

GREEN SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF METHOXY CHALCONES

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ABSTRACT

The purpose of this research is to develop the synthesis of chalcone through a green chemical approach that has the potential as an antibacterial. Chalcone synthesis was carried out using a grinding technique through a Claisen-Schmidt condensation reaction at room temperature for 30 minutes by grinding benzaldehyde (4-bromobenzaldehyde or 4-hydroxybenzaldehyde), 4'-methoxyacetophenone, and NaOH. The reaction product was purified by recrystallization through 96% ethanol. The purity of the compound was tested by TLC. Characterization of chalcones by FT IR, ¹H-NMR, ¹³C-NMR, and GC-MS showed that 4-hydroxy-4'-methoxy chalcone and 4-Bromo-4'-methoxy chalcone were formed. The antibacterial activity of Methoxy chalcone was determined in vitro by a disc diffusion test for gram-positive and gram-negative bacteria. The test results showed that the second compound chalcone had weak activity against *Staphylococcus aureus* and was not active against *Escherichia coli*.

Keywords: Chalcone, Claisen Schmidt, Grinding, Antibacterial

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INTRODUCTION

Control of these bacteria often creates a new problem, resistance to antibiotics. This resistance can occur due to several factors, such as changes in receptor sites on bacteria that cause drug binding to the target to be reduced or lost, the presence of microorganisms that produce enzymes that can damage drug activity, changes in the permeability of microorganisms, mutations, and genetic transfer. Modifying the structure of resistant antibacterial drugs must be developed to overcome the resistance problem. Therefore, one of the strategies for developing antibacterial is to find new compounds that can be antibacterial, namely chalcone compounds. Chalcone (1,3-diphenylpropenone) is an intermediate in synthesizing various heterocyclic compounds.² Claisen-Schmidt condensation reactions between aromatic aldehydes and aromatic ketones are often used to form chalcones. This condensation can occur with the help of an acid or base catalyst. The use of acid catalysts such as HCl, BF₃, and B₂O₃ in the condensation reaction of chalcone formation generally gives low yields (10-40%).² KOH catalyst has been used in the chalcone synthesis reaction through the Claisen-Schmidt reaction with a yield of 88-94%.³ Ba(OH)₂ used in the synthesis of chalcone derivatives was obtained with a yield of 88-98%,⁴ and the use of NaOH as a catalyst in the synthesis of chalcone gave a yield of 90-96%.⁵ The Claisen-Schmidt reaction in the chalcone synthesis with NaOH catalyst gave better yields (93-98%) than KOH, NaOAc, and NH₄OAc (81-85%).⁶ This reaction is very important in synthetic organic chemistry. Chalcone synthesis through the Claisen-Schmidt condensation reaction has been widely used.^{7,8,9,10,11} Susanti *et al.* (2014) synthesized hydroxy chalcone from hydroxyl acetophenone and methoxy benzaldehyde by conventional Claisen-Schmidt condensation using ethanol as a solvent. Previous studies have shown that hydroxy chalcone requires bases (NaOH 50%), long reaction (24 hours), and low yields (40-70%).¹² The development of chalcone synthesis through a green synthesis approach has been carried out using ultrasonic irradiation¹³, but there are still weaknesses such as the ultrasonic frequency is not universally the same and it is difficult to control the temperature. This research aims to develop a new design to synthesize two chalcones by grinding technique. This technique develops chalcone synthesis, which is profitable because it uses elementary equipment, namely a pestle and mortar. Various chalcone has been carried out for synthesis by grinding technique from 2-acetyl-1-naphthol and benzaldehyde.³ Rahman *et al.* (2012) synthesized chalcone with this technique without solvent in a short reaction time (4-8 minutes) with yields (84-95%).⁶ The chalcone from cyclohexanone and benzaldehyde were synthesized by grinding technique, giving 96-98%. Susanti *et al.* (2014) have also synthesized three

derivative compounds of hydroxyl chalcone with this technique and gave 70-84%.¹² However, chalcone synthesized by grinding has not been prepared from 4-methoxyacetophenone with substituted benzaldehyde. In this study, some chalcones derivatives were synthesized from 4-methoxyacetophenone with 2 benzaldehyde derivatives (4-hydroxybenzaldehyde and 4-bromobenzaldehyde) (Fig.-1).

