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Acetylation of Curcumin from Turmeric Rhizome (*Curcuma longa*) with Ni/SiO_2 and Pyridine Catalysts and Its Antibacterial Activity

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Abstract. Turmeric (*Curcuma longa*) has been known for its antibacterial activity because it contains active curcumin. Antibacterial activity can be amplified by reducing its polarity, one of the ways is by modifying –OH on the phenolic group of curcumin to an acetoxy group by acetylation. The curcuminoid compound was extracted and curcumin was separated by column chromatography. Curcumin was modified by acetylation with Ni/SiO_2 and pyridine catalyst. The products were then separated by column chromatography and all compounds were characterized using thin layer chromatography, FTIR, and UV-Vis. All compounds were tested for *Escherichia coli* and *Bacillus subtilis* bacteria. The results showed that acetylation curcumin with pyridine was more effective at 94 % conversion of di-*O*-acetylcurcumin compared to acetylation with Ni/SiO_2 catalyst which has 90 % conversion but still in a mixture of di-*O*-acetylcurcumin, mono-*O*-acetylcumin and curcumin residual. Di-*O*-acetylcurcumin showed the highest antibacterial activity against *E. coli* with inhibitory zone diameters at 2 mm while the mono-*O*-acethylcurcumin showed the highest antibacterial activity against *B. subtilis* with inhibitory zone diameters at 3 mm.

Keywords: *Curcuma longa*, curcumin, antibacterial, *Bacillus subtilis, Escherichia coli*, acetylation, di-*O*-acetylcurcumin, mono-*O*-acetylcurcumin

INTRODUCTION

Bacterial infections are the major cause of diseases in the world, especially in tropical countries such as Indonesia because of its warm and humid temperatures which support microbes to thrive. This situation is supported by the ease of transportation and poor sanitation which facilitate the infectious diseases to develop [1]. One effort to overcome infectious diseases caused by bacteria is by using antibiotics. However, irrational use of antibiotics can make the microbial growth resistant [2]. Moreover, antibiotics can sometimes give side effects such as hypersensitivity, decreased immunity, and allergic reactions [3]. Because of these side effects and bacterial resistance, a more effective and safer antibacterial agent is needed. One way to require this antibacterial agent is by developing the extract of active compounds isolated from medicinal plants [4].

Turmeric (*Curcuma longa*) is one of the species which has been used a long time as medicinal plant for antibacterial activity because it contains active curcumin. However, curcumin is not enough to make this compound widely used in the clinical field due to its low bioavailability [5]. Therefore, modification of curcumin is needed to increase its bioactivity especially as an antibacterial agent.

Non-polar compounds which are soluble in fat have larger lipophilicity that makes the compound easily penetrating the cell membrane with passive diffusion [6]. Based on these studies, curcumin can be increased by reducing the

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polarity. One way to reduce the polarity of curcumin is by modifying the hydroxyl group into acetoxy group through acetylation.

Zendrato & Margono [7] have modified the analogous curcumin, gamavuton, with acetylation using the pyridine catalyst to produce *O*-acetyl gamavuton with percent conversion of 98.24 %. Whereas Alam, et al. [8] also carried out acetylation to phenol and naphthol using Ni/SiO_2 catalyst to produce *O*-acetyl with percent conversion of 90 %. Therefore, it is known that the acetylation can be carried out either with Ni/SiO_2 or pyridine catalysts. Therefore, based on these previous researches, this experience will modify the curcumin structure by converting the hydroxyl group with an acetoxy group so that it can reduce polarity and increase lipophilicity by using two different catalysts, Ni/SiO_2 and pyridine. The use of these different catalysts was carried out to determine which catalyst is the most effective in acetylating the phenolic group from curcumin. The test of its antibacterial activity is carried out against *E. coli* and *B. subtilis*.

