SYNTHESIS AND ANTI-BACTERIAL ACTIVITY TEST OF CHALCONE ANALOGUE COMPOUNDS FROM 1-ACETYLNAPHTHALENE WITH FURFURAL

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Abstract: The development of antibacterials has become crucial due to the emergence of antibiotic-resistant bacteria. One such antibacterial is a chalcone analogue compound, known to possess various biological activities useful in medicine**.** This study aims to synthesize a compound of chalcone analogues and test for anti-bacterial activity. Chalcone analogue compounds from 1-acetylnaphthalene with furfural in this study have been successfully synthesized using a cross aldol condensation reaction with the *stirrer* method. The structure obtained is a chalcone analogue compound with a synthesis yield of 97%, and the target structure was confirmed by FTIR and NMR instruments. In this study, chalcone analogue compounds have anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria with the agar diffusion testing method. Results of the antibacterial test in this study showed a moderate analogue category for *S. aureus* bacteria and weak for *E. coli* bacteria. This is evidenced by the concentration of 40 mg/mL having an inhibition zone for *S. aureus* bacteria of 9.5 mm and *E. coli* bacteria of 0.5 mm. Chalcone compounds can be intermediate compounds that can be resynthesized with other compounds and have various biological activities. So, this study can be used as a reference for literacy to develop the theory of synthesis of chalcone analogue compounds as new drug candidates.
Keywords: Aldol condensation reaction, anti-bacterial, chalcone analogue compounds

Abstrak: Pengembangan antibakteri menjadi penting seiring dengan adanya bakteri yang resisten terhadap antibiotik. Salah satu antibakteri tersebut adalah senyawa analog kalkon yang dikenal memiliki berbagai aktivitas biologis yang berguna dalam pengobatan. Penelitian ini bertujuan untuk mensintesis senyawa analog kalkon dan menguji aktivitas antibakterinya. Senyawa analog kalkon dari 1-asetilnaftalena dengan furfural dalam penelitian ini telah berhasil disintesis menggunakan reaksi kondensasi aldol silang dengan metode pengadukan. Struktur yang diperoleh adalah senyawa analog kalkon dengan rendemen sebesar 97% dan struktur target dikonfirmasi oleh instrumen FTIR dan NMR. Dalam penelitian ini, senyawa analog kalkon memiliki aktivitas antibakteri terhadap bakteri *Escherichia Coli* dan *Staphylococcus Aureus* dengan metode pengujian difusi agar. Hasil uji antibakteri dalam penelitian ini menunjukkan kategori sedang untuk bakteri *S. aureus* dan lemah untuk bakteri *E. coli*. Hal ini dibuktikan dengan konsentrasi 40 mg/mL yang memiliki zona inhibisi untuk bakteri *S. aureus* sebesar 9,5 mm dan bakteri *E. coli* sebesar 0,5 mm. Senyawa kalkon dapat menjadi senyawa perantara yang dapat disintesis ulang dengan senyawa lain dan memiliki berbagai aktivitas biologis. Oleh

karena itu, penelitian ini dapat digunakan sebagai referensi untuk pengembangan teori sintesis senyawa analog kalkon sebagai kandidat obat baru**.**
Kata kunci: Reaksi kondensasi aldol, antibakteri, senyawa analog kalkon

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Introduction

Synthesis of chalcone analogue compounds in this study used starting materials in the form of 1-acetylnaphthalene and furfural. These two compounds have been widely used to form chalcone analogue compounds, but with different partners. The 1-acetylnaphthalene compound in the studies of Jasril et al., (2017) and Fitria et al., (2023) was shown to be able to form chalcone analogue compounds with various aromatic aldehydes. Previous studies of variations of aldehydes in the form of furfural were synthesized to form substituted chalcone analogue compounds with 2-hydroxy-3,4,6 trimethoxyacetophenone (Silva et al., 2021). Furfural also succeeded in forming chalcone analogue compounds with 4-aminoacetophenone compounds (Nogueira et al., 2020). The biological activity of chalcone analogue compounds is very much an attraction for scientists to find new structures of chalcone analogues. Some of the biological potentials of chalcone analogue compounds include, as antibacterial agents (Frimayanti, 2021).

