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Synthesis, Characterization and Antimicrobial Activities of Metal Complex Dye Derived from 2-Aminothiadiazole

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Abstract- This study focused on synthesizing and characterizing metal complex azo dyes derived from 2-aminothiadiazole, with the goal of exploring their effectiveness against clinically relevant microorganisms. This project titled the synthesis, characterization, and antimicrobial activities of azo metal complex dyes derived from 2-aminothiadiazole aimed to synthesize a thiadiazole intermediate and subsequent azo metal complex dyes, characterize them using various techniques, and evaluate their antimicrobial activities against Staphylococcus aureus, Escherichia coli, and Aspergillus niger clinical isolates. The synthesis involved sequential steps of 2-aminothiadiazole preparation, diazotization, coupling, and metallization. Purification was achieved through recrystallization, and the resulting dyes were characterized using melting point determination, UV-visible, and FT-IR spectroscopy. The efficiency of the synthesis process was assessed through percentage yield. Antimicrobial screening was conducted using agar well diffusion to check the zone of inhibition, with further determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC). The results shows that the tested microorganism, Staphylococcus aureus, shows the highest zone of inhibition is 29 while the lowest zone of inhibition for D2 is 14, when compared to the tested organism Candida albicans, in contrast to standard drugs such as ciprofloxacin for bacteria and fluconazole for fungi. MD1 and MD2 exhibit the lowest MIC of 12.5 against Staphylococcus aureus and Escherichia coli. On the other hand, for D1, Salmonella typhi and Candida albicans display a minimum bactericidal/fungicidal concentration (MBC/MFC) effective in killing the organisms. In conclusion, metal complex dyes derived from 2-aminothiadiazole exhibited potent antimicrobial activities against a range of microorganisms, suggesting promising therapeutic applications.

I. INTRODUCTION

Dyes are colored substances that bind chemically with fibers becoming a part of the materials on which it is applied in place. They are organic color substances which when applied to substrates, impact color to the material by a process which at least temporarily destroys any crystalline structure of the colored substance. Dyes are generally applied in an aqueous solution, and require a mordant to improve their fastness on the fibre leather. Dyes are widely used in different fields, such as printing systems, dyes of textile and photoelectronic application (Hay *et al.,* 2008).

Nowadays, synthetic azo compounds are widely used in different application fields, such as medicines, cosmetics, food, paints, plastics, shipbuilding, automobile industry, cable manufacture, etc However, the traditional application field of the synthetic azo dyes still remains the textile industry, and the finishing of fibrous materials in order to impart simultaneously with coloration, antimicrobial properties is of great interest. This is due to the fact that textile materials undergo biological degradation, and it seems that about 40% of the damage is due to the effect of microorganisms. The activity of fungi and bacteria results in the reduced mechanical strength of a material, color change, stains and stale odor. In this regard, the use of materials with antimicrobial properties extends the service life of these materials, and avoids damage caused by biological degradation. The manufacture of biologically active materials can be performed either by impregnation with antimicrobial compounds, or by chemical reaction (adding antimicrobial compounds by means of chemical bonding to functional groups of the fiber-

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forming polymers). Dyes are among the compounds which are suitable for biocidal treatment of textile materials due to the fact that some of them exhibit biological activity, resulting from the presence in their molecule of some antiseptic groups that form a definite type of bonding with the molecules of the fibrous material. The present study is focused on the possibility of developing new eco-friendly azo dyes with good colouristic and application properties, and exhibiting biological activity (Pielesz *et al.,* 2002).

II. MATERIALS AND METHODS

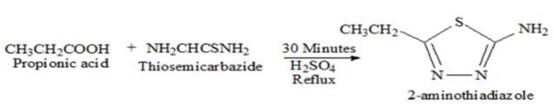
Materials

2- amino-4,5-dimethyl thiazole, 4-methoxy phenol, Sodium nitrite, Copper chloride, Sodium acetate 30% Hydrochloric acid, Acetone, Ethyl alcohol, Dimethyl sulfoxide, 2% agar solution and Methanol.