EXPERIMENTAL

Materials

Turmeric rhizome (*C. longa*) was obtained from Serpong market, ethanol, cooking oil, n-hexane, dichloromethane, chloroform, methanol, silica gel 60, $Ni(NO_3)_2 \cdot 6H_2O$, aquadest, acetic anhydride, acetonitrile, ethyl acetate, pyridine, agar nutrient (NA), beef extract, peptone, alcohol 70 %, amoxycillin, dimethyl sulfoxide (DMSO) and *B. subtilis* and *E. coli* bacteria.

Isolation of Curcumin from Turmeric Rhizome

Turmeric powder (40 g) was extracted by Soxhlet using ethanol solvent at 80 ºC. The curcuminoid extract was dried with a rotary evaporator, washed with n-hexane and dichloromethane, and curcumin was then purified using gravity chromatography columns with chloroform: methanol: n-hexane (19:1:1) eluent.

Synthesis of Ni/SiO_2 Catalyst

$Ni(NO_3)_2 \cdot 6H_2O$ (2.5 g) was dissolved in distilled water (20 mL) then added with 5.0 g of silica gel. The mixture was stirred with a magnetic stirrer for 2 h at room temperature and left overnight. The catalyst product was dried in an oven for 24 h at 120 ºC.

Curcumin Acetylation with Ni/SiO_2 Catalyst

Ac_2O (4 mmol) was added to curcumin (200 mg) in 15 mL acetonitrile then added with 15 % Ni/SiO_2 catalyst. The reaction mixture was stirred with a magnetic stirrer at a temperature of 70–80 ºC for 5 h. After the reaction was complete, the mixture was extracted using ethyl acetate (10 mL) and distilled water (10 mL) twice. The organic phase was then dried with Na_2SO_4 anhydrous. The reaction product was purified by gravity column chromatography with chloroform: methanol: n-hexane (20:1:24) eluent. TLC result of curcumin acetylation with Ni/SiO_2 catalyst is labelled as KA1.

Curcumin Acetylation with Pyridine Catalyst

Ac_2O (4 mmol) was added to curcumin (200 mg) in pyridine 2 mL. The reaction mixture was stirred with a magnetic stirrer at a temperature of 70–80 ºC for 5 h. After the reaction was complete, the mixture was extracted using ethyl acetate (10 mL) and distilled water (10 mL) twice. The organic phase was taken, dried with Na_2SO_4 anhydrous. TLC result of curcumin acetylation with pyridine catalyst is labelled as KA2.

Characterization of Curcumin and Acetylcurcumin

Identification of curcumin and acetylcurcumin compounds was carried out using TLC with chloroform: n-hexane (19:1) eluent while characterization was carried out using FTIR spectrometer and UV-Vis spectrophotometer.

Test of Antibacterial Activity

Antibacterial activity test was carried out by disc method using *E. coli* as Gram negative bacteria and *B. subtilis* as Gram positive bacteria. A total of 100 µL of bacterial suspension was put into a petri dish then 20 mL of liquid NA was added. NA media was allowed to solidify, then paper discs (6 mm diameter) which have been dipped in the tested compound solution was placed. Curcumin, mono-*O*-acetylcumin, and di-*O*-acetylcumin were varied in concentrations from 62.5; 125; 250; 500; and 1000 ppm in DMSO solvents. Amoxicillin was used as a positive control while DMSO solution was used as a negative control. Petri dishes were then incubated for 24 h in an incubator at 37 °C and then the inhibition zone diameter was measured.

RESULTS AND DISCUSSION

Curcuminoid Extraction

Curcumin was isolated using a gravity chromatographic column with chloroform:methanol:n-hexane eluent (19:1:12). From the results of the separation, there were 105 fractions obtained. Curcumin was in fraction 25-35 which perform curcumin Rf at 0.675. From 6 grams curcuminoids, the amount of curcumin that was successfully purified was 1.032 grams with 17.19 % yield.

