Synthesis of Schiff Base Compounds from 4-Formilpyridine and *P*-Anisidina Using Sonication Method as Antibacterial

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Abstract. Schiff base compounds are reported to have various biological activities, one of which is antibacterial. This study aims to determine the characterization of the Schiff base product with the sonication method and to determine its antibacterial activity. Schiff base products from 4formylpyridine and panisidine were characterized using FTIR, KG-SM, and 1H-NMR as well as antibacterial activity tests. The results of the characterization using FTIR produced a wave number of 1621 cm^{-1} which was thought to be the absorption of the C = N group which was the target compound, and the KG-SM characterization showed the presence of the target compound for the synthesis of 4-methoxy-N(pyridine-4-ylmethylene)aniline at m/ z 212. Meanwhile, the 1H-NMR characterization of the product showed the presence of a singlet proton imine signal at a chemical shift of 8.46 ppm (1H, s), as well as the presence of antibacterial activity that was effective in inhibiting bacterial growth at a concentration of 10,000 μg/mL.

Keywords: Schiff base · 4-Formylpyridine · p-Anisidine · Sonication Method · Antibacterial

1 Introduction

Health problems caused by bacteria are one of the problems faced by developing countries such as Indonesia. The tropical climate in Indonesia is very suitable for the development and growth of microbes, so that many diseases are caused by microbes such as bacteria. Antibiotics are types of drugs that are widely used as antibacterial agents [1, 2]. Antibacterial is a compound that can inhibit the work of bacteria. The general mechanism of antibacterial action is by damaging cell walls, interfering with protein synthesis, changing membrane permeability, and inhibiting enzymatic activity (Jawezt et al., 2007). Schiff base compound is one of the compounds that has the potential as an antibacterial agent [3].

4-formylpyridine is one of the derivatives of pyridine compounds having the molecular formula C6H5NO which is formed from pyridine aldehyde derivatives [4]. The compound 4-Formilpyridine has many benefits in the field of medicine [5]. In addition, pyridine compounds as heterocyclic compounds have many biological activities. Schiff's

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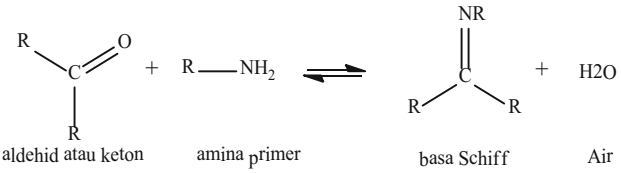

Fig. 1. Schiff base compound scheme

base compound which has a heterocyclic structure can produce greater antibacterial inhibition, whereas heterocyclic compounds have a pyridine core which can potentially be antibacterial [6].

Schiff bases are imine functional groups or azomethine groups of aldehydes or ketones in which the C=O group is replaced by an RHC=NR group, where R and R1 are alkyl, aryl, cycloalkyl or heterocyclic groups which can be substituted in various ways [7, 8]. It is usually formed by the condensation of an aldehyde or ketone with the primary amine shown in Fig. 1.

Schiff base compounds can be synthesized using the sonication method. The sonication method can be classified as the geen synthesis method which has the advantages of being environmentally friendly, the reaction process is faster, simpler, and the product purity is high and the yield obtained is more than other methods [9].

Schiff base compound has a C=N group which can act as an antibacterial where the amine group (NH2) has free electrons. The presence of an amine group that has a cationic charge that is able to bind to bacterial food sources so that it can inhibit nutrients (food) from entering the cell [10]. The presence of imine groups or azomethin groups in Schiff base compounds which have free electrons on their nitrogen atoms can involve the formation of hydrogen bonds with the active center of the cell which will disrupt normal cell processes, causing cell death [11].

2 Experimental

Melting points were determined using melthing point apparatus Squart type SMP 11. IR (KBr, cm-1) spectra were obtained on Shimadzu variant type 1000 spectrophotometer. 1H NMR spectra were recorded on type Agilent DD2 NMR at 500 MHz spectrometer using TMS as an internal reference (Chemical shifts in δ, ppm). Mass spectra were recorded on SM QP-2010 variant VF-5MS (70 ev) mass spectrometer. For sonication type Q500 Sonicator. Antibacterial using disk diffusion.

