The Pharma Innovation Journal 2022; 11(1): 28-32

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 28-32

www.thepharmajournal.com
Received: 14-11-2021
Accepted: 20-12-2021

Damasa Syurga Ode
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA) IPB University, Dramaga Bogor, Indonesia

Ayu Setyani Dwi
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok, Indonesia

Syahbirin Gustin
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA) IPB University, Dramaga Bogor, Indonesia

Utami Cahyaning Rahayu Dyah
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok, Indonesia

Dianhar Hanhan
Chemistry Study Program, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Negeri Jakarta, Jakarta, Indonesia

Sugita Purwantiningsih
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA) IPB University, Dramaga Bogor, Indonesia

Corresponding Author:
Sugita Purwantiningsih
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA) IPB University, Dramaga Bogor, Indonesia

Secondary metabolite isolated from Indonesian white turmeric (*Curcuma zedoaria*) rhizomes and its potential as antibacterial agent

Damasa Syurga Ode, Ayu Setyani Dwi, Syahbirin Gustin, Utami Cahyaning Rahayu Dyah, Dianhar Hanhan and Sugita Purwantiningsih

Abstract
White turmeric (*Curcuma zedoaria*) is widely grown in Southeast and South Asia including Indonesia and as a spice and vegetable. It is commonly used traditionally for the treatment of various diseases. The objective research is to isolate alkaloids and terpenoids from *C. zedoaria* rhizomes from Bogor, West Java, Indonesia, and reviewed by literature studies its potency as an antibacterial agent. *C. zedoaria* rhizomes were extracted by maceration under methanol then followed by increasing solvent polarity. the n-Hexane fraction was further purified by using chromatography techniques to afford a simple fraction. The simple fraction then was analyzed by LCMS/MS and the result showed that it was as a mixture content of alkaloids and terpenoids types, such as trichostachine, piperine, curzerenone, and curcumenol. The literature studies revealed that *C. zedoaria* rhizomes extract could be developed as an antibacterial agent.

Keywords: *Curcuma zedoaria*, antibacterial, alkaloid and terpenoids types

1. Introduction
Curcuma zedoaria is a traditional medicine that originated in the Himalayas and India and is now widely used across Asia, including China, Vietnam, Japan, and Indonesia. The outside of the zedoary rhizome resembles ginger (wrinkled gray, ash-colored) while the inside resembles turmeric (brownish red-yellow). It has a milder scent that falls in between turmeric and mango. Zedoary rhizome extracts exhibit anticancer [1, 2], anti-inflammatory [3, 4], analgesic [4, 5], antiallergic [6], antioxidants [7], anti-angiogenesis [8], antibacterial and antifungal activities[9].
Thirty-six compounds in essential oils have been identified from *C. zedoaria*, including 17 terpenoids, 13 alcohols, and 6 ketones [10], flavonoids [11], terpenoids, especially sesquiterpenoids [11, 12, 13, 14], alkaloids and saponins [15]. Crude extract *C. zedoaria* of Indian with diethyl ether contained elemena (3%), isolongifolen (9%), methyl sterolate (24.94%), and isocurcumenol (25.24%) compounds [16]. Lipifoli-1(6)en-4-ol-5-on, benzotazola-2-thiol, -siperon, madolin A, 1,6-dimethyl-9-91-metiletiliden)-5.12-dioxatricylododecan-8-one, and benzo [1,2-b: 4,3-b'] dipiran-3,6-dion-2,2-dimethyl compounds were found in methanol extract of *C. zedoaria* from Bogor [17]. Monoterpenoids (15–20%) and sesquiterpenoids (80–85%) make up the majority of *C. zedoaria* rhizome oil. Curdione (7.0–19.6%), curzerene (10.4%), and epicurzerene (19.0–46.6%) [10, 18, 19], curzerenone (22.3–31.6%) [20, 21], debromofiliforminol (31.5%) [22], p-cymene (18.4%), 1,8-cineole (18.5–40.8%), α-phellandrene (14.9%), curcumenene (18.7%), and β-sesquiphellandrene (21.5%) [23, 24] were reported as major components of *C. zedoaria* rhizome essential oil. Essential oil of *C. zedoaria* rhizome from Thailand contained curzerenone (13.7%) and 1,8-Cineol (37.6%) [25]. Some secondary metabolites that are alleged to have antibacterial activity include epicurzenone and curdione from Chinese essential oils of *C. zedoaria* [19].
Previous research, from methanol crude extract of *C. zedoaria* from Bogor, West Java, Indonesia was extracted based on increasing solvent polarity using n-hexane, EtOAc, and MeOH, respectively. EtOAc fraction of *C. zedoaria* rhizome revealed dimethoxycurcumin, 7-methoxycumarin, and 3,5,7-trihydroxy-4-methoxyflavone (kaempferide) [26]. All of the substances tested negative for acne-causing bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* in antibacterial tests. Therefore, this study focused to isolate secondary metabolites in n-hexane fractions and literature studies of their potential as antibacterial agents.