Synthesis of Ni/SiO_2 Catalyst

In the synthesis of Ni/SiO_2, Ni particles are incorporated into SiO_2. This is done to make nickel have thermal stability and more porous in the presence of silica oxide as support so that it can improve the catalytic performance of nickel. FTIR characterization of Ni/SiO_2 catalyst is shown in Fig. 1. The absorption identified at wavelengths of ~975 cm^{-1} indicates the presence of Si-O-Ni vibrations, at wavelengths of 1102 cm^{-1} indicates the Si-O-Si asymmetry vibrations, and at the wavelength of 3600–3000 cm^{-1} shows the vibration of the -OH group from Si-OH [9].

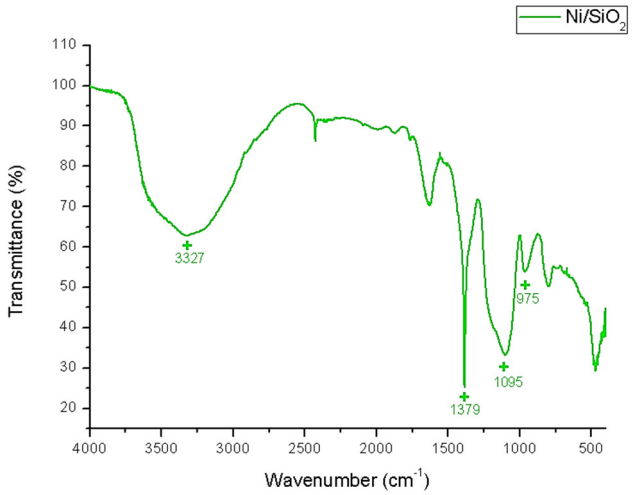


FIGURE 1. FTIR spectrum of Ni/SiO_2.

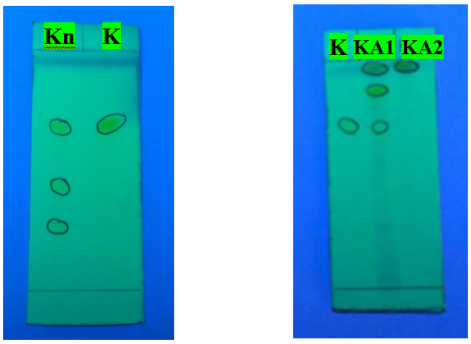
TLC Analysis of Curcuminoid Extract, Curcumin, and Acetylcurcumin

Analysis of TLC separation from curcuminoids and isolated curcumin on silica plate using eluent (19:1) are shown in Fig. 2a. All spots were shown to fluorescence under UV light. In the TLC results, curcuminoids have 3 spots sorted by polarity, Rf 0.250 = bisdemetoxycurcumin; Rf 0.425 = demethoxycurcumin; and Rf 0.675 = curcumin, whereas the isolated curcumin only has 1 spot, Rf 0.675 = curcumin.

The TLC analysis with chloroform: methanol (19:1) eluent of acetylcurcumin and curcumin are shown in Fig. 2b. The acetylation of curcumin with pyridine catalyst produces only 1 product with % conversion of 94 %, while using Ni/SiO_2 catalyst produces 2 products with % conversion of 90 % and curcumin residual. Product 1 (Rf = 0.95) is predicted as di-*O*-acetylcurcumin because its highly nonpolar structure, followed by product 2 (Rf = 0.85) which predicted as mono-*O*-acetylcurcumin because it is more polar. These products 1 and 2 were then separated with column chromatography gravitation with chloroform:methanol:n-hexane (24:1:20) eluent. Rf data of all compounds are shown in Table 1.

