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Phytochemical characteristics of white turmeric rhizome (*Curcuma zedoaria* (Berg.) Roscoe) essential oil from Lembang, West Java, Indonesia

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Abstract White turmeric (*Curcuma zedoaria*) rhizome is widely used as a cooking and medicinal ingredient. It is important to determine the essential oil standard that is abundant in white turmeric to prevent adulteration. The results showed that the yield concentration of essential oil from *C. zedoaria* was 0.66% v/w with the following characteristics: brownies colour, spicy taste, as well as sharp and specific aroma. The phytochemical screening result indicated that the essential oil contained alkaloid, flavonoid, triterpenoid, sesquiterpenoid, and quinone. Other phytochemical characteristics included specific gravity value, optical rotation, refractive index, and sapofinication value which were 0.989 g/cm^3, (+)15.19, 1.501, and 28.05 respectively. *C.zedoaria* essential oil was soluble in 80% and 90% ethanol but not soluble in 50%, 60%, and 70% ethanol. Using GC-MS, twenty-one compounds were identified in the essential oil, of which camphor was the marker compound.

Keywords: *C. zedoaria*; Camphor; Essential oil; Phytochemical analysis; Biomarker

Introduction

Indonesia is one of the countries with tropical rain forests that is rich in flora and fauna, therefore it is potential to become a producer of medicinal plants in the world (Salim and Munadi, 2017). Along with other Asian countries, such as China and India, Indonesia is one of the biggest medicinal plant consumers in the world (Yassir and Asnah, 2018). The 'back to nature' lifestyle has become a new trend in the world by utilizing natural resources for medicinal purposes which are relatively safer compared to synthetic medicine (Yulina, 2017).

Curcuma zedoaria or white turmeric is one of plants from Curcuma genus that are widely used as a medicine or cooking ingredients. White turmeric has also been utilized by many ethnic groups in Indonesia, Malaysia, and India.

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Traditionally, white turmeric is used to tread vomiting, menstrual disorder, and dyspepsia (Lobo *et al.*, 2009). *C. zedoaria* is a perennial herb with 1 meter height, oval-shaped main rhizome, and pale yellow inside the tuber (Sirirugsa *et al.*, 2007). White turmeric leaf has length of 80 cm, visible purple spots along the midrib on both leaf surfaces. On a young (small) plant, the colour of its rhizome is similar to Curcuma aeruginosa and Curcuma manga (Hamdi *et al.*, 2014).

White turmeric contains many chemical compounds, such as essential oil, curcumin, tetrahydrodemethoxy curcumin, dihydrocurcumin, polyphenol, trimethoxyflavon, tetramethoxyflavon, and flavonoids which are pharmacologically beneficial. The benefits of white turmeric that have been tested include anticancer, antifungal, antiamebic, larvicidal, antimicrobial, antioxidant, antiplasmodial, antiallergic, and analgesic (Putri, 2014). However, white turmeric cultivated at different region may have different phylogenetic classification which influences its medicinal application (Pangestika *et al.*, 2015). Analysis of essential oil is one of important assay to determine its quality to make it feasible and optimally beneficial when used. Phytochemical analyses of essential oil start with physical properties determination, including specific gravity, optical rotation, refractive index, solubility in alcohol, followed by chemical properties determination using Thin Layer Chromatography and Gas Chromatography-Mass Spectrophotometry to standardize essential oil (Guenther, 1987). Furthermore, chemical content of white turmeric can also be influenced by its cultivation area (Dosoky and Setzer, 2018). Therefore, this research was done to characterize white turmeric (*C. zedoaria*) essential oil which cultivated in Lembang, West Java.

Materials and methods

The main material observed in this research was white turmeric (*C. zedoaria*) rhizome with age of 7-10 months, obtained from Manoko Research Plantation, Lembang, West Java. Rhizomes were cleaned, sorted, dried and made into powder. Before essential oil was isolated, phytochemical screening and measurement of essential oil content were performed on white turmeric sample. Afterwards, isolation of essential oil from white turmeric sample, phytochemical analyses, and identification of essential oil chemical content were done.