2. Materials and Methods

2.1 Materials

In January 2019, *C. zedoaria* rhizomes were harvested from the Trop BRC garden LPPM-IPB in West Java, Indonesia. Pro analysis (Merck) grades of chemicals were employed for the isolation process, including methanol (MeOH), ethyl acetate (EtOAc), dichloromethane (DCM). n-hexane, and acetone. LC-MS/MS was utilized for structure analysis, employing an LC system called Ultra Performance Liquid Chromatography (UPLC) and a mass spectrometer called the XEVO-G2SQTOF (Waters, USA). The following conditions were used column: C18 (1.8 µm 2.1×100 mm) HSS, temperature: 50°C (column) and 25°C (room), flow rate: 20 µl/min (gradient-step) running 23 min, injection volume: 250 µL with MS system of ES (electrospray ionization) in ion positive mode, de-solvation temperature of 350 °C and detection at 50 eV and mobile phase: water + 5mM $HCOONH_4$ and CH_3CN + 0.05% HCOOH.

2.2 Extraction, Isolation and Compounds Identified from C. *zedoaria*

Two kg of dried *C. zedoaria* rhizomes were pulverized and extracted four times with one liter of MeOH at room temperature, yielding a 203-g extract. Under reduced pressure, the extract was evaporated, yielding a solid residual mass. The extract was evaporated under reduced pressure and a solid residual mass was obtained. The methanol extract was obtained using liquid-liquid extraction with solvents of n-hexane, then was added with distilled water. A total of 180 g of methanol extract was dissolved in 100 mL methanol, then extracted with n-hexane solvent in the ratio (1:1) and allowed to form 2 phases. The extraction process was carried out until the n-hexane solvent nit in color. The n-hexane fraction was concentrated using a rotary vaporizer to obtain concentrated extracts and was weighed. The n-hexane insoluble fraction was continued by another experiment. The crude methanol extracts and n-hexane fraction were produced according to the protocols[27] for phytochemical analysis. After that, the n-hexane fraction (7.0 g) was impregnated with 14,0 g silica gel 60 (0,2-0,5 mm), evaporated, and vacuum liquid chromatography (VLC) was performed. The first eluent for eluting used n-hexane, then followed by n-hexane increased polarity by increasing percentages of ethyl acetate (50:1(2x), 25:1 (3x); 15:1 (3x); 9:1 (2x); 5:5 (2x), v/v), ethyl acetate and ultimately methanol afforded 10 fractions (J1-J10). J7 fraction (500 mg) was further separated using RC with a gradient solvent of n-hexane 100%, then n-hexane: EtOAc which increased its polarity to a ratio of 18.5: 1.5, EtOAc 100%, and 100% methanol to wash, yielded six fractions (J7.1-J7.6). J7.1 fraction was purified by preparative TLC with eluent n-hexane:EtOAc (17.5: 2.5) resulted 3 sub fractions, crystal J71.1 and 2 oily J71.2 and J71.3). TLC profile, J71.1 appeared one spot with Rf value 0.46, but profiling with LC-MS appeared 4 peaks. Therefore, the J71.1 fraction was then purified using preparative TLC with a solvent ratio of n-hexane: DCM: MeOH (7:2:1). to yield curcumenol based on analyzed by FTIR, GCMS, and 1D and 2D NMR. Curcumenol is a minor compound

3. Result and Discussion

Recent literature revealed that *C. zedoaria* is commonly used for the treatment of numerous ailments and is known to be a rich source of terpenoids, so it was chosen for the research investigation. Terpenoids have the potential to be used as an antibacterial agent. According to a qualitative phytochemical analysis of *C. zedoaria*, the n-hexane fraction contained alkaloids and terpenoids, whereas the MeOH crude extract contained flavonoids in addition to alkaloids and terpenoids. Figure 1 showed that the chemical structures of separated alkaloids and terpenoids from *C. zedoaria* rhizomes discovered by LC-MS/MS. Figure 2 illustrated the chromatogram of fraction J71.1. The chromatogram of fraction J71.1 showed four peaks at the retention time of 7.71, 8.64, 10.35, and 18.06 minutes with a high abundance area of 6.59%, 38.90%, 22.12%, and 4.28%, respectively. A peak at 7.71 and 8.64 minutes belonged to alkaloid compounds 1 and 2, respectively. Furthermore, a peak at 10.35 and 18.06 minutes belonged to terpenoid compounds 3 to 4, respectively.