FTIR Characterization of Curcumin and Acetylcurcumin

Figure 3a shows the FTIR characterization of curcumin and acetylcurcumin and (Table 2) shows the functional group and its vibration. The isolated curcumin showed a typical group of curcumin vibration such at 3200–3600 cm^{-1} corresponds to hydroxyl (O-H) vibration, at 3000–2800 cm^{-1} to methyl ($-CH_3$) and methylene ($-CH_2$) symmetric and asymmetric stretching vibration, at ~1624 cm^{-1} to C=O absorption, though for ester compound, this vibration shifts to ~1770 cm^{-1}. Vibration at ~1602 cm^{-1} corresponds to alkenes (C=C), at ~1517 cm^{-1} is assigned to aromatic skeletal stretching vibration, and at ~1027 cm^{-1} due ether (C-O-C) vibration. In product 1 which is predicted as di-*O*-acetylcurcumin has no vibration of hydroxyl (O-H) but ester (COO) vibration at ~1199 cm^{-1} has appeared which shows that all hydroxyl groups have been acetylated. In product 2 which is predicted as mono-*O*-acetylcurcumin still has vibration of hydroxyl (O-H) but with lower absorption compared to the curcumin spectrum and there is also an ester vibration at ~1199 cm^{-1} which shows that only 1 hydroxyl group is acetylated.



(a) (b)

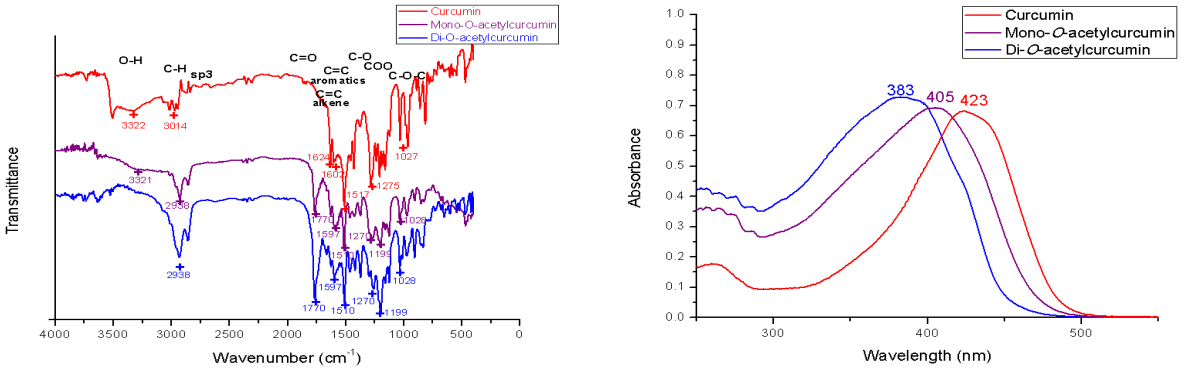
FIGURE 2. (a) TLC analysis of curcuminoid (Kn) and isolated curcumin (K), (b) TLC analysis of curcumin (K); synthesis of acetylcurcumin with Ni/SiO_2 catalyst (KA1); and synthesis of acetylcurcumin with pyridine catalyst (KA2).

TABLE 1. Rf data of curcuminoid and acetylcurcumin.

Rf data	Compound
0.250	Bisdemethoxycurcumin
0.425	Demethoxycurcumin
0.675	Curcumin
0.85	Mono-*O*-acetylcurcumin (Product 2)
0.95	Di-*O*-acetylcurcumin (Product 1)

UV-Vis Characterization of Curcumin and Acetylcurcumin

Figure 3b shows the UV-Vis characterization of curcumin and acetylcurcumin. In UV-Vis absorption, the spectrum of curcumin has maximum absorption at 423 nm wavelength. This wavelength is suitable with the curcumin standard [10]. The product 1 and product 2 has a shift at the lower maximum absorption at 383 and 405 nm wavelength, respectively compared to curcumin. This wavelength shift is caused by the replacement of the -OH group into the -$OCOCH_3$ group on one or both sides of the phenolic group on curcumin as the change of this functional group makes the resonance of benzene ring less stable caused by withdrawal of electron density and therefore the energy will become higher. The structure of curcumin, mono-*O*-acetylcurcumin, di-*O*-acetylcurcumin is shown in Fig. 4.



(a) (b)

FIGURE 3. (a) FTIR and (b) UV-vis characterization of curcumin and acetylcurcumin.