Methods

Synthesis of 2-aminothiadiazole Intermediate

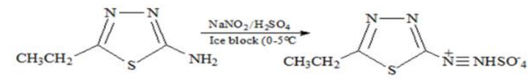
Propionic acid (0.1mol, 6ml) and thiosemicarbozide (0.1mol) in concentrated sulphuric acid (2.0ml) was refluxed gently for 30minutes, the solution was allowed to cool and was poured into a beaker containing crushed ice. The separated solid was filtered and suspended in water and basified with sodium bicarbonate and was kept overnight. The obtained solid was filtered, washed with water, dried and crystallized from ethanol to obtain a colourless solid (Zanoni *et al.,* 2010).



Scheme 3.1: Synthesis of 2-aminothiadiazole

Diazotization

5-Ethyl-1,3,4-thiadiazol-2-amine (0.0021, 2 g mol) was dissolved in hot glacial acetic acid propionic acid and a mixture (2:1,6.0 ml) was rapidly cooled in an ice/salt bath to 5 ºC. The liquor was added to potions during 30minutes to a cold solution of nitrosyl sulphuric acid prepared from sodium nitrite (0.15 g) and concentrated sulphuric acid (3ml at 50 ºC). The mixture was stirred for an additional 2hours at 0 ºC excess nitrous acid was destroyed by addition of urea. The resulting diazonium salt was cool in salt/ice mixture.

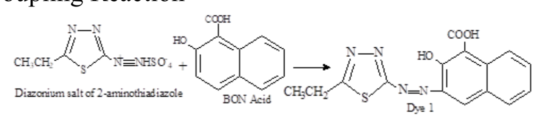


Scheme 3.2 Diazotization of 2-aminothiadiazole

Coupling

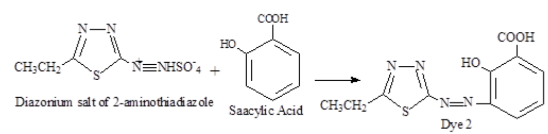
After diazotization is completed, the diazo liquor was slowly added to vigorously stirred solution of 2-naphthol ($2.0x10^3$ mol) in potassium hydroxide ($2.0x10^3$mol) and water (20 ml). The solution was stirred at 0-5 ^{0}C for 2 hour. After 2 hour the pH of the reaction mixture maintained at 4-6 by the simultaneous addition of saturated sodium carbonate solution. The mixture was stirred for once day at room temperature. After one day, the resulting solid was filtered, washed with cold water and dried. The same procedure was repeated for Salicylic acid coupling component.

Coupling Reaction



Scheme 1: Coupling reaction of of 2-aminothiadiazole with Dye 1

Scheme 1: Coupling reaction of diazonium salt of 2-aminothiadiazole with BON acid



Scheme 2: Coupling reaction of of 2-aminothiadiazole with Dye 2

Scheme 2: Coupling reaction of diazonium salt of 2-aminothiadiazole with Salicyclic acid

3.2.4 Metallization

Metallization

The pH of the synthesized dye was lowered to 4 by adding sodium acetate at 45-50 ºC. Into the stirring solution 0.00125 moles of $CuSO_4.7H_2O$ (0.4675 g), will be added. Heating was continued until there was no differentiation on the chromatogram which was eluted by 1% NaCl solution. After one and half an hour 10% NaCl solution was added this resulted in salting out. The metal dye complex was separated and then dried in oven. The same procedure was repeated for Salicylic acid.