2.1 Preparation of Schiff Base Compounds 4-Methoxy-N-(Pyridine-4-Ylmethylene)Aniline

4-formylpyridine and p-Anisidina Were Dissolved in Water. Mix the Two Solutions in a Beaker, then Put in Sonication for 7 min. The Product Formed is Filtered and Dried in a Desiccator Until a Constant Mass is Weighed. Then Further Tested, Namely the Characterization.

2.2 Antibacterial Test of Schiff Base Compounds

Sterile MHA Media Was Poured into a Petri Dish and Allowed to Solidify. Then the Results of the Bacterial Inoculum Were Scratched (Spread Plate Method) on the Media Until Evenly Distributed Using a Sterile Cotton Swab. The Paper Disc Was Placed on the Surface of the Agar Medium that Had Been Inoculated with Bacterial Suspension, then Incubated in an Incubator at 37 °C for 18–24 h, After Which the Diameter of the Inhibition Area (Clear Zone) Growth Around the Paper Disc Was Measured. Determination of Antibacterial Activity Was Carried Out 3 Times with Concentrations of 10,000, 5,000, 1,000, 500 and 100 μg/mL (in DMSO Solvent). The Controls Used Were DMSO (Negative Control) and Gentamicin (Positive Control).

3 Results and Discussion

Based on Table 1, it can be seen that there are physical differences in the product compared to the reaction. The difference in physical characteristics is one indication of the formation of a new compound, namely the base compound Schiff 4-methoxy-N-(pyridine-4-ilmethylene)aniline. The formation of Schiff base compounds is also due to the difference in melting points between the products of synthesis and the reactants. The results of the synthesis of the base compound Schiff 4-methoxy-N-(pyridine-4-ilmethylene)aniline by sonication method within 7 minutes resulted in a yield of 95.08%. The effect of the yield may be caused by the condition of the materials used and environmental conditions. One of the Schiff base reactants, namely 4-formylpyridine reactant has properties that are sensitive to room temperature, air and light [12].

Figure 2 shows the IR spectra of the product of the Schiff base compound synthesis of 4-formylpyridine and p-anisidina having a different functional group absorption when compared to the two reactants, it can be suspected if the target compound has been formed. This is based on the absence of a typical absorption C group. =O aldehyde of 4-formylpyridine and -NH group of primary amine from p-anisidina with strong intensity at wave number 1712 cm-1 and 3322-3348 cm-1 in the product compound spectra. Furthermore, this assumption was further strengthened by the visible absorption of the typical C=N group (imine/Schiff base) of the product compound that occurred at a wave number of 1621 cm-1.

Table 1. Physical characterization of the Schiff base products compared to the reactants

Parameter	**Hasil Pengamatan**		
	R1	**R2**	**Produk**
Form	Liquid	Solid	Solid
Colour	Light yellow	Dark brown	Yellowish Green
Massa (gram)	0,5521	0,62207	1,0083
Rendemen (%)	-	-	95,08
Melthing point ^{o}C	-4[(a)]	56–59[(a)]	90–91

Description: a. Sigma-aldrich (2022) R1 = 4-formyl pyridine R2 = p-anisidina

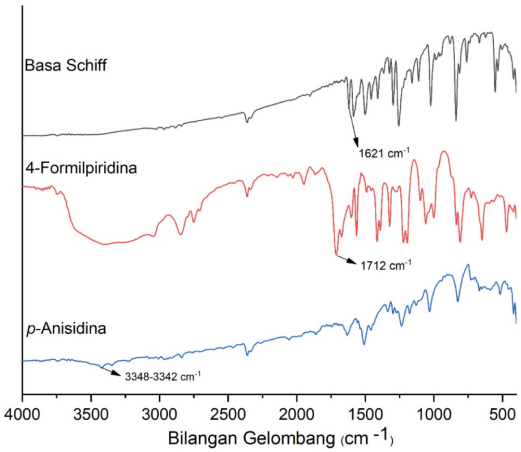

Fig. 2. IR spectra of 4-formylpyridine, p-anisidina and Schiff base compound synthesis products