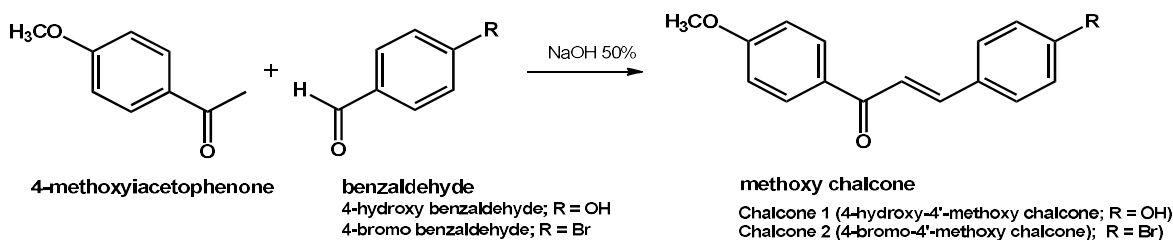


Fig.-1: Synthesis Reaction of Methoxy Chalcone

EXPERIMENTAL

Material and Instrumentations

The materials used in this study have an analytical grade quality from E-Merck, including 4-Bromo benzaldehyde, 4-hydroxy benzaldehyde, 4-methoxyacetophenone, sodium hydroxide, hydrochloric acid, acetone, ethyl acetate, ethanol, n-hexane, chloroform, methanol, Thin layer chromatography (TLC) aluminum plates 20x20 cm coated by silica gel 60 F254 (Merck), and anhydrous sodium sulfate. The equipment was laboratory glassware, analytical balance (Libror EB330 Shimadzu), magnetic stirrer, desiccator, magnetic stirrer plate, 254 nm UV lamp, Whatman paper no. 1, Infrared spectra Shimadzu Prestige-21 using KBr discs., proton core magnetic resonance spectrometer (¹H-NMR, 500 MHz), carbon (¹³C-NMR, 125 MHz) JEOL-MY500, and GC-MS-2010 S-SHIMADZU.

General Procedure to Chalcone Synthesis

The chalcone was synthesized by grinding 4-methoxyacetophenone with 4-bromobenzaldehyde (or 4-hydroxybenzaldehyde) in a mortar and pestle at room temperature for several minutes. The completeness of the reaction was monitored by Thin Layer Chromatography (TLC). The reaction mixture was then diluted with cold water, neutralized with a cold solution of HCl 10% (v/v), then filtered. Purification was carried out by recrystallization. The synthesized chalcone was then characterized by FT IR, ¹H-NMR, ¹³C-NMR, and GC-MS.

Antibacterial Evaluation

The antibacterial activity from synthesized chalcone was tested by the filter paper disc diffusion method (the filter paper plate diffusion method). The test bacteria used were *E. coli* and *S. aureus*. The medium used was Mueller Hinton (MH) agar and tetracycline was used as the standard for antibiotics. All equipment used for antibacterial testing must be sterilized by inserting tools that have been wrapped in aluminum foil in an autoclave. Muller-Hinton agar was prepared according to the manufacturer's instructions, then autoclaved immediately and cooled to 45-50°C. Pour as much as 20-25 mL of agar into a 150 mm Petri disk. The media sterility test was carried out by incubating the media for 24 hours at 30-35°C. Finally, the media is ready to be used for the antibacterial test. The selected bacterial culture was mixed with sterile NaCl solution, so that turbidity of about 0.5 CFU/mL (colony-forming units per milliliter) was obtained. A Petri plate containing 20 mL of MH agar was used as a test bacteria medium. The inoculum was spread over the surface of the solid MH medium. Whatman disc filter paper no. 1 (6 mm diameter) was dripped with the sample (20 µL/disc), then placed on the surface of the plate. Chloramphenicol is a positive control, and DMSO is a negative control. Plates that have been inoculated with bacteria were incubated at 37 °C for 24 hours. The inhibition zone's diameter was shown by forming a clear zone around the filter paper of more than 2 mm, indicating a positive inhibition.¹⁴

