Acetylation of curcuminoids extract from Turmeric Rhizomes (Curcuma longa) as antibacterial compounds against S. aureus and E. coli

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Abstract. Curcuminoids are active compounds of turmeric rhizome that widely known to have antibacterial activities. The low activity of curcuminoid as antimicrobial may be due to the presence of phenol moiety and hence it has low lipophilicity and low bioavailability. Antibacterial activities of curcuminoid derivatives may be improved by increasing their lipophilicity, one of which is replacing the hydrogen atom on the phenol groups with acetyl group by acetylation. The curcuminoids were extracted from turmeric rhizomes by Soxhlet method yielded of 10.24 %. This curcuminoids were structurally modified by acetylation using acetic anhydride with Ni/SiO₂ catalyst. The products were separated through column chromatography then characterized using thin layer chromatography (TLC), UV-Vis, FTIR and LC-MS spectrometers. The results showed that the best condition of this reaction found when 15 % (w/w) catalyst was used with product conversion of 90.44 %. Diameter of inhibitory zone of acetyl curcuminoid derivatives showed the highest antibacterial activity at a concentration of 500 ppm against S. aureus of 18 mm and against E. coli of 13 mm. On the other hand, at the same concentration, the curcuminoid had inhibitory zone of 7.5 mm and 8 mm against S. aureus and E. coli, respectively. Therefore, the acetylation of curcuminoids has increased their antibacterial activities against Gram positive bacteria S. aureus up to 2.4 fold, while against Gram negative bacteria E. coli by only 1.6 fold.

Keywords: Curcuminoid, acetylation, Ni/SiO₂ catalyst, antibacterial, Staphylococcus aureus, Escherichia coli

1. Introduction
Curcumin with the chemical formula of ((E,E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the main yellow compound of phytophenol isolated from Curcuma sp. [1, 2]. Curcumin in nature is found together with two other compounds, demethoxycurcumin and bis-demethoxycurcumin, known as curcuminoids. Curcumin has pharmacological activities as antibacterial, anti-inflammatory, antioxidant, antiprotozoal, antitumor, the immune system enhancer (immunomodulators), neuroprotective and antiaging [3, 4].

Infectious diseases such as respiratory tract and diarrhea or disorders are one of the biggest causes of death in developing countries such as Indonesia, caused by pathogenic microorganisms [5]. Common treatments for infectious diseases are the use of antibiotics. However, irrational use of antibiotics can
make pathogenic microbes become resistant and develop a major cause of infection [6]. The increasing number of bacterial resistances to antibiotics today, has triggered the need to find antibacterial that are more effective. Curcumin has been shown to have antibacterial activities against *S. aureus* with MIC value of 187.5 μg/mL [7], against *E. coli* with MIC values of 250 μg/mL [8]. These low antibacterial activities may be due to the presence of the phenol moiety which cause it difficult to be transported across the membrane due to its low lipophilicity and therefore has a low bioavailability. Converting the phenol group to its less polar derivatives, mono-O-acetylcurcumin and di-O-acetylcurcumin, using pyridine catalyst proved to increase curcuminoid antimicrobial activities against *M. tuberculosis* with MIC values of 50 and 100 μg/mL, respectively [9]. Here, we report the modification of curcuminoids by acetylation using a heterogeneous catalyst, Ni/SiO2. As heterogeneous catalyst, Ni/SiO2 has many advantages over homogeneous catalysts commonly used for esterification of hydroxy groups. It is environmentally friendly, economical, easy to be separated, reusable and avoid the use of high temperatures to minimize thermal risk [10].

2. **Materials and method**

2.1. **Materials and apparatus**

Turmeric (*Curcuma longa*) was obtained from a traditional local Market in Depok, West Java, Indonesia. n-hexane, ethanol, dichloromethane, acetone, diethyl ether, ethyl acetate, Ni(NO3)2.6H2O, silica gel and DMSO were purchased from E. Merck Indonesia, amoxicillin was a gift from Faculty of Pharmacy, UI, *Staphylococcus aureus*, *Escherichia coli* (from Biochemistry Laboratory, Department of Chemistry UI). Nutrient broth and agar were purchased from Sigma. The instruments used were FT-IR spectrometer Shimadzu IR Prestige21, UV-VIS spectrophotometer Shimadzu UV-2450 and LC/MS/MS ABSCIEX 3200 Q TRAP.

2.2. **Preparation of Ni/SiO2 catalysts**

Nickel nitrate hexahydrate (2.51 g) was dissolved in distilled water (20.0 mL) and silica gel (5.0 g) was added and stirred for 2 h using a magnetic stirrer at room temperature. The mixture was left to stand at room temperature overnight. The excess water was removed by heating the mixture on an oil bath at 100 °C and using a rotary evaporator under vacuum to evaporate the water. The catalyst material was dried in an oven at 100–120 °C for 12 h.

