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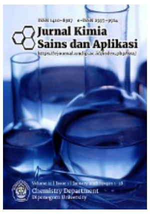

Microwave-assisted synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one and its activities as an antioxidant, sunscreen, and antibacterial

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Abstract

Chalcone analogs have been reported to have a variety of exciting biological activities, such as anticancer, anti-inflammatory, antioxidant, photoprotective, antibacterial, and antidiabetic activities. Therefore, analogs of these compounds have been widely synthesized as intermediate compounds or target molecules in the discovery of bioactive compounds to be applied in the pharmaceutical field. The purpose of this study is to synthesize chalcone analog, (E)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one under microwave irradiation and explore some of the biological activities of this compound, including testing the antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, in vitro sunscreen activity by microplate method, and antibacterial activity by disk diffusion method. DPPH test results indicate that the compound has weak antioxidant activity, with an IC50 value of 300.43 µg/mL. However, the compound showed excellent potential as a UV B and UV A filter at a concentration of 150 µg/mL with a %Te value of 0.73±0.10% (sunblock), %Tp value of 0.07±0.00% (sunblock), SPF value of 21.10±1.46 (ultra-protection) and potentially better than benzophenone-3 as a standard sunscreen. Then, disk diffusion testing showed that the compound had weak antibacterial activity against Staphylococcus aureus and did not show antibacterial activity against Escherichia coli at test concentrations of 30, 60, and 120 µg/disk.

1. Introduction

Chalcone (1,3-diphenylprop-2-en-1-one) is a precursor of flavonoid compounds that can be found in several types of plants. This compound is known to have a variety of exciting biological activities. For example, Broussochalcone A isolated from the plant *Broussonetia papyrifera* Vent is reported to have free radical scavenging activity stronger than α-tocopherol as a comparative antioxidant [1]. Some other natural chalcone analogs are also reported to have antibacterial activity; for example, the lichochalcone E. This compound is reported can inhibit the growth of several strains of *Staphylococcus aureus* with a minimum inhibitory concentration of 1-4 µg/mL [2]. Some hydroxy-substituted synthetic chalcone analogs are also reported to have inhibitory properties against *Escherichia coli* and *Staphylococcus aureus* bacteria [3]. Then, some nitro-substituted [4] and hydroxy-substituted [5] synthetic chalcone analogs have also been patented regarding their potential use as active ingredients in some cosmetic products, because they possessed good sunscreen activity. Other studies have also shown that hydroxy and methoxy substituted chalcone analogs, such as *(E)*-1-(2-hydroxyphenyl)-3-(4-methoxy phenyl)prop-2-en-1-one shows the maxima absorption close to the dibenzoyl methane as one of the active ingredients of sunscreen [6]. Then, the latest research shows that the analog chalcone *(E)*-1-(3-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one has the potential to be developed as an active ingredient in sunblock preparations at a concentration of 200 μg/mL with an SPF value of 30.61±0.38 [7].

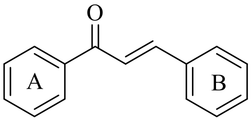

Figure 1. Structure of chalcone

Besides showing a variety of potential biological activities, chalcone analogs have also been used extensively as intermediates in the synthesis of their derivatives [8]. Various chalcone-derived compounds, such as flavonols [9] and pyrazolines [10], have been reported to have a variety of biological activity potentials. The diverse biological activities of chalcone analog compounds and their derivatives have made many researchers interested in synthesizing and exploring the potential use of these compounds in the context of discovering bioactive compounds as drug candidates.

Chalcone analog compounds can be synthesized by several conventional methods, such as the grinding method [11], stirring with magnetic stirrer [8], and reflux [12]. In some cases, this conventional method has several disadvantages, such as low reaction selectivity [13] and requires a relatively long reaction time [9]. On the other hand, synthesis by the microwave irradiation method has been widely reported can increase reaction selectivity and also shorten the reaction time [14].

Synthesis using conventional methods and anticancer potential of chalcone analog *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one has been reported by several previous researchers [15, 16, 17, 18]. However, the application of microwave irradiation to synthesize this compound has never been reported. Therefore, in this study, we are interested in applying the microwave irradiation method to synthesize this chalcone analog, then evaluate some other biological activities, including antioxidants, sunscreen, and antibacterial activities in vitro to explore the potential use of this compound widely. Because, in many cases, the fact is found that a chalcone analog possessed various biological activities. For example, xanthohumol, besides showing antidiabetic activity, this compound also has anti-cancer, antioxidant, and anti-inflammatory activity. In addition, isobavachalcone, besides showing anticancer activity, it also shows antibacterial and antifungal activity [19].

