Antibacterial activity of curcuminoid derivatives resulted from hydrogenation reaction with Pd-C catalyst from turmeric (Curcuma longa) rhizomes extract

L D Sudewo, E Saepudin and D U C Rahayu
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
Corresponding author’s email: endang.saefudin@sci.ui.ac.id

Abstract. Turmeric (Curcuma longa) is one of the most commonly used plants for traditional medicine especially for antibacterial activity. However, because of its color, it is necessary to conduct a transformation reaction that can change the color of curcuminoid into colorless but still have antibacterial activity. The aim of this research was to analyze the antibacterial activity of tetrahydrocurcuminoid, derived from hydrogenation of curcuminoid isolated from turmeric extract towards Staphylococcus aureus and Fusobacterium nucleatum. Hydrogenation reaction was carried out using H₂ gas with Pd-C catalyst to remove the conjugated double bond in curcuminoid to form tetrahydrocurcuminoid. Tetrahydrocurcuminoid then was purified by using silica gel column chromatography and characterized using UV-Vis and FTIR spectral data. Antibacterial activity of curcuminoid and tetrahydrocurcuminoid were tested against S. aureus and F. nucleatum using disc diffusion method. The antibacterial activity showed that tetrahydrocurcuminoid had a better antibacterial activity against S. aureus and F. nucleatum with average diameter of clear zone were 5.5 and 2.75 mm, respectively, than curcuminoid with 2 and 2.5 mm, respectively.

Keywords: turmeric (Curcuma longa), hydrogenation, antibacterial, Staphylococcus aureus, and Fusobacterium nucleatum

1. Introduction
The floral resources in Indonesia are estimated around 30–40 thousand species which are categorized as medicinal plants [1]. On some areas in Indonesia, turmeric is widely used as herbs or traditional medicine. Over the past two-decade, the research on curcuminoid as an active ingredient for several diseases has been conducted. Among these studies, various studies reported that curcumin contained in turmeric proved a series of health benefits such as anti-inflammatory, antitumor, anti-cancer, and anti-bacterial [2-5].

Curcuminoid is diarylheptanoid derivatives which commonly exists in turmeric as three main components, namely curcumin, demethoxycurcumin, and bis-demethoxycurcumin. Curcumin belongs to polyphenol compound class that was well-known to have antibacterial activity based on altering membrane permeability leading to a nutritional leakage in bacterial cells [6]. However, the only weakness of turmeric is the yellow dye from curcuminoid that inhibits this plant in the utilization as an
alternative for herbal mouthwash. Therefore, some modification should be carried out to treat the color of turmeric. In this research, hydrogenation reaction was carried out using Pd-C catalyst to remove double bonds on curcuminoid structures and then further purified using silica gel column chromatography. The result of purified hydrogenation reaction was tetrahydrocurcuminoid which was characterized using FTIR and UV-Vis. Evaluation of its ability to inhibit the growth of *Staphylococcus aureus* and *Fusobacterium nucleatum* was also conducted using disc diffusion method. From this research, it is expected to use tetrahydrocurcuminoid as an alternative herbal mouthwash for further application.

2. Experimental method

2.1. Materials
The materials used in isolation were turmeric (*Curcuma longa*) rhizomes, ethanol, silicon oil, n-hexane, and dichloromethane. For hydrogenation reaction, the required materials were anhydrous sodium sulfate (Na₂SO₄), nitrogen gas (N₂), hydrogen gas (H₂), Pd-C 10 % (Sigma Aldrich), and acetone. For separation and purification, the materials used were ethyl acetate, CHCl₃, methanol, and silica gel 60 (0.063–0.200 mm, 230 mesh ASTM Merck), while the chemicals used in characterization were methanol pro analysis and potassium bromide (KBr). The materials used for antibacterial activity test were aquadest, nutrient agar medium (NA) (Microbiology Nutrient Agar Nutritive, Merck), beef extract, peptone (peptone from casein pancrecially digested for microbiology, Merck), 70% alcohol, mouthwash (Listerine), dimethylsulfoxide (DMSO), denaturated alcohol, *Staphylococcus aureus* (Department of Chemistry, Universitas Indonesia), and *Fusobacterium nucleatum* (Dental Laboratory, Universitas Indonesia).

2.2. Extract preparation
Turmeric rhizomes were obtained from Fresh Market Kota Wisata (Cibubur, West Java). Fresh rhizomes were cleaned, cut into small pieces, air-dried for 7 days, and ground into powder.

