Microwave-assisted synthesis of 7-hydroxy-4-methyl coumarin and its bioactivity against acne-causing bacteria

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Abstract. Acne is a problem that often occurs in adolescents, especially during puberty phase. One of the acne-causing factors is proliferation of Propionibacterium acnes and Staphylococcus epidermidis bacteria. Acne is usually treated with antibiotics, but the resistance of antibiotics is also found. Therefore, research on new antibacterial compounds continues to be developed. One of the compounds reported to have antibacterial activity is coumarin derivatives. In this research, 7-hydroxy-4-methyl coumarin had been successfully synthesized from resorcinol and ethyl acetoacetate with the presence of p-toluene sulfonic acid (PTSA) as catalyst and solvent free reaction using 800W microwave-assisted method. This compound obtained in 48.36 % in yield with the reaction condition as follow: resorcinol/ethyl acetoacetate molar ratio of 1:1, 10 mol% PTSA, and 150s reaction time. Synthesized compound was further elucidated using FTIR, UV-Vis, and 1H-NMR spectral data. Unfortunately, study on the ability to inhibit the growth of P. acnes and S. epidermidis showed that the compound was inactive towards both acne-causing bacteria. Therefore, it can be concluded that 7-hydroxy-4-methyl coumarin did not have potential antibacterial activity against acne-causing bacteria.

Keywords: Microwave-assisted synthesis, 7-hydroxy-4-methyl coumarin, antibacterial, acne-causing bacteria

1. Introduction
Acne is a problem that often occurs in adolescents, especially during puberty phase. Acne infects at least 85 % prevalence in the 12–24 years age range and can also cause long-term scarring and have a large psychological impact on individuals [1]. Several factors causing acne are inflammation and proliferation of Propionibacterium acnes. Not only the P. acnes, but also there is another acne-causing bacterium, i.e. Staphylococcus epidermidis which is classified as opportunistic pathogenic bacteria [2]. Tetracycline is a bacteriostatic agent with a broad-spectrum antimicrobial activity against gram-positive and negative bacteria both aerobic and anaerobic [3]. Based on its structure, tetracycline has a conjugated multicyclic framework with a lot of hydroxyl groups, at least five hydroxyl groups on simple tetracycline. In order to figure out the analogue compound of tetracycline, in terms of its structure, coumarins have a similar structural framework compared with tetracycline.

Previous study on nine standard natural products, coumarin modified with triterpenoid showed a strong resistance to the growth of P. acnes [4]. Coumarins are usually synthesized in a laboratory scale...
having substitution at C4 and C7 by Pechmann condensation using an acid catalyst with several methods [5-8]. Among all methods, microwave irradiation is proven as a simple and powerful method in preparing coumarins in a good yield with a shorter reaction time [8]. Following our interest on microwave-assisted synthesis and searching for potential antibacterial compound [9-14], herein we describe the microwave-assisted synthesis using p-toluene sulfonic acid (PTSA) catalyst and structure elucidation of 7-hydroxy-4-methyl coumarin. We also evaluate the ability of its compound using disk diffusion method against acne-causing bacteria in human skin tissue, i.e. *P. acnes* and *S. epidermidis*.

2. Materials and method

2.1. General
Domestic microwave oven Samsung ME-731K/XSE was used for microwave irradiation. Coumarin was measured for its melting point by using Electrothermal Melting Temp Apparatus and elucidated for its structure by using UV-Vis Shimadzu UV-2450 spectrophotometer, FTIR Shimadzu IR Prestige 21 spectrometer, and 1H-NMR Agilent 500 MHz spectrometer. The chemicals used for synthesis were purchased from Merck, i.e. resorcinol, ethyl acetoacetate, PTSA, ethanol, *n*-hexane, ethyl acetate, and TLC plates (Si 60 GF254, 0.25 mm thickness), while for antibacterial activity, nutrient agar, DMSO, clindamycin, and tetracycline were used. Acne-causing bacteria (*P. acnes* and *S. epidermidis*) were obtained from Biochemistry Laboratory, Department of Chemistry, FMIPA Universitas Indonesia.

