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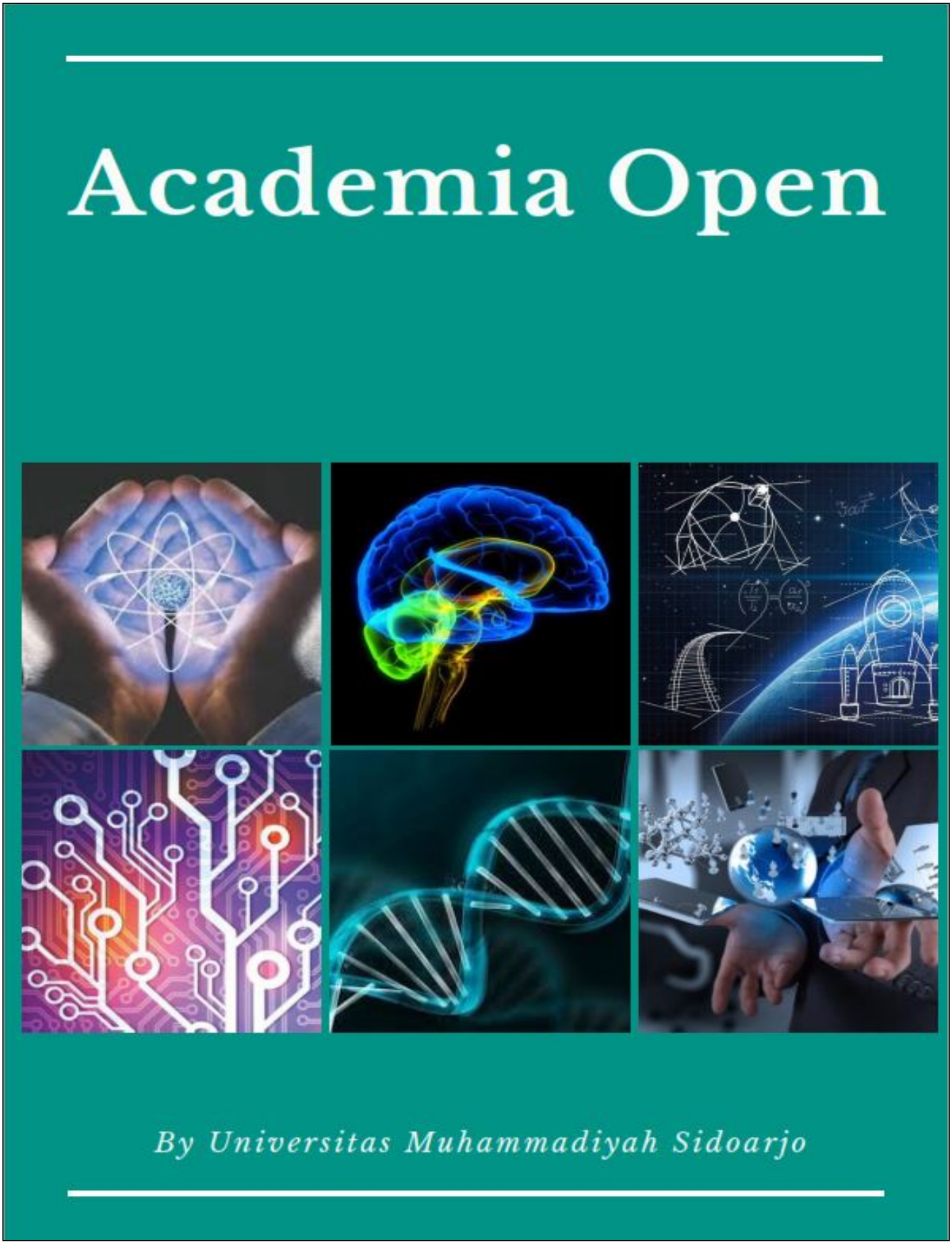

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Anti-Bacterial Activity of Procaine_4_ Hydroxycoumarin - Aqueous Copper Chloride