RESULTS AND DISCUSSION

Synthesis of Chalcone

Chalcone synthesis by grinding technique was carried out by grinding 4-methoxyacetophenone, benzaldehyde (4-hydroxybenzaldehyde and 4-bromobenzaldehyde), and solid NaOH in a mortar. This

grinding was carried out for 30 minutes at room temperature. In this process, frictional energy was generated from local heat due to collisions between reactants which accelerated the reaction to form products. Monitoring of the results of the synthetic compounds was carried out using TLC. The results of TLC of chalcone 1 showed two stains, indicating that the resulting product was not pure, so recrystallization was necessary. The recrystallization obtained a crystal weight of 0.675 grams. The crystals were checked for purity using TLC (n-hexane: ethyl acetate (6:4) as eluents). Crystal TLC results showed one stain, indicating that the synthesized compound was pure and could be further characterized using FT-IR. The characterization of the synthesized compounds using FT-IR. In these spectra, there was C=O absorption in the synthesized compound that appeared at a wavenumber of 1646 cm^{-1} . This absorption shifted to a lower wavenumber region than the absorption of C=O 4-methoxy acetophenones, which appeared at 1664 cm^{-1} , indicating that the chalcone had been formed. The appearance of C=O absorption at a lower wavenumber is due to the C=O conjugation system with C=C alkenes and C=C aromatic rings in chalcone compounds. This is reinforced by the presence of absorption at 1581 and 1553 cm^{-1} (C=C aromatic), and absorption at 1600 cm^{-1} (C=C aliphatic). A broad absorption band at $3,450\text{ cm}^{-1}$ indicates the presence of an OH group, supported by a band in the $1,268\text{ cm}^{-1}$ (C-O-C) region. The absorption at 3025 cm^{-1} indicates the presence of $\text{Csp}^2\text{-H}$ while at 2966 cm^{-1} of the presence of $\text{Csp}^3\text{-H}$. Further characterization of chalcone 1 was carried out using a $^1\text{H-NMR}$ spectrometer. The $^1\text{H-NMR}$ spectra of the chalcone are presented in Fig.-2, showing 14 protons in the synthesized compound. The peak chemical shift (δ) of 3.85 ppm was presumably the proton absorption from the Methoxy group with a singlet appearance and three integrations. The singlet appearance indicated no neighboring protons coupling with these protons. On the other hand, the absorption of the doublet appearance at a chemical shift of 6.84 ppm was a signal from protons from C3 and C5 (aromatic ring B). This doublet appeared because the protons in C3 and C5 had 1 proton neighbor, respectively. The absorption band on the chemical shift of 7.06 ppm had a doublet appearance, presumably coming from the proton signals of C3' and C5' (aromatic ring A) because the protons at C3' and C5' had the same neighborhood of 1 neighboring proton. The peak with the same appearance also occurred at the chemical shift of 7.74 ppm, namely the absorption of protons at C2 and C6. The protons of α , β -unsaturated ketones were observed at chemical shifts of 7.64 and 7.75 ppm with $J=9$ and 15 Hz , showing that the formed chalcone had a trans structure. The peak at 10.09 ppm chemical shift had a singlet appearance which was an unprotected hydroxy proton absorption due to the induction of electronegative O atoms.

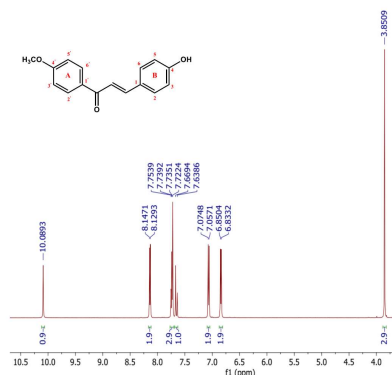


Fig.-2. $^1\text{H-NMR}$ Spectra of Chalcone 1

The structure of the synthesized compound was further characterized using $^{13}\text{C-NMR}$. The results of the $^{13}\text{C-NMR}$ spectra (Fig.-3) showed 12 signals indicating the presence of 12 different carbons. A distant chemical shift (deshielding area) at 187.28 ppm chemical shift showed the carbonyl band (C=O). The carbonyl carbon of chalcone usually appeared in the area of $170\text{--}194.6\text{ ppm}$.¹¹ The carbon was bonded directly to the O atom, which had a large electronegativity so that the nucleus of the C atom was increasingly unprotected by the electrons around the C atom due to the attraction of the O atom.

Carbon with the same environment appeared at 1 peak, such as the peak at the chemical shift of 113.9 ppm , which was the absorption of C3' and C5'. The same thing happened to absorption at 115.8 ppm (C3 and C5), 130.8 ppm (C2 and C6), and 114.09 ppm (C2' and C6'). The absorption of Methoxy carbon was observed at a chemical shift of 55.65 ppm , while the absorption of $\text{C}\alpha$ and $\text{C}\beta$ was observed at 118.4 and

143.7 ppm, respectively. Further characterization of the synthesized chalcone by GC-MS is shown in a chromatogram of 1 peak at 50 minutes tR with 100% purity (Fig.-4). This peak was identified as chalcone 1. Presence of a molecular ion (M^+) at m/z 254 which corresponds to chalcone 1 molecular weight.

