MICROWAVE-ASSISTED SYNTHESIS OF 4-METHYL COUMARINS, THEIR ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT
Coumarins are heterocyclic compounds naturally found in plants. Coumarin-derived compounds have been commonly synthesized and some have exhibited a wide range of bioactivities. In searching for coumarins-based antioxidants and antibacterial, in this study, various 4-methyl coumarins have been synthesized by reacting ethyl acetoacetate with several phenol compounds, e.g. resorcinol, catechol, hydroquinone, α-naphthol, and β-naphthol, catalyzed by multiple Lewis acids, e.g. SnCl2, SnCl2·2H2O, ZnCl2, and AlCl3, using microwave irradiation via the Pechmann condensation. The antioxidant activities of synthesized compounds were tested using the DPPH method, while the antibacterial activities were measured using the disc diffusion method. Of the five phenol and four Lewis acid variations, only resorcinol catalyzed by SnCl2·2H2O and AlCl3 produced the targeted coumarin, 7-hydroxy-4-methyl coumarin, in a pure compound. The synthesized compound was obtained with an optimum yield of 55.25% by reacting resorcinol/ethyl acetoacetate 1:1 (mol/mol) and 10 mol% SnCl2·2H2O for 260 s reaction time under solvent-free conditions. This compound was identified by TLC, melting point apparatus, and then further characterized using FTIR, UV-Vis, and NMR instruments. The antioxidant activity of 7-hydroxy-4-methyl coumarin showed moderately active towards DPPH with an IC50 value of 99.69 ppm. This compound also displayed moderate activity against E. coli with 9 mm of inhibition zone in 125 ppm. However, 7-hydroxy-4-methyl coumarin was inactive against S. aureus at all tested concentrations. This study reveals that various phenols and Lewis acids can affect coumarins-based antioxidant and antibacterial synthesis.

Keywords: Antibacterial, Antioxidant, Coumarin, Microwave Irradiation, Pechmann Condensation.

INTRODUCTION
Coumarins are a group of natural phenolics consisting of fused benzene and α-pyrone rings. Classifying as an essential family of oxygen-containing heterocycles, coumarins display a remarkable array of potential applications in pharmaceutical, food, fragrance, cosmetic, agrochemical, and color technology.1,2 Coumarin derivatives can be obtained via Pechmann, Perkin, Knoevenagel, Claisen, Reformatsky, Suzuki, Wittig, Baylis-Hillman, and Vilsmeier-Haack reactions.3-5 However, the Pechmann reaction is the most frequent method due to its simplicity and directly one-step synthesis method.6 This reaction involves the reaction of activated phenols and β-ketoesters utilizing acid catalysts.7 In some cases, coumarin synthesis consumes long reaction times along with the formation of undesired by-products. Subsequently, further development of milder reaction conditions is urgently needed.8 Moreover, as coumarins have been established to be effective pharmacophores, demand for their synthesis is also increasing.5 Therefore, the microwave-assisted synthesis method has been employed to overcome the problems.9 Besides, this method provides the possibility to be carried out in solvent-free conditions, making an environmentally-friendly reaction.10
Coumarins have captured great attention as possible therapeutic agents in various medical problems.\textsuperscript{11,12} The antioxidant properties of these compounds are due to their strong radical scavenging effects on reactive nitrogen species (RNS) and reactive oxygen species (ROS).\textsuperscript{13,14} The coumarin-based antioxidant plays an important role in health maintenance, neurodegenerative disease treatment, and aging-related disease protection. Furthermore, antioxidants can reduce cancer risk, heart disease, and stroke.\textsuperscript{15} On the other hand, these compounds are also recognized as outstanding antibacterial agents since the establishment of Novobiocin and Chlorobiocin.\textsuperscript{16-18} However, little information has considered the structure-antioxidant and antibacterial activities relationship of coumarins. Among coumarin moieties, 4-methyl coumarin is deemed to have several valuable pharmacological properties, which are extremely challenging in medicinal chemistry.\textsuperscript{19-20} Therefore, this study aims to synthesize various structures of 4-methyl coumarins via Pechmann condensation under microwave irradiation as well as examine its antioxidant properties using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and antibacterial activities using the disc-diffusion protocol. Moreover, herein we also explain the effect of phenols and catalytic activity of Lewis acid catalysts in the described reaction. A study on coumarins' structures and functional properties could help the strategies further design coumarins-based antioxidants and antibacterial.

