Phytochemical and antiacne investigation of Indonesian White Turmeric (*Curcuma zedoaria*) Rhizomes

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Abstract. White turmeric (*Curcuma zedoaria*) is one of plant species belongs to Zingiberaceae from Himalaya, India. Previous research showed that the rhizomes of this plant contained several secondary metabolites such as alkaloid, phenolic, and terpenoid which commonly known to have antibacterial activities. This study aims to investigate the phytochemical of white turmeric rhizomes growth in Indonesia and evaluate its antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis* as acne-causing bacteria. In this study, secondary metabolites from Indonesian white turmeric rhizomes were successfully extracted in methanol and partitioned in polarity increase of several organic solvents. Furthermore, crude and all partitioned extracts were subjected to phytochemical investigation. The ability of crude and ethyl acetate extracts to inhibit the growth of *P. acnes* and *S. epidermidis* were evaluated using disk diffusion method. According to the antibacterial activity results, all of samples did not exhibit antibacterial activity against both acne-causing bacteria. The results of this study will provide a useful information regarding the phytochemical and antiacne investigation of Indonesian white turmeric rhizomes.

Keywords: White turmeric rhizomes, *Curcuma zedoaria*, Zingiberaceae, antiacne, antibacterial

1. Introduction

Acne is a chronic inflammatory disease of pilosebaceous which commonly occurs in adolescence. Acne in teenagers occupies a prevalence rate of 85 % with the age range of 12–25 years [1]. In Indonesia, acne ranks at the third most diseases according to the number of visitors in Department of Skin Health and Venereology in several hospitals and skin clinics. One of factors causing acne is bacterial infection of *Propionibacterium acnes* and *Staphylococcus epidermidis* which belong to a normal flora bacteria on skin and Gram-positive bacteria. These two bacteria are pathogenic and often resistant to various types of antibiotics. *P. acnes* was resistant to doxycycline and minocycline in 100 % usage, while 10 % usage was found to be resistant to erythromycin, clindamycin, and tetracycline. Moreover, the side effect from using antibiotics such as tetracycline can cause liver damage for pregnancy women [2]. Therefore, research in discovering new compounds having potential as antibacterial activity, especially derived from natural products, are urgently needed.

White turmeric (*Curcuma zedoaria*) is one of the plant species belongs to Zingiberaceae from Himalaya, India. In Indonesia, white turmeric is usually used as herbal medicine, especially its rhizomes
which is commonly well-known to have several bioactivities such as antioxidant, anti-inflammatory, anticancer, and antibacterial activities [3]. These bioactivities in white turmeric rhizomes are due to its secondary metabolites. Previous study had been reported secondary metabolites from white turmeric rhizomes grown in Faisalabad, Pakistan consisted of terpenoid [4]. Flavonoid and curcuminoid were also reported in Indonesian white turmeric rhizomes from Jimbaran, Bali and Batu, East Java [5-6]. Furthermore, antibacterial activity of white turmeric rhizomes extract was previously reported against various bacteria i.e. S. aureus, Escherichia coli, Streptococcus pyogenes, Symphoricarpos albus, Pseudomonas aeruginosa, and S. mutans [7-8]. However, research on phytochemical investigation of Indonesian white turmeric rhizomes from Bogor, West Java together with its bioactivity towards acne-causing bacteria are not yet reported. To the best of our knowledge, differences in geographical location will influence the differences in secondary metabolites [9]. Therefore, the aims in this present study are to investigate the phytochemical and evaluate antiacne activity of Indonesian white turmeric rhizomes. This study was aligned with our interest on Indonesian medicinal plants during the recent years [10-16].