Phytochemical screening of C. zedoaria rhizome

Phytochemical screening was done according to method by Farnsworth (1996). Screening was done which included alkaloid, flavonoid, tannin, polyphenol, saponin, sesquiterpene, steroid, terpenoid, and quinone.

Isolation of essential oil from C. zedoaria rhizome

Isolation of essential oil from *C. zedoaria* rhizome was done using Stahl distillation unit (Mustarichie *et al.*, 2017). About 100 gram of sample powder was mixed with 600 ml of distilled water at 70^{o}C for 72 hours. Moreover, isolation was done several times until a sufficient amount of essential oil for analysis was obtained.

Physicochemical analyses of C. zedoaria rhizome essential oil

Physichochemical analyses included organoleptic analysis (colour, aroma, taste), specific gravity using pycnometer, optical rotation, refractive index using Refractometer-Abbe, solubility of essential oil in ethanol, and saponification value analysis according to the procedures on Farmakope Indonesia, 4th edition (DepkesRI, 1995).

Optical rotation analysis was done by using polarimeter. About 1 ml dehydrated essential oil was dissolved in 9 ml of n-hexane. Afterwards, the mixture was put in 100 mm polarimeter tube that was placed between polarimeter and analyzer. Analyzer was then rotated slowly and observed until the similar intensity of light to its light source was obtained. Subsequent rotation direction was determined i.e. (+) or dextro rotatory if the analyzer rotated clockwise from zero and (-) or levo rotatory if the analyzer rotated counterclockwise. Analyzer was rotated until line position between two area could be clearly and sharply observed. The angle was then recorded (Shabbir *et al.*, 2009). In this analysis, 8 replications were done. The rotation angle was determined using formula (1) as follows:

$$\theta_{solvent} = b_{solvent} - a_{solvent}$$
$$\theta_{solution} = b_{solution} - a_{solution}$$
$$\theta_{sample} = \theta_{solution} - \theta_{solvent} \qquad (1)$$

Notes:
θ = rotation angle
a = degree of bright center position
b = degree of dark center position

Solubility of essential oil in ethanol was analyzed using various alcohol concentration in screw cap test tubes. About 0.1 ml of essential oil was put into a test tube and small amount of alcohol with different concentrations (50%, 60%, 70%, 75%, 80%, and 90%) was slowly added into the tubes. Volume of added alcohol (less than 1 ml) required to obtain clear solution was then recorded (Alam et al., 2018). If clear solution was not obtained until 1 ml of

alcohol added, then experiment was continued using higher concentration of alcohol until clear solution was obtained. Solubility degree of essential oil in each alcohol concentration was recorded.

Identification of chemical compositions of essential oil from C. zedoaria rhizome

Identification of chemical compounds in essential oil was done using Thin Layer Chromatography (TLC) and Gas Chromatograhy-Mass Spectrophotometry (GC-MS) (Sukrasno *et al.*, 2012). TLC was done using GF 254 silica gel with toluene:ethyl acetate (93:7) and n-hexane:ethyl acetate (9:1) as the eluents. Visible light, 254 nm UV light and vanillin-sulfuric acid were used to view the spots.

GC-MS analysis was done using Shimadzu, type Class 5000 GC-MS instrument with DB-17 capillary column with length of 30m. Temperatures used in experiment are as follows: injector temperature of 200^{o}C, detector temperature of 250^{o}C, column temperature of 40^{o}C per 2 min/ 8^{o}C per min/250^{o}C per 10 min with Helium with speed of 1.3 ml/min as carrier gas. The amount of sample added into the instrument was 1 µL, with multiplier electron voltage of 1.2 kV.

Results

Phytochemical screening results of C. zedoaria rhizome

Results of phytochemical screening of *C. zedoaria* rhizome is presented on Table 1. It showed that phytochemical screening results of *C. zedoaria* rhizome revealed *C. zedoaria* rhizome contained alkaloid, flavonoid, triterpenoid, sesquiterpenoid, and quinone.