2.3. **Curcuminoids isolation**

Dried powdered of turmeric (40 g) was wrapped in a filter paper and placed into the Soxhlet apparatus with ethanol as a solvent. The Soxhlet was heated on an oil bath for 9 h. The extract was then concentrated in the rotatory evaporator to obtain a brownish red curcuminoid extract. The curcuminoid extract was washed with n-hexane followed by dichloromethane. The curcuminoid derivatives then were characterized using TLC on silica plate and eluted with chloroform: methanol (19:1). The curcuminoid derivatives were then analyzed by using FTIR dan UV-Vis.

2.4. **Acetylation of curcuminoids**

The curcuminoid (0.5 g) was placed in a two-neck round bottom flask. Acetic anhydride (6.5 mmol) was added to the flask, then Ni/SiO2 catalyst was added with varying amounts of catalyst. A condenser was attached to the flask and the reaction mixture was stirred with a magnetic stirrer at 70 °C for 5 h. The reaction was checked every hour using TLC to see if all the starting materials have been converted to their acetyl derivatives. Upon completion, the mixture was transferred into a separatory funnel and extracted with ethyl acetate twice and the water phase was discarded. The organic phase was washed with distilled water three times. The solution was placed in a round bottom flask and the remaining water was removed with the addition of anhydrous Na2SO4. The solution was filtered and the solvent was removed using a rotary evaporator.
2.5. Separation with column chromatography
Acetyl curcuminoids were separated from the unreacted curcuminoids using column chromatography on silica gel and eluted with n-hexane: ethyl acetate (8:2). The fractions were collected in 5 mL vials. Each fraction was monitored by TLC analysis and visualized under UV light at 366 nm.

2.6. Antibacterial activity test
Antibacterial activity of curcuminoid extract and their acetyl derivatives were evaluated using disc diffusion methods. The suspension of test bacteria was transferred aseptically into a petri dish. Warm liquid medium (± 40 °C) was poured into the petri dishes. Petri dishes containing bacteria and media were stirred slowly, so the bacteria can diffuse evenly. The media was then allowed to solidify. Sterile disc papers (6 mm diameter) was placed on the surface of the media. Each compound to be tested (31.25, 62.5, 125, 250 and 500 ppm) was dropped into the disc paper. The petri dish and its contents were incubated for 24 h in an incubator at 37 °C.

3. Results and discussion
3.1. Isolation of curcuminoids from Turmeric Rhizomes
Turmeric rhizome (8 kg), after chopping, grinding, and drying, resulted only 364 g of turmeric powder. The dried turmeric powder was then extracted using the Soxhlet extraction method. The initial extract result was obtained in dark red. After 9 h, the extraction was stopped, and the extract was concentrated in rotary evaporator followed by washing with a mixture of hexane and dichloromethane to remove unwanted materials. Curcuminoids extract was obtained as much as 20.487 g with a yield % of 10.24 % out of 200 g of turmeric powder.

3.2. Characterization of curcuminoids
The curcuminoid extract was characterized by TLC analysis. Eluent used was chloroform: methanol (19: 1) visualized under UV light at $\lambda = 254$ nm. The TLC result of the curcuminoid extract showed 3 major compounds, which is consistence with the one that has been reported elsewhere [3, 4, 9] (consisting of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

FTIR spectrum shows a broad peak around 3300 cm$^{-1}$ which corresponds to the stretch vibration of hydroxyl groups (figure 1). The carbonyl, ethylene, and conjugated aromatic double bond showed peaks at 1626, 1587 and 1513 cm$^{-1}$, respectively. Other absorption bands show the vibration of the C-O-C ether bond at 1032 cm$^{-1}$ and an enol C-O bond absorption occurs at a wave number of 1283 cm$^{-1}$, which

![Figure 1](a) FTIR and (b) UV spectra of curcuminoids.
are in accordance with the structure of curcuminoids. In the UV-Vis spectrophotometer the absorption peak is shown at a maximum wavelength of 425 nm.

3.3. Acetylation of Curcuminoids with Ni/SiO$_2$ catalyst

Ni/SiO$_2$ was chosen as a heterogenous catalyst in the acetylation reaction of curcuminoids for its advantages in catalyst separation, which is easier, environmentally friendly, economical, reusable and to avoid the use of high temperatures to minimize thermal risk. In addition, the operational procedures performed is simple, safe and energy efficient (figure 2). The Ni/SiO$_2$ catalyst, apparently, plays a major role to maximize the activation of acetic anhydride.