**EXPERIMENTAL**

**General Procedures**

The reagents used for synthesis were resorcinol, catechol, hydroquinone, α-naphthol, β-naphthol, ethyl acetoacetate, SnCl\textsubscript{2}, SnCl\textsubscript{2}.2H\textsubscript{2}O, ZnCl\textsubscript{2}, AlCl\textsubscript{3}, ethanol, acetonitrile, n-hexane, ethyl acetate, methanol, acetone, and distilled water. All reagents were obtained from Merck without any purification before use. Microwave irradiation synthesis was conducted using domestic microwave Samsung ME-731K/XSE, and infrared thermometer GM320 was used to observe the reaction's temperature. Reactions were observed using thin-layer chromatography (TLC) on precoated Si 60 GF\textsubscript{254} plates of 0.25 mm thickness (Merck), and TLC spots were envisioned under ultraviolet light UVP UV GL 55 (254 and 366 nm). For the antioxidant test, DPPH (Sigma Aldrich) and methanol (Merck) were used with ascorbic acid (Merck) as a positive control. Whereas for the disc-diffusion assay, nutrient agar, nutrient broth, and DMSO from Merck and ciprofloxacin HCl monohydrate\textsuperscript{®} from PT. Bernofarm Pharmaceutical Company as a positive control was used. In addition, the bacteria culture of *Escherichia coli* and *Staphylococcus aureus* were gained from the Biochemistry Laboratory, Department of Chemistry, Universitas Indonesia. The melting point was determined using Electrothermal Melting Point Apparatus 9100 for product identification, while UV-Vis Shimadzu UV2450 in methanol spectroscopic grade (Merck), FTIR Shimadzu IR Prestige 2 in KBr pellet (Merck), and 500 MHz NMR Bruker Avance operating at 500 MHz (\textsuperscript{1}H) and 125 MHz (\textsuperscript{13}C) in acetone-\textit{d}_\text{6} (Merck) were used for structural elucidation.

**Microwave-Assisted Synthesis of 4-Methyl Coumarin Derivatives**

The Pechmann condensation using resorcinol and ethyl acetoacetate was chosen as a reference for the optimization of reaction conditions. To the different mole ratios of resorcinol and ethyl acetoacetate (1:1, 1:1.125, and 1:1.25, mol/mol), several amounts (0; 5; 10; 15; and 20 mol\%) of various Lewis acids (SnCl\textsubscript{2}, SnCl\textsubscript{2}.2H\textsubscript{2}O, ZnCl\textsubscript{2}, and AlCl\textsubscript{3}) were added into a crucible. The mixture then was irradiated using a 600-800W microwave for several reaction times (3-30 min) depending on the catalyst used under the solvent-free condition or with the solvents (ethanol and acetonitrile). The temperature was regularly checked during the reaction using an infrared thermometer. The reaction was also monitored using TLC with different solvent systems in order to have the best melting point: n-hexane: ethyl acetate (6:4, v/v). After completing the reaction, the mixture was then worked up by cooling to room temperature, washing with ethanol-water (1:1, v/v), and recrystallizing with hot ethanol. Furthermore, the dried synthesized compound was weighed, observed for its melting point, and subjected to structural elucidation using UV-Vis, FTIR, and NMR instruments. Similar reactions were carried out using various phenols, e.g. catechol, hydroquinone, α-naphthol, and β-naphthol, by applying the optimum condition obtained for resorcinol with some modifications.