Table 1. Phytochemical screening results of *C. zedoaria* rhizome

No	Chemical Compounds	Results
1	Alkaloid	+
2	Flavonoid	+
3	Tannin	-
4	Polyphenols	-
5	Saponin	-
6	Steroid and Terpenoid	+
7	Monoterpene and Sesquiterpenoid	+
8	Quinone	+

Note: (+): detected, (-): not detected

Physichochemical characteristics of essential oil from C. zedoaria rhizome

Physichochemical analysis of essential oil plays a significant role to give an overview about purity and quality of oil. Results from this research can be determined the standard for *C. zedoaria* rhizome essential oil, especially *C. zedoaria* that is cultivated in Indonesia. Results of physicochemical analyses on *C. zedoaria* rhizome essential oil can be observed on Table 2.

Table 2. Physicochemical characteristics of *C. zedoaria* rhizome essential oil

Parameter	Unit	Amount (% v/w)
Essential oil yield	%v/w	0.66±0.014
Specific gravity	g/cm^3	0.989±0.000
Optical rotation	θ	(+)15.19±1.50
Refractive index	-	1.501±0.000
Solubility	-	Insoluble in 50%, 60%, 70% ethanol, soluble in 80% and 90% ethanol
Saponification value	-	28.05
Colour	-	Brownish
Aroma	-	Sharp and characteristic
Taste	-	Spicy

Identification of chemical composition of essential oil from C. zedoaria rhizome

Identification of chemical compounds in essential oil was done using Thin Layer Chromatography (TLC), followed by Gas Chromatograhy-Mass Spectrophotometry (GC-MS). Identification of chemical compounds could be done qualitatively using TLC to obtain TLC profile from compounds separation based on their polarity properties. TLC profile from certain eluent could be used as a reference for initial identification of essential oil from *C. zedoaria* rhizome. Thin Layer Chromatography (TLC) was provided relatively different patterns of the compound when beams with different wavelengths or specific dye reagents are used. Results of TLC in this research can be observed in Tables 3 and 4.

TLC identification was then continued by Gas Chromatography-Mass Spectrophotometry (GC-MS) to obtain peak spectra of compounds which are clearly separated from each other. Gas chromatogram pattern of essential oil from *C. zedoaria* rhizome can be observed on Figure 1. Furthermore, the chemical components of essential oil from *C. zedoaria* rhizome can be observed in Table 5.

Table 3. TLC analysis result with eluent of toluene:ethyl acetate (93:7) mixture

No	Rf	Colour		
		With visible light		With 254 nm UV light
		Reactant*	Without reactant	
1	0.15	Blue	-	Light purple
2	0.43	Purple	-	Light purple
3	0.47	-	-	Light purple
4	0.51	-	-	Light purple
5	0.64	Red	-	Light purple

*Reactant: vanillin-sulfuric acid

Table 4. TLC analysis result with eluent of n-hexane:ethyl acetate (9:1) mixture

No	Rf	Colour		
		With visible light		With 254 nm UV light
		Reactant*	Without reactant	
1	0.02	Grey	-	Light purple
2	0.18	Purple	-	Light purple
3	0.28	-	-	Light purple
4	0.33	Blue	-	Light purple
5	0.41	-	-	Light purple
6	0.83	Red	-	Light purple
7	0.88	-	-	Light purple