2.3. Isolation of curcuminoi
Turmeric powder (40 g) was put in a porous container which is made from filter paper and stuffed with cotton, then in a 250 mL round bottom flask ethanol (260 mL) was added. The mixture was extracted using Soxhlet system at 80 °C for 8–10 h. The filtrates then were concentrated using reduced pressure using a rotatory evaporator. The extract was next separated using n-hexane and dichloromethane. The total curcuminoi portion extracted from turmeric rhizomes was identified by using TLC and analyzed by FTIR and UV-Vis.

2.4. Synthesis of tetrahydrocurcuminoids with hydrogenation reaction using Pd-C catalyst
The isolated curcuminoid (0.5 g) was dissolved in acetone (25 mL) with subsequent addition of 10 % Pd/C (25 mg) [6-7]. The mixture then was poured in the reactor and H₂ gas (1 atm) was passed in different time intervals (30, 60, 90, 120 and 150 min). Filtration was applied to the mixture, and the result was concentrated using N₂ gas to produce a crude product. The crude product was purified by using silica gel column chromatography to acquire the tetrahydrocurcuminoid. The total tetrahydrocurcuminoid was also identified by TLC and analyzed by FTIR and UV-Vis.

2.5. Anti-bacterial assay using disc diffusion method

2.5.1. Cultivation of microorganism. Bacteria cultures were grown on nutrient broth at concentration 10⁶ CFU/ml which was monitored by using UV-Vis, kept at 37 °C for 24 h, then preserved and maintained on nutrient agar slants at 37 °C before usage [8].
2.5.2. Antibacterial activity test. Samples (curcuminoid and tetrahydrocurcuminoid) were dissolved in DMSO in various concentrations of 62.5, 125, 250, 500 and 1000 ppm [9]. Sterile disc having 6 mm diameter was impregnated with 10 mL of each serial dilution of extract solution. The impregnated discs which contained various concentrations were added onto nutrient agar surface spread with 0.1 mL of bacterial culture (standardized to 0.5 McFarland standards (10^6 CFU/ml). The plates were incubated at 37 °C for 24 h. The results were registered by determining the growth inhibition zone around the discs [10]. Negative control was DMSO while positive control was standard antibacterial mouthwash (Listerine). The experiments were conducted in duplicate.

3. Results and discussion

The isolated curcuminoid was obtained as reddish-yellow gummy. TLC was used to determine the content of each component of curcuminoid before and after addition of n-hexane and dichloromethane. The results of TLC are summarized in table 1. The \( R_f \) value intense spot obtained was corresponded to \( R_f \) value of the reported curcumin [11]. Moreover, the other spots were observed as demethoxycurcumin and bis-demethoxycurcumin analyzed by a functional group of each structure. The chemical structure of curcuminoid is illustrated in figure 1 which gives an idea about various functional groups presents the compound.

|              | Before | After |
|--------------|--------|-------|
| Bis-demethoxycurcumin | 0.57   | 0.57  |
| Demethoxycurcumin     | 0.67   | 0.67  |
| Curcumin (intense spot) | 0.80   | 0.82  |

**Figure 1.** Chemical structure of curcuminoid.

**Figure 2.** (a) FTIR spectrum and (b) the UV-Vis spectrum of isolated curcuminoid.
Identification of isolated curcuminoid was further confirmed by FTIR spectral data (figure 2a). FTIR spectrum of curcuminoid showed a unique stretching band of O-H at 3296 cm\(^{-1}\). Furthermore, the peak at 1600 cm\(^{-1}\) was assigned to C=C symmetric aromatic ring stretching while the peak at 1512 cm\(^{-1}\) and 1282 cm\(^{-1}\) represent C=O and enol C-O, respectively. The FTIR spectrum of the curcuminoid showed a similarity with FTIR spectrum reported in previous research [11]. The isolated curcuminoid was also analyzed by using UV-Vis (figure 2b) and showed similarity with UV-Vis spectrum reported in the previous study [12]. The high absorbance caused by the existence of the conjugated bonds in the form of double bonds intermittent with a single bond this also affects the yellow color of the compound of curcuminoid, where later electron \(\phi^*\) will be delocalized advanced so that the energy level of \(\phi^*\) decreases and reduces anti-bond [13].