2. Methodology

2.1. Equipment and Materials

The equipment used for synthesis is the Samsung microwave (ME109F). The progress of the synthesis reaction was observed by TLC analysis using GF_{254} silica gel plates (Merck). The spots on the TLC plates were observed under UV lamps 254 and 366 nm (Camag^{TM}). HPLC analysis was carried out with UFLC Prominence-Shimadzu LC Solution, using a Shim-pack VP-ODS column (250 x 4.6 mm), a mixture of methanol and water used as mobile phase in a gradient elution system, with a flow rate of 0.75 mL/min, detected by a UV detector SPD 20AD and analysis time for 25 minutes. The melting point of the synthesized compound was measured by Stuart Melting Point (SMP-11). The UV spectrum was measured by UV-Vis Spectrophotometer (Genesys 10S UV-VIS v4.002 2L9N175013). The FT-IR spectrum was measured by FT-IR Spectrophotometer (Shimadzu, IR Prestige-21). The ^1H NMR spectrum was measured by the NMR spectrometer (Agilent, 500 MHz). The high-resolution mass spectrum is measured by premier XE mode positive LCT water. Antioxidant activity was evaluated with a 96-wells microplate reader (Berthold), and the sunscreen activity was evaluated with a 96-wells microplate reader (Epoch Biotech).

The materials used are 4'-hydroxy acetophenone (Merck), 4-methoxy benzaldehyde (Merck), potassium hydroxide (Merck), hydrochloric acid (Merck), distilled water (Bratachem), universal indicators (Merck), filter papers (Whatman 42), 1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich), ascorbic acid (Merck), benzophenone-3 (Cosmetic Grade), Nutrient Agar (Merck), physiological NaCl (Otsuka), chloramphenicol 30 μg/disk antibiotic (Oxoid), and some organic solvents such as *n*-hexane (Merck), ethyl acetate (Merck), chloroform (Merck), dichloromethane (Merck), methanol (Merck), absolute ethanol and dimethylsulfoxide (Merck).

2.2. Synthesis of Chalcone Analog

Synthesis of chalcone analog (*E*)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one as can be seen in Figure 2, was carried out by modifying the previous synthesis method [15, 16, 17, 18]. As much as 20 mmol 4-hydroxy acetophenone was dissolved in 20 mL absolute ethanol in an erlenmeyer, then added with 50 mL of 6N KOH solution and homogenized. 20 mmol of 4-methoxy benzaldehyde was added to the solution, and then the reactant mixture was irradiated in the microwave using 180 W power. The progress of the reaction was observed every 2 minutes by TLC analysis. After the reaction was complete, the reaction mixture was neutralized by adding 3N hydrochloric acid solution to form a precipitate. The precipitate was filtered with a Buchner funnel, and the solid was dried in a desiccator. The crude product solid obtained was tested by TLC to determine its purity and purified by recrystallization technique in hot ethanol. Then, the purity and physical properties of the recrystallized product are determined by the TLC test, melting point measurement, and HPLC analysis. The structure of the pure compound was determined through UV, FT-IR, 1H NMR, and HRMS spectroscopic analysis.

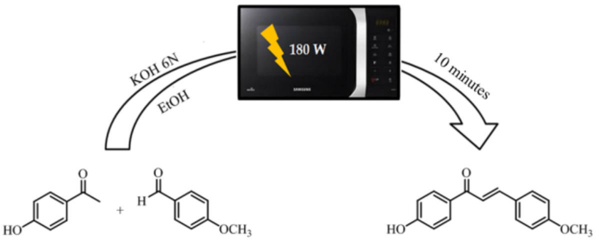

Figure 2. The synthesis route of chalcone analog

(E)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl) prop-2-en-1-one. Yellow solids. Yield 89%. Melting point 184-185 °C. TLC chromatogram: Rf 0.45 (*n*-hexane:ethyl acetate = 6:4). HPLC Chromatogram: t_R = 11.382 minutes. UV Spectrum (MeOH) (λ_{max}, nm): 203, 235 and 345. FT-IR spectrum (KBr) (ν, cm^{-1}): 3161, 3089, 2975, 2941, 2908, 1643, 1601, 1508, 1466, 1466, 1466 and 1037. The ^{1}H NMR spectrum (acetone-d$_6$, 500 MHz) (δ, ppm): 9.23 (*br-s*, ^{1}H, Ar-4-OH); 8.09 (*d*, 2H, Ar-2 ', 6'-H, *J* = 8.5 Hz); 7.79 (*d*, 2H, Ar-2,6-H, *J* = 9.0 Hz); 7,74 (*s*, 2H, H_α, H_β), 7.02 (*d*, 2H, Ar-3,5-H, *J* = 9.0 Hz); 6.98 (*d*, 2H, Ar-3', 5'-H, *J* = 8.5 Hz); 3.87 (*s*, 3H, Ar-4-OCH$_3$). ^{1}H NMR spectrum based on literature [20] (polysol, 300 MHz) (δ, ppm): 10.10 (*s*, ^{1}H, Ar-4-OH), 8.00 (*d*, 2H), 7.69 (*d*, ^{1}H, H_β), 7.66 (*d*, 2H), 7.58 (*d*, ^{1}H, H_α), 6.93 (*d*, 4H), 3.80 (*s*, 3H). ^{1}H NMR spectrum based on literature [21] (CDCl$_3$, 200 MHz) (δ, ppm): 7.99 (*d*, 2H, *J* = 8.6 Hz), 7.78 (*d*, ^{1}H, H_β, *J* = 15.6 Hz), 7.60 (*d*, 2H, J = 8.6 Hz), 7.41 (*d*, ^{1}H, H_α, J = 15.6 Hz), 6.93 (*d*, 4H, *J* = 7.2 Hz), 5.85 (*s*, ^{1}H, Ar-4-OH), 3.86 (*s*, 3H, Ar-4-OCH$_3$). Mass spectra (HRMS, ES+): mass was calculated as $C_{16}H_{15}O_3$ $[M + H]^+$: 255.1021 m/z and found at m/z = 255.1010 m/z.