TABLE 2. Functional group and its vibration of curcumin, mono-o-acetylcurcumin, and di-o-acetylcurcumin.

Functional Group	Vibration (cm^{-1})		
	Curcumin	Product 1 (Di-*O*-acetylcurcumin)	Product 2 (Mono-*O*-acetylcurcumin)
-OH	3322	-	3321
C-H Stretching	3014	2938	2938
C=O conjugate	1624	1770	1770
C=C alkenes	1602	1597	1597
C=C aromatics	1517	1510	1510
C-O enol	1275	1270	1270
COO ester	-	1199	1199
C-O-C ether	1027	1028	1028

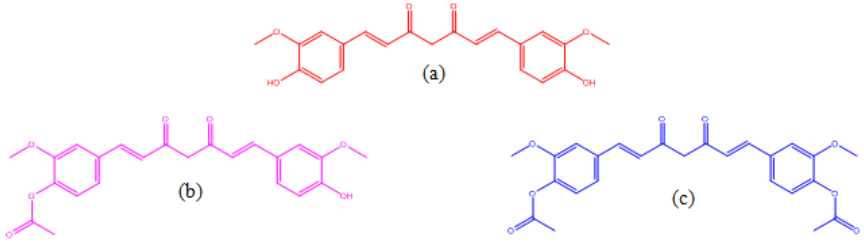


FIGURE 4. The structure of (a) curcumin, (b) mono-*O*-acetylcurcumin and (c) di-*O*-acetylcurcumin.

Mechanism of Reaction

Mechanism of Curcumin Acetylation with Ni/SiO_2 Catalyst

Nickel based catalysts has been widely used in various reactions because it is easier, safer, environmentally friendly, economical, and can be reused. Figure 5 shows the proposed mechanism for acetylation with Ni/SiO_2 catalysts. When acetic anhydride is added to the Ni/SiO_2 catalyst, asylinum ions will be produced on the surface of the silica through the Lewis acid character which increases the reaction speed [9]. First of all, with the support of SiO_2, Ni will form Ni^{2+} and interact with oxygen from the carbonyl group of acetic anhydride to form NiO. Then, the double bond C=O will resonate into a single bond which causes the acetyl ion to resonate to complete the ion from the electropositive C atom and form the Ni-acylium ion. Furthermore, the oxygen atom from the curcumin compound will attack C which is electropositive and break the acetate group. The interaction between the catalyst Ni/SiO_2 and the acetyl group will resonate and the O atom will attack the H atom which has been easily released due to the positive charge of O at the curcumin to form acetic acid as a byproduct and mono-*O*-acetylcurcumin as the main product. The reaction goes on until acetic anhydride and Ni/SiO_2 can form acetyl groups on the other hydroxyl group on curcumin to form di-*O*-acetylcurcumin.

Mechanism of Curcumin Acetylation with Pyridine Catalyst

Pyridine acts as a nucleophile in the acetylation [11]. The use of pyridine-base catalysts will catalyze the reaction of esters but not catalytic against other reactions [12]. Pyridine as a tertiary amine has a role as a primary base and nucleophile for the acetylation. Aside from being a catalyst, pyridine can also act as a good organic solvent. The acetylation mechanism with pyridine catalyst is shown in Fig. 6. First, pyridine will attack hydrogen in the phenol group of curcumin to form phenoxide ions and pyridinium ions. Then, the negative charge O atom attacks one of the C carbonyl in the acetic anhydride which is partially positive and causes the acetic anhydride to resonate and break the acetate bond and then form a mono-*O*-acetylcurcumin. The O atom in the negative charge acetate attacks the H atom in the pyridinium ion to form acetic acid to form pyridine and acetic acid. The reaction goes on until it forms di-*O*-acetylcurcumin.