Utilizing the curcuminoid as a substrate for hydrogenation reaction was carried out to perform a reaction with an accompanying change in color (figure 3). The result of the hydrogenation reaction in different interval time reaction is represented in table 2. According to table 2, it can be seen that there was another spot corresponded to tetrahydrocurcuminoid which was slightly higher than curcuminoid spot. However, hydrogenation reaction results did not eliminate the yellow color completely. The result of the hydrogenation reaction after 150 min was still remained yellow. This indicates that the results of the hydrogenation reaction of curcuminoid were not all converted to tetrahydrocurcuminoid. Data of the TLC analysis indicated that tetrahydrocurcuminoid has been formed after 90 min of hydrogenation, evidenced by the presence of the new spot above the curcuminoid spots. It can be concluded that the minimum time required for producing tetrahydrocurcuminoid was within 90 min. The results from hydrogenation were also analyzed by FTIR and UV-Vis spectral data (figure 4).

FTIR spectrum (figure 4a) supported UV-Vis spectrum (figure 4b) which both spectrum showed differences in 90 min of reaction. According to figure 4(a), in 30 and 60 min no peak at 2900 cm\(^{-1}\) which correspond to C-H sp\(^3\) resulted from the loss of double bonds on curcuminoid (figure 3).

![Figure 3. Hydrogenation reduction reaction of curcuminoid into tetrahydrocurcuminoid.](image)

| Time Interval | 30 min | 60 min | 90 min | 120 min | 150 min |
|---------------|--------|--------|--------|---------|---------|
| Change of color | Intense reddish brown | Intense dark red | Clear reddish orange | Clear orange | Clear orange slightly yellow |
| TLC Analysis (n-hexane: EtOAc = 1:1) under UV 254 nm | ![Image](image) | ![Image](image) | ![Image](image) | ![Image](image) | ![Image](image) |

![Table 2. Comparison of time and TLC analysis results.](image)
Figure 4. (a) FTIR spectrum and (b) UV-Vis spectrum of tetrahydrocurcuminoid.

had been formed. Furthermore, based on figure 4b, the 30 and 60 min of reaction resulted UV-Vis spectrum there was still a similar peak to curcuminoid 418 nm (figure 2b) while the 90, 120 and 150 min reaction it can be seen that there was a shift to a lower wavelength of 240–280 nm. This UV-Vis spectral data was correlated with TLC analysis (table 2) that the tetrahydrocurcuminoid begins to form at 90 min reaction time and the changes continue to increase within the time. Subsequently, separation using silica gel column chromatography with n-hexane:EtOAc (8:2) was conducted to separate tetrahydrocurcuminoid with the unreacted curcuminoid.

FTIR spectrum (figure 4a) supported UV-Vis spectrum (figure 4b) which both spectrum showed differences in 90 min of reaction. According to figure 4a, in 30 and 60 min no peak at 2900 cm\(^{-1}\) which correspond to C-H sp\(^3\) resulted from the loss of double bonds on curcuminoid (figure 3) had been formed. Furthermore, based on figure 4b, the 30 and 60 min of reaction resulted UV-Vis spectrum there was still a similar peak to curcuminoid 418 nm (figure 2b) while the 90, 120 and 150 min reaction it can be seen that there was a shift to a lower wavelength of 240–280 nm. This UV-Vis spectral data was correlated with TLC analysis (table 2) that the tetrahydrocurcuminoid begins to form at 90 min reaction time and the changes continue to increase within the time. Subsequently, separation using silica gel column chromatography with n-hexane:EtOAc (8:2) was conducted to separate tetrahydrocurcuminoid with the unreacted curcuminoid.

The results of column chromatography yielded 50 fractions. Among them, fraction 26–41 showed tetrahydrocurcuminoid spot when identified using TLC. Therefore, further characterization was conducted by using FTIR and UV-Vis (figure 5). According to figure 5a, FTIR spectrum showed that tetrahydrocurcuminoid after separation had more visibility of CH-sp\(^3\) peak at 2900 cm\(^{-1}\) due to the absence of curcuminoid. This result was also strengthened with UV-Vis spectrum (figure 5b). It can be concluded that the separation using the column chromatography can purify tetrahydro-curcuminoid from the mixture of unreacted curcuminoid.

Antibacterial activities of both curcuminoid and tetrahydrocurcuminoid were evaluated against *S. aureus* and *F. nucleatum* by using disc diffusion method (table 3). According to table 3, tetrahydrocurcuminoid had a larger mean inhibitory zone diameter than curcuminoid towards *S. aureus* and *F. nucleatum*. The best antibacterial activity effect was shown by tetrahydrocurcumin at 1000 ppm against *S. Aureus* (5.5 mm). Tetrahydrocurcuminoid was one of the curcuminoid derivatives which exhibited more nonpolar than curcuminoid. Because it was influenced by the loss of double bonds contained in the compound, so it can be more easily used in the use of drugs for human,
Figure 5. FTIR spectrum of tetrahydrocurcuminoid (a) before and (b) after separation.