2.3. Measurement of antioxidant activity by DPPH method

The *in vitro* antioxidant activity assay was conducted by modifying the previous procedure [22]. A sample solution (1000 µg/mL) and DPPH solution (80 µg/mL) were prepared in a methanol solvent. A total of 100 µL of 1000 µg/mL sample solution was pipetted to row A wells on 96-wells microplate. Then, the sample solution in line A was diluted by the two-fold serial dilution method by filling wells in line B-G with 50 µL of methanol and followed by transferring 50 µL of the solution from line A to line B. Then as much as 50 µL of the solution from line B was pipetted to line C, and so on up to line F. Furthermore, a total of 50 µL of the solution from row F was pipetted and discarded, so that several sample solutions were obtained with a concentrations of 1000 µg/mL (line A), 500 µg/mL (line B), 250 µg/mL (line C), 125 µg/mL (line D), 62.5 µg/mL (line E), and 31.25 µg/mL (line F). Ascorbic acid solutions as standard antioxidant were prepared in the same way, but with a variety of concentrations of 100; 50; 25; 12.5; 6.25; and 3,125 µg/mL. Then, as much as 80 µL of DPPH solution was pipetted into lines A to line G and as much as 130 µL of methanol as a blank was pipetted to line H. The mixture on 96-wells microplate was then incubated for 30 minutes in a dark room [23]. Then, the absorbance of the solutions was measured with a 96-wells microplate reader using a 520 nm filter [24], and the percentage of free radical scavenging activity (percentage of inhibition) was calculated based on equation 1 below.

$$\text{Inhibition}(\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}\, x\; 100\% \qquad (1)$$

Inhibition Concentration 50 (IC$_{50}$) value is calculated based on a linear regression equation (y = ax + b) obtained from the curve by plotting the value of Ln concentration on the x-axis and the percentage inhibition value on the y-axis. Antioxidant activity is categorized as very strong if the IC$_{50}$ value <50 µg/mL, strong if the IC$_{50}$ value is between 50-100 µg/mL, while if the IC$_{50}$ value is between 101-250 µg/mL, it is weak if the IC$_{50}$ value is between 251-500 µg/mL, and not active as an antioxidant if the IC$_{50}$ value> 500 µg/mL [25].

2.4. *In vitro* sunscreen activity test

2.4.1. Determination of %Te and %Tp values

Determination of %Te and %Tp values were conducted by modifying the previous method [7]. A total of 10 mg of sample was dissolved in 10 mL absolute ethanol to obtain the mother liquor with a concentration of 1000 µg/mL. Then the mother liquor was diluted to obtain a 200 µg/mL test solution. As much as of 120, 90, 60, 30, and 15 µL of the 200 µg/mL test solution were pipetted successively into the wells in lines A, B, C, D, and E using multichannel micropipettes. Then as much as 30, 60, 90, and 105 µL absolute ethanol was pipetted into wells in rows B, C, D, and E using a multichannel micropipette. In order to obtain a sample concentration of 200 µg/mL (row A), 150 µg/mL (line B), 100 µg/mL (line C), 50 µg/mL (line D), 25 µg/mL (line F). The benzophenone-3 solutions as standard sunscreen were prepared in the same way as the sample solutions. The wells in line F were filled with 120 µL of absolute ethanol as blank. A 96-wells microplate reader measured the absorbance of the solution in each well at the wavelengths that cause erythema (293-318 nm) and pigmentation (323-373 nm) with 5 nm intervals. Then, to calculate the %Te and %Tp values, the absorbance value (A) was first converted to the transmittance percentage value (% T) using the following equation.

$$A = -\log\, \text{T} \qquad (2)$$

Next, the % T value obtained is entered into equation 3 to calculate the %Te value and entered into equation 4 to calculate the %Tp value. In this case, Fe is the erythema flux value, and Fp is the pigmentation flux. Fe and Fp values are constants and have been explained in previous literature [26].

$$\%\text{Te} = \frac{\Sigma(\text{T x Fe})}{\Sigma\text{Fe}} \qquad (3)$$

$$\%\text{Tp} = \frac{\Sigma(\text{T x Fp})}{\Sigma\text{Fp}} \qquad (4)$$

Sunscreen is categorized as a sunblock if it has %Te <1% and %Tp of 3-40%, extra protection if it has %Te of 1-6% and %Tp of 42-86%, standard suntan if it has %Te of 6-12% and %Tp of 45-86%, fast tanning if it has %Te of 10-18% and %Tp of 45-86% [26].

