Reduction of curcuminoid extract from turmeric (Curcuma longa) rhizomes and its antibacterial activities

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Abstract. Curcuminoid is an active compound of turmeric rhizomes commonly used as yellow pigment. In some cases, the presence of pigment in beauty product is not desirable due to its aesthetics effect. In this study, yellow color removal of curcuminoid was carried out by two steps hydrogenation, i.e. using LiAlH₄ followed by Pd-C catalyst to form dihydrocurcuminol and hexahydrocurcuminol derivatives, respectively. Dihydrocurcuminol derivatives had successfully synthesized by observing a wavelength shift from 419 to 428 nm in UV-Vis spectra and FTIR spectra also showed a reduction intensity of the carbonyl groups at 1600 cm⁻¹. Moreover, hexahydrocurcuminol derivatives were identified at 283 nm in UV-Vis spectra while in FTIR spectra showed an absorption band of C-H sp³ at 2942 cm⁻¹. Curcuminoid and hexahydrocurcuminol derivatives were evaluated for their antibacterial activities against Staphylococcus aureus with 2 and 2.5 mm and Fusobacterium nucleatum with 4 and 2.75 mm inhibitory zone diameter, respectively. It can be concluded that curcuminoid and hexahydrocurcuminol derivatives had a weak antibacterial activity against both bacteria tests.

Keywords: hydrogenation, curcuminoid, turmeric, antibacterial activities

1. Introduction

Turmeric (Curcuma longa) is a herb plants belongs to Zingiberaceae family that commonly used as herbal and traditional medicine for various diseases such as rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments [1]. Curcuminoid, an active compound contained in turmeric, is known to have antioxidants, antitumor, anticancer, antimicrobial, anti-Alzheimer’s, and antitoxic activities [2]. According to Muhammad et al., spices and herbs contain antibacterial compounds [3]. Turmeric contains some active compounds that can be used as an antibacterial agent such as essential oil, alkaloid, flavonoid, tannin, curcuminoid, and terpenoid derivatives [4].

Due to its color, curcuminoid is rarely used as antibacterial in some beauty product, such as toothpaste, mouthwash, and soap. To decolorize its color, structure transformations by reduction of carbonyl ketone group and double bond alkene group should be conducted to remove the conjugated double bond. Furthermore, tetrahydrocurcuminoid, hexahydrocurcuminoid, and octahydrocurcuminoid (hexahydro-curcuminol) as the reduced form of curcuminoid are more hydrophilic than curcuminoid. Therefore, they have better stability in aqueous media [5] and this property is good for antibacterial and antioxidant activities.
In this research, curcuminoid extract from turmeric rhizome was successfully isolated using Soxhlet. Reduction of curcuminoid extract was performed with LiAlH₄ followed by Pd-C catalyst. Curcuminoid and hexahydrocurcuminol derivatives were then tested for their antibacterial activities towards mouth bacteria, i.e. *Staphylococcus aureus* and *Fusobacterium nucleatum*. Hexahydrocurcuminol derivatives were expected to have a better antibacterial activity than curcuminoid derivatives due to their better stability [6]. In addition, hexahydrocurcuminol derivatives were also expected to have a wider application due to the loss of the conjugated double bond which caused curcuminoid derivatives losing their colors. Therefore, it can be used as an active substance in cosmetic products, toothpaste, and mouthwash for further application.

2. Material and methods

2.1. Apparatus and materials

Turmeric (*Curcuma longa*) was obtained from Kramatjati Market, East Jakarta in August 2017. The chemicals used were purchased from Sigma Aldrich i.e. LiAlH₄ and 10 % (w/w) Pd-C while the rest were obtained from Merck i.e. *n*-hexane, ethanol, dichloromethane, acetone, diethyl ether, ethyl acetate, thin layer chromatography (TLC) plate, chloroform, methanol, dimethylsulfoxide (DMSO), sodium bromide, H₂ gas, and N₂ gas. For antibacterial activities, the chemicals used were Listerine®, *Staphylococcus aureus* (Biochemistry Laboratory, Department of Chemistry, Universitas Indonesia), *Fusobacterium nucleatum* (Microbiology Laboratory, Faculty of Dentistry, Universitas Indonesia), and nutrient broth and agar from Merck. The instruments used were general laboratory glass equipment, rotatory evaporator, Soxhlet, vacuum pump, hydrogenation equipment, hotplate, analytical scales, magnetic stirrer, Shimadzu IR Prestige 21 spectrometer, Shimadzu UV-Vis spectrophotometer, petri dish, test tube, filter paper, vortex, water bath, and TLC equipment.