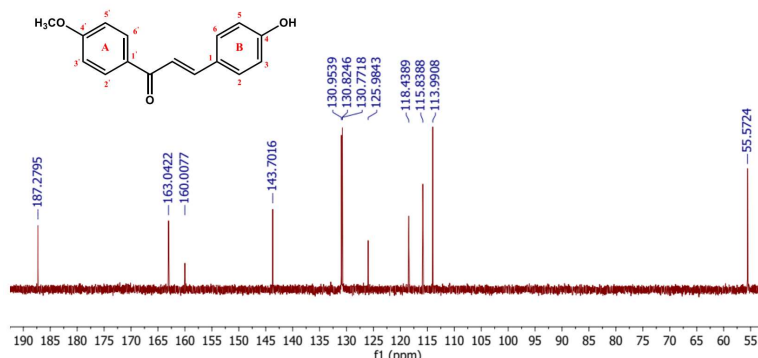


Fig.-3: ^{13}C -NMR Spectra of Chalcone 1

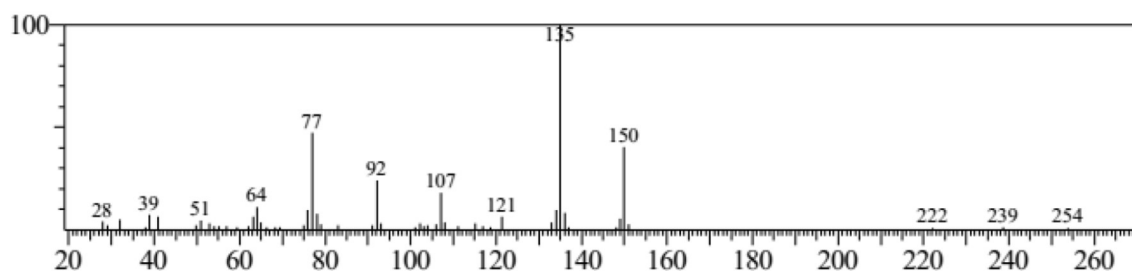


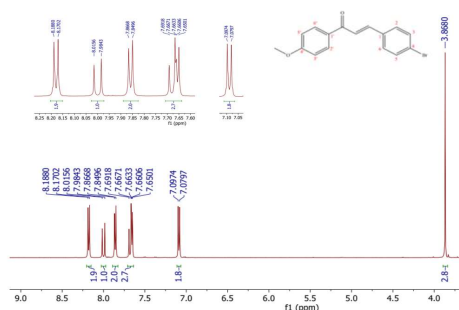
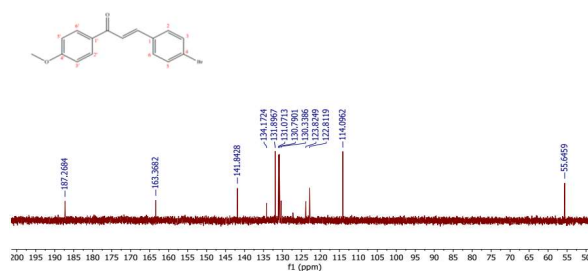
Fig.-4. Mass Spectra of Chalcone 1

Analyses of FT-IR, GC-MS, ^1H -, and ^{13}C -NMR on the synthesized compounds showed that the Claisen-Schmidt condensation reaction between the 4-methoxy acetophenone and 4-hydroxy benzaldehyde had occurred and formed a chalcone 1, namely 4-hydroxy-4'-methoxy chalcone.