Aktivitas Anti-Bakteri dari Procaine_4_ Hydroxycoumarin - Tembaga Klorida Berair

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Abstract

Background: The presence of a -N=N- double bond with two substituents, often aromatic rings, is what distinguishes azo compounds. Because of their exceptional qualities, azo dyes and their derivatives have become promising antibacterial substances. Materials and methods: The procaine_4_ hydroxycoumarin - aqueous copper chloride {C9H6O3-C13H20N2O2 -CUCI2.H2O} was prepared by many reactions, and tested as an antibacterial compound against (Pseudomonas aeruginosa, Staphylococcus sp., Proteus mirabilis, Shegilla sp, and Escherichia coli) according to the agar diffusion method. Results: The compound showed antibacterial activity against all bacterial isolates, two of which were resistant to antibiotics such as amikacin and amoxicillin. Conclusion: The ligand and its metal complex are known as biologically antibacterial

Highlights:

1. Azo compounds with aromatic rings exhibit promising antibacterial properties.
2. Procaine_4_hydroxycoumarin-copper chloride tested via agar diffusion method.
3. Effective against bacteria, including antibiotic-resistant strains like E. coli.

Keywords: Anti-bacterial, Azo dye, procaine_4_ hydroxycoumarin- aqueous copper chloride

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Introduction

Azo compounds and azo dyes are organic compounds that have special colors when they absorb light from the spectrum in the visible and ultraviolet regions, jugated double bond (Ganjoo et al., 2022).

These compounds are of great importance, and they are called azo compounds they are used on many sides and they are sometimes used as a dye to dye many fabrics, but on the other hand, they have a negative effect as they are considered water pollution (Zafar et al., 2022).

Azo compounds consist of two homogeneous or heterogeneous groups that are linked through coupling reactions, as the ability to couple in such compounds shifts towards a higher wavelength that reaches the visible region between (400-800 nm), and the reason for calling them. The azo compound is the presence of the azo group (-N = N) - as the nitrogen atoms are linked to the sp2-hybridized carbon atoms. Synthetically prepared organic compounds that can color fibers (Ganjoo et al., 2022), Have wide use as antibacterial and anti-inflammatory (Razali and Jamain, 2023).

It participates in most biological reactions as an inhibitor of DNA. RNA in the body and also as an anti-diabetic, and its most important uses are in experiments that take place in laboratories as reagents in analytical chemistry (Aljamali and Hassen, 2021). It is widely used as a surface disinfectant and sterilizer. It is used in the manufacture of medicines and cosmetics, the manufacture of paper and dyeing leather, and their use as drugs because of their effect in inhibiting germs (Abdul-Ra'aoof, Tiryag, and Atiyah, 2024).

Bacterial antibiotic resistance is a serious problem and it is necessary to discover new compounds to fight resistant bacteria (Abdul-Ra'aoof et al., 2024).

Methods

1- preparation of procaine_4_ hydroxycoumarin - aqueous copper chloride {C9H6O3-C13H20N2O2 -CUCI2.H2O}

The compound was prepared by dissolving 1.3g of procaine in 2ml of concentrated hydrochloric acid and adding 5 ml of water C13H20N2O2 free of any ions where it was placed in an ice bath so that the temperature reached 5 degrees Celsius, then 0.4 grams of sodium nitrite was dissolved in 3ml of water free of any ions where it has placed in an ice bath, then sodium nitrite was added slowly in form of drops to the first solution (cold)v with Continuous stirring and keeping the temperature below 5 C. After that, 0.8 grams of 4-hydroxycoumarin were dissolved in (ethanol + sodium acetate) in the presence of sodium acetate dissolved in 5 ml of ethyl alcohol. After that, the material prepared in the previous step was added to the step of preparing the diosinium salt for Addition drop by drop with continuous stirring for an hour. This solution was left for (24) hours in the refrigerator to complete the precipitation of the crystals. After that, the dye solution was neutralized from the basic form to the acid form by adding a solution of hydraulic acid and then leaving the precipitate to dry.