Research conducted by Ferraz et al., (2020) that the chalcone analogue compound of furfural has antibacterial and antibiotic activity. According to Gudapati et al., (2024) stated that chalcone analogue compounds and their derivatives have antibacterial activity. Many antibacterial drugs are unable to control infectious diseases due to the emergence of antibacterial resistance (Shabani et al., 2023). Increasing antibacterial resistance due to various factors drives the search for new active compounds against pathogens against many drugs (Xu et al., 2019). Antibacterial agents can be obtained by synthesizing chalcone analogue compounds and their derivatives (Sepvianti et al., 2021). The synthesis of chalcone analogue compounds and their derivatives is a group of secondary metabolites in the form of flavonoids. Chemically, chalcone analogue compounds consist of unsaturated ketone groups with two phenyl rings (Ngameni et al., 2021).

Synthesis of chalcone analogue compounds in this study using the stirrer method. The synthesized compounds were then analyzed using Fourier Transform Infrared *(*FTIR) and Nuclear Magnetic Resonance (NMR) to confirm the target structure (Jasril et al., 2017). Chalcone analogue compounds were tested as anti-bacterial agents against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria. (Gudapati et al., 2024). Chalcone analogue compounds are very efficient to be re-synthesized with other compounds and can be used in designing and modeling new anti-bacterial drug agents. So, this paper can be used as literature

and reference to find structures of chalcone analogues and to find new anti-bacterial drug agents.

Methodology

Tools and Materials

The chemicals used in this study were furfural (Merck), 1-acetylnaphthalene (Sigma Aldrich), filter paper, pH paper, TLC plate F_{254} (Merck), and sodium hydroxide pellets (Merck), Nutrient Agar (NA), Nutrient Broth (NB), colonies of *E. coli* and *S. aureus* bacteria. Solvents used include ethyl acetate (Merck), n-hexane (Merck), methanol (Merck), ethanol (Merck), and distilled water.

Methods

Dissolve 5 mmol each of 1-acetylnaphthalene and furfural in 5 mL of absolute methanol under cold conditions. Add 5 mmol of KOH base catalyst in 10 mL of methanol/aquadest (50%, v/v). After that, mix the three ingredients in one erlenmeyer and stir using a *magnetic stirrer* regularly under cold conditions. The mixture was stirred using the stirrer method for 24 hours, and TLC was performed every 2 hours. After 24 hours, add 15 mL of cold distilled water. Then the mixture was neutralized by adding 1 N HCl solution and measured using a pH indicator (Arora et al., 2012). In this study, no solids were obtained, so extraction was carried out to separate the product from the solvent. This extraction process by adding 25 mL of ethyl acetate for 2 repetitions using a separating funnel. The product will be characterized using FTIR and NMR instruments. (Fitria et al., 2023). Then, tested for anti-bacterial activity by agar diffusion method (Trisnawita et al., 2020). This research was conducted at the Faculty of Science and Technology Laboratory of Sulthan Thaha Saifuddin State Islamic University, Jambi. FTIR analysis was carried out using a Thermo Scientific-Nicolet iS50 FT-IR Spectrometer at the Jambi University Faculty of Science and Technology Laboratory. ^{1}H-NMR and ^{13}C-NMR analyses were performed using a Bruker Avance Neo 500 MHz and 126 MHz Nuclear Magnetic Resonance Spectrometer at the ILRC Laboratory, University of Indonesia.

Results and Discussion

Synthesis of Chalcone Analogue Compounds

Synthesis of chalcone analogue compounds derived from aromatic ketones with aromatic aldehydes using Claisen-Schmidt condensation reaction or commonly called cross aldol condensation. This research uses the compounds 1-acetylnaphthalene as an aromatic ketone and aromatic aldehyde in the form of furfural shown in **Figure 1**. This research has successfully used the strirer method to synthesize chalcone analogue compounds from 1-acetylnaphthalene and furfural. This method is commonly used in synthesizing chalcone analogue compounds because it is more flexible and cheaper (Mirzaei et al., 2020).

