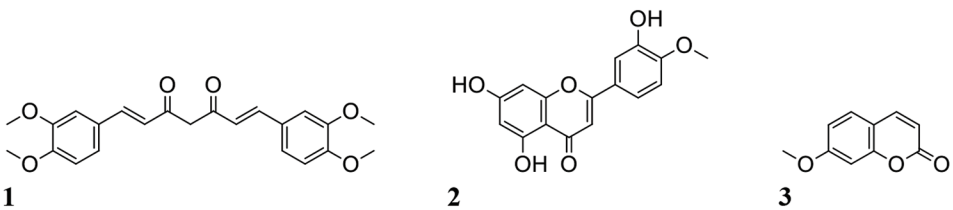
RESULTS AND DISCUSSION

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 dimethoxycurcumin (DiMC, **1**) and Z2 fraction which was obtained in a mixture of 3,5,7-trihydroxy- 4'-methoxyflavone (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 sp^2, C=C aromatic, and C-O-C ether, respectively. In addition, this compound also showed peaks for C-H sp^3, 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 curcuminoid 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

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

Reagents	Cinnamoyl (nm)		Benzoyl (nm)		Remarks
	Initial	Shift	Initial	Shift	
MeOH	323	–	275	–	Flavonol
MeOH/NaOH	369	+46	273	−2	3-OH, no 4'-OH free
MeOH/$AlCl_3$	323	0	275	0	No *o*-diOH on A and B ring
MeOH/$AlCl_3$/HCl	383	+60	322	−1	3-OH (with or without 5-OH)
MeOH/NaOAc	323	0	281	+6	7-OH
MeOH/NaOAc/H_3BO_3	323	0	275	0	No *o*-diOH on A and B ring