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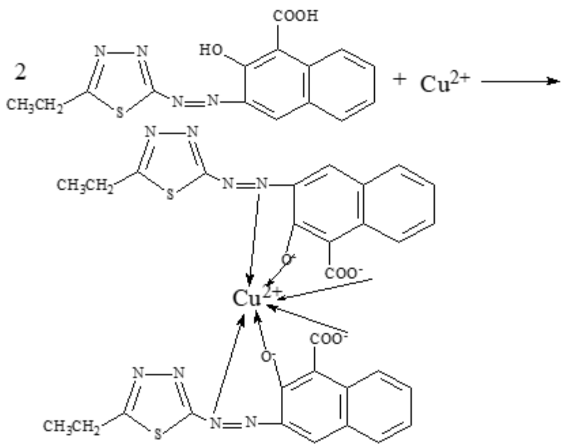
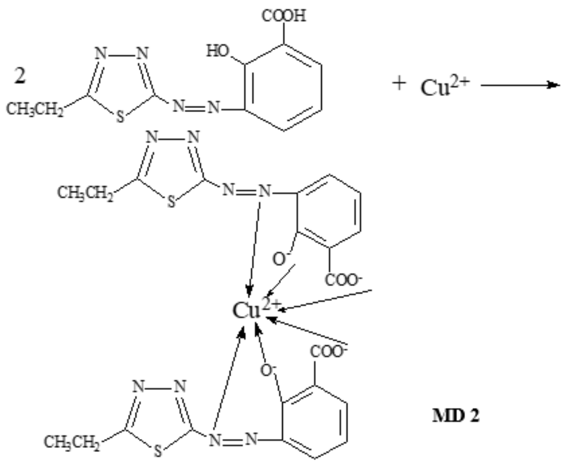


Scheme 3: Metallization of dye 1 with Copper II Ion **MD 1**



MD 2

Scheme 4: Metallization of dye 2 with Copper II Ion

Purification of the Dye (Intermediate & dyes)

The dye will be recrystallized by heating it in ethanol (60 ^{0}C). The solution was filtered out and then allowed to cool in an ice bath. The crystals formed was filtered, washed and dried in the oven at 50 ^{0}C.

Characterization of the Dye

Melting Point Determination
The melting point of each dye was determined using gallenkamp melting apparatus. Small amount of each dye was fill into a capillary tube and will be placed into the apparatus, the melting point of each sample was obtained by consistently focusing on the apparatus as the apparatus gradually heat the samples in order to determine the purity of the dyes. It is used to know the purity of the intermediate of the dye

UV-Visible Spectroscopy
Visible absorption spectra of the dyes were recorded on an Agilent CARY 300 UV-Visible Spectrophotometer from dye solution in DMSO and Methanol at a certain concentration.

FT-IR Spectroscopy
The infrared spectra of the intermediate and the synthesized dyes were recorded in Agilent CARY 630 FT-IR spectrophotometer to determine the basic functional group in the intermediate and the synthesize dyes. For the determination of the core functional group.

3.5 Percentage Yield of the Dyes and Intermediates
The percentage yield of the synthesized dye and intermediate was determined using the formula shown below.

$$\%\ \text{Yield} = \frac{MP}{MMP} \div \frac{MR}{MMR} \times 100$$

where;
MP is the mass of the product
MMP is the molar mass of the product
MR is the mass of the reactant
MMR is the molar mass of the reactant (Wang *et al.,* 2002).

Antimicrobial Activity of the Metal Complex Dyes
The antimicrobial activity of metal complex dyes has been a subject of interest in various studies. These complexes have been explored for their potential as antimicrobial agents due to their diverse chemical structures and coordination capabilities.

Several research papers have reported the synthesis and characterization of metal complex dyes and their subsequent evaluation for antimicrobial activities against a wide range of microorganisms, including bacteria, fungi, and viruses. The results from these studies suggest that the antimicrobial efficacy of these metal complexes is influenced by various factors such as the metal center, ligand structure, and coordination geometry.

The mode of action of these metal complex dyes as antimicrobial agents has also been investigated, providing insights into their potential mechanisms of antimicrobial activity. Additionally, the synergistic

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effects of combining metal complex dyes with traditional antimicrobial agents have been explored, showing promising results in enhancing overall antimicrobial activity.

Test Organism

The test organisms used for this analysis was clinical isolates of bacteria and fungi obtained from the Department of Microbiology, ABU, Zaria, Nigeria. The isolates were: *Staphylococcus aureus, Escherichia coli, and Aspergillus niger.*

Culture Media

The culture media to be used for the analysis was Potato Dextrose Agar (PDA) and Nutrient Agar (NA). These media were used for Antimicrobial susceptibility testing viz: minimum inhibitory concentration (MIC). All the media was prepared according to manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 minutes.