This equation explains the method of preparing the compound. Procaine -4-Hydroxycoumarin (Chen et al., 2021).

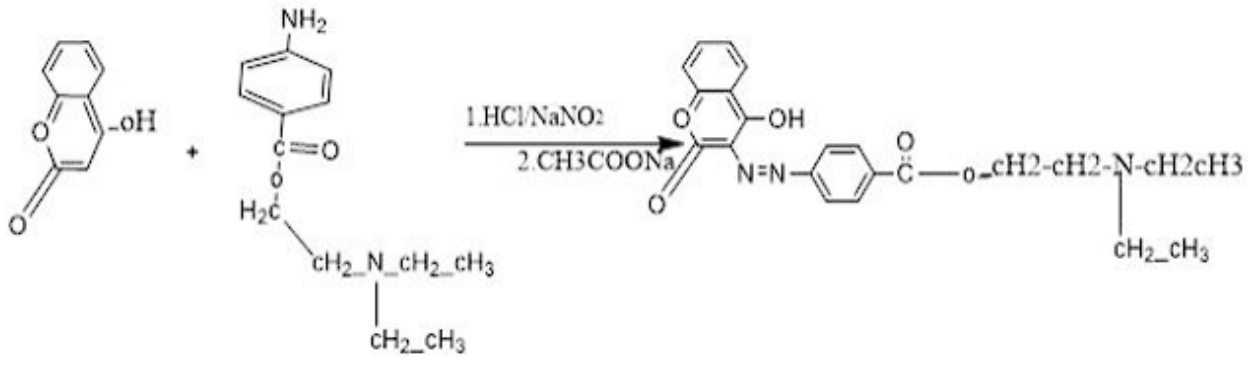

Figure 1. *Chemical equation for the preparation of the compound procaine- 4-Hydroxycoumarin.*

After this step, the complex of the compound is prepared by adding 0.4 grams of this compound to 30 ml of ethanol, then 0.069 grams of aqueous copper chloride salt was added to it, and this product is ascended for six hours in the reflex device, then the mixture is left. To cool and precipitate the solid complex, it is filtered and washed several times, after which it is recrystallized with hot ethanol, and then it is dried in an electric oven at a temperature of 60C (Aljamali & Hassen, 2021).

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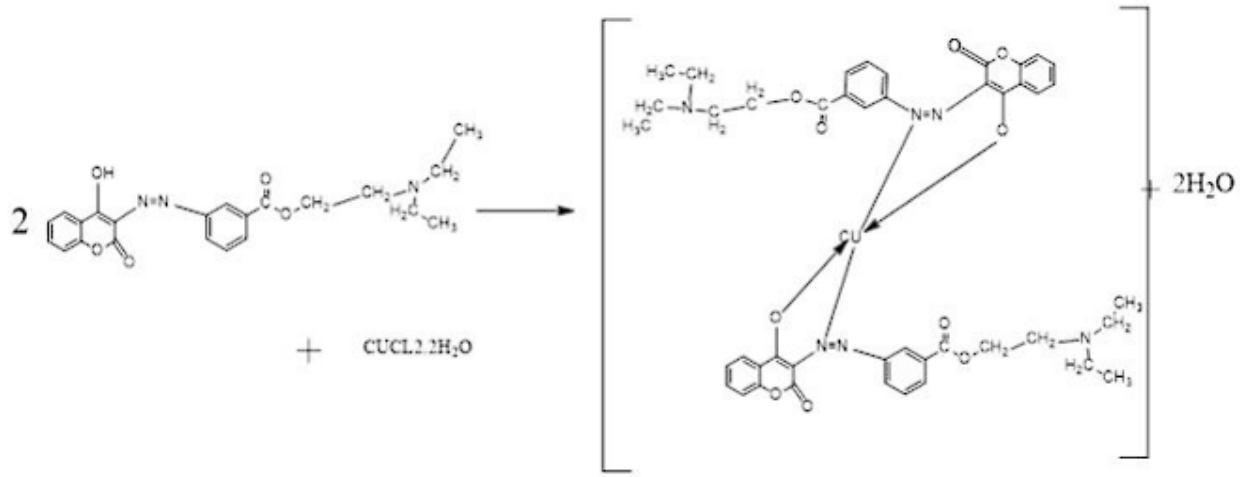