*Reactant: vanillin-sulfuric acid

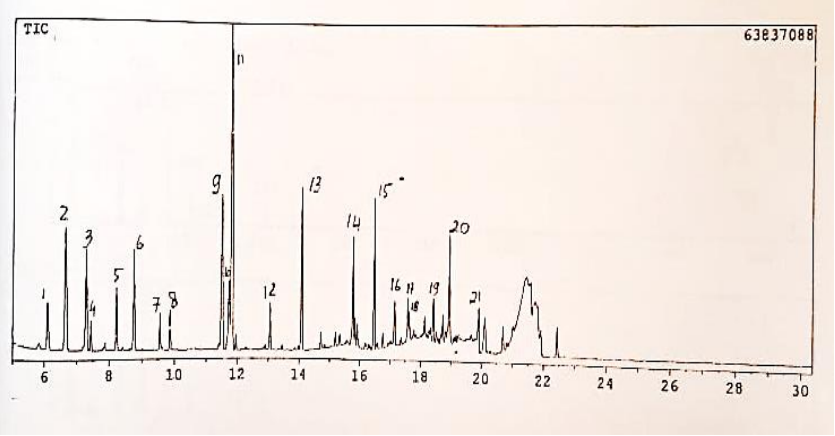

Figure 1. Gas chromatogram pattern of essential oil from *C. zedoaria* rhizome

Table 5. Chemical components of essential oil from *C. zedoaria* rhizome

No	Component	Percentage (%)
1	Linalyl acetate	1.62
2	Camphene	3.17
3	2- β -pinene	2.18
4	Beta myrcene	0.19
5	1-limonene	1.04
6	Eucalyptol	1.94
7	2-nonanol	0.64
8	2-nonanon	0.67
9	Isoborneol	3.21
10	Borneol	1.5
11	Camphor	6.62
12	Delta element	0.79
13	Beta element	2.38
14	Germacrene D	1.98
15	Furanodiene	2.11
16	Germacrene B	0.92
17	Viridiflorol	1.45
18	Elemol	0.83
19	Spatulenol	0.94
20	Epi-β-santalol	2.07
21	Germacrone	1.02

Chromatogram pattern of camphor from *C. zedoaria* essential oil rhizome was further analyzed using mass spectrophotometer and the results can be seen in Figure 2.

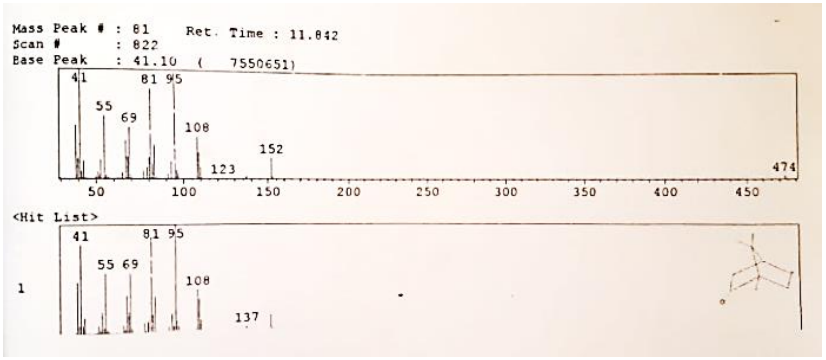

Figure 2. Mass spectrophotometry chromatogram pattern of camphor compound from essential oil from *C. zedoaria* rhizome

Discussion

Results of phytochemical screening of *C. zedoaria* rhizome (Table 1) are comparable to a previous research by Sumathi *et al.* (2013) who reported that methanol extract of fresh and dry rhizome of *C. zedoaria* contain alkaloids, phenolics, flavonoids, saponins, glycosides, steroids, and terpenoids. This result also shows that *C. zedoaria* rhizome is potential to be used for medicine, because some sesquiterpenes from *C. zedoaria*, such as ar-turmerone was reported to have antimicrobial activities against Gram-positive and Gram-negative bacteria (Hong *et al.,* 2001). Some sesquiterpenes that has been identified from *C. zedoaria* rhizome included curcumol, curcolone, procureumenol, isocurcumenol, furadiene and its iso-derivative, such as curcumadiol, dehydrocurdione and zederon (Joy *et al.,* 2002).

Essential oil of *C. zedoaria* rhizome was obtained by extraction using distilled water (hydrodistillation) because organic solvents can be toxic for human and can induce loss of volatile compounds and extraction of some non-volatile compounds, which later influences the change in essential oil's quality and effectiveness (Berka-Zougali *et al.,* 2012). Furthermore, extraction of *C. zedoaria* rhizome using water resulted in higher yield compared to ethanol (Marliani *et al.,* 2017).

Essential oil of *C. zedoaria* rhizome had brownish colour, sharp and characteristic aroma and spicy taste. In this research essential oil content of white turmeric rhizome analyzed by Stahl distillation method was 0.66 ± 0.014 %v/w, lower than (Angel *et al*., 2014), which was 1.4 ml/100 g fresh weight and Joy *et al.* (2002), which was 1.05% on dry weight basis, but higher than *C. zedoaria* leaves essential oil, which was 0.33% w/w (Rahman *et al.,* 2014). The amount of essential oil in rhizome is influenced by several factors, include condition in which the plant grows, such as climate, temperature, humidity, soil and nutrient conditions, growing method, and plant maintenance. Other factors include age of plant, preparation, and processing of raw materials (Guimarães *et al.*, 2020).