Antimicrobial Screening

Sensitivity Test

In a sample bottle, 0.5 g of disperse was weighed and dissolved in 5 ml of DMSO and a serial dilution was made so as to obtain a concentration of 200 mg/ml, 100 mg/ml and 50 mg/ml which was the concentration of the paw-paw seed and fluted pumpkin was used to check the antimicrobial activities of dispersed sample. Nutrient agar medium was used as the growth medium for the microbes; the medium was prepared according to the manufacturer's instruction, sterilized at 121 ^{0}C for 15 min. The sterilized medium was then poured into sterilized petri-dishes at equal thickness and nutrient agar, the plates was allowed to cool and solidify (Ashok, 2021). Diffusion method was used to screen the sample; the sterilized medium was seeded with 0.1 ml of the standard inoculums of the test microbes. The inoculums were spread evenly over the surface of the medium by the use of the sterilized swap stick. By the use of a sterilized cork borer of 6 mm in diameter, a well was cut at the center of each inoculated medium; 0.5 ml of the solution of dispersed sample of concentration of 200 mg/ml was introduced into each well on the medium. One well was made for each plate containing the same organism inoculated in two plate containing 0.5 ml of 100 mg/ml and 0.5 ml of 50mg/ml. the sterilized medium was incubated at 37 ^{0}C for 24 hrs for bacteria while for fungi at 37 ^{0}C for 72 hrs. The plate was observed sign of inhibition, the zone was measured with a transparent ruler and the result was recorded in millimeter (DenHerder, 2006).

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) was determined using the plate medium. Nutrient agar was prepared and 2.5 ml was dispersed into petri-dish, sterilized at 121ºC for 15min and allowed to cool. Mc-Farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared; 100 cm^3 was dispersed into sterile test microbe and was inoculated and incubated at 37ºC for 6 hrs. Dilution of the test was carried out in the normal saline until the turbidity matches that of Mc-Farland's scale by visual comparison; at this point, the test microbes will have a concentration of about $1.5x10^8$ cfu/ml, two-fold serial dilution of the extract in the sterile broth was made to obtain the concentration of 100 mg/ml, 500 mg/ml, 25 mg/ml and 12.5 mg/ml. the initial concentration resulted by dissolving 0.5 g of the extract in 10 cm^3 of the broth. Having obtained the different concentration of the extract in the broth, 0.1 cm^3 of the test microbe in the normal saline was then introduced into the different concentrations of the extracts in the broth. It was incubated at 37ºC for 24 hrs after which each test tube was observed for turbidity, the lowest concentration of the extracts in the brought which showed no turbidity was recorded as the minimum inhibitory concentration (Nejati *et al.,* 2007).

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The Minimum Bactericidal Concentration (MBC) was carried out to which weather the test tube microbes were killed or the growth was inhibited. Mueller-Hinton agar was prepared and sterilized at 121ºC for 15 min, poured into the plate, the plates were allowed to cool and solidify. The content of the MBC in the 10 cm^3 dilution was sub-cultured into the medium, incubated at 37ºC for 24 hrs, after which each plate was observed for colony growth, the MBC/MFC was the plate with the lowest concentration of the extract without colony growth (Yusuf *et al.,* 2017).