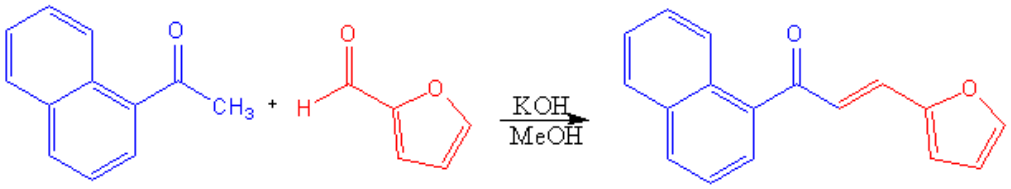

Figure 1. Synthesis of chalcone analogue compounds

Based on **Figure 1,** the percentage yield of chalcone analogue compounds in this study is 97%. The percentage yield result is calculated from the number of experimental product results divided by the number of theoretical products then multiplied by one hundred percent. According to Chen et al, (2023) that the higher the yield of a product, the higher the success rate of a reaction. The result of this study was the chalcone analogue structure named (E)-3-(furan-2-yl)-1-(naphthalen-1-yl)prop-2-en-1-one.

The synthesis process of chalcone analogue compounds requires a catalyst to accelerate the compounds formation reaction. (Tanpure et al., 2020). The catalysts used to synthesize chalcone analogue compounds can be acidic, basic, or heterogeneous catalysts. (Hadi et al., 2023). In this study, the catalyst used was KOH base catalyst. This research refers to research conducted by Arora et al. (2012) and Fitria et al. (2023), who synthesized acetylnaphthalene derivatives using KOH catalyst. The synthesis process of the chalcone analogue in the study requires a *stirrer* time of 24 hours under cold conditions. (Arora et al., 2012). The results of the formation of chalcone analogue compounds are shown in the results of the TLC test in **Figure 2.**

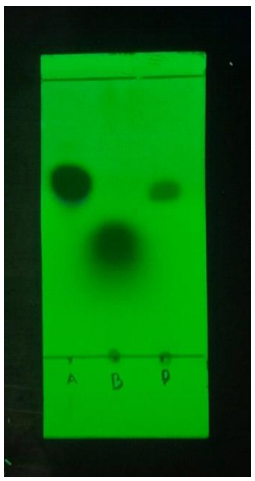

Figure 2. TLC results of the products, stain (A) is the compounds 1-acetylnaphthalene, stain (B) the compounds furfural, and stain (P) of the chalcone analogue compounds.

The compounds progress of the ongoing synthesis can be observed from the TLC test results. Chalcone analogue compounds have been formed if there is one

stain on the product and the *Retardian Factor* (Rf) value of the product is not the same as the starting material (Fitria et al., 2023). The results of the TLC test of the chalcone analogue compounds are shown in **Figure 2** that the stain on the product (P) has a different Rf value both with stain A and stain B of 0.8. The Rf value is obtained by dividing the distance of the product by the distance of the eluent calculated from the bottom line. Eluent in the TLC test used a ratio of n-hexane:ethyl acetate (9:1) (Silva et al., 2021). Besides being able to see the progress of a compound synthesis, the TLC test can also determine the purity of the compounds. The purity results of the chalcone analogue compounds with the TLC test are in **Figure 3.**

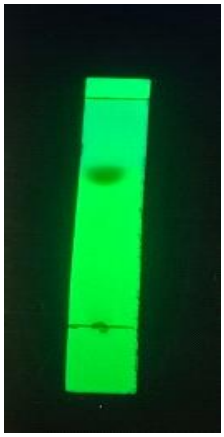

Figure 3. Purity test results TLC with eluent ratio of n-hexane: ethyl acetate (9:1)

Based on **Figure 3,** there is only one stain on the TLC plate and the stain are clean from impurities. This shows that the chalcone analogue compounds in this study is pure. The purity test of the chalcone analogue compounds besides using the TLC test can also be observed through the melting point. A pure compound has a small melting point range equal to two (≤2°C) from melting to complete melting. The melting point of the chalcone analogue compounds in this study is 66.5-67.8°C using the capillary method. Similar research conducted by Jasril et al., (2017) stated that the chalcone analogue compounds from 1-acetylnaphthalene which is subsumed with 2-florobenzaldehyde has a melting point of 41-42°C.