2.2. Microwave-assisted synthesis of 7-hydroxy-4-methyl coumarin
7-Hydroxy-4-methyl coumarin was synthesized by reacting resorcinol (1 mmol) with ethyl acetoacetate (1 mmol) and PTSA (10 mol% of resorcinol) in solvent-free. The mixture then was irradiated in 800 W microwave for 150 s. The reaction temperature was controlled using an infrared thermometer and maintained at ±80 °C. The mixture was purified by recrystallization using warm ethanol and identified by TLC analysis. The product then was measured for its melting point and characterized for its structure using FT-IR, UV-Vis, and 1H-NMR.

2.3. Antibacterial activity assay against acne-causing bacteria using disk diffusion method
Into a sterile petri dish, 200 μL of suspension for each bacterium test (1 × 10⁸ cells/mL, 0.5 McFarland standard) were added with 20 mL nutrient agar at ±45–50 °C. The petri dish then was shaken until homogeneous mixture was produced then left to freeze. Sterile disc papers were placed on the media and 4 μL coumarin in various concentrations was dropped onto disc paper. The petri dish then was incubated at 37 °C for 24 hours. Inhibition zones indicated by clear areas around paper discs were measured in diameter. As a positive control, clindamycin 1 % and tetracycline 1 % were used, while DMSO was used for negative control. Antibacterial activity was repeated twice.
precursors in n-hexane: ethyl acetate (3:2, v/v) with \( R_f \) of resorcinol, ethyl acetoacetate, and product were 0.52, 0.80, and 0.46, respectively. Melting point measurement showed the value of 188–189 °C, in line with the previous study [16].

3.2. FTIR characterization of 7-hydroxy-4-methyl coumarin
The results of FTIR characterization of synthesized compound are presented in figure 2. According to figure 2, FTIR spectrum showed the presence of several functional groups, i.e. hydroxyl (-OH) group at 2400–3500 cm\(^{-1}\) with typically broad-peak, carbonyl (C=O) group at 1705–1725 cm\(^{-1}\), and alkene double bond (C=C) at 1620–1680 cm\(^{-1}\). The presence of alkene double bond peak is an evidence that the synthesized product has been formed, since both precursors and catalyst do not have this functional group. Therefore, the alkene double bond is a unique functional group in the product.

3.3. UV-Vis characterization of 7-hydroxy-4-methyl coumarin
Figure 3 exhibits the UV-Vis spectrum in methanol of synthesized compound. Based on figure 3, the product has a maximum wavelength at 322 nm corresponds to cinnamoyl chromophore. Compared with the maximum wavelength of both precursors (data not shown), the maximum wavelength of product is shifted to bathochromic due to the extended conjugated system. Therefore, it leads to the formation of more stable products [17]. This data is also strengthened by previous study on 7-hydroxy-4-methyl coumarin synthesis having the maximum absorbance at \( \lambda_{\text{max}} \) 337 nm [16].

3.4. \(^1\)H-NMR characterization of 7-hydroxy-4-methyl coumarin
Characterization using \(^1\)H-NMR (500 MHz, acetone-\(d_6\)) was conducted to ensure the formation in the synthesized product. Table 1 summarizes the \(^1\)H-NMR data in comparison to the previous study [16].

Figure 1. Reaction scheme for the formation of 7-hydroxy-4-methylcoumarin compound.

Figure 2. FTIR spectrum of 7-hydroxy-4-methyl coumarin.
Figure 3. UV-Vis spectrum of 7-hydroxy-4-methylcoumarin.

Table 1. $^1$H-NMR data of 7-hydroxy-4-methyl coumarin compared with previous literature.

| No. | C          | Synthesized product (acetone-$d_6$) | 7-Hydroxy-4-methyl coumarin (DMSO-$d_6$) |
|-----|------------|------------------------------------|------------------------------------------|
|     |            | $\delta$ ppm (int, $m$, $J_{coupling}$) | $\delta$ ppm (int, $m$, $J_{coupling}$)  |
| 3   | 3          | 6.09 (1H, s)                         | 6.10 (1H, s)                              |
| 5   | 5          | 7.62 (1H, $d$, 8.7 Hz)               | 7.56 (1H, $d$, 8.7 Hz)                    |
| 6   | 6          | 6.75 ppm (1H, $d$, 2.4 Hz)           | 6.69 (1H, $d$, 2.4 Hz)                    |
| 8   | 8          | 6.87 ppm (1H, $dd$, 8.7; 2.4 Hz)     | 6.78 (1H, $dd$, 2.4; 8.7 Hz)              |
| 11  | 11         | 2.43 ppm (3H, s)                     | 2.34 ppm (3H, s)                         |
| 7-OH| 7-OH       | 9.41 ppm (1H, s)                     | 10.51 (1H, s)                            |