2. Materials and method

2.1. General
White turmeric (C. zedoaria) rhizomes were obtained as dried powder from Pusat Studi Biofarmaka Tropika (Trop. BRC), Institut Pertanian Bogor. Organic solvents used for extraction and partition were in technical grade i.e. methanol, n-hexane, and ethyl acetate (EtOAc). Extracts were monitored by using thin layer chromatography (TLC) and performed on pre-coated silica gel 60 GF 254 plates (Merck). Chemicals used for phytochemical screening were purchased from Merck, i.e. NaOH, Mg, HCl, NH4OH, chloroform, (CH3CO)2O anhydrous, H2SO4, FeCl3, Wagner, and Dragendorff reagents. Moreover, reagents and equipment used for antibacterial activity assay using disk diffusion method were also purchased form Merck, i.e. paper disk (diameter ± 6 mm), beef extract, peptone, nutrient agar, and dimethyl sulfoxide (DMSO), while clindamycin was purchased from drug store in Depok, West Java. Acne-causing bacteria such as Propionibacterium acnes and Staphylococcus epidermidis were obtained from Biochemistry Laboratory, Department of Chemistry, Universitas Indonesia.

2.2. Extraction and partition of white turmeric rhizomes (C. zedoaria)
Dried and powdered white turmeric rhizomes (2 kg) were extracted at 3 x 24 h in methanol at room temperature. After filtering the residue, filtrate was evaporated to remove the solvent under reduced pressure at 40°C using rotatory evaporator yielded the crude extract. The crude extract then was partitioned by using organic solvents with increasing polarity from n-hexane, EtOAc, and methanol. Each partition extract was also subjected under similar work up to remove the solvent. Both crude and partition extracts then were calculated for its yields following previous report [17] and subjected to analyze for its compounds using TLC with n-hexane:EtOAc = 7:3 (v/v) as mobile phase.

2.3. Phytochemical investigation of crude and all partitioned extracts
Phytochemical screening such as flavonoid, tannin, saponin, alkaloid, terpenoid, and steroid tests was conducted for crude and all partitioned (n-hexane, EtOAc, and methanol) extracts of white turmeric rhizomes following standardized procedures [18]. Flavonoid test was carried out using NaOH and Mg-HCl while tannin test was conducted using FeCl3. Moreover, the presence of alkaloid was studied using Wagner and Dragendorff reagents after adding chloroform, NH4OH, and HCl to the extracts. Terpenoid and steroid tests were investigated using (CH3CO)2O and H2SO4 after dissolving the extracts with chloroform. The color changing on flavonoid, tannin, alkaloid, terpenoid, and steroid test were observed after each reagent was added. Furthermore, saponin was also identified by shaking the extracts after dissolving in aquadest and observed when the stable foam was formed.
2.4. Antibacterial activity assay of crude and EtOAc extracts
The ability to inhibit acne-causing bacteria growth of crude and EtOAc extracts were carried out with various concentrations of 1000, 500, 250, 125 and 62.5 ppm using disk diffusion method following established procedure [19]. Clindamycin (250 ppm) was selected as positive control while DMSO was used for negative control.

3. Results and discussion

3.1. Extraction and partition of white turmeric rhizomes (C. zedoaria)
In this present study, 2 kg of white turmeric rhizomes were extracted by maceration in methanol for 3 x 24 h. Maceration involved soaking plant material in container with solvent for a minimum period of 3 days with frequent agitation to soften and break the plant’s cell wall to release soluble phytochemicals [20]. Methanol filtrate was separated from the residue and subjected to evaporate to obtain crude extract. Crude extract was established as blackish brown gummy with the weighted mass of 73.68 g with 3.684 % in yield.

A total 65 g of crude extract then was partitioned by using n-hexane, EtOAc, and methanol, successively. Table 1 summarizes the partition data together with the weighted mass and yield. According to table 1, the highest mass was obtained in EtOAc extract with the weighted mass of 30.59 g (47.06 %). This result indicated that compounds contained in white turmeric rhizomes were classified as semi polar compounds. It was also proven by TLC analysis showed in figure 1. Based on figure 1, EtOAc extract exhibited the most spot (compounds) among others, with semi polar compounds.

| Solvent   | Extract (g) | Yield (%) |
|-----------|-------------|-----------|
| n-Hexane  | 7.17        | 11.03     |
| EtOAc     | 30.59       | 47.06     |
| Methanol  | 4.31        | 6.63      |

Table 1. The partition data of crude extract from white turmeric rhizomes.