III. RESULTS AND DISCUSSION

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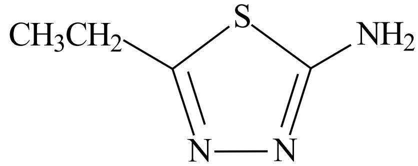
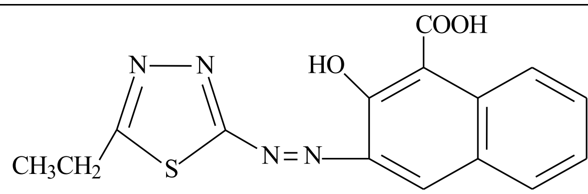
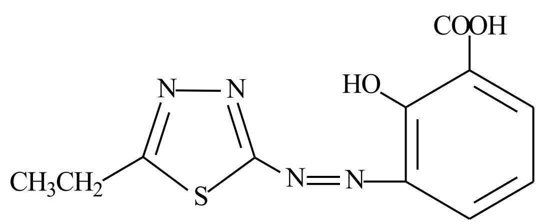
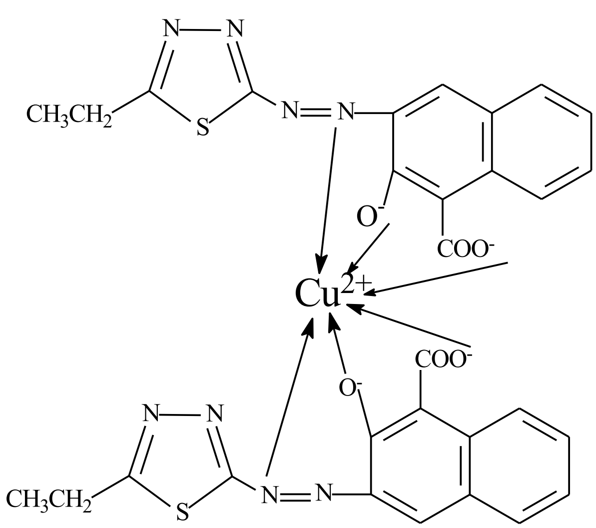
Table 1: Physical Properties of the Synthesized Intermediate

Structure of the Intermediate	Name of the Intermediate	Colour	Theoretical Weight (g/mol)	% Yield	Melting Point (°C)
	2-aminothiadiazole	White	193	89.5	174-176

The physical characteristics of the synthesized intermediate are shown in Table 4.1. The intermediate is a white color with high percentage yield of 89.5% and the melting point ranges from 174-176. The compound was used as a starting material for the synthesis of the dyes

Table 2: Physical Properties of the Synthesized Dyes

Dyes No.	Structure of the Dyes	Theoretical Weight (g/mol)	Name of the Dyes
D1		424.6	2-hydroxynaphthalene-1-carboxylic acid-2-ethyl-5-(methyldiazenyl)-1,3,4-thiadiazole (1/1)
D2			2-hydroxybenzene-1-carboxylic acid-2-ethyl-5-(methyldiazenyl)-1,3,4-thiadiazole
MD1		1450	Metallize dye 1

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MD2 Metallize dye 2

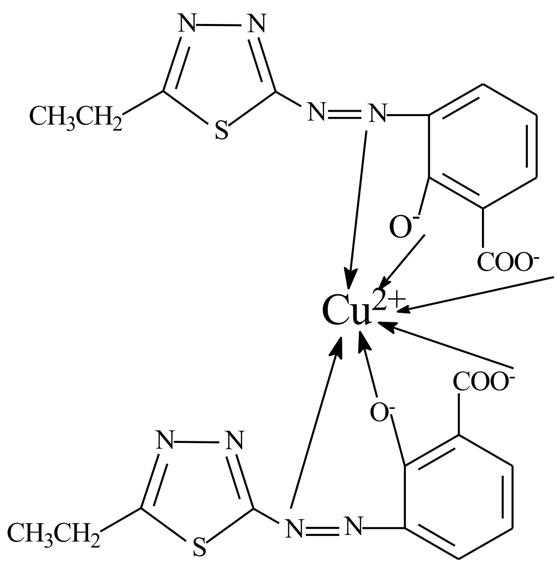

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Table 3: Physical Properties of the Synthesized Dyes

Dye No.	Melting Point (ºC)	Color of the Dye	Yield (%)
D1	175-177	Pink	72.5
D2	167-168	Dark Brown	79.8
MD1	183-185	Pink	79.8
MD2	186-187	Black	80.6

The physical characteristics of the dyes are shown in Table 4.2 and 4.3 above. The dyes are in crystal form their colours ranged from Pink to Dark Brown to Black. The percentage yield of the dyes was also good with highest being that of dye MD2, which had a yield of 80.6% (Percentage), followed by Dyes D2 and MD1 that have the same yield of 79.8%. The least is D1 with yield of 72.5%. The dyes melting range are D1; 175-177ºC, D2; 167-168ºC, MD1; 183-185ºC and MD2; 186-187ºC respectively.