Synthesis of Chalcone 2

Chalcone 2 was prepared in almost the same way as chalcone 1; in this case, the benzaldehyde used was bromobenzaldehyde. The synthesized compound in white crystals forms 0.8 g (32.5% yield) was obtained after the recrystallization process using ethanol. The FT-IR spectrum showed absorption at 3013 cm^{-1} , an aromatic C-H bond vibration, while the absorption at 2968 cm^{-1} was an aliphatic C-H absorption. A sharp absorption at 1656 cm^{-1} indicated the presence of the C=O group. Absorption at 1603 and 1508 cm^{-1} indicated the presence of an aromatic C=C double bond, and at 591 cm^{-1} was a C-Br bond vibration. The ^1H -NMR spectra of chalcone 2 NMR (Fig.-5) showed 13 protons in the synthesized compound. The absorption peak at the chemical shift (δ) of 3.86 ppm was presumably a proton signal from the Methoxy group with a singlet appearance and three integrations. The singlet appearance indicated no neighboring protons coupling with these protons. On the other hand, the absorption at 7.08 ppm chemical shift with a doublet appearance was a signal of protons from C3 and C5 in the aromatic ring A. This doublet appeared because the protons in C3 and C5 had 1 proton neighbor, respectively. The chemical shift at 7.65 ppm was a proton signal of C3' and C5' (aromatic ring B), supported by the peak at 7.65 ppm with a doublet appearance, indicating protons at C3' and C5' having the same environment, namely 1 neighboring proton. The peak with the same appearance also occurred at the chemical shift of 7.85 ppm, namely protons at C6' and C2'. The olefin protons of α , β -unsaturated ketones were observed at chemical shifts of 7.69 and 8.01 ppm with $J=11.95$ and 15.65 Hz , showing that the formed chalcone had a Trans structure. The compound structural characterization was confirmed by ^{13}C -NMR analysis (Fig.-6) which indicated 12 signals and 12 different carbons. The absorption for methoxy carbon was observed at a chemical shift of 55.65 ppm. The carbonyl band (C=O) is shown in a distant chemical shift (deshielding area) at 187.27 ppm chemical shift. As stated by Mostahar *et al.* (2007), the carbonyl carbon of chalcone usually appears in the 170-194.6 ppm

area.¹¹ The carbon was bonded directly to the O atom which had a large electronegativity so that the nucleus of the C atom was increasingly unprotected by the electrons around the C atom due to the attraction of the O atom. Carbon with the same environment appeared as at 1 peak, such as the peak at the chemical shift of 131.89 ppm which is the absorption of C3' and C5'. The same thing happened to the absorption at 131.07 ppm (C2 and C6), 130.34 ppm (C2' dan C6'), and 114.09 (C3 and C5). C α absorption was observed at a 122.81-ppm chemical shift while C- β was at 141.84 ppm. The C- β peak appeared more downfield than the C- α atom as shown by Mostahar *et al.* (2007) that the absorption of C- β in chalcone appeared at a more remarkable chemical shift than C- α .

Fig.-5: ¹H-NMR Spectra of Chalcone 2Fig.-6: ¹³C-NMR Spectra of Chalcone 2

The GC chromatogram of chalcone 2 (Fig.-7) showed a very sharp peak at 47.491 min with 100% percent area, and the mass spectra gave m/z 317 corresponding to the molecular weight of 4'-Bromo-4-methoxy chalcone.

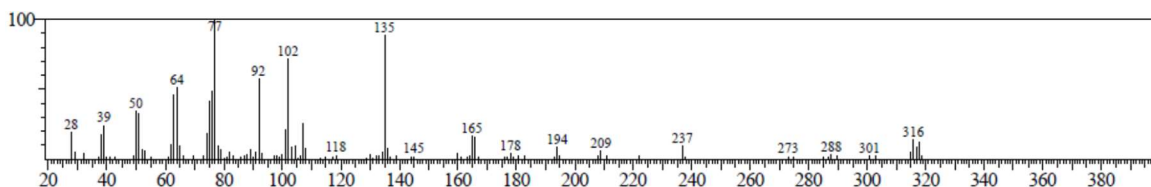


Fig.-7. Mass Spectra of Chalcone 2

Based on analyses of FT-IR, ¹H-NMR, ¹³C-NMR, and GC-MS, chalcone 2, namely 4'-Bromo-4-methoxy chalcone has been formed because of the Claisen-Schmidt condensation between 4-methoxyacetophenone and 4-methoxyacetophenone-bromobenzaldehyde. The synthesis product was in the form of white crystals. The reaction for chalcone formation presumably followed the mechanism of the aldol condensation reaction. The reaction started from an acid-base reaction, where the base took a proton from the carbon of 4-methoxyacetophenone to form a resonance stabilized enolate ion. The nucleophilic addition of the carbanion of 4-methoxyacetophenone occurred to the carbonyl carbon of 4-bromobenzaldehyde, followed by the release of a water molecule with acid to form a double bond (Fig.-8). The synthesis of chalcone using the grinding technology is a very strategic breakthrough because the principle of green chemistry, namely reducing using of solvents in the synthesis process, is considered. Solvents in the synthesis of many compounds are toxic and cause environmental problems. Therefore, it is crucial to develop a method for

synthesizing compounds without solvents. In the grinding process, mortar all reactants were ground in a mortar, resulting in the reactant's collisions and local heat of friction energy which accelerated the reaction to form a chalcone.