2.2. Curcuminoid derivatives isolation

Dried and powdered turmeric (40 g) was wrapped in filter paper and inserted into Soxhlet apparatus with ethanol as the solvent. The Soxhlet apparatus was heated at 60 °C in an oil bath for 9 hours. The extract then was concentrated using rotatory evaporator to obtain a brownish-red curcuminoid extract. The curcuminoid extract was washed with *n*-hexane followed by dichloromethane. The curcuminoid extract then was analyzed using TLC with silica as a stationary phase and chloroform: methanol (19:1, v/v) as a mobile phase. The curcuminoid extract was characterized by using FTIR dan UV-Vis.

2.3. Reduction of curcuminoid extract using LiAlH₄

Curcuminoid extract (0.5 g) and LiAlH₄ (0.05 g) were dissolved in diethyl ether (31.25 mL). 3 h stirring at room temperature was applied to the mixture. The reaction was stopped by adding aquadest into the solution. The solution then was extracted two times to separate the organic and inorganic phase. The organic phase was collected and diluted with 0.5 M HCl to obtain neutral condition. The product, dihydrocurcuminol derivatives, then was analyzed using TLC with silica as a stationary phase and chloroform: methanol (19:1, v/v) as a mobile phase. The curcuminoid extract was characterized by using FTIR dan UV-Vis.

2.4. Hydrogenation of dihydrocurcuminol derivatives using Pd-C 10 % (w/w) catalyst

Dihydrocurcuminol derivatives (0.5 g) were dissolved in 25 mL acetone and Pd-C 10 % (w/w, 25 mg) was added into 100 mL two-necked round bottom flask in H₂ atmosphere. The mixture then was stirred and maintained in a water bath at a constant temperature of 30 °C for 2.5 hours. After the reaction was completed, Pd-C catalyst was filtered from the mixture. The product, hexahydrocurcuminol derivatives, then was analyzed using TLC with silica as a stationary phase and *n*-hexane:ethyl acetate (1:1, v/v) as a mobile phase. The product then was further characterized using FTIR and UV-Vis.
2.5. Antibacterial activity test using disc diffusion method
Antibacterial activity of curcuminoid extract and hexahydrocurcuminol derivatives were evaluated using disc diffusion method [7]. Suspension of bacteria test was poured aseptically into a petri dish followed by adding the media at temperature ± 40 °C. Petri dishes containing bacteria and test media were stirred slowly and allowed to solid. Sterile disc paper with diameter of 6 mm was put in the petri dish and each tested compound (62.5, 125, 250, 500 and 1000 ppm) was dropped into disc paper and incubated at 37 °C for 24 hours. Listerine® was used as positive control while DMSO was used as negative control.

3. Results and discussion
3.1. Curcuminoid derivatives isolation
Curcuminoid extract (3.306 g, 8.27 % yield) was obtained as brownish-red gummy after Soxhlet extraction of turmeric rhizomes followed by washing with n-hexane and dichloromethane. TLC analysis of crude extract, n-hexane-soluble, dichloromethane-soluble fractions, and curcuminoid extract (figure 1) indicated that after washed with n-hexane and dichloromethane, the spot of curcuminoid derivatives in the extract can be clearly seen.

UV-Vis and FTIR spectra of curcuminoid extract are shown in figure 2. According to figure 2a, curcuminoid extract showed an absorption peak at a maximum wavelength of 419 nm. The resulting curcuminoid extract has a theoretical conformity with the maximum wavelength of standard curcuminoid at 428 nm [8]. FTIR spectra of curcuminoid extract (figure 2b) exhibited the absorption band for hydroxyl, carbonyl, double bond aromatic, and ether groups at the wavenumber of 3296, 1639, 1512, and 1282 cm⁻¹, respectively. All functional groups in FTIR spectra are related to curcuminoid structure.

3.2. Reduction using LiAlH₄
Reaction scheme of curcuminoid extract reduction using LiAlH₄ is illustrated in figure 3. LiAlH₄ is a reductive agent that converts carbonyl group into hydroxyl group. An orange solid was obtained (0.210 g, 48 % yield) and subjected to TLC analysis with n-hexane:ethyl acetate (9:7, v/v) as a solvent and further characterized using FTIR and UV-Vis (figure 4). TLC analysis exhibited that Rf value of curcuminoid and dihydrocurcuminol derivatives were 0.625 and 0.375, respectively. The lower value of dihydrocurcuminol Rf is due to the increase of polarity.