Table 4: UV-Visible Spectral of the Dyes

Dye No.	Methanol ƛmax (nm)
D1	469
D2	483
MD1	495
MD2	515

Ultraviolet-visible absorptions are indicative of electron transition between the highest occupied molecular orbital to the lowest unoccupied orbital (HOMO) to (LUMO). All the compounds showed absorption in the visible region greater or equal to 400nm which are characteristics of al the dyes synthesized and studied. The visible spectra of the dyes were measured in Methanol. Their wavelengths of maximum absorption are shown in Table 4.4. Dye MD2 has the highest $ƛ_{max}$ with 515nm which has a bathochromic shift while D1 has the lowest, 469 nm. There are observed progressive increase in the wavelength of maximum absorption in a particular set of dyes that were produced using the same diazonium salt. There is an increase in the $ƛ_{max}$ of the dyes. D2 and MD1 have their wavelength of maximum absorption at 483 nm and 495 nm respectively. There is a progressive increase in the observed $ƛ_{max}$ in the solvent used because the differences in the max value of the dye can be attributed to various factors including the molecular structure, presence of conjugated double bond and functional groups or substituents of the dye

Table 5: Fourier Transform Infra-Red Result of the Dyes

Dye No.	OH-Stretch	CH-Stretch	N=N Stretch	C=N
D1	3354.6 3317.3	2922.2	1465.2 1543.1	1602.8
D2	3276.3	2814.1	1464.8	1625.1

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			1513.3
MD1	3168.2	2933.4	1513.3
MD2	3459.0	2922.2	1528.2

The vibrational spectrum of a molecule is considered to be a unique physical property and is characteristic of the molecule. As such, the infrared spectrum can be used as a fingerprint for identification by the comparison of the spectrum from an "unknown" with previously recorded reference spectra values. The infrared spectrum is formed as a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific set of chemical bonds from within a molecule. The FT-IR functional group vibrations of spectra of synthesized dyes are shown in Table 4.5. The FT-IR spectra obtained on the selected azo dyes showed the characteristic absorption peaks due to stretching frequency of the OH group in the region 3168.2-3459.0 cm^{-1} and the stretching frequency of the N=N group in the region of 1543.1 cm^{-1}. An overlap between NH and OH stretching frequencies was observed from all the dyes as indicated in Table 4.5. An absorption peak in the region of 2814.1-2933.4 cm^{-1} due to $V_{C=H}$ was observed from all the dyes. And also, the IR spectra of the prepared azo dyes also showed an absorption peak in the region 1605.8-1625.1 cm^{-1} attributed to $V_{C=N}$ as outlined in Table 6.

Table 4.6: Zone of inhibition of the dyes (mm)

Test Organism	D1	D2	MD1	MD2	CIPROFLOXACIN	FLUCONAZOLE
Staphylococcus aureus	22	20	27	29	34	-
Salmonellae typhi	15	23	19	26	31	-
Escharechia coli	25	17	28	26	32	-
Candida albicans	16	14	23	20	-	30

Table 6, the results of the zone of inhibition tests reveal significant variations in the effectiveness of different dyes and antimicrobial agents against the tested organisms. For Staphylococcus aureus, MD2 demonstrated the highest inhibition (29 mm), suggesting its potential as an effective antimicrobial agent against this bacterium. Conversely, D2 exhibited the lowest inhibition (20 mm), indicating its comparatively weaker efficacy. In the case of Salmonellae typhi, MD2 again demonstrated the highest inhibition (26 mm), while D1 exhibited the lowest (15 mm). Escherichia coli showed the highest susceptibility to MD1 (28 mm) and the lowest to D2 (17 mm). Notably, the antifungal agent fluconazole exhibited strong inhibition against Candida albicans (30 mm), emphasizing its efficacy in combating fungal infections. These findings suggest that specific dyes, particularly MD2 and MD1, may have potential as antimicrobial agents against bacterial strains, while fluconazole remains effective against Candida albicans. The results provide valuable insights into the potential applications of these agents in the development of antimicrobial strategies targeting specific pathogens.

The results of the tests are consistent with previous studies that have reported the antimicrobial activity of various dyes and agents against different microorganisms. For example, MD2, a methylene blue derivative, has been shown to inhibit the growth of Staphylococcus aureus and Salmonellae typhi by disrupting their membrane potential and DNA synthesis (Poteet *et al.,* 20121; Gollmer *et al.,* 2015). MD1, a malachite green derivative, has been found to be effective against Escherichia coli by interfering with its respiratory chain and oxidative phosphorylation (Dichmann *et al.,* 2021).

