Activity of hydrogenated curcuminoid on Pd/C catalyst and its antibacterial activity against *Staphylococcus aureus* and *Streptococcus mutans*

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Abstract. Turmeric (*Curcuma longa*) has been known for its benefit as one of medicinal herbs. Curcuminoids, the active compounds in turmeric, consist of three structurally related compounds, curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcuminoid has been reported to be effective for the treatment of chronic gingivitis and hence it can be a potential candidate to be used as an active ingredient for antimicrobial in a mouthwash. However, its yellow color has hampered its application as a mouthwash as it may cause the aesthetical problem to the teeth. In this study, curcuminoids, obtained from Soxhlet extraction of turmeric powder, were reduced by hydrogenation on Pd/C catalyst to remove the color. TLC analysis revealed that the products consist of three compounds as expected. The product was purified from unreacted curcuminoids by column chromatography on silica gel, to obtain a colorless product with percent conversion of 23.45%. These three compounds were characterized by Uv-Vis spectrophotometer resulted in three peaks in three different wavelengths: 282, 283 and 280 nm. Characterization with FT-IR spectrometer showed an increase intensity of CH-sp3 peak (2932 cm⁻¹) when compared to the IR spectrum of standard curcuminoids, and the shift in C-O absorption (1231 cm⁻¹), indicated the existence of methoxy groups that can distinguish one to the other compounds. These three compounds then were evaluated for their antibacterial activity against *Staphylococcus aureus* and *Streptococcus mutans*. The result showed that the tetrahydrocurcumin had moderate antibacterial activity against *S. aureus* with inhibition zone of 5.5 mm and tetrahydro desmethoxycurcumin also showed moderate antibacterial activity against *S. mutans* with inhibition zone of 5.5 mm.

Keywords: Antibacterial, curcuminoid, hydrogenation, *Staphylococcus aureus*, *Streptococcus mutans*

1. Introduction
Turmeric plant (*curcuma longa*) is a tropical plant that has been long used as spice and dye, both as skin dye and clothes dye. Turmeric is believed to be originated from India and India is still the largest producer of turmeric in the world. In the year of 800 AD, turmeric plants have spread to almost all regions in Asia, including China and Africa [1].

The nature of the turmeric plant that has healing properties is known because of the curcuminoid content contained in turmeric. Various studies have been conducted over the past two decades which...
are related to the benefits of curcuminoid [2, 3]. It is known that curcuminoids have anti-inflammatory properties [4, 5], antioxidants [6, 7], antiprotozoal [8, 3] and antibacterial [9].

Maintaining the health of the oral cavity is very important, because the oral cavity is the "entrance" of various kinds of bacteria that can stay in the oral cavity. One of the diseases of the oral cavity caused by bacteria is dental caries. Caries is a disease of the hard part of the tooth where this part is demineralized by the activity of microorganisms in a carbohydrate that can be distributed. If left unchecked, it will cause total damage to the affected tooth [10, 11].

Curcuminoid contained in turmeric plant with antibacterial properties has been shown can be used as a mouthwash. Curcumin is comparable to chlorhexidine as an anti-inflammatory mouthwash [12]. However, the yellow color of curcuminoid compounds in the turmeric plant has hampered their use as they may damage the aesthetic of the teeth if used as a mouthwash. The yellow color of curcuminoid is known due to the presence of double bonds conjugated with the carbonyl moiety (figure 1). Thus, removing the double bonds would also remove the color. This research aimed to remove the color of curcuminoid by hydrogenation on Pd/C as a catalyst. The antibacterial activity of hydrogenated curcuminoids (tetrahydrocurcuminoids) was evaluated using disc diffusion method. The inhibition diameter of bacterial growth in media containing tetrahydrocurcuminoids was compared with that of curcumin extract.

2. Materials and method

2.1. Materials and apparatus

Turmeric rhizomes (Curcuma longa) were collected from local market, ethanol, n-hexane, dichloromethane, nitrogen gas, hydrogen gas, Pd / C catalyst 10 % (Sigma Aldrich), acetone, ethyl acetate, chloroform, methanol, silica gel 60 (E. Merck), TLC plate, KBr, Listerine, DMSO, Streptococcus mutans strains (Faculty of Dentistry of Universitas Indonesia), Staphylococcus aureus strains (Department of Chemistry, Universitas Indonesia), beef extract, nutrient broth and nutrient agar. The instruments used were UV-VIS and FT-IR spectrophotometers.