Characterization of chalcone analogue compound

Characterization of synthesized chalcone analogue compounds with several instruments to confirm the structure of the target compounds. The synthesized compounds were characterized with FTIR and NMR instruments. FTIR will confirm the groups present in the structure of the chalcone analogue compounds. The working principle of FTIR is to analyze the presence of compounds through vibrations or bond vibrations. Compounds have vibrational energy in accordance with the infrared region of the electromagnetic spectrum. In certain infrared radiation regions, peaks will appear that can determine the functional groups

present in a compounds. The wave numbers in FTIR start from the highest to the lowest, namely 4000 cm^{-1} to 400 cm^{-1} (Nanjundaswamy et al., 2022). The FTIR results of the chalcone analogue compounds in this study are shown in **Figure 4.**

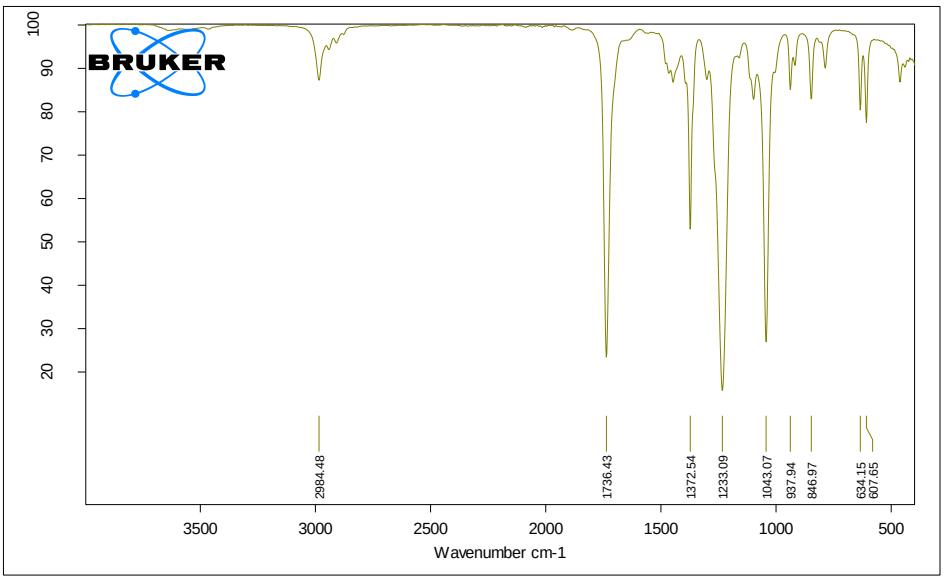

Figure 4. FTIR results of chalcone analogue compounds 3-(furan-2-yl)-1-(naphthalene-1-yl)prop-2-en-1-on

Based on **Figure 4,** the FTIR spectrum shows peaks at certain wave numbers. The chalcone analogue compounds is identical to the C=C, C-C, and C=O groups with consecutive positions. Other groups that appear in the FTIR spectrum provide additional information in confirming the target structure, such as C-O and C-H functional groups. The wavelength of 2.984.48 cm^{-1} shows the vibrations of C-H sp^3 groups derived from aromatic C-H and olefinic C-H. Wavelength at 1.736.43 cm^{-1} indicates the C=O functional group. Wavelengths at 1.233.09 cm^{-1}, 1.372.54 cm^{-1}, and 1.043,07 cm^{-1} showed vibrations of the C-O-C stretching in cyclic ethers. Wavelength 937.94 cm^{-1} indicates out-of-plane bending of the aromatic ring C-H. 846.97 cm^{-1} out-of-plane bending of naphthalene C-H while 634.15 cm^{-1} and 607.65 cm^{-1} in-plane bending of the ring in naphthalene. In addition to using FTIR, chalcone analogue compounds were further confirmed through NMR instruments.