2.4.2. Determination of SPF value

The determination of the SPF value was performed by modifying the previous method [7]. A total of 10 mg of sample was dissolved in 10 mL absolute ethanol to obtain the mother liquor with a concentration of 1000 µg/mL. Then the mother liquor was diluted to obtain a 200 µg/mL test solution. As much as of 120, 90, 60, 30, and 15 µL of the 200 µg/mL test solution were pipetted successively into the wells in lines A, B, C, D, and E using multichannel micropipettes. Then, as much as 30, 60, 90 and 105 µL absolute ethanol is pipetted into wells in rows B, C, D, and E using a multichannel micropipette, so that the concentration of the test solution obtained in row A (200 µg/mL), B (150 µg/mL), C (100 µg/mL), D (50 µg/mL), and E (25 µg/mL). The benzophenone-3 solutions as standard sunscreen were prepared in the same way as the sample solutions. The wells in line F were filled with 120 µL of absolute ethanol as blank. A 96-wells microplate reader measured the absorbance of the solution in each well at the wavelength range of 290-320 nm. The absorbance value of the sample at each test concentration was recorded, and then the SPF value was calculated using the Dutra equation [27].

$$\text{SPF} = \text{CF x} \sum_{290}^{320} EE(\lambda)\ x\ I\ (\lambda)\ x\ Abs\ (\lambda) \tag{5}$$

In this case, CF is a correction factor of 10. EE is Erythemal Effect, and I is the intensity of the sun. The value of EE x I is a constant, as explained elsewhere [27]. Then Abs is the absorbance of the sample after deducting the absorbance of the blank. The protective strength of a sunscreen is categorized as ultra-protection if the SPF value is ≥ 15, the maximum protection is if the SPF value is 8-≤ 15, extra protection is if the SPF value is 6-≤ 8, moderate protection is if the SPF value is 4-<6 and minimal protection is if the SPF value is 2-<4 [28].

2.4.3. Determination of antibacterial activity by the disk diffusion method

The antibacterial activity test was carried out by modifying the previous procedure [29, 30]. Before testing, the equipment has been sterilized, and the bacteria used were also rejuvenated. The bacterial suspension was made in a physiological NaCl solution with a %T of 25% at a wavelength of 580 nm. A total of 10 mg of chalcone analog was weighed and dissolved in 10 mL DMSO, then diluted to obtain test solutions with concentrations of 120, 60, and 30 µg/disk. A total of 300 µL of bacterial suspension was put into a petri dish and added with 15 mL of NA media, then homogenized and then allowed to stand until it solidified. As much as 10 µL of each concentration of test solutions was pipetted and dropped on each disk paper. Then, the disk paper was placed on a compacted inoculum media and incubated for 24 hours at 37°C by turning the petri dish upside down. After 24 hours, the formed inhibition zone around the disk was measured. The 10 µL of DMSO was used as a negative control, and 30 µg chloramphenicol antibiotic disk was used as a positive control. The strength of the sample antibacterial activity was expressed as the percentage of the inhibitory power by comparing the sample inhibitory diameter with the inhibitory diameter of the positive control following equation 6 below.

$$\text{inhibitory power (\%)} = \frac{\text{Inhibitory diameter of the sample}}{\text{Inhibitory diameter of the positive control}}\ x\ 100\% \tag{6}$$

Antibacterial activity is categorized as strong if the percentage of inhibition power ≥ 70%, as moderate if the percentage of inhibition ranges from 50-70% and as weak if the percentage of inhibition < 50% [31].

3. Results and Discussion

3.1. Chalcone analog synthesis

The chalcone analog compound, *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one has been successfully synthesized by the microwave irradiation method through the Claisen-Schmidt condensation reaction between 4'-hydroxy acetophenone and 4-methoxy benzaldehyde. The microwave irradiation method was chosen because it has various advantages, such as faster reaction time, more comfortable to control, more energy-efficient, and can increase product yield, but not under all reaction conditions [32]. In this study, the use of the microwave irradiation method was proven to accelerate reaction times and increase the yield of synthesized compounds when compared to the literature [15, 17]. However, the yield obtained in this study is slightly lower when compared to the literature [21], as can be seen in Table 1.