At 7.71 minutes of retention time, the mass spectrum revealed a peak of 272 $[M+H]^+$, which belonged to the chemical formula of $C_{16}H_{17}NO_3$, compound 1, and a peak of 286 $[M+H]^+$, which belonged to the molecular formula of $C_{17}H_{19}NO_3$, compound 2. Both have a primary fragmented peak at 201, 135, 115, and 70 [28] and included alkaloids group. Compounds 1 and 2 have never been isolated in *C. zedoaria* or other Zingiberaceae plants, according to the literature, however, compound 2 is present in small amounts in *Zingiber officinale* of the Zingiberaceae family[29]. The compound 2 is the major component of black pepper (*Piper nigrum*) [30, 31]. Figure 3 illustrated the fragmentation scheme of 2 prediction.

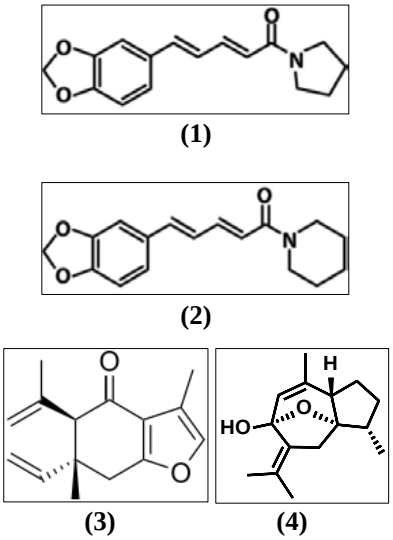

Fig 1: Alkaloids (1 and 2) and terpenoid (3 and 4) from n-hexane fraction of C. zedoaria rhizomes

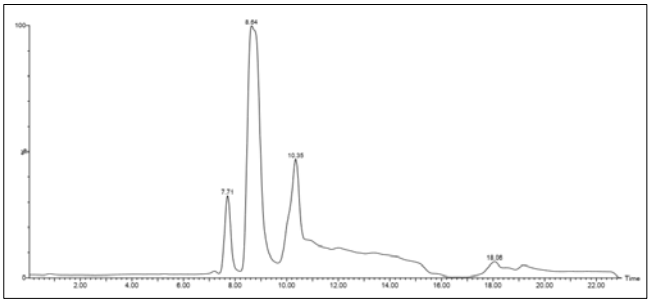

Fig 2: The chromatogram of fraction J71.1 of n-hexane extract from crude extract methanol of *C. zedoaria*

The mass spectrum at 10.35 min of retention time showed a peak of 231 $[M+H]^+$, belonged to the molecular formula of $C_{15}H_{18}O_2$, compound 3. The fragmented peak of compound 3 produced ions at m/z 231, 213, 185, 157, 142, 128, 115, 105, 91. The physically of compound 3, oil yellowish and belonged to curzerenone [31]. Curzerenone is a elemane type structure and displayed $[M+H]^+$ ions at m/z 231, 213, 185, 157, 142, 128 [32]. Li *et al.* [33] reported that curzerenone has major fragment ions at m/z 203.1448 and 185.1334 by successive losses of ethylene $[M+H+-C_2H_4]^+$, ethylene, and water $[M+H^+-C_2H_4-H_2O]^+$, respectively. Elemanes are a sesquiterpene that belongs to a limited group of sesquiterpenes. This category could be used to describe artifacts created during isolation. In the genus Curcuma, 12 elemane-type sequiterpenes have been discovered, including β -elemene, γ-elemene, δ-elemene, curzerene, and curzerenone, which are widely dispersed across *Curcuma species*. Compound 3 had been reported from *C. zedoaria* and it is the largest component found in white turmeric oil [3, 20, 21, 25, 34]. Besides that, it had been also reported in *C. aromatica* essential oils [22, 35], *C. inodora* Blatt [36], and *C. kwangsiensis* [37].