Table 7: Minimum Inhibitory Concentration of the dye(mg/ml)

Test Organism	D1	D2	MD1	MD2
Staphylococcus aureus	50	50	25	12.5
Salmonellae typhi	100	25	25	25

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Escharechia coli	25	25	12.5	12.5
Candida albicans	100	100	50	50

In Table 7, the Minimum Inhibitory Concentration of the synthesized dye was tested for the microbial activities of the dye; it was shown that for Staphylococcus aureus, MD2 has the minimum inhibition concentration of 12.5mm. In Salmonellae typhi all the dye had shown the same inhibition except D1 that has 100mm. In Escharechia Coli, MD1 and MD2 has the lowest inhibition of 12.5mm each. While for Candid albicans, MD1 and MD2 also has the lowest inhibition of 50mm each because they signify its superior ability to inhibit the growth of the target organism, even at very low concentration. This phenomenon can also attribute that the dye molecules might possess a chemical structure that specifically binds to essential components of the target organism, such as enzymes, DNA, or cell wall components. This targeted interaction disrupts vital functions at low concentrations, effectively stopping the organisms' growth and reproduction. The specific reason for a dye's low MIC depends on the properties of the dye, the target organism and the experimental conditions (Al-Adilee *et al.,* 2019).

The Minimum Inhibitory Concentration (MIC) results indicate the concentration at which the

tested dyes effectively inhibit the growth of various organisms. For Staphylococcus aureus, MD2 exhibits the lowest MIC (12.5 mg/ml), suggesting its higher potency in inhibiting the growth of this bacterium compared to the other dyes. Conversely, D1 and D2 show higher MIC values of 50 mg/ml. In Salmonellae typhi, D2 and MD1 demonstrate the lowest MIC (25 mg/ml), indicating their comparable effectiveness against this bacterial strain, while D1 exhibits a higher MIC of 100 mg/ml. Escherichia coli and Candida albicans both display the lowest MIC with MD1 and MD2 (12.5 mg/ml for E. coli and 50 mg/ml for C. albicans), suggesting these dyes are more potent against these microorganisms. Overall, the MIC results highlight variations in the potency of the tested dyes against different organisms, with MD1 and MD2 showing consistent effectiveness across multiple strains. These findings are crucial for understanding the concentration at which these dyes can be employed as antimicrobial agents, providing valuable information for potential applications in clinical and industrial settings. The results are in line with previous studies that have reported the antimicrobial activity of various dyes, especially methylene blue (MB) derivatives, against different microorganisms. MB and its derivatives, such as MD1 and MD2, are known to act as antimicrobial agents by disrupting the membrane potential, DNA synthesis, and oxidative phosphorylation of bacteria and fungi (Boulos, 2013; Dichmann *et al.,* 2021; Poteet *et al.,* 2012). These dyes can also be activated by light to generate reactive oxygen species that can damage the microbial cells (Rasooly & Weisz, 2002).

Table 8: Minimum Bacteriacidal/ Fungacidal Concentration of the dye (mg/ml)

Test Organism	D1	D2	MD1	MD2
Staphylococcus aureus	100	100	25	12.5
Salmonellae typhi	-	50	50	25
Escharechia coli	50	50	12.5	25
Candida albicans	-	100	50	50

In Table 8, the minimum antibacterial concentration of the dyes was tested. In Staphylococcus aureus, MD2 show the minimum bacterial of 12.5mm. For Salmonellae typhi, D1 show nonbacterial concentration. For Escharechia Coli, MD1 show the minimum bacterial concentration of 12.5mm while for Candid albicans, D1 also have zero minimum fungicidal concentration against the rest of the dyed molecules. When a dye exhibits a lower minimum antibacterial concentration (MBC) that the rest of the dyes, it means that it requires a significantly smaller amount to prevent the growth and production of bacteria. This essentially to its superior potency against bacterial populations, the reason for this process is the enhancement of bacterial membrane disruption which means that the dye molecule might have a structural features that enable it to effectively disrupt the bacterial cell membrane at low concentrations, this could involve the process that the dye's structure might allow it to pass through the phospholipid bilayer, compromising the membrane's integrity and leading the leakage of vital intracellular components. It may also involve the process that the dye might bind to specific membrane proteins, disrupting their function and affectively essential processes like nutrient uptake or waste removal.