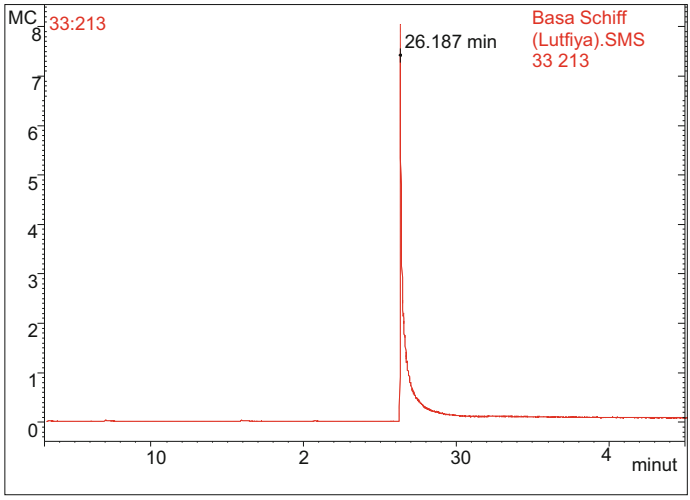

Fig. 3. Synthetic product chromatogram results

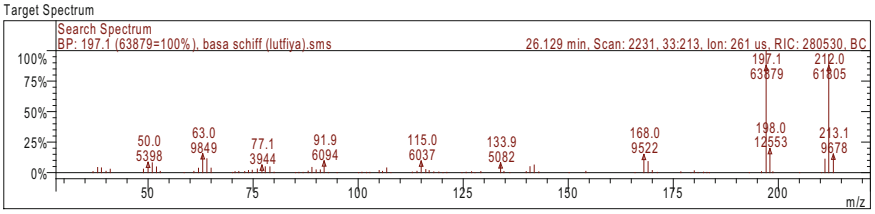

Fig. 4. The peak mass spectra of the synthesis product

The chromatogram shown in Fig. 3 shows a peak with a retention time of 26.187 minutes and an area of 100%. This indicates that the resulting product contains one pure compound. While the results of the mass spectra in Fig. 4 can be seen that the compound product of the synthesis has a molecular ion (M+) m/z 212 which corresponds to the molecular weight of the target compound, which is 212 g/mol. This can strengthen the suspicion of the formation of the basic compound Schiff 4-methoxy-N(pyridine-4-ilmethylene)aniline.

Based on Fig. 5. The results obtained, it shows that there is one proton methoxy signal (–OCH3) at a chemical shift of 3.83 ppm (3H, s). Benzene ring proton signals appear at chemical shifts of 7.72-7.73 ppm (2H, d) and 6.93-6.95 ppm (2H, d). Furthermore, several pyridine ring proton signals appeared at chemical shifts of 7.27-7.29 ppm (2H, d) and 8.72-8.73 ppm (2H, d). Then, there is a proton signal indicating imine proton (-C=N-) at a chemical shift of 8.46 ppm (1H, s). If the total number of protons corresponds to the target compound, the Schiff base 4-methoxy-N(pyridine-4-ilmethylene)aniline has 13 protons.

Based on Table 2, it can be seen that the diameter of the inhibition zone for the growth of Staphylococcus aureus and Escherichia coli bacteria increased with increasing Schiff base concentration. This shows that the increase in concentration is directly proportional to the increase in the inhibition zone of Staphylococcus aureus and Escherichia coli bacteria. However, the diameter of the inhibition zone of Staphylococcus aureus was greater than the value of the inhibition zone of Escherichia coli because the two test bacteria had different cell wall compositions. Staphylococcus aureus which is a gram-positive bacterium has a simple cell wall structure (low lipid content) compared to Eschericia coli which is a gram-negative bacterium which has a more complicated cell wall structure (complex high lipid content), so that the walls of gram-negative bacteria are more difficult to penetrate. by antibacterial agents [13].