Table 1. Comparison of the synthesis results of compound *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one by conventional methods from several references and microwave irradiation methods

Synthesis Method	Reaction Conditions	Yield (%)	Melting Point (°C)	Ref.
Stirrer	Room Temperature	86	182	[17]
		86	180-182	[15]
		90	184-185	[21]
Microwave irradiation	180 W power	89	184-185	This work

Based on Table 1, it can be observed that the melting point of the synthesized compound is reached at a temperature of 184-185°C. The melting point range of ≤ 2°C indicates that the solid product obtained was pure. This conclusion was supported by a TLC chromatogram that showed the single spot with Rf 0.45 and HPLC chromatogram, which also showed a single peak at a retention time of 11.38 minutes.

The structure of synthesized chalcone analog was confirmed based on the analysis of UV, FT-IR, ^{1}H NMR, and HRMS spectrum. The UV spectrum of chalcone analog shows absorption at wavelengths of 203, 235, and 345 nm. This absorption indicates the characteristics of conjugated double bonds found in the conjugation system of benzoyl and cinnamoyl of chalcone analog. The presence of a hydroxy group (OH) in chalcone analog is confirmed by the appearance of a broad absorption band in the FT-IR spectrum, at a wavenumber of 3162 cm^{-1} which is supported by the appearance of a broad singlet peak (δ 9.23 ppm) with the integration of one proton in the ^{1}H NMR spectrum. The presence of a methoxy group (OCH_3) is confirmed by the appearance of several absorption bands in the FT-IR spectrum at wavenumbers of 2975-2908 cm^{-1} (aliphatic C-H) and 1037 cm^{-1} (C-O), which is supported by the appearance of a singlet peak in the ^{1}H NMR spectrum with the integration of three protons at δ 3.87 ppm. In addition, the presence of a carbonyl group (C = O) of ketone that conjugated with an aromatic ring A and with α, β double bond (keto ethylene group) is confirmed by the appearance of the absorption band at the wavenumber of 1643 cm^{-1}. Then, the presence of phenyl groups is confirmed by the appearance of absorption bands at wavenumbers of 1601, 1508, and 1466 cm^{-1} (aromatic C=C) and 3089 cm^{-1} (aromatic C-H and alkene C-H) in the FT-IR spectrum, which is supported by the appearance of peaks in the ^{1}H NMR spectrum with a total integration of 10 protons in the chemical shift area of 8.01-6.97 ppm, as can be seen in Figure 3.

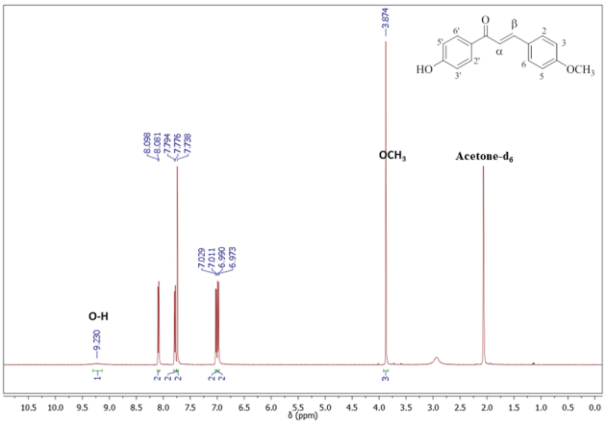

Figure 3. ^{1}H NMR spectrum (acetone-d_6, 500 MHz) of chalcone analog *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one

Generally, the signals of α and β protons appear as two doublet signals (1H), with a coupling constant of 15-16 Hz. Nevertheless, in this case, the signals of protons α and β appear as a singlet (2H) signal. This can be caused by the effect of the solvent used in the measurement of the spectrum so that the chemical environment of α and β protons in chalcone analog becomes equivalent. As a result, the spectrum does not show a spin splitting, even though the protons are not equivalent, structurally. When measured with polysol solvent [20], there is a slight spin splitting between α and β protons because the chemical environment becomes slightly different, but the 3‘/5’ and 3/5 proton signals were overlapped and appear as a doublet (4H) signal. When measured with the $CDCl_3$ solvent [22], the difference in the chemical environment of α and β protons becomes greater, as a result, the two doublet signals become further apart, but the 3'/5' and 3/5 proton signals still overlap, so that they appear as a doublet (4H) signals. Another difference can be observed from the hydroxy (OH) proton signal. In the acetone-d_6 solvent, the hydroxy proton signal appears at a chemical shift of 9.23 ppm as a broad singlet. However, when measured in polysol and $CDCl_3$ solvents, the hydroxy proton signal appears as a singlet at a chemical shift of 10.10 ppm and 5.85 ppm, respectively. Comparison of ^{1}H NMR spectrum data of synthesized chalcone analog as measured in an acetone-d_6 with ^{1}H NMR spectrum data of reference compounds measured in polysol and $CDCl_3$ has been shown in section 2.2. Then the comparison of the three ^{1}H NMR spectra of this compound in the aromatic chemical shift region can be seen in Supplementary Materials. Based on the comparison, it can be concluded that the type of solvent used in the ^{1}H NMR analysis can affect the chemical shift of protons of chalcone analog.