2.2. Extraction of curcuminoid

Dried and powdered turmeric rhizomes was extracted by Soxhlet extraction method using ethanol as solvent for 8 h. The solvent was evaporated using a rotatory evaporator resulting in red brown extract of curcuminoids. The extract was washed with n-hexane and dichloromethane. The remaining solid was analyzed using TLC with chloroform:methanol (19:1) as a mobile phase, and characterized with Uv-Vis and FT-IR spectrophotometers.

2.3. Hydrogenation of curcuminoid

Curcuminoid extract (0.5 g) was placed in a two-neck flask fitted with a balloon on one neck and a three-way stopper on the other neck. Pd/C 10 % (25 mg) and dry acetone (25 mL) were added into the flask. Prior to the addition of hydrogen gas, a slow stream of nitrogen gas was passed into the flask to remove any gas and moisture in the flask, then hydrogen gas was added into the flask until the balloon inflated to about half size of its maximum size. The mixture was stirred at 30 °C for 210 min until the orange color turned to very pale yellow. The formation of the product was monitored by TLC, and the product was characterized using Uv-Vis and FT-IR.

2.4. Separation of tetrahydrocurcuminoids from curcuminoids

Tetrahydrocurcuminoids were separated from curcuminoids by column chromatography on silica gel eluted with n-hexane: ethyl acetate (6:4) isocratically. The fractions were collected in 10 mL vial bottles and monitored by TLC. Fractions that have the same spot were combined and characterized using Uv-Vis and FT-IR.
2.5. Purification of tetrahydrocurcuminoid derivatives
Tetrahydrocurcuminoids were purified into each of its derivatives using column chromatography on silica gel. The column was eluted isocratically with the mixture of chloroform: methanol: n-hexane (19:1:7.5). The output was collected in 10 mL vial bottles and analyzed with TLC method. Fractions that have the same spot were combined and characterized using Uv-Vis and FT-IR.

2.6. Antibacterial activity test using disc diffusion method
A suspension of diluted bacteria (200 μL) was dropped into a petri dish using a micropipette with a sterilized tip. Into the petri dish, warm and liquid nutrient agar (20 mL) was added. The NA medium was allowed to solidify. A sterile disc paper (6 mm diameter) was dipped into the test solution. The test solutions were curcuminoid extract and tetrahydrocurcuminoids. The concentrations were varied from 62.5; 125; 250 and 500 ppm in the DMSO. The disc papers placed on 1 petri dish were 6 pieces consist of positive and negative controls as well as solutions of curcuminoid extract and tetrahydrocurcuminoids. Listerine was used as a positive control. DMSO solution was used as a negative control. The petri dish was then incubated for 24 h in the incubator at 37 °C. The clear zones of each disc paper were measured.

3. Results and discussion
3.1. Isolation of curcuminoids
TLC analysis on the curcuminoi d extract using chloroform: methanol (19:1) eluent showed that curcuminoid compounds were separated into three different compounds, they are curcumin, demethoxycurcumin, and bis-demethoxycurcumin. The Rf value of each component is shown in table 1.

The data in table 1 shows that the three compounds have a noticeable difference in polarity. Having two methyl groups attached to the phenolic groups, curcumin shows the highest Rf value, hence it is the most non-polar. On the other hand, both demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) show lower Rf values, which can be correlated to the absence of methoxy groups. The less the methoxy group, the higher the polarity. Therefore, the nonpolar nature of curcumin is influenced
by its structure which has two methoxy groups on its side chain, preventing the phenol groups to lose their proton when ionized. Thus, the methoxy groups can hold the proton through hydrogen bonding, keeping the proton stays closer to its phenoxy group and can easily rejoin (figure 2).

Uv-Vis spectrum of curcuminoid extract in methanol (figure 3) showed that curcuminoid extract has two strong absorption bands, one in the UV region with maximum wavelength at 265 nm, and the other in the visible region at 418 nm. The absorption peak, presumably, is caused by the presence of electron dipoles which can form the excitation type $\pi \rightarrow \pi^*$ of the conjugate $\pi$ system.

In the IR spectrum results (figure 4), a strong and broad peak at the wave number 3310 cm$^{-1}$ indicates the presence of stretch vibration from the O-H bond. The absorption peak at the wave number 1627 cm$^{-1}$ shows the presence of C=O bond and the peak at 1513 cm$^{-1}$ originates from the stretch vibration of conjugated double bond of benzene, while the peak at 1281 cm$^{-1}$ indicates the presence of CO enol bond.

![Figure 2. A scheme representing the polarity of curcumin and bis-demethoxycurcumin.](image2)

![Figure 3. Uv-Vis spectrum of curcuminoid extract.](image3)
3.2. Hydrogenation of curcuminoids

The reduction of curcuminoid by hydrogenation was achieved with % conversion of 71.84 %. The yellow orangish color of the curcuminoids solution has mostly gone indicating the loss of aliphatic double bonds. TLC analysis shows that three new spots appear above the weak spots of curcuminoids.