The workings of Schiff base compounds can involve the formation of hydrogen bonds through the C=N group with the active center of the cell which results in disruption of

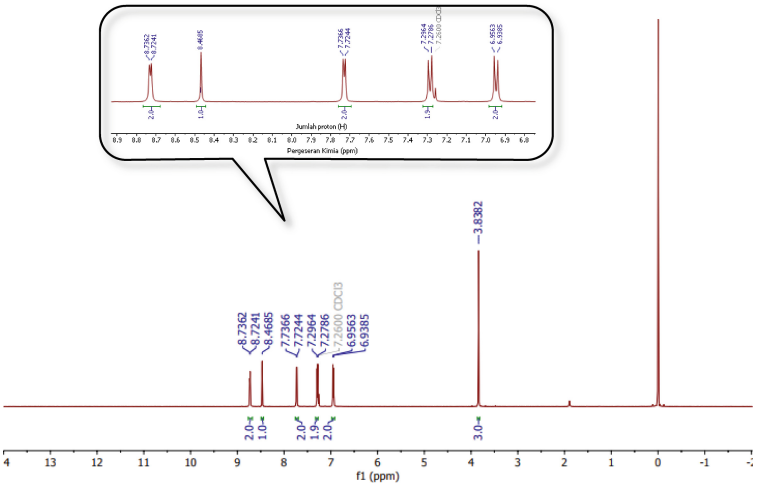

Fig. 5. 1H-NMR spectrum of Schiff base compound synthesis product

Table 2. The results of the inhibition zone of control against Staphylococcus aureus and Escherichia coli bacteria.

Test Bacteria	Treatment	Concentration (μg/mL)	Bacteria Inhibition Zone (mm)
Sthapylococcus aureus	Negative control (DMSO)	-	0
	positive control (Gentamicin)	100	4,6
Escherichia coli	Negative control (dmso)	-	0
	positive control (Gentamicin)	100	12,5

Table 3. Inhibition zone results from variations in the concentration of Schiff base compound 4-methoxy-N-(pyridine-4ilmethylene) aniline

Test Bacteria	Concentration (μg/mL)	Bacteria Inhibition Zone (mm)			
		U1	U2	U3	Average
Sthapylococcus aureus	100	2,3	2,1	2	2,13[a]
	500	2,6	2,8	2,4	2,60[a]
	1.000	3,25	3,4	3	3,21[a]
	5.000	6,4	5,15	6,4	5,98[b]
	10.000	11,25	12,4	12,55	12[c]
Escherichia coli	100	1,4	1,3	1,8	1,50[a]
	500	1,7	2	2,9	2,20[ab]
	1.000	2,5	2,9	3,2	2,86[bc]
	5.000	3,7	3,5	3,95	3,71[c]
	10.000	6,6	6,85	6,8	6,75[d]

Description: U= Deuteronomy; and different letters indicate there is a significant (significant) difference, whereas if the letters are the same then vice versa

normal cell processes. This is related to damage to the structure of the bacterial cell wall, as well as by inhibiting the synthesis mechanism of the peptidoglycan layer which is responsible for maintaining the bacterial organism [11, 14]. The highest antibacterial effectiveness based on the results of the One Way ANOVA statistical test was at a concentration of 10,000 μg/mL (Table 3).

4 Conclusion

Based on the Results of the Study, Which Resulted in the FTIR Characterization, There Was a Wave Number of 1621 cm-1 Which Was Suspected to Be a Typical Absorption of the C = N Group. Then the Results of GC-MS Detected 1 Peak at a Retention Time of 26.187 min, and the Molecular Ion M/z 212 Was Detected Which Corresponded to the Molecular Weight of the Schiff Base Compound 4-Methoxy-N-(Pyridine-4-Ilmethylene)aniline. Supported by the 1H-NMR Results of the Synthesis Product Which Showed the Presence of 6 Proton Environments, and There Was a Chemical Shift of 8.46 ppm with Singlet Absorption Which Could Strengthen the Suspicion of the Presence of an Imine Group that Binds to the H Atom (N = C-H). The Results of the Antibacterial Test of Schiff Base Compounds 4-Methoxy-N-(Pyridine-4-Ilmethylene)aniline Against Staphylococcus Aureus and Escherichia Coli Using Disc Diffusion Method Produced the Largest Inhibition Zone at a Concentration of 10,000 μg/mL

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