Figure 1. TLC analysis of crude and all partitioned extracts using n-hexane:EtOAc = 7:3 (v/v) visualized under UV 254 nm. C: crude extract, H: n-hexane extract, E: EtOAc extract, and M: methanol extract.
3.2. Phytochemical investigation of crude and all partitioned extracts

Preliminary phytochemicals screening of crude and all partitioned extracts are summarized in table 2. According to table 2, crude extract of white turmeric rhizomes was found to be composed by alkaloid, saponin, terpenoid, flavonoid, and tannin. Partition of crude extract distributed the compounds in three organic solvent having difference in polarity. For example, n-hexane extract contained terpenoid and flavonoid, EtOAc extract contained alkaloid, terpenoid, and flavonoid, while methanol extract contained saponin, flavonoid, and tannin. In comparison with phytochemical analysis from other regions of white turmeric rhizomes, summarized results of several literature will be further described.

Previous reported showed that phytochemical analysis of dichloromethane extract of Brazilian white turmeric rhizomes revealed the presence of terpenoid as dominance compound and confirmed by FTIR and NMR spectral data as curcumenol and mixture of phytosterols [21]. Essential oil obtained from hydro-distillation of Northeast Indian white turmeric rhizomes consisted of curzerenone, 1,8-cineole, and germacrone after analyzing with GC/MS [22]. Moreover, another white turmeric rhizomes essential oil from India also reported to have predominate terpenoid such as 1,8-cineole, cymene, α-phellandrene, and β-eudesmol [23]. Meanwhile, essential oil from Indian white turmeric leaves exhibited mainly mono- and sesquiterpenoid [24].

3.3. Antibacterial activity assay of crude and EtOAc extracts

Antibacterial activity assay was carried out on crude and EtOAc extracts against acne-causing bacteria, i.e. *P. acnes* and *S. epidermidis*. Clindamycin was chosen as positive control since it classified as lincomycin derived antibiotics which can inhibit protein synthesis at 50S ribosome affecting the initial phase of peptide formation in bacteria [25]. The results of antibacterial activity assay are exhibited in table 3. According to table 3, both crude and EtOAc extracts did not showed antibacterial activity.

| Phytochemicals | Crude extract | n-Hexane extract | EtOAc extract | Methanol extract |
|----------------|---------------|------------------|---------------|-----------------|
| Alkaloids      | Present       | Absent           | Present       | Absent          |
| Saponins       | Present       | Absent           | Absent        | Absent          |
| Terpenoids     | Present       | Present          | Present       | Absent          |
| Flavonoids     | Present       | Present          | Present       | Present         |
| Tannins        | Present       | Absent           | Absent        | Absent          |
| Steroids       | Absent        | Absent           | Absent        | Absent          |

| Concentrations (ppm) | Average diameter of *P. acnes* bacteria inhibition zone (mm) | Average diameter of *S. epidermidis* bacteria inhibition zone (mm) |
|----------------------|-------------------------------------------------------------|-------------------------------------------------------------|
|                      | Crude | EtOAc extract | Crude | EtOAc extract |
| 62.5                 | 0     | 0             | 0     | 0             |
| 125                  | 0     | 0             | 0     | 0             |
| 250                  | 0     | 0             | 0     | 0             |
| 500                  | 0     | 0             | 0     | 0             |
| 1000                 | 0     | 0             | 0     | 0             |

*The average diameter of clindamycin inhibitory zone (+) for *P. acnes* and *S. epidermidis* were 9.5 and 9.0 mm, respectively. Meanwhile, the average diameter of DMSO inhibitory zone (-) for both two bacteria were 0 mm*
towards both acne-causing bacteria, as proven by the absence of clear zone produced around the paper disc. These results indicated that all of samples cannot inhibit the growth of acne-causing bacteria. However, previous study had been reported that methanol extract of white turmeric rhizomes showed a medium antibacterial activity against \textit{S. aureus} with inhibitory zone of 13 mm [26].

4. Conclusion
Secondary metabolites from Indonesian white turmeric rhizomes (\textit{C. zedoaria}) can be extracted through maceration method in methanol with 3.684 % in yield. Further partition processes of crude extract gave three partitioned extracts with EtOAc extract which is having the most compounds and weighed mass. However, crude and EtOAc extracts exhibited inactive in inhibiting the growth of acne-causing bacteria, i.e. \textit{P. acnes} and \textit{S. epidermidis}. Further research should be carried out to fractionate the extract together with examine its antibacterial activity in other bacteria.