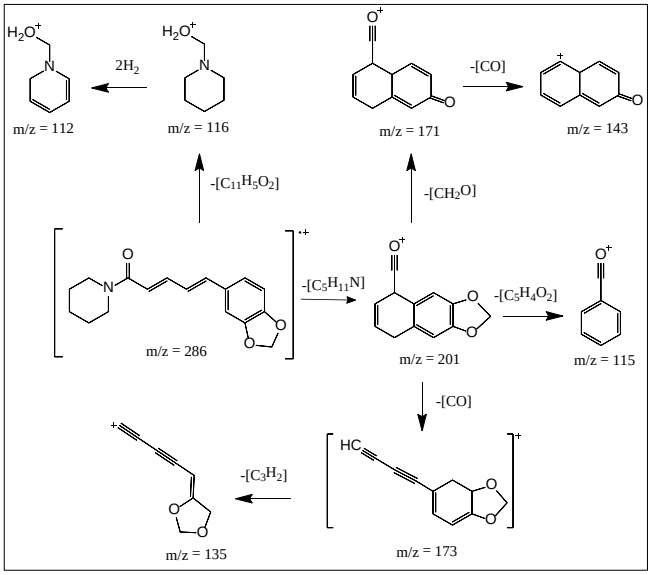

Fig 3: Fragmentation scheme of 2 [38]

The mass spectrum at 18.06 min of retention time showed a peak of 235 $[M+H]^+$ belonged to the molecular formula of $C_{15}H_{22}O_2$, compound 4. The fragmented peak of the MS spectrum for compound 4 displayed ions at m/z 235, 189, 161, 147, 135/133, 121/119, 107/105, 91. It has similarly fragmented peak at m/z 189, 147, 145, 133, 121, 105, and 91 [23, 39] and Human Metabolome DataBase (HMDB0033960) at m/z 217, 191/189, 175, 161, 81/79. Curcumenol compound is a sesquiterpenoid group which Guaiane-type sesquiterpenes, that was successfully identified by Lee *et al.* [40] in white turmeric from Korea. The second-largest group of sesquiterpenes found in *Curcuma species* is guaiane-type sesquiterpenes. The dried rhizomes of *C. zedoaria* contain the most guaiane-type sesquiterpenes [12, 41]. Besides that, compound 4 had also been reported from *C. aeruginosa* [42, 43, 44], *C. aromatic* [45, 46], *C. heyneana* [47], and *C. kwangsiensis*, *C. wenyujin, C. phaeocaulis, and C. longa* [39, 48, 49, 50].

Members of the Zingiberaceae family are known to contain terpenoids, flavonoids, phenypropanoids, and sesquiterpenes, according to literature studies. *Curcuma species* have edible, medicinal, and economic properties. Because of the differences in chemical makeup, diverse biological activities of the same plant species from different regions are possible. The antibacterial activity of *C. zedoaria* essential oil makes it an excellent option for application in the pharmaceutical and cosmetic industries. *C. zedoaria's* volatile constituents included a wide range of volatile monoterpenes, sesquiterpenes, and other aromatic chemicals. *C. zedoaria* rhizome had antibacterial activity in petroleum ether, hexane, chloroform, acetone, and ethanol. Two Gram-negatives *Klebsiella pneumoniae*, *Proteus mirabilis*, and two Gram positives *Micrococcus luteus*, *Bacillus subtilis* were highly active against the hexane and acetone extracts. These extracts had MIC values of 0.01 mg/mL[9]. The essential oil of *C. zedoaria* tubers prevented the growth of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*[24], whereas the essential oil of *C. zedoaria* rhizome inhibited the growth of four Gram-negatives (*Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *E. coli*, and *Salmonella typhimurium*) and two Gram-positive (*S. aureus* and *Bacillus cereus*) bacteria [19].

4. Conclusions

Two alkaloids and terpenoids, trichostachine (1), piperine (2), curzerenone (3), and curcumenol (4), were successfully identified from the *n*-hexane fraction of *C. zedoaria* rhizomes. However, purification of these four compounds and bioactivity testing will require additional work. In addition, Indonesian Curcuma contains terpenoids with varying degrees, which could be developed as an antibacterial compound.

5. Acknowledgments

Through an Agreement No. 1/E1/KP.PTNBH/2020 and Amendment No. 1/AMD/E1/KP.PTNBH/ 2020, this work was supported by a grant from the Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) scheme.

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