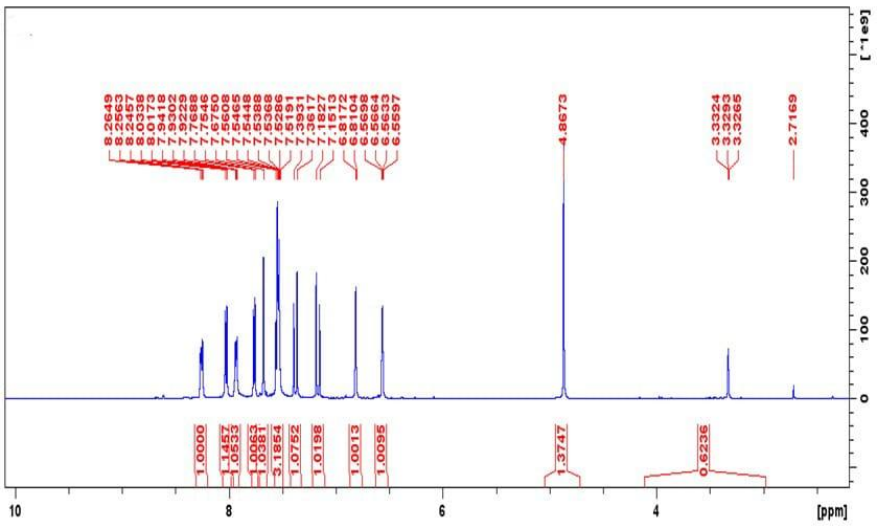

Figure 5. [1]H-NMR analysis results of the chalcone analogue compounds

The analysis results from the [1]H-NMR spectrum can be observed in **Figure 5.** Based on the spectrum of [1]H-NMR, the chalcone analogue compounds has a chemical shift value between 6-8 ppm which indicates the presence of aromatic protons (Fitria et al., 2023). The amount of integration in the [1]H-NMR spectrum data in this study is in accordance with the number of protons in the target structure, namely 12 protons. Analysis of the chemical environment on protons is seen from the number of peaks of each integration in the H-NMR spectrum (Kosela, 2010). Chemical shifts of 6.8144 (d, 1H) and 7.3771 (d, 1H) which are in the C-2 and C-3 positions. Chemical shifts at 8.2582 ppm (d, 1H), 7.5360 ppm (m, 1H); 7.5360 ppm (m, 1H); 7.6844 ppm (s, 1H); 8.0238 ppm (d, 1H); 7.5360 ppm (m, 1H); and 7.9342 ppm (dd, 1H) each chemical shift indicates the presence of protons in the C-3', C-4', C-5', C-6', C-8', C-9', and C-10' with different peaks. Furthermore, chemical shifts of 7.1615 ppm (d, 1H) C-4", 6.5723 ppm (m, 1H) C-3", and 7.7618 ppm (d, 1H) C-2" indicate the furfural ring.