**Physical Properties and Spectral Data of Synthesized Compound**

7-Hydroxy-4-methyl coumarin: C\textsubscript{10}H\textsubscript{8}O\textsubscript{3}; a pale-yellow solid; R\textsubscript{f} 0.46 (n-hexane: ethyl acetate 6:4, v/v); MP (°C): 187-188; FTIR (KBr pellet) ν\textsubscript{max} (cm\textsuperscript{-1}): 3157-3500 (O-H), 3079-3116 (C-H sp\textsuperscript{2}), 3028-3078 (C-H sp\textsuperscript{2}), 2936-2854 (C-H aliphatic), 1603-1662 (C=O), 1461-1530 (C-H aromatic), 1373-1388 (C-O), 1157-1223 (C-O), 1054-1068 (C-O), 880-895 (C-O), 782-798 (CH aromatic).
H sp³), 1640-1690 (C=O), 1594-1605 (C=C), and 1145 (C-O-C); UV-Vis (methanol) \( \lambda_{\text{max}} \) (nm): 218.5 and 323 nm; \(^1\)H-NMR (500 MHz, acetone-d\(_6\) \( \delta \) (ppm): 9.36 (1H, s, 7-OH), 7.60 (1H, d, J = 8.5 Hz, H-5), 6.85 (1H, dd, J = 8.5, 2.5 Hz, H-6), 6.73 (1H, d, J = 2.5 Hz, H-8), 6.07 (1H, d, J = 1.5 Hz, H-3), and 2.41 (1H, d, J = 1.5 Hz, H-11); and \(^{13}\)C-NMR (125 MHz, acetone-d\(_6\) \( \delta \) (ppm): 161.8 (C-7), 161.0 (C-2), 156.3 (C-9), 153.8 (C-4), 127.3 (C-5), 113.7 (C-10), 113.5 (C-6), 111.8 (C-3), 103.3 (C-8), and 18.5 (C-11).

**DPPH Assay for Antioxidant Activity Test**

The antioxidant activity test was carried out using the DPPH method by adding 3 ml of 0.1 mM DPPH in methanol into 1.5 ml of a sample in methanol with different concentrations (5-250 ppm). After mixing thoroughly, the mixture was incubated in the dark for 30 min at RT. The absorbance at 517 nm then was measured using methanol as a baseline correction. As a control, DPPH in methanol solution was prepared without using a test sample. The result was presented as an IC\(_{50}\), which means a sample concentration that afforded 50% of radical scavenging activity (RSA) of the DPPH radical. Ascorbic acid was used as a positive control in this study.

**Disc Diffusion Assay for Antibacterial Activity Test**

The disc diffusion method for the antibacterial activity test was conducted by spreading a 200 \( \mu \)L of *E. coli* and *S. aureus* into a nutrient agar in a Petri dish. A 3 mm paper disc was immersed in various concentrations of a sample (62.5-1000 ppm) before being placed above the agar. The Petri dish then was incubated at 37\(^\circ\)C for 24 h. The inhibition zone was observed and measured in mm. As a positive and negative control, ciprofloxacin and DMSO were selected in this study, respectively.

**RESULTS AND DISCUSSION**

In this work, 4-methyl coumarins have been synthesized via Pechmann condensation assisted by microwave irradiation. Pechmann condensation is a valuable and regular method to afford coumarin scaffolds with simple precursors and good yields.\(^{21}\) This reaction involves a three-step consecutive mechanism, including trans-esterification, intramolecular electrophilic aromatic substitution, and elimination of water.\(^{19}\) Furthermore, coumarin synthesis following Pechmann condensation can generally be accelerated using organic and inorganic acid catalysts, either Brønsted or Lewis acid.\(^3\) The acid catalyst was found to play an essential role energetically in carbonyl activation of β-keto-ester as an initial reaction mechanism.\(^{6,8}\) Reaction conditions have been optimized based on the reaction between resorcinol and ethyl acetoacetate as a model reaction to obtain 7-hydroxy-4-methyl coumarin (Scheme-1).

![Scheme-1: Reaction Scheme of 7-hydroxy-4-methyl Coumarin Synthesis](image)