Figure 2. *Chemical equation for the preparation of the compound procaine- 4-Hydroxycoumarin aqueous copper chloride.*

2- Screening for Antibacterial Activity

Antibacterial activity against (Pseudomonas aeruginosa, Staphylococcus sp., Proteus mirabilis, Shegilla sp, and E. coli) was evaluated according to the agar diffusion method. The surface of the nutritional agar was equally coated with the bacterial inoculum (108 cfu/ml in comparison to McFarland tube No. 1). 100 µl of procaine-4-Hydroxycoumarin aqueous copper chloride was added to the hole in each dish using a pure cork punch with a diameter of 4mm.

The reference antibiotics: Amikacin (AK 20 µg), Amoxicillin (AX 10 µg), and Ciprofloxacin (CIP 5 µg) [TM MEDIA, were used as positive controls. The inoculated plates were incubated for 24 h at 37°C, and then the region of the inhibition zone resulting from the effect of the compound was measured by the ruler in mm

Result and Discussion

Through a variety of processes, including DNA intercalation, membrane disruption, enzyme inhibition, and reactive oxygen type production, azo dyes provide antibacterial action (Li-Xia et al., 2006).

In this research, the chemical Azo compound with copper was prepared to determine the extent of its effect on inhibiting pathogenic bacteria.

However, depending on elements like the kind of compound, metal ion, metal complex shape, lipophilicity, and pharmacokinetic agents, these drugs' efficacy may differ (Li-Xia et al., 2006).

In our study, the compound showed antibacterial activity against all bacterial isolates, two of which were resistant to amikacin and amoxicillin (table 1) and Figure (2).

Inhibition zone (mm)				Bacterial isolates
CIP (5 µg)	AX (10 µg)	AK (20 µg)	The compound	
15	-	-	13	Pseudomonas aeroginosa
38	26.5	29	16	Staphylococcus sp.
37	-	12.5	14	Proteus mirabilis
29.5	-	15.5	15	Shegilla sp.
15	-	-	15	E. coli

Table 1. *antibacterial activity of the compound compared with standard antibiotics*

Several previous studies involving the synthesis of azo compounds have shown antibacterial activity consistent with our present study (El-Ghamry et al. 2018; Waheeb and Al-Adilee, 2021; Al-Saidi et al., 2022; Hosny., 2022; Abdul-Ra'aoof et al., 2024).