Then, a high-resolution mass spectrum measurement is performed to confirm the molecular mass of the synthesized chalcone analog. Based on the results of mass spectrum analysis, the molecular ion peak $[M+H^+]$ of chalcone analog in the MS spectrum was found at m/z 255.1010 with a mass difference of 0.0011 from the calculated mass. This small difference in mass shows that the compound obtained has excellent purity. Thus, the overall results of the spectroscopic analysis showed that the compound obtained had the structure as expected.

3.2. Biological Activity Assay

3.2.1. Measurement of Antioxidant Activity by DPPH Method

In this study, the measurement of antioxidant activity was carried out by the DPPH method. This method has several advantages, including being more accessible and cheaper compared to other antioxidant activity testing methods. Therefore, this method is widely used to predict the potential antioxidant activity of a pure compound or plant extract. The activity of capturing free radicals by chalcone analog and ascorbic acid as a standard antioxidant can be observed visually through the color changes that occurred in the test solution. DPPH solution has maximum absorbance in the visible light region, which is in the wavelength range of 515-520 nm. When the sample stabilizes a dark purple DPPH free radical through a proton or electron donor mechanism, the DPPH radical will be reduced to its stable form, namely 1,1-diphenyl-2-picrylhydrazine (DPPH-H) which is yellow [22], as can be seen in Figure 4.

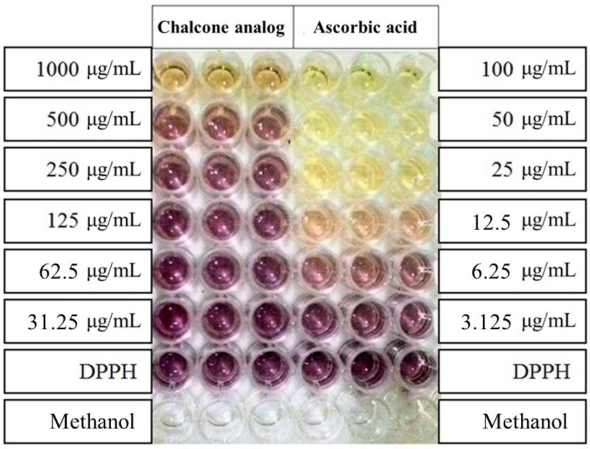

Figure 4. Color changes in antioxidant activity test with the DPPH method

The results of the antioxidant activity assay of chalcone analog by the DPPH method can be seen in Table 2. Based on these results, the higher the test concentration, the higher the DPPH free radical scavenging activity. A hydroxy-substituted chalcone analog can donate its hydroxyl proton to the DPPH free radical because the conjugation system in this compound will direct resonance into the aromatic ring. Then, the presence of electron-donating groups such as methoxy in this compound can also increase the stability of produced aryloxy radicals through electron delocalization [33]. The mechanism of proton donation of chalcone analog to DPPH free radical can be seen in Figure 5.

Table 2. DPPH test result of chalcone analog

Compounds	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Chalcone analog	1000	77.61±1.84	300.43
	500	59.61±1.71	
	250	46.29±0.62	
	125	30.09±0.45	
	62.5	15.62±0.66	
	31.25	1.44±0.87	
Ascorbic acid	100	98.63±0.12	9.45
	50	85.03±0.76	
	25	69.83±0.76	
	12.5	57.24±1.14	
	6.25	40.39±1.69	
	3.125	27.07±0.45	

Based on the calculation of IC_{50} values, the antioxidant activity of this chalcone analog is included in the weak category [25], with an IC_{50} value of 300.43 µg/mL. In contrast, ascorbic acid as a standard antioxidant shows extreme free radical scavenging activity, with an IC_{50} value of 9.45 µg/mL. This is assumed because the chalcone analog has only one proton that can be donated to DPPH free radical, the hydroxyl proton which is located in the *para* position of the ring A, while ascorbic acid can donate two protons rapidly to DPPH free radicals [34] as can be seen in Figure 6.

Some literature also supports this explanation that the phenolic compound antioxidant activity was affected by the position of the hydroxyl group and also by the number of hydroxyl groups attached to the aromatic ring. The more hydroxyl groups that are bound to a phenolic compound will increase the ability of the compound to donate its protons to DPPH free radicals, thereby increasing the potential for antioxidant activity [35]. For example, compound (*E*)-1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one containing two hydroxy substituents in the aromatic ring has a stronger antioxidant activity (IC_{50} = 47.45 µM) [36].