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References
[1] Dreno B 2017 \textit{J. Eur. Acad. Dermatol. Venereol.} \textbf{31} 8-12
[2] Sitohang I B S, Fatgan H, Effendi E and Wahid M 2019 \textit{Med. J. Indones.} \textbf{28} 21-7
[3] Marliani L, Budiana W and Anandari Y 2017 \textit{Indones. J. Pharm. Sci. Technol.} \textbf{4} 57-63
[4] Tariq S, Imran M, Mushtaq Z and Asghar N 2016 \textit{Lipids Health Dis.} \textbf{15:39} 1-10
[5] Ashfahani E D, Wiratmini N I and Sukmaningsih A A S A 2010 \textit{Jurnal Biologi Udayana} \textbf{14} 20-3
[6] Ongko N X, Chiuman L and Ginting C N 2019 \textit{Am. Sci. Res. J. Eng. Technol. Sci.} \textbf{55} 69-74
[7] Chachad D P, Talpade M B and Jagdale S P 2015 \textit{Int. J. Sci. Res.} \textbf{5} 938-40
[8] Wirahmi S D, Busman and Edrizal 2019 \textit{Menara Ilmu} \textbf{13} 19-28
[9] Sugita P, Anggraini D, Syahbirin G, Rahayu, D U C and Ilmiawati A 2019 \textit{J. Indones. Chem. Soc.} \textbf{2} 114-20
[10] Rahayu D U C, Adilah S N and Sugita P 2018 \textit{Eur. J. Pharm. Med. Res.} \textbf{5} 582-8
[11] Sugita P, Octaviana N, Wukirsari T and Rahayu D U C 2018 \textit{J. Pharm. Res.} \textbf{12} 293-7
[12] Rahayu D U C, Hartono and Sugita P 2018 \textit{Rasayan J. Chem.} \textbf{11} 762-5
[13] Sugita P, Firdaus S O, Ilmiawati A and Rahayu D U C 2018 \textit{J. Chem. Pharm. Res.} \textbf{10} 68-75
[14] Purwantiningsih, Hartono, Firdaus S O, Kurniawanti, Rahayu D U C, Nurhayati L, Ilmiawati A and Wukirsari T 2019 \textit{Drug Invent. Today} \textbf{11} 386-90
[15] Rahayu D U C, Nurfadhilah D and Sugita P 2019 \textit{Int. J. Phram. Sci. Res.} \textbf{10} 3354-8
[16] Purwantiningsih, Jannah N and Rahayu D U C 2020 \textit{Rasayan J. Chem.} \textbf{13} 322-6
[17] Cespedes C L, Avila J G, Martinez A, Serrato B, Calderon-Mugica J C and Salgado-Garciglia 2006 \textit{J. Agric. Food. Chem.} \textbf{54} 3521-7
[18] Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H 2011 \textit{Internationale Pharmaceutica Sciencia} \textbf{1} 98-106
[19] Dogan B, Bektebore E, Karabacak E and Ozuyt M 2017 \textit{Turkderm-Turk. Arch. Dermatol. Venereol.} \textbf{51} 32-6
[20] Azwanida N N 2015 \textit{Med. Aromat. Plants} \textbf{4} 1-6
[21] Navarro D de F, de Souza M M, Neto R A, Golin V, Niero R, Yunes R A, Monache F D and Filho V C 2002 \textit{Phytomedicine} \textbf{9} 427-32
[22] Purkayastha J, Nath S C and Klinkby N 2011 \textit{J. Essent. Oil Res.} \textbf{18} 154-5
[23] Singh G, Singh O P and Maurya S 2002 \textit{Prog. Cryst. Growth Charact. Mater.} \textbf{45} 75-81
[24] Garg S N, Naquvi A A, Bansal R P, Bahl J R and Kumar S 2005 *J. Essent. Oil Res.* **17** 29-31
[25] Smilack J D, Wilson W R and Cockerill F R 1991 *Mayo Clin. Proc.* **66** 1270-80
[26] Das K and Rahman M A 2012 *Int. J. Pharm. Pharm. Sci.* **4** 322-8