At first, reactions were set to select the best Lewis acid catalyst using 800W domestic microwave, 1:1 (mol/mol) resorcinol/ethyl acetoacetate molar ratio, and 10 mol% Lewis acid under solvent-free conditions (Table-1). Based on Table-1, among four Lewis acid catalysts, SnCl\(_2\).2H\(_2\)O and AlCl\(_3\) revealed the best catalyst to facilitate Pechmann condensation with moderate-high yield. This reaction was also found to be efficient since the by-products were not observed. While other catalysts, i.e. SnCl\(_2\) and ZnCl\(_2\), required a longer reaction time with trace yield and the presence of side products. SnCl\(_2\).2H\(_2\)O afforded the highest yield with a shorter reaction time due to its presence as hydrates. The hydrates around Sn\(^{2+}\) tend to increase the Lewis acid sites and lead to more active involvement in the proton transfer of water, which is an important step in Pechmann condensation.\(^6\) In addition, the presence of water as a polar molecule can also help to absorb microwave radiation and convert it into heat energy.\(^9,22\) On the other hand, AlCl\(_3\) is a strong Lewis acid and established catalyst of Friedel Craft-like reaction.\(^{23}\) Further experiments were carried out to attain the optimum conditions for 7-hydroxy-4-methyl coumarin synthesis catalyzed by SnCl\(_2\).2H\(_2\)O and AlCl\(_3\).
Table-1: Lewis Acid Variations as a Catalyst in The Synthesis of 7-hydroxy-4-methyl coumarin

| Catalyst       | Reaction time (min) | Yield (%) |
|----------------|---------------------|-----------|
| SnCl$_2$       | 6                   | Traces    |
| SnCl$_2$.2H$_2$O | 4                   | 36.72     |
| ZnCl$_2$       | 8                   | Traces    |
| AlCl$_3$       | 10                  | 18.37     |

*Reaction conditions: MW 800W, resorcinol/ethyl acetoacetate molar ratio (1:1, mol/mol), 10 mol% Lewis acid, and solvent-free.

Table-2: Optimization Reaction Condition of Microwave-Assisted for The Synthesis Of 7-hydroxy-4-methyl coumarin Catalyzed By SnCl$_2$.2H$_2$O

| Entry | Molar ratio of R/EAA$^a$ (mol/mol) | SnCl$_2$.2H$_2$O (% mol) | Reaction time (s) | Solvent | Yield$^b$ (%) |
|-------|-----------------------------------|--------------------------|-------------------|---------|---------------|
| 1     | 1:1                               | 10                       | 220               | -       | 37.95         |
| 2     | 1:1                               | 10                       | 240               | -       | 36.72         |
| 3     | 1:1                               | 10                       | 260               | -       | 55.25         |
| 4     | 1:1                               | 10                       | 280               | -       | 46.41         |
| 5     | 1:1.125                           | 10                       | 260               | -       | 17.45         |
| 6     | 1:1.25                            | 10                       | 260               | -       | 2.04          |
| 7     | 1:1                               | 10                       | 260               | Ethanol | 26.96         |
| 8     | 1:1                               | 10                       | 260               | Acetonitrile | Traces |
| 9     | 1:1                               | 0                        | 260               | -       | -             |
| 10    | 1:1                               | 5                        | 260               | -       | Traces        |
| 11    | 1:1                               | 15                       | 260               | -       | 25.09         |