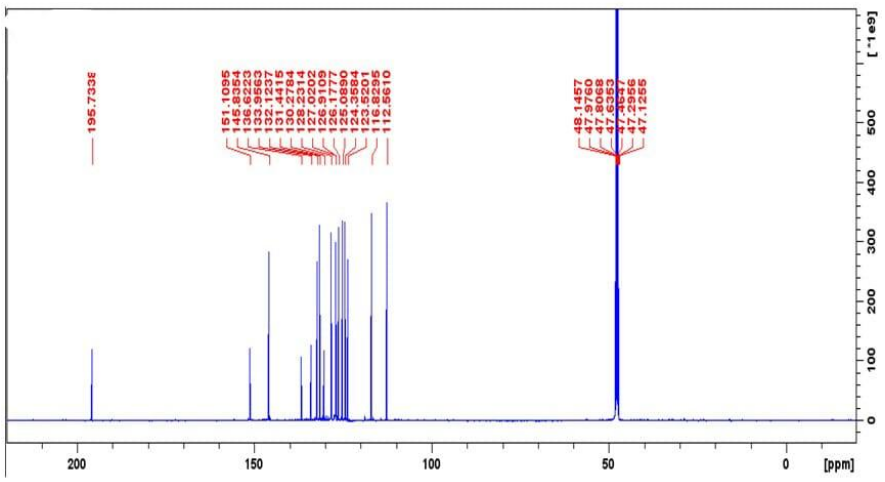

Figure 6. [13]C-NMR analysis results of chalcone analogue compounds

Confirming the structure of the target compounds further by analyzing the ^{13}C-NMR spectrum shown in **Figure 6.** Based on the results of the spectrum of ^{13}C-NMR there are peaks that indicate the number of carbons as many as 17 carbon atoms. According to Kosela, (2010) that the range of chemical shifts in ^{13}C-NMR ranges from 0-230 ppm is greater than that of ^{1}H-NMR which has a range of 0-10 ppm. Chemical shift C carbonyl (C=O) derived from ketone is at 195.73 ppm. This chemical shift is the highest shift in the analysis of^{13} C-NMR shown by C-1. Chemical shifts of chalcone analogue compounds are then shown at shifts of 145.83 ppm, 136.62 ppm, 133.95 ppm, and 151.10 ppm, each of which shows the C-1', C-2', C-7', and C-1'' groups. In the position of the group shows a low intensity, this indicates a chemical shift from the quaternary C.

Chemical shifts at 126.17 ppm and 124.35 ppm indicate carbon at C-2 and C-3. Furthermore, there are chemical shifts that indicate the presence of carbon in both aromatic rings, namely 123.52 ppm and 131.44 ppm. These two chemical shifts indicate the presence of carbon at C-3' and C-4'. Chemical shifts of the furfural ring at 116.82 ppm, 112.56 ppm, and 132.12 ppm indicate the presence of carbon at C-2', C-3', and C-4'. Finally, the aromatic ring shifts are 125.08 ppm, 127.02 ppm, 130.27 ppm, 126.91 ppm, and 128.23 ppm. Corresponding data of ^{13}C-NMR and ^{1}H-NMR spectra results are shown in **Figure 7** and **Table 1**.

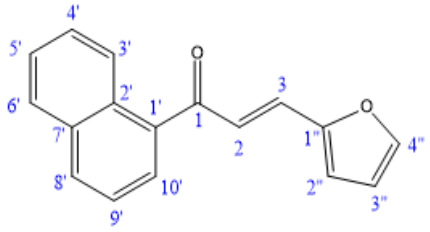

Figure 7. Compounds 3-(furan-1-yl)-1-(naphthalen-1-yl)prop-2-en-1-on) (*ChemDraw Ultra* 12.0)

Table 1. Interpretation of ^{13}C-NMR and ^{1}H-NMR data of compounds 3-(furan-1-yl)-1-(naphthalene-1-yl)prop-2-en-1-on

Atomic Number	δ_H (ppm)	δ_C (ppm)
1	-	195,73
2	6.8144 (d, 1H)	126,17
3	7.3771 (d, 1H)	124,35
1'	-	145,83
2'	-	136,62
3'	8.2582 (d, 1H)	123,52
4'	7.5360 (m, 1H)	131,44
5'	7.5360 (m, 1H)	125,08
6'	7.6844 (s, 1H)	127,02
7'	-	133,95
8'	8.0238 (d, 1H)	130,27
9'	7.5360 (m, 1H)	126,91

Atomic Number	δ_H (ppm)	δ_C (ppm)
10'	7.9342 (d, 1H)	128,23
1''	-	151,10
2''	7.1615 (d, 1H)	116,82
3''	6.5723 (m, 1H)	112,56
4''	7.7618 (d, 1H)	132,12

Based on **Table 1, the** results of the interpretation of target compounds from[1] H-NMR and [13]C-NMR can be adjusted to the chalcone compounds in **Figure 7**. [13]C-NMR analysis through peaks in groups can be seen from the number of adjacent protons plus one (n + 1). If there is a difference between the theory and the peak results in the analysis of [1]H-NMR, this is due to spin-spin coupling or interaction between neighboring protons or adjacent protons (Kosela, 2010). So, the data analyzed using [1]H-NMR and [13]C-NMR proved to be able to confirm the suitability of the proton-potron position with the target compounds structure. The results of NMR analysis data are in accordance with the structure of the target compounds, $C_{17}H_{12}O_2$.