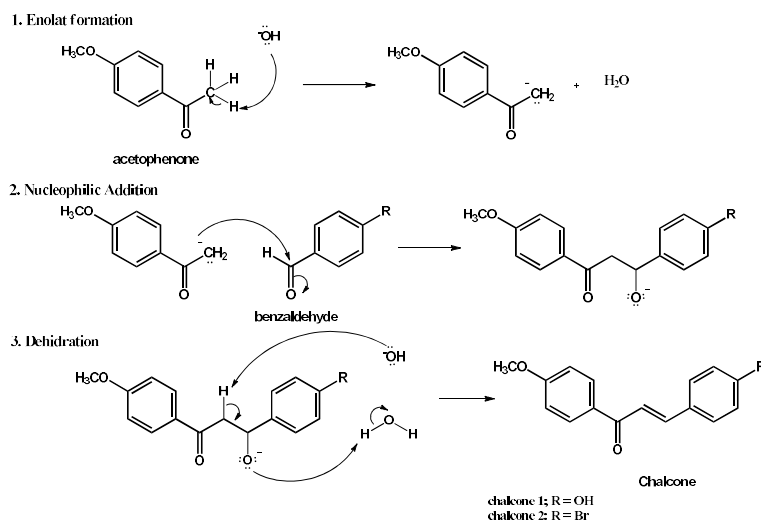


Fig-8: Mechanism of Chalcone Synthesis Reaction

Antibacterial Evaluation

An antibacterial test was carried out on the synthesized chalcone to inspect it as an antibacterial. Antibacterial activity in vitro was carried out by the filter paper disc diffusion method. This method uses placing filter paper on top of Mueller Hinton (MH) agar media inoculated with bacteria.¹³ Bacterial growth can be observed after incubating for 24 hours at 37°C by observing the presence or absence of inhibition around the filter paper. There were two bacteria used, *E. coli*, and *S. aureus*. The synthesized chalcone was solid, so it had to be dissolved using DMSO (Dimethyl Sulfoxide). Chloramphenicol 30 mg is a standard antibiotic or positive control, while DMSO is a negative control. The inhibition zone diameter observed in the antibacterial test of the synthesized chalcone against the 2 test bacteria is presented in Table-1. Table-1 indicated that the synthesized chalcone had medium inhibition against *E. coli* and was inactive against *S. aureus*. David and Stout (1971) classified the zone of inhibition (ZOI) in four intensities corresponding to ZOI diameters: >20 mm, very strong; 10-20 mm, strong, 5-10 mm, medium; and <5 mm, no response.¹⁴ The results of the antibacterial activity test showed that, in general, the synthesized chalcone was more likely to inhibit gram-negative bacteria than gram-positive bacteria. Nikaido (2003) revealed that the difference in sensitivity of gram-negative and gram-positive bacteria is related to the bacterial cell wall.

Table-1: Diameter of Chalcone Inhibition Zone

Bacteria	Compound	Inhibition Zone (mm)
<i>Escherichia coli</i> ATCC 25922	4-bromo-4'-metoksi chalcone	8.23
	4-hidroksi-4'-metoksi chalcone	7.88
	chloramphenicol	29.49
	DMSO	0
<i>Staphylococcus aureus</i> ATCC 25923	4-bromo-4'-metoksi chalcone	0
	4-hidroksi-4'-metoksi chalcone	0
	chloramphenicol	29.49
	DMSO	0

The peptidoglycan layer of Gram-positive bacteria is an ineffective permeable barrier. While gram-negative bacteria have an additional outer membrane consisting of lipopolysaccharide compounds, consequently, the cell wall is impermeable to lipophilic solutes and porins as a selective barrier against hydrophilic solutes.¹⁶

CONCLUSION

Eco-friendly synthesis methods should be developed continuously. The use of grinding technology in chalcone synthesis is the potential to be developed. Researchers have succeeded in synthesizing 4-Bromo-4'-methoxy chalcone and 4-hydroxy-4'-methoxy chalcone through the grinding technique. The antibacterial activity test on both chalcones showed that the synthesized chalcone had medium inhibition against *E. coli* and was inactive against *S. aureus*.

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