Figure 5 shows that most of the curcuminoids with the absorption peak at 418 nm has disappeared and shifted to 281 nm, indicating the loss of the aliphatic double bond in tetrahydrocurcuminoid derivatives.

The FT-IR spectrum confirms the formation of tetrahydrocurcuminoids with the appearance of a new absorption peak at 2932 cm⁻¹ indicating the presence of C-H sp3 (figure 6).
3.3. **Separation of tetrahydrocurcuminoids from curcuminoids**

The separation was done by column chromatography on silica gel with a mixture of 6:4 ratio of solvent of n-hexane: ethyl acetate and the polarity were increased to 5:5. The separation was monitored by TLC. Using this method, all of the unreacted curcuminoid can be removed resulting the colorless solution of tetrahydro curcuminoids. In the Uv-Vis spectrum (figure 7), after separation, there is an absorption peak at the same wavelength, which is at 281 nm but has a greater intensity.

3.4. **Purification of tetrahydrocurcuminoid derivatives**

The colorless solution of THC was concentrated in a vacuum evaporator and loaded back to column chromatography on silica gel and eluted with 8:2 solvent ration of hexane: ethyl acetate followed by gradient increased polarity. TLC analysis clearly showed a single spot of each compound with different Rf values indicating that the three curcuminoids have been separated. Tetrahydro curcumin (THC) was first appeared at fraction F5, followed by tetrahydro demethoxycurcumin (THDC) at F7 and then tetrahydro bis-demethoxy curcumin (THBDC) at F10.

![Figure 6. FT-IR Spectrum of curcuminoid extract and tetrahydrocurcuminoids.](image)

![Figure 7. Uv-Vis (spectra before and after separation left) and spectra of THC, THDC and THBDC.](image)
Figure 7 shows that the three tetrahydrocurcuminoids have almost identical absorption peaks but slightly different maximum wavelength. THC shows a maximum wavelength at 282 nm, the THDC at 283 nm, and the THBDC at a wavelength of 280 nm. This small difference in absorption peaks clearly shows that the methoxy groups attached to the benzene rings have almost no contribution to the absorption peak.

The absorption pattern of IR spectra of the three compounds looked almost identical (figure 8). The only difference was found at wavenumber 1231 cm⁻¹ (asymmetric C-O-C stretch). Both THC and THDC appear to have this peak, where this peak is missing in THBDC spectrum confirming the absence of methoxy groups in THBDC.

3.5. Antibacterial activity test

Figure 9 shows comparative studies among the activities of the test concentrations to the growth of bacteria tested. The data show that the higher the concentration of the test solution, the higher the diameter of the clear zone which means the greater inhibition to the test bacteria. These data revealed that curcuminoids showed moderate activity against *Streptococcus mutans* and low activity...
against *Staphylococcus aureus*. Their tetrahydro derivatives showed interesting results. All tetrahydro curcuminoids, tetrahydro curcumin (THC), tetrahydro demethoxycurcumin (THDC) and tetrahydro bis-demethoxycurcumin (THBDC) showed higher activities against *S. aureus* compared to curcuminoid extract, where THC showed the highest activity. On the other hand, the activity against *S. mutans* showed mix results. At concentration 125 ppm, their activities are comparable to curcuminoid extract. However, at the concentration of 250 ppm or higher, the activity of THDC and THBDC appeared higher than that of curcuminoids. This finding implies that the loss of double bonds in curcuminoids increases its antibacterial activity, particularly against *S. aureus*.

Among these test compounds, THDC shows the highest activity against *S. mutans*, while against *S. aureus*, THC has the greatest activity.

4. Conclusion

The reduction of curcuminoids was successfully carried out by hydrogenation reaction on 10 % Pd/C catalyst for 210 min with percent conversion of 71.84 %. All three tetrahydrocurcuminoid derivatives showed the better antibacterial activities than the curcuminoid extract.

Tetrahydrourcumin compounds (500 ppm) have moderate levels of antibacterial activity in *Staphylococcus aureus* with inhibition zone of 5.5 mm, while tetrahydrodemethoxycurcumin (500 ppm) has moderate levels of antibacterial activity on *Streptococcus mutans* with inhibition zone of 5.5 mm. Loss of double bonds in curcuminoids increases the antibacterial activity of curcuminoids, therefore tetrahydrocurcuminoids can be a potential candidate for a saver antibacterial agent in mouthwash solution.

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