$^a$R/EAA: resorcinol/ethyl acetoacetate
$^b$Reaction using MW 800W

The optimizations of reaction parameters for the microwave-assisted synthesis of 7-hydroxy-4-methyl coumarin (Scheme-1) catalyzed by SnCl$_2$.2H$_2$O were summarized in Table-2. According to Table-2, the optimum condition for a model reaction was obtained by reacting 1:1 (mol/mol) of resorcinol/ethyl acetoacetate with 10 mol% SnCl$_2$.2H$_2$O for 260 s under solvent-free conditions and 800W microwave irradiation (Table-2, entry 3). The decreasing reaction time reduced an isolated yield (Table-2, entries 1 and 2), while an increase in the reaction time could not improve the yield (Table-2, entry 4). The formation of by-products causes the reduction of yield at a longer reaction time. In our conditions, the various molar ratios of precursors (1:1.125 and 1:1.25 mol/mol of resorcinol/ethyl acetoacetate) significantly reduced yields (Table-2, entries 5 and 6). The solvent effect on reaction yield was also optimized in the model reaction using SnCl$_2$.2H$_2$O catalyst. Ethanol was selected to represent polar protic solvent, while acetonitrile exemplified polar aprotic solvent. The best results were achieved under solvent-free conditions (Table-2, entry 3). On the other hand, the ethanol addition afforded a half reduction in yield (Table-2 entry 7), whereas the acetonitrile addition led to a lower result even further (Table-2, entry 8). The lower yield in addition to solvent is caused due to the competition between reactants and solvents at the catalyst’s active site, which led to a different interaction between solvent and catalyst. Subsequently, the catalytic activity of SnCl$_2$.2H$_2$O was observed by varying the amount of catalyst. The highest yield was obtained at 10 mol% of SnCl$_2$.2H$_2$O (Table-2, entry 3). There was no observed product outcome from the uncatalyzed reaction (Table-2, entry 9) indicates that this reaction requires an acid catalyst. Furthermore, 5 mol% SnCl$_2$.2H$_2$O showed traces in yield (Table-2, entry 10), while increasing the catalyst amount to 15 mol% could not raise the yield (Table-2, entry 11). The yield dropping at the increase of catalyst amount is probably due to the selectivity reduction in the catalyst’s active site. Table-3 recapped reaction parameters optimizations for the described reaction (Scheme-1) using AlCl$_3$ as a catalyst. Based on Table-3, the optimum condition was achieved at the solvent-free condition of 1:1 (mol/mol) of resorcinol/ethyl acetoacetate with 10 mol% AlCl$_3$ for 25 min under 600W microwave irradiation (Table-3, entry 3) the use of microwave power of 600W due to by-product minimization in AlCl$_3$-catalyzed reaction. Moreover, similar trends with model reaction catalyzed by SnCl$_2$.2H$_2$O in reaction time (Table-3, entries 1, 2, and 4), the molar ratio of precursors (Table-3, entries 5 and 6), and no addition of or lower catalyst's amount (Table-3, entries 7 and 8) were also
observed. Surprisingly, by increasing the amount of AlCl$_3$ to 15 mol%, the yield was increased (Table-3, entry 9). However, the more amount of catalyst added (20 mol%) tends to give a lower yield (Table-3, entry 10), similar to the previous trend using SnCl$_2$.2H$_2$O as a catalyst. Therefore, it led to other reaction conditions optimizations using 15 mol% of catalyst. Nevertheless, a decreasing reaction time offered a lower yield (Table-3, entry 11), while the increasing reaction time decreased the yield further (Table-3, entries 12 and 13).

Table-3: Optimization Reaction Parameter of Microwave-Assisted for the Synthesis of 7-Hydroxy-4-Methyl Coumarin Catalyzed by AlCl$_3$

| Entry | Molar ratio of R/EAA$^a$ (mol/mol) | AlCl$_3$ (% mol) | Reaction time (min) | Yield$^b$ (%) |
|-------|----------------------------------|-----------------|---------------------|--------------|
| 1     | 1:1                              | 10              | 10                  | 18.37        |
| 2     | 1:1                              | 10              | 20                  | 24.66        |
| 3     | 1:1                              | 10              | 25                  | 53.67        |
| 4     | 1:1                              | 10              | 30                  | 16.5         |
| 5     | 1:1.125                          | 10              | 10                  | 13.51        |
| 6     | 1:1.25                           | 10              | 10                  | 13.04        |
| 7     | 1:1                              | -               | 10                  | -            |
| 8     | 1:1                              | 5               | 10                  | 15.21        |
| 9     | 1:1                              | 15              | 10                  | 33.08        |
| 10    | 1:1                              | 20              | 10                  | 20.56        |
| 11    | 1:1                              | 15              | 5                   | 20.53        |
| 12    | 1:1                              | 15              | 15                  | 18.86        |
| 13    | 1:1                              | 15              | 20                  | 15.43        |