Activity Test as Anti-bacterial of Chalcone analogue compounds

Anti-bacterial activity test uses two types of bacteria to represent gram-negative and gram-positive pathogens. This aims to see the response of chalcone analogue compounds as anti-bacterial on each pathogen. The difference between gram negative and gram positive lies in the cell wall. Gram-negative cell walls have a thin peptidoglycan layer and a complex outer membrane. While gram-positive cell walls have a thick peptidoglycan layer compared to gram-negatives. So, that the inhibition process by chalcone analogue compounds against the two pathogens is different (Shekhar et al., 2024). This study used *S. aereus* bacteria as gram positive and *E. coli* as gram negative. The inhibitory activity of the bacteria is shown in **Table 2**.

Table 2. Anti-bacterial activity test results of compounds 3-(furan-1-yl)-1-(naphthalen-1-yl)prop-2-en-1-on

Concentration	Bacterial zone of inhibition *S. aureus.*	Bacterial zone of inhibition *E. coli*
30 mg/mL	5 mm	0.5 mm
40 mg/mL	9.5 mm	0.5 mm
50 mg/mL	1.5 mm	0.5 mm
Positive control	30 mm	45 mm
Negative control	-	-

Results of the calculation of the clear zone diameter as the inhibitory ability in the anti-bacterial test are listed in Table 2. The inhibitory ability against *S. aureus* bacteria is higher than that of *E. coli* bacteria. So that the ability of inhibition against *S. aureus* bacteria includes the ability of moderate inhibition and the ability of

inhibition against *E. coli* bacteria including the ability of weak inhibition. According to Trisnawita et al. (2022), the classification of the diameter of the inhibitory ability is very strong (20-30 mm), strong (10-20 mm), moderate (5-10 mm), and weak (<5 mm).

Inhibitory ability against *S. aureus* bacteria (gram positive) at a concentration of 40 mg/mL showed the highest inhibitory ability, which was 9.5 mm compared to a concentration of 30 mg/mL and a concentration of 50 mg/mL. The concentration of antibacterial compounds that is too high, the penetration of secondary metabolites into the bacterial cell wall can be disrupted, thereby reducing the effectiveness of antibacterial compounds in inhibiting bacteria (Chamidah & Rohmawati, 2022). The inhibitory ability against *E. coli* bacteria (gram negative) from all concentrations (30,40, and 50 mg/mL) has an average inhibitory ability of 0.5 mm. So, the inhibitory ability against *E. coli* bacteria is included in the weak inhibitory ability. The difference in the inhibition zone is partly due to the composition of the cell wall of gram-positive bacteria which has a low lipid content. In contrast to the cell wall of gram-negative bacteria which has a high lipid content in its cell wall.

Conclusion

Chalcone analogue compounds was successfully synthesized from 1-acetylnaphthalene compounds with furfural through aldol condensation reaction with stirrer method and obtained yield of 97%. The synthesized compounds was characterized using FTIR and NMR instruments to ensure its suitability with the target structure, namely 3-(furan-1-yl)-1-(naphthalen-1-yl)prop-2-en-1-on. Chalcone analogue compounds was successfully proven its anti-bacterial activity using *E. coli* and *S. aureus* bacteria. The inhibition results at a concentration of 40 mg on *S. aureus* bacteria are included in the medium inhibition zone of 9.5 mm and the *E. coli* inhibition zone has a weak inhibition of 0.5 mm.

Conflict of Interest

The first author was responsible for conducting the laboratory experiments, collecting data, and preparing the initial draft of the manuscript. The second author, as the first supervisor, contributed to the conceptualization of the research, providing methodological direction, and critical review of the manuscript's content. The third author, as the second supervisor, contributed to the analysis of the test data and the final editing of the article. All authors declare that the use of artificial intelligence (AI) in this study was limited to assisting with grammatical corrections and translation of several sections of the manuscript to enhance readability, without affecting the substance of the data or the scientific findings of the study.

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Department of Chemistry Research Laboratory, Faculty of Science and Technology, Sultan Thaha Saifuddin Jambi State University.

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