Antibacterial Activity Test

The average inhibition zone diameter of curcumin, mono-*O*-acetylcurcumin, and di-*O*-acetylcurcumin against *B. subtilis* and *E. coli* bacteria are shown in Fig. 7a and Fig. 7b. The negative control (DMSO) shows no inhibition zone diameter which means it has no antibacterial activity and has no interference in the result of the tested compound. As for control positive (Amoxicillin), the inhibition zone diameter for *B. subtilis* and *E. coli* bacteria are 3.6 mm and 1.5 mm respectively. Curcumin, mono-*O*-acetylcumin, and di-*O*-acetylcurcumin have the highest antibacterial activity at concentration of 1000 ppm for both tested bacteria. The highest antibacterial activity against *B. subtilis* was shown in mono-*O*-acetylcurcumin with an average inhibition zone diameter of 3 mm, whereas the highest antibacterial activity against *E. coli* was shown in di-*O*-acetylcurcumin with an average inhibition zone diameter of 2 mm. As for curcumin, the antibacterial activity against *B. subtilis* and *E. coli* are 2.5 mm and 1 mm, respectively. The modified curcumin into acetylcurcumin produces more nonpolar structure and becomes more lipophilic. The more lipophilic a compound is, the easier that compound to interact with the phospholipid membrane and diffuse passively into the bacterial cell wall [6].

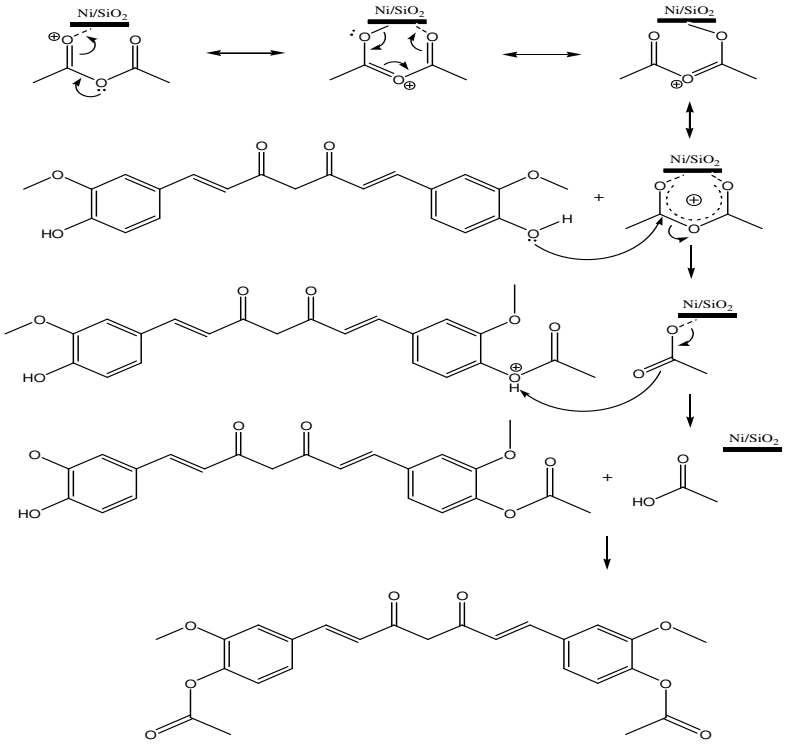


FIGURE 5. Proposed mechanism for the acetylation with Ni/SiO_2 catalysts.