$^a$R/EAA: resorcinol/ethyl acetoacetate
$^b$Reaction using MW 600W

7-Hydroxy-4-methyl coumarin was obtained as a pale-yellow solid with R$_f$ 0.46 in n-hexane: ethyl acetate 6:4 (v/v). The melting point of this compound was measured at 187-188°C, which was similar to the previous report. FTIR spectrum of 7-hydroxy-4-methyl coumarin exhibited typical peaks of coumarins at a wavenumber of 3079-3116, 1640-1690, 1594-1605, and 1145 cm$^{-1}$ indicated C-H sp$^2$, C=O, C=C, and C-O-C, respectively. In addition, hydroxyl and methyl groups as additional substituents on coumarin scaffold were identified by peaks for O-H and C-H sp$^3$ at a wavenumber of 3157-3500 and 2936-2854 cm$^{-1}$, respectively. The FTIR spectrum of this compound was found by previous results. Furthermore, the UV-Vis spectrum of this compound showed the maximum wavelength of 218.5, and 323 nm belonging to lactone carbonyl and cinnamoyl chromophores, respectively, indicating the characteristic peaks of coumarins. NMR characterization of 7-hydroxy-4-methyl coumarin in 1D ($^1$H and $^{13}$C) and 2D (HSQC and HMBC) were recorded to support FTIR and UV-Vis analysis. $^1$H-NMR spectrum (500 MHz, acetone-$d_6$) exhibited six proton signals that indicated α-benzopyrone skeleton with methyl and hydroxyl groups attached to the backbone. Proton signals of α-benzopyrone consisted of one proton signal of alkene at a chemical shift of 6.07 ppm (1H, $d$, $J$ = 1.5 Hz, H-3) and three proton signals of trisubstituted benzene ring following ABX system at the chemical shifts of 7.60 (1H, $d$, $J$ = 8.5 Hz, H-5), 6.85 (1H, $dd$, $J$ = 8.5, 2.5 Hz, H-6), and 6.73 ppm (1H, $d$, $J$ = 2.5 Hz, H-8). Furthermore, methyl and hydroxyl groups as substituents appeared at chemical shifts of 2.41 (1H, $d$, $J$ = 1.5 Hz, H-11) and 9.36 ppm (1H, $s$, 7-OH), respectively. In addition, the $^{13}$C-NMR spectrum (125 MHz, acetone-$d_6$) showed ten carbon signals including one lactone carbonyl at a chemical shift of 161.0 ppm (C-2); six-carbon of a benzene ring, i.e. 161.8 (C-7), 156.3 (C-9), 127.3 (C-5), 113.7 (C-10), 113.5 (C-6), and 103.3 ppm (C-8); two-carbon of alkene, i.e. 153.8 (C-4) and 111.8 ppm (C-3); and one methyl carbon at 18.5 ppm (C-11). All the peaks of $^1$H- and $^{13}$C-NMR spectral data were in close agreement with previous reports, except for the assignment of carbon chemical shifts for C-2, C-6, C-7, and C-10. Herein, we revised the chemical shifts of C-2, C-6, C-7, and C-10 by analyzing HSQC and HMBC experiments (Fig.-1) which previous researchers did not conduct. According to Fig.-1, the chemical shift of C-2 was established by the HMBC correlation between proton alkene at C-3 ($d$$_{C}$ 6.07 ppm) with C-2 ($d$$_{C}$ 161.0 ppm). Moreover, the chemical shift of C-6 was recognized from the HSQC correlation between H-6 ($d$$_{H}$ 6.85 ppm) and HMBC correlation between the hydroxyl group ($d$$_{H}$ 9.36
ppm) with C-6 (δ C 113.5 ppm). Other HMBC correlations between H-6 and hydroxyl group with C-7 (δ C 161.8 ppm) and H-3 and H-6 with C-10 (δ C 113.7 ppm) were used to identify chemical shifts in C-7 and C-10, respectively.

Fig.-1: Key HSQC and HMBC Correlation of 7-hydroxy-4-methyl Coumarin

The reaction scope using the optimum conditions was studied for various phenol’s series of reactions (Scheme-2). In the Pechmann reaction, phenol as a nucleophile plays a vital role in intramolecular electrophilic aromatic substitution. Therefore, the reactivity of phenols will affect the reaction. The study on the phenols' reactivity was carried out via a similar condition to the model reaction, and the results were presented in Table-4. The highest yield was attained from the model reaction using resorcinol (Table-4, entry 1). Different catalysts, i.e. SnCl₂·2H₂O and AlCl₃, showed similar yields but under other conditions. A reaction catalyzed by SnCl₂·2H₂O requires a shorter reaction time (260 s) than AlCl₃ as a catalyst (25 min), which revealed the higher reactivity of SnCl₂·2H₂O instead of AlCl₃ as mentioned earlier.