The product formation is indicated by the presence of three proton signals in aromatic ring chemical shift around 6–8 ppm. The peaks at chemical shift of 6.75 ppm (1H, $d$, $J=2.4$ Hz); 6.87 ppm (1H, $dd$, $J=8.7$; 2.4 Hz); and 7.62 ppm (1H, $d$, $J=8.7$ Hz) belongs to proton signals from trisubstituted benzene ring following ABX system for C8, C6, and C5, respectively. The resulted coupling constant also confirmed that the synthesized compound has ABX system with values of 2.4 and $8.7$ Hz for positions 1,3 (meta) and 1,2 (ortho), respectively. A peak at 6.09 ppm (1H, s) is a proton at C3 which specifically known for proton bonded to alkenes. Singlet multiplicity of proton at C3 means that it has no neighbour protons and trisubstituted alkene. Moreover, a peak at chemical shift of 2.34 ppm (3H, s) revealed the deshielding proton signal typically for protons across the alkene double bond (H-C-C=C). The presence of one substituted hydroxyl group in C7 is indicated by the presence of a signal in 9.41 ppm (1H, s). According to $^1$H-NMR spectrum, these six proton signals deduce a benzopyrone skeleton with double bond alkene at C3 with the presence of a methyl group at C4 and a hydroxyl group at C7. Based on table 1, it can be confirmed that the synthesized product matched with 7-hydroxy-4-methyl coumarin. Slightly differences in some of chemical shifts were found due to different solvent used in the sample measurement.

3.5. Antibacterial activity assay against acne-causing bacteria using disk diffusion method

The ability of the synthesized compound in inhibiting the growth of acne-causing bacteria, i.e. *P. acnes* and *S. epidermidis*, was conducted in this study using disk diffusion method. The various of coumarin
Table 2. The average diameter of 7-hydroxy-4-methyl coumarin inhibition zone against acne-causing bacteria.

| Concentration (mg/mL) | The average diameter of the zone of inhibition of bacterial growth (mm)* |
|-----------------------|---------------------------------------------------------------|
|                       | P. Acnes             | S. Epidermidis      |
| 5                     | 0                    | 0                   |
| 7.5                   | 0                    | 0                   |
| 10                    | 0                    | 0                   |
| 12.5                  | 0                    | 0                   |
| 15                    | 0                    | 0                   |
| 20                    | 0                    | 0                   |

* Inhibition zone of both clindamycin and tetracycline as positive control were 10 and 40 mm, respectively, for both acne-causing bacteria. Negative control (DMSO) did not show any activity against all bacteria.

concentrations (5, 7.5, 10, 12.5, 15 and 20 mg/mL) with DMSO as negative control, clindamycin 10 mg/mL and tetracycline 10 mg/mL as positive control were used in this assay. Clear area produced around the disc paper indicates the antibacterial activity of the compound [11].

The average diameter of synthesized compound inhibition zone against both acne-causing bacteria is summarized in table 2. According to table 2, 7-hydroxy-4-methyl coumarin did not show any antibacterial activity against P. acnes or S. epidermidis at all concentrations. This result indicated that the coumarin-derived synthesized compound cannot inhibit the growth of P. acnes and S. epidermidis bacteria. However, recent study reported that coumarin derivatives, i.e. 6-methyl and 6,7-dihydroxy coumarin exhibited an antibacterial activity towards gram-negative bacteria, i.e. Escherichia coli and Pseudomonas aeruginosa, and gram-positive bacteria, i.e. S. aureus and Bacillus cereus [18].

4. Conclusion

In conclusion, 7-hydroxy-4-methyl coumarin was successfully synthesized from resorcinol and ethyl acetooacetate with PTSA 10 mol % under 800 W microwave irradiation at 150 s reaction time with 48.36 % yield. However, this compound did not exhibit an activity towards the acne-causing bacteria. Further research is needed to modify its structure together with evaluation on its antibacterial activity for other bacteria.

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