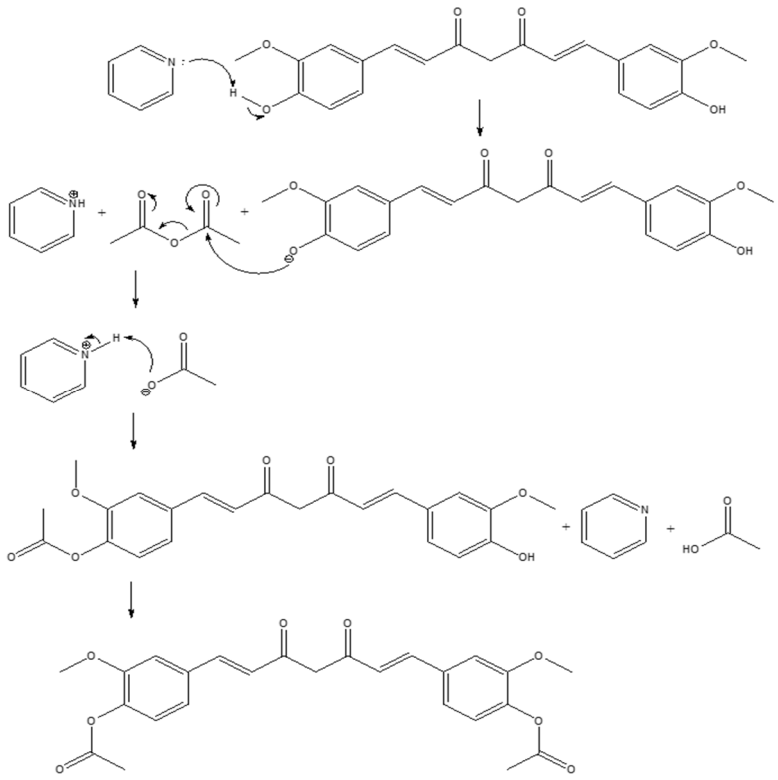


FIGURE 6. Proposed mechanism for the acetylation with pyridine catalysts .

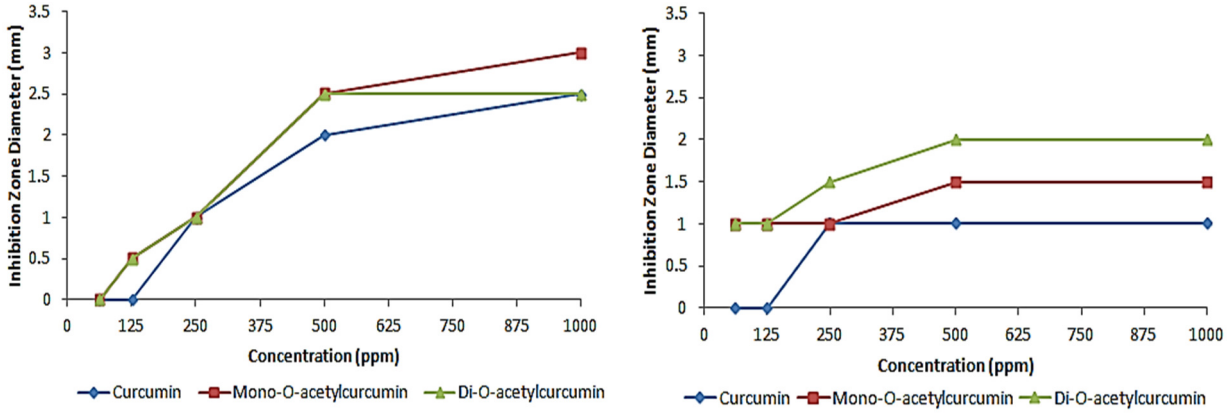


FIGURE 7. Average inhibition zone diameter of curcumin, mono-o-acetylcurcumin and di-o-acetylcurcumin against (a) *B. subtilis* bacteria (b) *E. coli* bacteria.

CONCLUSION

Acetylation of curcumin with Ni/SiO2 catalyst produced 90 % conversion of mono-*O*-acetylcurcumin, di-*O*-acetylcurcumin, and curcumin while with pyridine catalyst produced 94% conversion of di-*O*-acetylcurcumin so the use of pyridine catalyst for acetylation is more effective. All compounds have the highest antibacterial activity at concentration of 1000 ppm. Di-*O*-acetylcurcumin has the highest antibacterial activity against *E. coli* bacteria while mono-*O*-acetylcurcumin has the highest antibacterial activity against *B. subtilis* bacteria. The modified curcumin into mono-*O*-acetylcurcumin and di-*O*-acetylcurcumin increased the antibacterial activity of curcumin against Gram positive bacteria *B. subtilis* and Gram negative bacteria *E. coli*.

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