Scheme-2: Reaction Scheme of 4-methyl Coumarins under Neat Condition

To investigate the effect of dihydroxy position, synthesis was conducted using catechol and hydroquinone following the optimum condition for SnCl₂·2H₂O (Table-4, entries 2 and 3). Under our condition, the reactions obtained the desired products in the mixtures with by-products. The side products were formed due to the high temperature of the reaction, which leads to the decomposition of the main product. However, slight modifications by varying microwave power and reaction time did not affect the products. Moreover, other reactions to explore the effect of phenyl position in phenol were also observed. A similar trend in yielding by-products was perceived in the reaction with α-naphthol using the optimum condition for AlCl₃ (Table-4, entry 4). In addition, the reaction between β-naphthol and ethyl acetoacetate failed to give the targeted product (Table-4, entry 5). The failure of the synthesis using β-naphthol is due to the lack of electronic activation of the aromatic ring. This is also confirmed by the previous trial from Dianhar et al., which also failed to afford coumarin from phenol. These findings revealed that the meta position of the electron-donating group (EDG), such as the hydroxyl group of phenols, greatly influences the reaction's activation. Furthermore, this EDG at the meta position plays a key role in ring closure at C10, active-directed by another hydroxyl as an EDG substituent in C7. Nevertheless, previous research succeeded in affording the corresponding coumarin as the only product using more robust acid catalysts, such as heteropoly acids and sulfonated catalysts. In the absence of this critical EDG at the meta position, the phenyl acetoacetate ester might have been formed, but it was not the primary concern of this study. All synthesized compounds were measured for their antioxidant activity through the DPPH assay and listed in Table-5. DPPH assay is the most commonly used method for antioxidant assay since it facilitates rapid, inexpensive, and straightforward methods. This assay was conducted based on the scavenging capacity measurement of hydrogen and electron transfer from a molecule or an antioxidant to DPPH•. Ascorbic acid was used as a positive control in this assay since ascorbic acid can donate hydrogen and electrons, which can be detected in UV-Vis measurement. According to the data in Table-5, by increasing the concentration, %RSA tends to be improved. The IC₅₀ value of 7-hydroxy-4-methyl coumarin and the mixture of 7,8-benzo-4-methyl coumarin were 99.69 (moderate) and 16.26 ppm (strong), respectively. The ability of 7-hydroxy-4-methyl coumarin as an antioxidant is due to the presence of hydroxyl group (-OH) in the aromatic ring (C7), and carbonyl (C=O) lactone, and phenyl ring. The weak O-H bonding will react with a stable free radical DPPH• to yield reduced DPPH and a stable free radical of 7-hydroxy-4-methyl...
coumarin due to its conjugation. Moreover, the carbonyl lactone and phenyl ring also allow single electron transfer from its oxygen atom and double bond to neutralize a DPPH•, respectively. The strong antioxidant activity of the 7,8-benzo-4-methyl coumarin mixture is caused by the contribution of the hydroxyl group in α-naphthol as an unreacted precursor besides carbonyl lactone in 7,8-benzo-4-methyl coumarin. Previous research had reported that α-naphthol showed similar activity with Trolox (a vitamin E analogue) at 100 mM with a %RSA value of 80% with DPPH assay.

Table-4: Pechmann Condensation on 4-methyl Coumarin Derivatives Synthesis using Various Phenols and Lewis Acids with Ethyl Acetoacetate

| Entry | Phenol | Lewis acid | Desired product | Isolated yield (%) |
|-------|--------|------------|----------------|--------------------|
| 1     | ![Phenol Image] | a. SnCl₂.2H₂O  
b. AlCl₃ | ![ Desired Product Image] | a. 55.25ᵃ  
b. 53.00ᵇ |
| 2     | ![Phenol Image] | SnCl₂.2H₂O | ![ Desired Product Image] | n.d.ᵃ |
| 3     | ![Phenol Image] | SnCl₂.2H₂O | ![ Desired Product Image] | n.d.ᵃ |
| 4     | ![Phenol Image] | AlCl₃ | ![ Desired Product Image] | n.d.ᵇ |
| 5     | ![Phenol Image] | AlCl₃ | ![ Desired Product Image] (expected) |ᵇ |