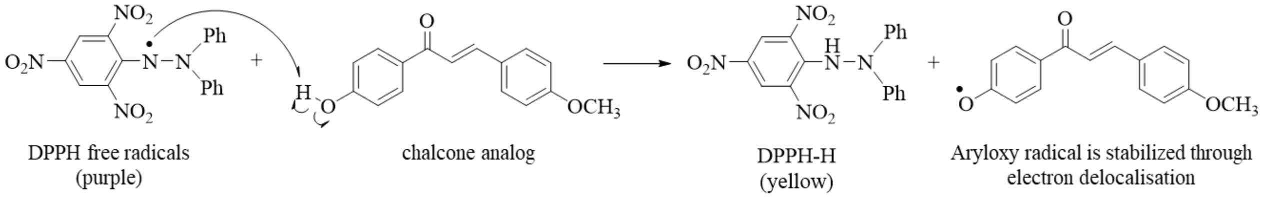

Figure 5. Proton donor mechanisms of chalcone analog to DPPH free radicals

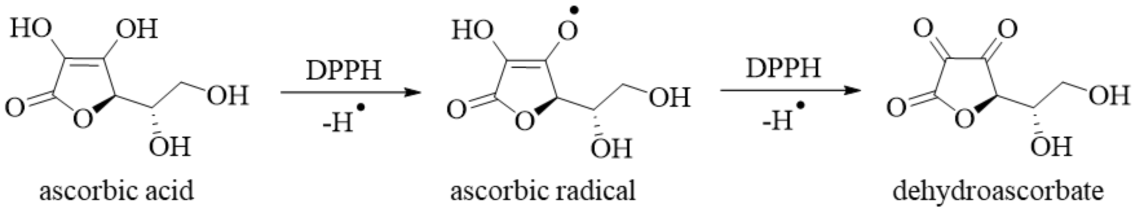

Figure 6. Proton donor mechanisms of ascorbic acid to DPPH free radicals

3.2.2. *In vitro* Sunscreen Activity Test

Sunscreen activity testing was carried out *in vitro* using a 96-wells microplate reader. This test includes determining the %Te, %Tp, and SPF values. The %Te value represents the ability of a molecule to protect the skin from UV B rays (290-320 nm), which are transmitted and can cause redness of the skin (erythema). While the %Tp value illustrates the ability of a molecule to protect the skin from UV A rays (320-375 nm), which are passed and can cause darkening of the skin (pigmentation) [37]. The lower the %Te and %Tp values of a substance indicates that the smaller the intensity of UV rays transmitted to the skin. In other words, the better the potential of the substance acts as a UV B or UV A filter.

Table 3. Results of determining the %Te and %Tp values of chalcone analog in various concentrations

Compounds	Concentration (µg/mL)	%Te values	UV B Filter Categories	%Tp values	UV A Filter Categories
Chalcone analog	200	0.19±0.01	Sunblock	0.05±0.00	Sunblock
	150	0.73±0.10	Sunblock	0.07±0.00	Sunblock
	100	3.29±0.19	Extra protection	0.27±0.12	Sunblock
	50	21.74±1.40	-	4.03±1.68	Sunblock
	25	42.78±0.13	-	16.24±0.63	Sunblock
Benzophenone-3	200	0.30±0.01	Sunblock	9.72±0.58	Sunblock
	150	0.85±0.03	Sunblock	13.04±0.23	Sunblock
	100	5.73±0.66	Extra protection	22.82±0.23	Sunblock
	50	21.09±2.04	-	39.93±2.58	Sunblock
	25	44.49±1.99	-	60.97±3.05	Fast tanning

Based on sunscreen activity test, as can be seen in Table 3, the results show that at the lowest test concentration (25 µg/mL), chalcone analog, *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one has been able to provide proper protection from UV A rays, but it has not been able to protect from UV B rays. The chalcone analog can only provide proper protection from UV A and UV B rays at a test concentration of 150 µg/mL with %Te and %Tp values of 0.73±0.10 and 0.07±0.00 %, respectively. At these concentrations, the protective strength by chalcone analog can be categorized as sunblock [26]. When compared with a standard sunscreen compound (benzophenone-3 or oxybenzone), the chalcone analog proved to be slightly better in acting as a UV B filter, which is shown by the %Te value which is slightly lower than the %Te value of benzophenone-3 at a test concentration of 100-200 µg/mL. The chalcone analog also possessed better potential as UV A filter. Because, at the lowest test concentration (25 µg/mL), the chalcone analog can already act as UV A filter in the sunblock category, while benzophenone-3 at the same concentration still causes the fast tanning effect. This is caused by the presence of the conjugation system of p-methoxy cinnamoyl in chalcone analog. The presence of a more extended conjugation system causes the chalcone analog to has a better ability to absorb UV light at higher wavelengths compared to benzophenone-3, so that, the intensity of UV A light transmitted to the skin (%Te) is lower compared to benzophenone-3. Therefore, the chalcone analog can act better as a UV A filter.

Then, the SPF values measurement is performed to determine the ability of a sunscreen to protect the skin from sun exposure. The SPF value states how many times the natural endurance of a person's skin is doubled so that it is safe in the sun without getting burnt [38]. Based on measurements of the SPF values, as can be seen in Table 4. The results show that at a concentration of 100 µg/mL, the chalcone analog was able to provide ultra-protection as indicated by an SPF value ≥ 15 [29], while the benzophenone-3 can provide protection ultra at concentrations above 100 µg/mL. Thus, it can be concluded that the chalcone analog has better potential in protecting the skin from UV B and UV A radiation compared to benzophenone-3.