Table 3. Average measurement of inhibitory zone of curcuminoid and tetrahydrocurcuminoid towards \textit{S. aureus} and \textit{F. nucleatum} together with its classification$^a$.

|          | \multicolumn{2}{c|}{\textit{S. aureus}} | \multicolumn{2}{c}{\textit{F. nucleatum}} |
|----------|----------------|----------------|----------------|----------------|
|          | Tetrahydro- | Curcuminoid | Tetrahydro- | Curcuminoid |
| ppm      | curcuminoid |            | curcuminoid  |            |
| 1000 ppm | 5.5 (M)     | 2 (W)       | 2.75(W)      | 2.5(W)       |
| 500 ppm  | 3.25(W)     | 1.5(W)      | 2.25(W)      | 1.5(W)       |
| 250 ppm  | 2.25(W)     | 1.0(W)      | 1.75(W)      | 1.0(W)       |
| 125 ppm  | 1.75(W)     | 0.5(W)      | 1.25(W)      | 0.8(W)       |
| 62.5 ppm | 0.5(W)      | 0.3(W)      | 0.75(W)      | 0            |
| Listerine (+) | 6     | 0.5 | 4.5 | 0.5 |
| DMSO (-) | 0 | 0 | 0 | 0 |

$^a$Classification of clear zone [13-14]: > 20 mm (SS: super strong), 10–20 mm (S: strong), 5–10 mm (M: medium), and < 5 mm (W: weak)

the solubility of organic compounds in fat relates to the easy or not to penetrate the cell membrane. Non-polar compounds are fat-soluble, having a large fat/water partition coefficient value that easily penetrates cell membranes in a passive diffusion [14-15]. Supported by the loss of yellow dye from the compound and leaving no marks or stains that are detrimental to humans, tetrahydrocurcuminoid can be explored as alternative herbal mouthwash for further application.

4. Conclusion
Tetrahydrocurcuminoid can be produced through the hydrogenation reaction step using Pd-C catalyst in which the compound was separated by using column chromatography. Tetrahydrocurcuminoid showed medium and weak antibacterial activity towards \textit{S. aureus} and \textit{F. nucleatum}, respectively, at 1000 ppm with the average diameter of clear zone were 5.5 and 2.75 mm, respectively. This study
found that tetrahydrocurcuminoid synthesized from curcuminoid showed a better antibacterial activity than curcuminoid which can be used as an alternative herbal mouthwash for further application.

Acknowledgments
This work was financially supported by Universitas Indonesia under research grant PITTA 2018 with grant contract number 2265/UN2.R3.1/HKP.05.00/2018.

References
[1] Wijayakusuma M H 2007 Penyembuhan dengan Temulawak (Jakarta: Sarana Pustaka Prima)
[2] Fadus M C, Lau C, Bikhchandani J and Lynch H T 2016 J. Tradit. Complement. Med. 7 1-8
[3] Sirohi V K, Popli P, Sankhwar P, Kaushal J B, Gupta K, Manohar M and Dwivedi A 2017 J. Nutr. Biochem. 44 60-70
[4] Aggarwal B B, Kumar A and Bharti A C 2003 Anticancer Res. 23 363-98
[5] Pangemanan A, Fatimawali and Budiarso F 2016 EBIOMEDIK 4 81-5
[6] Oomah B D and Mazza G 1997 LWT – Food Sci. Technol. 30 135-40
[7] Singh R P and Jain D A 2012 J. Pharm. Res. 3 650-3
[8] Hudzicki J 2016 MBio 1-23
[9] Chairandy C M, Seaforth C, Phelps R H, Pollard B P S and Khambey 1999 J. Ethnopharmacol. 64 265-70
[10] Teow S Y, Liew K, Ali S A, Khoo A S B and Peh S C 2016 J. Trop. Med. 1-10
[11] Pawar H, Karde M, Mundle N, Jadhav P and Mehra K 2014 J. Med. Chem. 4 588-91
[12] Waranyoupalin R, Wongnawa S, Wongnawa M, Pakawatchai C, Panichayupakaranant P and Sherdshoongpase P 2009 Cent. Eur. J. Chem. 7 388-94
[13] Paiva P M G, Pontual E V, Coelho L C B B and Napoleao T H 2013 FORMATEX 641-9
[14] Bauer A W, Kirby W M M, Sherris J C and Turk M 1966 Am. J. Clin. Pathol. 45 493-6
[15] Davis W W and Stout T R 1971 Appl. Microbiol. 22 659-65