According to the mechanism, the formation of Ni-acylium ion was facilitated by the hydroxyl group presents on silica surface [10]. Presumably, the reaction proceeds via an acyl-oxygen cleavage followed by nucleophilic attack of the hydroxyl group of phenol moiety leading to formation of the product (figure 3).

![Figure 2. Reaction scheme of curcuminoids acetylation.](image)

![Figure 3. The proposed mechanism of acetylation.](image)
TLC analysis on silica plate with eluent of dichloromethane : methanol (98:2), viewed under UV light at $\lambda = 366$ nm, showed that after 5 h of reaction, almost all the starting materials have been converted to their acetyl derivatives indicating by their greater $r_f$ values (figure 4). After the reaction has completed, the solid catalyst was separated by filtration. Water was added to the solution and the product was extracted with ethyl acetate.

When the amount of catalyst was varied at 5, 10, 15 and 20 %, the 15 % of catalyst produced the highest product conversion as much as 90.44 %.

The FTIR spectrum of acetylation reaction results showed that the hydroxyl group of curcuminoid at wave number regions of 3200–3600 cm$^{-1}$ have disappeared compared to the curcuminoid spectrum, indicating that the acetylation of curcuminoid in the present of Ni/SiO$_2$ catalyst has successfully replaced the hydroxyl groups of curcuminoid side chain (figure 4).

The UV-Vis spectrum of the products with variations in the amount of catalyst of 5 %, 10 % and 15 % showed a shift to the lower maximum wavelength when compared to the starting material (curcuminoid extract with a maximum wavelength of 425 nm) (figure 5). The spectrum of the product of 15 % catalyst showed the highest intensity and the least noticeable shoulder peak at 425 nm, attributable to the highest conversion of the reaction.

![Figure 4](image1.png)

**Figure 4.** The analysis results of (a) TLC and (b) FTIR spectra on acetylated reaction products with varying amounts of catalyst (c) the two IR spectra showing the disappearance of the hydroxyl group after acetylation.

![Figure 5](image2.png)

**Figure 5.** The UV-Vis spectra of curcuminoid acetylation with varying amount of catalysts.
Table 1. Inhibitory zone of acetyl curcumin and curcuminoids against S. aureus and E. coli.

| Concentration (ppm) | Acetyl curcumin | Curcuminoids |
|---------------------|-----------------|--------------|
|                     | Average inhibitory Zone (mm) | S. aureus | E. coli | S. aureus | E. coli |
| 31.25               | 7.5             | 6            | 6        | 6         | 6       |
| 62.5                | 9.5             | 6            | 6        | 6         | 6       |
| 125                 | 12.5            | 6            | 6.5      | 6         |         |
| 250                 | 15.5            | 8            | 7        | 7.5       |         |
| 500                 | 18              | 13           | 7.5      | 8         |         |

3.4. Purification using column chromatography
The product of acetylation reaction was purified by column chromatography on silica gel to remove the unreacted starting material. The column was eluted isocratically with the mixture of solvent [n-hexane: ethyl acetate (8:2).

3.5. Antibacterial activity test
Disk diffusion method was used to test the antibacterial activity of curcuminoid and acetyl curcumin. Various concentrations of 31.25; 62.5; 125; 250 and 500 ppm were used to see the inhibition of the compounds to the growth of S. aureus and E. coli by measuring the diameter of the clear zone. The results of the test showed that diacetyl curcumin exhibited a higher inhibitory zone than that of curcuminoids both on E. coli and S. aureus, where S. aureus experienced higher inhibition compared to the growth of E. coli.

Although it is premature to generalize this statement, our finding (table 1) showed that the diacetyl curcuminoid seems to have a greater effect on Gram positive bacteria (S. aureus) than that of Gram negative bacteria (E. coli). In all concentrations, diacetyl curcuminoid exhibited linear inhibitory zones on S. aureus, the higher the concentration, the greater the inhibitory zones, while on E. coli, significant increase of inhibitory zones was only noticeable at the concentration of 250 ppm or higher. The data on table 1 also tell us that the acetylation of curcuminoids has increased the antibacterial activity of the compounds. At 500 ppm, the increase was 2.4 fold against S. aureus and 1.6 fold against E. coli.

4. Conclusion
Acetylation of curcuminoids using Ni/SiO₂ catalyst was successfully carried out to produce acetyl curcumin with optimum conditions at 15 % (wt.) catalyst with conversion of 90.44 %. Acetylation of curcuminoids has improved the antibacterial activity of curcuminoid by 2.4-fold against Gram-positive S. aureus and 1.6-fold against Gram-negative bacterium E. coli.

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