Specific gravity, optical rotation, and refractive index are some analyses that are performed to determine physical constants of essential oil (Bousbia *et al.,* 2009). Essential oil of *C. zedoaria* rhizome had specific gravity of 0.989 ± 0.000 g/cm^3, refractive index of 1.501 ± 0.000, and optical rotation of $(+)15.19 \pm 1.50$. Furthermore, essential oil of *C. zedoaria* rhizome had saponification value of 28.05 and were soluble in 80% and 90% ethanol, but insoluble in 50%, 60%, and 70% ethanol.

The n-hexane:ethyl acetate (9:1) was a better eluent with 7 spots detected with 254 nm UV light and 7 spots with visible light. Meanwhile,

toluene:ethyl acetate (93:7) mixture gave 5 spots with 254 nm UV light and 3 spots with visible light. Results in Table 4 indicates that there are some compounds present in *C. zedoaria* rhizome essential oil. Rf value of 0.18 might indicate the presence of camphor because camphor has Rf value of 0.2. Rf value of 0.33 might indicate the presence of isoborneol (Rf =0.33) (Croteau *et al*., 1981), and Rf of 0.83 might indicate the presence of camphene (Rf = 0.85) and will have brown colour with vanillin sulfate reactant (Salman, 2009). In Table 3, Rf 0.64 might indicate linalyl acetate (Rf = 0.63) (Kreis and Mosandi, 1992).

From essential oil of *C. zedoaria* rhizome obtained in this research, there were 21 compounds detected. Compared to other similar research, there were 37 compounds detected by Purkayastha *et al.* (2006), 36 compounds identified by Mau *et al.* (2003), and 24 compounds identified (Rahman *et al.*, 2014) from essential oil of *C. zedoaria* rhizome from Northeast India, China, and Bangladesh, respectively. Furthermore, the highest peak spectrum showed the main compound in white turmeric rhizome essential oil in this research was camphor. Mau *et al.* (2003) reported that compounds that had the highest amount in *C. zedoaria* rhizome essential oil were epicurzerenone and curzerene, meanwhile Purkayastha *et al.* (2006) reported that major component in *C. zedoaria* rhizome essential oil was curzerenone. Dosoky and Setzer (2018) also mentioned that the main component of essential oil from *C. zedoaria* cultivated in Kerala (India) and Thailand was 1,8-cineol, while the ones that were cultivated in Colombo (Sri Lanka) have debromofiliforminol as their main component. The difference in the major compounds may be influenced by difference in cultivation regions (Tsai *et al.*, 2011).

Result showed that the main component of white turmeric (*C. zedoaria*) was camphor, which is one of cyclic terpenoids that gives characteristic aroma to white turmeric. A previous research by Dosoky and Setzer (2018) stated that generally camphor is not a main compound in *C. zedoaria* rhizome, and only present in *C. zedoaria* rhizome cultivated in Sri Lanka. Therefore, result from this research showed that camphor might be a characteristic compound from white turmeric in Lembang, West Java. Other compounds that can be considered area camphene (3.17%) and 2-β-pinene which are presented in *C. zedoaria* rhizome essential oil for about 2.18%, different from other species from *Curcuma* species which only contain less than 0.05% of β-pinene. Besides, Germacrene D and Germacrene B are compounds that only presented in *Curcuma* (Dosoky and Setzer, 2018), with amount of 1.98% and 0.92%, respectively.

In conclusion, White turmeric (*C. zedoaria*) rhizome that was cultivated in Lembang, West Java contains 0.66% of essential oil (v/w) with specification

of brown colour, spicy taste, and characteristic aroma, specific gravity of 0.989 g/cm3, optical rotation (+) of 15.19, refractive index of 1.501, soluble in 80-90% ethanol, and saponification number of 28.05. Identification results of essential oil of white turmeric rhizome from GC-MS showed that a component that can be considered as marker compound is camphor. To enrich the references to determine standard for white turmeric rhizome essential oil, analyses on white turmeric essential oil from different cultivation area in Indonesia should be done. Therefore, comparison of its physichochemical properties and marker compound content could be further investigated.

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