1 **2** **3**

Fig. 1: Phenolics from MeOH crude extract of *Curcuma zedoaria* rhizomes

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

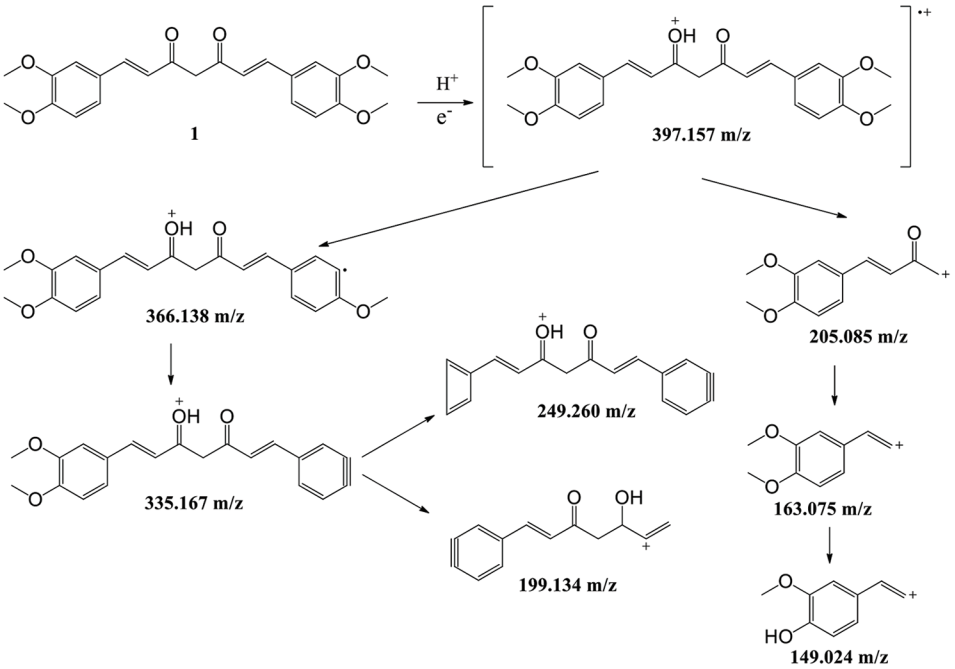


Fig. 2: Fragmentation scheme of **1**

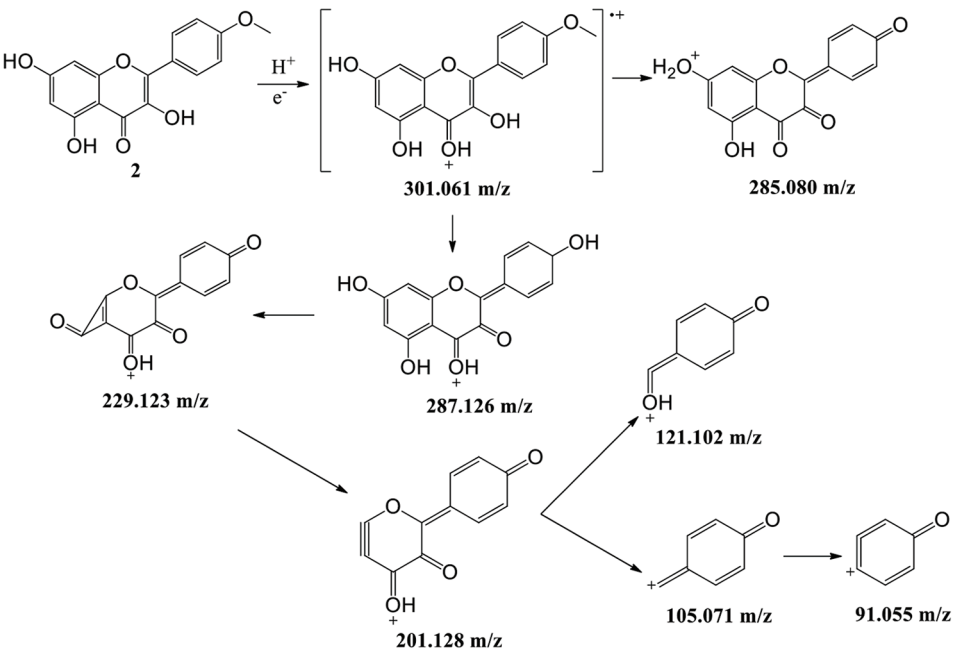


Fig. 3: Fragmentation scheme of **2**

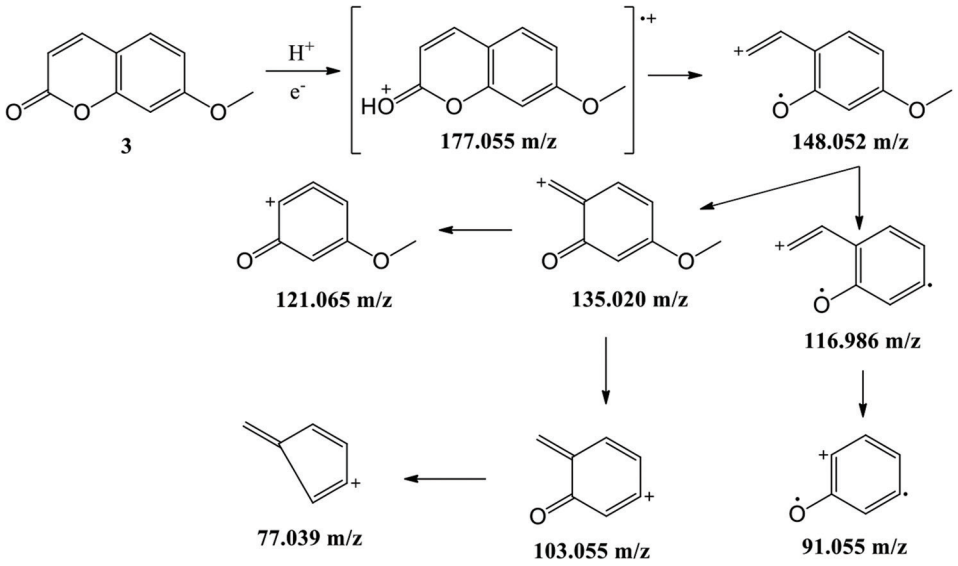


Fig. 4: Fragmentation scheme of **3**

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 (-OCH_3) substituent. Furthermore, there were no *o*-dihydroxyl groups in both ring A and B in flavonol. Since the fraction was still in a mixture proven by the appearance of a λ_{max} 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 $C_6H_{12}O_6$ and fragmented peaks at 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** ($C_{10}H_8O_3$), revealing the molecular ion of 177.055 $[M+H]^+$ as base peak and fragmented peaks of 148.052, 135.020, 121.065, 116.986, 103.055, 91.055, and 77.039. 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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