ᵃReaction conditions: MW 800W, solvent-free, phenolic/ethyl acetoacetate molar ratio of 1:1 (mol/mol), 260 s reaction time, and 10 mol% of SnCl₂.2H₂O  
ᵇReaction conditions: MW 600W, solvent-free, phenolic/ethyl acetoacetate molar ratio of 1:1 (mol/mol), 25 min reaction time, and 10 mol% of AlCl₃  
n.d: not determined, still in a mixture  
⁻: no reaction occurs

Antibacterial activity of selected compound towards *E. coli* (gram-negative) and *S. aureus* (gram-positive) was observed using the disc diffusion method and presented in Table-6. This method is the most widely used protocol for preliminary antibacterial screening since it provides simple and inexpensive methods. Disc diffusion assay measures a clear inhibition zone around the paper disc. Ciprofloxacin was used as a positive control since it exhibits a C₆C₃ skeleton similar to coumarin. Based on Table-6, 7-hydroxy-4-methyl coumarin was found to have moderate activity (6-9 mm of inhibition zone) against *E. coli* at all tested concentrations rather than *S. aureus*, which showed inactive results. Theoretically, increasing concentration will directly inhibit the bacteria's growth. However, in this study, raising the concentration above 125 ppm resulted in a lower inhibition zone area. This phenomenon can be occurred due to the rate of diffusion differences on agar and the inequality amount of the sample used.
Table 5: Antioxidant Activity Based on DPPH Radical Scavenging of 4-methyl coumarin Derivatives

| Sample                              | Concentration (ppm) | RSA (%) | IC₅₀ (ppm) | Remark     |
|-------------------------------------|---------------------|---------|------------|------------|
| 7-Hydroxy-4-methyl coumarin         | 5                   | 29.81   |            |            |
|                                     | 10                  | 30.36   |            |            |
|                                     | 25                  | 35.86   |            |            |
|                                     | 50                  | 36.58   |            |            |
|                                     | 100                 | 44.65   | 99.69      | Moderate   |
|                                     | 250                 | 86.59   |            |            |
| A mixture of 7,8-benzo-4-methyl coumarin | 5                  | 35.67   |            |            |
|                                     | 10                  | 37.04   |            |            |
|                                     | 25                  | 65.14   |            |            |
|                                     | 50                  | 98.49   | 16.26      | Strong     |
| Ascorbic acid                       | 5                   | 48.97   |            |            |
|                                     | 10                  | 62.36   |            |            |
|                                     | 25                  | 94.72   | 5.06       | Very strong|

DPPH assay: IC₅₀ < 10 ppm, very strongly active; 10-50 ppm, strongly active; > 50-100 ppm, moderately active; > 100-250 ppm, weakly active; > 250 ppm, inactive.

Table 6: Antibacterial Activities of 7-hydroxy-4-methyl coumarin Towards E. Coli and S. Aureus

| Compound                     | Inhibition zone (mm) in various concentration (ppm)²³ |
|------------------------------|--------------------------------------------------------|
|                              | E. coli | S. aureus |
| 7-Hydroxy-4-methyl coumarin  |         |           |
| 62.5                         | 7       | 9        |
| 125                          | 7       | 7        |
| 250                          | 7       | 7        |
| 500                          | 6       |          |
| 1000                         | 0       | 0        |
| Remark                       |         | Inactive |
|                             | Moderate |         |

CONCLUSION

Pechmann condensation using ethyl acetoacetate, various phenolics, and catalyzed by different Lewis acids to afford coumarin-scaffold was successfully carried out under microwave-assisted methods. Resorcinol was the most reactive phenol, while both SnCl₂·2H₂O and AlCl₃ were shown moderate to high catalytic activity among other Lewis acids used in this study. In addition, both antioxidants towards DPPH and antibacterial activity against E. coli of 7-hydroxy-4-methyl coumarin showed moderately active with an IC₅₀ value of 99.69 ppm and inhibition zone of 9 mm at 125 ppm, respectively. Further research needs to be conducted to synthesize other coumarin-based antioxidants and antibacterials.

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