Figure 1. TLC analysis in chloroform:methanol (19:1, v/v) of crude curcuminoid (A), n-hexane-soluble (B), dichloromethane-soluble (C), curcuminoid extract (D) visualized under UV 254 nm.
According to UV-Vis spectra (figure 4a), $\lambda_{\text{max}}$ of dihydrocurcuminol was appeared at 428 nm. Comparing with curcuminoid extract ($\lambda_{\text{max}}$ 419 nm), it indicates that there is a bathochromic shift due to the addition of hydroxyl groups as auxochrome. Therefore, it can be concluded that dihydrocurcuminol derivatives had been formed.

FTIR spectra of the compared curcuminoid and dihydrocurcuminol (figure 4b) showed the vibration peaks at wavenumber of 3446 cm$^{-1}$ from the intramolecular bond of hydroxyl group. In
addition, the increasing intensity of hydroxyl group peak compared with curcuminoid, it is estimated that more hydroxyl group will be produced. Moreover, the reduced intensity for carbonyl group at the wavenumber of 1690–1760 indicated that carbonyl group is also transformed to hydroxyl group. Based on FTIR spectra, it can be concluded that dihydroxycumurcinol had been formed.

3.3. Hydrogenation using Pd-C 10% (w/w) catalyst

The hydrogenation process is the addition reaction of double bond C=C to yield single bond C-C which illustrated in figure 5. After the hydrogenation, hexahydrocurcuminol was obtained a colorless to yellow solid (0.187 g, 91.21 % yield). Hexahydrocurcuminol derivatives then were analyzed by TLC using n-hexane:ethyl acetate (1:1, v/v) as a solvent. TLC analysis was also performed by comparing with dihydrocurcuminol. The $R_f$ value of hexahydrocurcuminol was found at 0.2 while dihydrocurcuminol showed $R_f$ value of 0.25. Based on $R_f$ value, it can be concluded that hexahydrocurcuminol is more polar compound compared to dihydro-curcuminol.

Subsequent characterization was performed using UV-Vis (figure 6a) to measure wavelength absorbed by hexahydrocurcuminol derivatives. According to Figure 6a, the maximum wavelength of hexahydrocurcuminol was 285.4 nm. Compared with dihydrocurcuminol and curcuminoid, there was a hypsochromic shift which signifies the lost of conjugate double bond. Moreover, FTIR spectra of hexahydrocurcuminol (figure 6b) was performed to identify the changes of functional groups in this reaction. Based on figure 6b, hexahydrocurcuminol derivatives were successfully formed by the marked increase of C-H sp$^3$ intensity at the wave number of 2942 cm$^{-1}$ and the reduced increase of the C-O bond of alcohol due to the loss of the conjugated double bond.

3.4. Antibacterial activity of curcuminoid extract and hexahydrocurcuminol

The antibacterial activity of curcuminoid and hexahydrocurcuminol were examined using disc diffusion method in various concentrations of 62.5, 125, 250, 500 and 1000 ppm (figure 7).
Disc diffusion method was intended to evaluate the inhibitory zone diameter of \textit{S. aureus} and \textit{F. nucleatum} growth compared to the negative control (DMSO) and positive control (Listerine®). According to figure 7, the greater concentration of the compound, the greater inhibitory zone will be produced. Hexahydrocurcuminol was found to be more effective than curcuminoid extract in terms of its antibacterial properties. It can be concluded that hexahydrocurcuminol can inhibit the development of gram-positive bacteria (\textit{S. aureus}) and gram-negative bacteria (\textit{F. nucleatum}).

4. Conclusion
Curcuminoid extract was successfully isolated from turmeric using Soxhlet extraction with 8.26 \% yield (3.306 g). Carbonyl groups on curcuminoid derivatives were successfully reduced to hydroxyl groups using LiAlH$_4$ (3 h, 30 °C) to yield dihydrocurcuminol derivatives (0.210 g, 42 \% yield). Further reduction of dihydrocurcuminol derivatives using Pd-C 10 \% (w/w) catalyst (2.5 h, 30 °C) was carried out to obtain hexahydrocurcuminol derivatives (0.187 g, 91.21 \% yield). Further separation and characterization need to be carried out to purify the compounds and establish the certain structures. Antibacterial activities of both curcuminoid and hexahydrocurcuminol derivatives were evaluated against \textit{S. aureus} with 2 and 2.5 mm, and \textit{F. nucleatum} with 4 and 2.75 mm inhibitory zone diameter, respectively. It can be concluded that the curcuminoid and hexahydrocurcuminol derivatives had a weak antibacterial activity against both bacteria tests.

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