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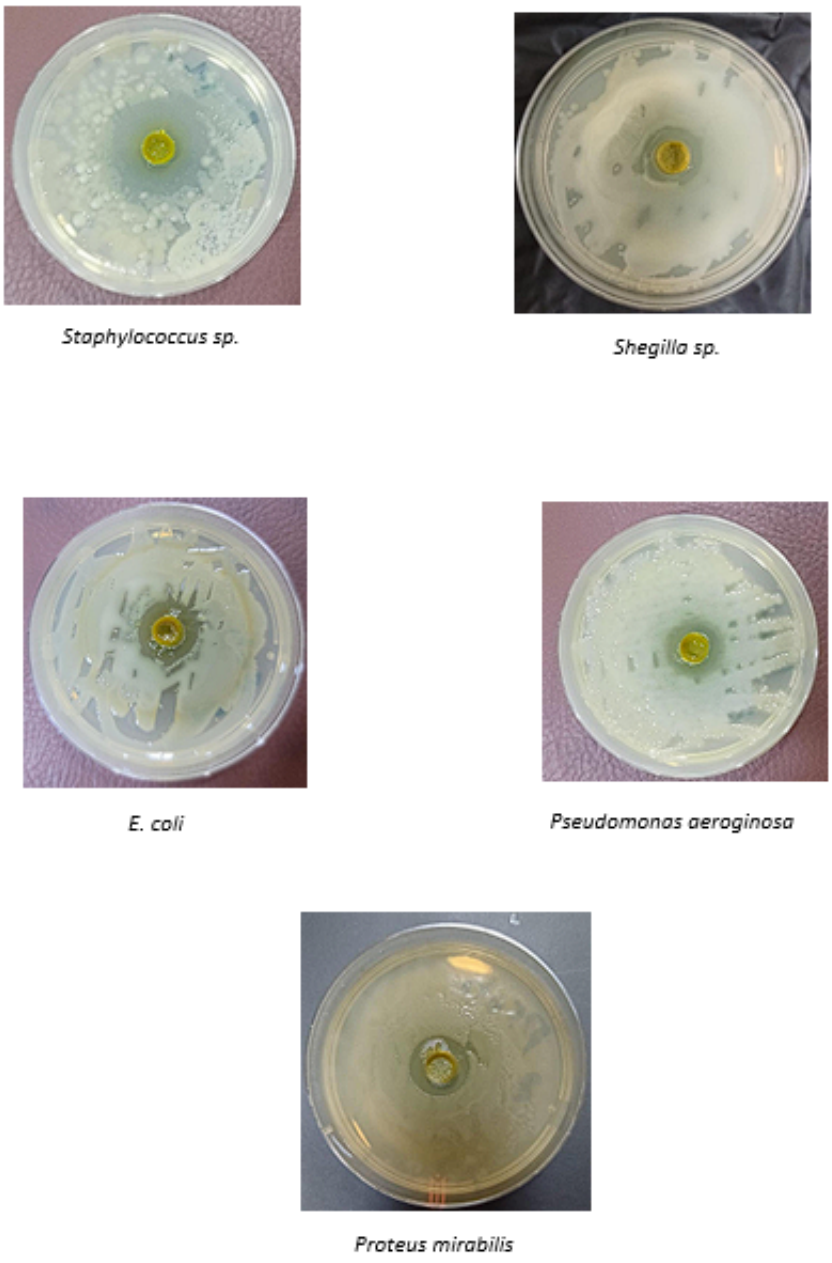

Figure 3. *Antibacterial activity of procaine_4_ hydroxycoumarin - aqueous copper chloride.*

Abbas N.F and Abbas A.K. (2020) examined the ligand (5-(4-methoxy benzene azo)-thiobarbituric acid) and its metal complexes' antibacterial activity against four different bacterial species, including gram-positive bacteria like S. aureus and B. anthracis and gram-negative bacteria like E. coli and Pseudomonas. As a reference medication, amoxicillin is frequently used. Of all the bacterial strains, the Cu complex shows the strongest activity.

Synthesis, structure characterization and molecular modeling of binary metal azo dye: DNA interactions and antimicrobial and anticancer activities (Al-Saidi et al., 2018).

Conclusion

The study successfully synthesized and characterized the azo compound procaine-4-hydroxycoumarin aqueous copper chloride and evaluated its antibacterial activity against a range of pathogenic bacteria, including Pseudomonas aeruginosa, Staphylococcus sp., Proteus mirabilis, Shigella sp., and E. coli. The compound exhibited significant antibacterial activity against all tested isolates, including strains resistant to standard antibiotics such as amikacin and amoxicillin. This highlights the potential of the ligand and its copper complex as promising antibacterial agents, likely due to their ability to disrupt bacterial membranes, intercalate DNA, and inhibit

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essential enzymes. The findings align with previous studies emphasizing the efficacy of azo compounds in combating microbial resistance. These results underline the necessity for further research to optimize the structure and pharmacokinetic properties of azo-based metal complexes, assess their toxicity, and explore their mechanisms of action to advance their application in antimicrobial therapy.

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