Table 4. Results of determining SPF values of chalcone analog in various concentrations

Compounds	Concentrations (µg/mL)	SPF values	Protection categories
Chalcone analog	200	29.60±0.55	Ultra
	150	21.10±1.46	Ultra
	100	15.83±2.28	Ultra
	50	7.99±0.66	Extra
	25	3.94±0.15	Minimum
Benzophenone-3	200	26.71±0.31	Ultra
	150	19.98±0.52	Ultra
	100	12.10±1.44	Maximum
	50	8.49±1.95	Maximum
	25	2.85±0.50	Minimum

3.2.3. Antibacterial Activity Test with Disk Diffusion Method

The results of the antibacterial activity test of the chalcone analog, *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one with the disk diffusion method can be seen in Figure 7 and Table 5. Based on these results, it can be observed that at test concentrations of 30, 60, and 120 µg/disk, this compound only shows antibacterial activity against Gram-positive bacteria, *Staphylococcus aureus* but does not show inhibitory zones against Gram-negative bacteria, *Escherichia coli.*

As presented in Table 5, it can be observed that the higher the concentration of the test, the greater the diameter of the inhibition zone against Gram-positive bacteria, *Staphylococcus aureus*. However, based on literature [31], the antibacterial activity produced at all concentrations of the test is still relatively weak, with a percentage of inhibition <50% compared to the inhibition produced by chloramphenicol as a positive control.

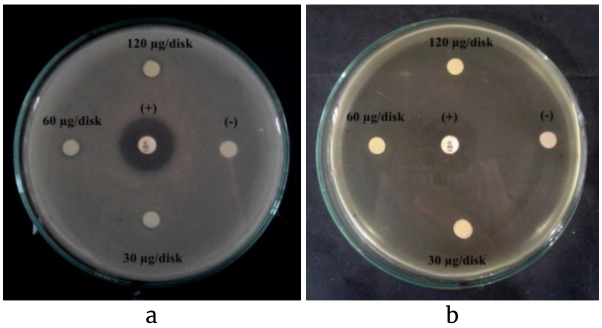

Figure 7. Results of antibacterial activity test of chalcone against (a) *Staphylococcus aureus*, (b) *Escherichia coli*

Table 5. Results for antibacterial activity test of chalcone analog

Bacteria	Treatments	Inhibition zone diameters (mm)				Inhibition powers (%)
		1	2	3	Average	
Staphylococcus aureus	Positive control	20.2	20.9	19.7	20.3±0.6	100
	Negative control	0	0	0	0	0
	30 µg/disk	7	6.5	6.5	6.7±0.3	33
	60 µg/disk	7.3	7	7	7.1±0.2	35
	120 µg/disk	7.5	7.5	7.5	7.5±0.0	37
Escherichia coli	Positive control	20.3	20.1	19.6	20.0±0.4	100
	Negative control	0	0	0	0	0
	30 µg/disk	0	0	0	0	0
	60 µg/disk	0	0	0	0	0
	120 µg/disk	0	0	0	0	0

Based on previous research on the antibacterial mechanism of another chalcone analog with a similar structure (hydroxy substituent located in the *meta* position), the chalcone analog exhibits antibacterial mechanisms by damaging the cell wall of *Staphylococcus aureus* bacteria [39]. To damage the bacterial cell wall, a compound must be able to interact with bacterial cell wall components. Based on the literature, the cell wall structure of Gram-negative bacteria is more complex and dynamic than Gram-positive bacteria. The cell wall of Gram-negative bacteria has an outer membrane above a thin layer of peptidoglycan. This structure causes Gram-negative bacteria to have a strong, robust, and elastic cell wall [40]. Therefore, the molecule size, the capacity of the electron-withdrawing and donating groups, and the hydrophilicity of a molecule will determine its antibacterial activity. Besides, the antibacterial activity of chalcone analog is also affected by the hydrophobicity of the bacterial cell surface. Based on previous research, chalcone analog compound with a hydroxy substituent in the *meta* position provide better antibacterial activity against bacteria with more hydrophobic cell surfaces. According to the study, the surface of *Staphylococcus aureus* cells is more hydrophobic compared to *Escherichia coli* [39]. This might cause chalcone analog *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one only shows inhibition against *Staphylococcus aureus* but does not show inhibition against *Escherichia coli* at the test concentrations.

4. Conclusion

In this study, chalcone analog *(E)*-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one has been successfully synthesized using a microwave irradiation method with shorter time and more yield high compared to some previous synthesis methods. Based on bioactivity testing that has been done, the chalcone analog compound shows a better potential for sunscreen activity compared to the standard sunscreen, benzophenone-3. However, the compound has no potential as an antioxidant and antibacterial. Further studies are needed to determine the stability of this compound under UV radiation and also its toxicity to human skin.

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