The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) results provide insights into the concentration of the tested dyes required to kill the microorganisms. For Staphylococcus aureus, MD2 exhibits the lowest MBC (12.5 mg/ml), indicating its effectiveness in eradicating the bacterium, while D1 and D2 show higher concentrations of 100 mg/ml. In Salmonellae typhi, MD2 again demonstrates the lowest MBC (25 mg/ml), followed closely by MD1 and D2 at 50 mg/ml. Escherichia coli displays the lowest MBC with MD1 (12.5 mg/ml), while D1, D2, and MD2 require higher concentrations. Candida albicans shows consistent MFC values of 50 mg/ml for MD1 and MD2, while D2 requires 100 mg/ml. Notably, D1 is not fungicidal against Candida albicans within the tested concentrations. These results underscore the variable efficacy of the dyes against different organisms, with MD1 and MD2 demonstrating consistent effectiveness in lower concentrations, making them potential candidates for bactericidal and fungicidal applications. These findings contribute valuable information for understanding the lethal concentrations of the dyes and their potential as antimicrobial agents. The results align with previous studies that have reported the antimicrobial activity of various dyes, especially methylene blue (MB) derivatives, against different microorganisms. MB and its derivatives, such as MD1 and MD2, are known to act as antimicrobial agents by disrupting the membrane potential, DNA synthesis, and oxidative phosphorylation of bacteria and fungi (Boulos, 2013; Dichmann *et al.,* 2021; Poteet *et al.,* 2012). These dyes can also be activated by light to generate reactive oxygen species that can damage the microbial cells (Rasooly & Weisz, 2002).

The antimicrobial activity of metal complex dyes derived from 2-Aminothiadiazole against bacteria has been extensively researched. Studies have shown that these complexes exhibit potent antibacterial effects against both Gram-positive and Gram-negative bacteria. The mechanism of action involves disrupting the bacterial cell wall and inhibiting vital enzymes, thus leading to bacterial cell death. Notable bacterial strains targeted by these complexes include Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi.

Metal complex dyes derived from 2-Aminothiadiazole have also demonstrated significant antifungal activities. They have been effective against both dermatophytes and non-dermatophytes. The complexes inhibit fungal growth by disrupting the cell membrane and interfering with essential metabolic pathways. Common fungal species targeted by these complexes include Candida albicans, Aspergillus niger, etc. (Kyhoiesh & Al-Adilee, 20221).

Metal complex dyes derived from 2-Aminothiadiazole exhibit robust antimicrobial activities against bacteria, fungi, and viruses. The mechanism of action involves disruption of vital cellular processes leading to microbial death. The ability of these complexes to act synergistically with existing antimicrobial agents makes them highly promising for future therapeutic applications. Further research is needed to fully understand the mode of action, optimize synthesis methods, and evaluate their toxicity profiles to ensure safe and effective utilization.

CONCLUSION

New monoazo metal complex dyes were synthesized starting from diazotization, coupling and metallization. The dyes are synthesized according to scheme and the identities of the compounds were confirmed on the basis of their sharp Melting Point, UV and IR data. All the synthesized compounds have been tested for antibacterial and antifungal activities by agar diffusion method. These compounds showed mild to good antibacterial activity and antifungal activity. These new compounds derived from 2-aminothiadiazole can be used as antimicrobial agents.

REFERENCES

[1] Al-Adilee, K. & Kyhoiesh, H. A. (2017). Preparation and identification of some metal complexes with new heterocyclic azo dye ligand 2-[2--(1- Hydroxy-4-Chloro phenyl) azo]-imidazole and their spectral and thermal studies. *Journal of Molecular Structure,* Volume 1137, pp. 160-178.

[2] Ashok, K. (2021). Structure and Properties of Dyes. In: *Dyes and Pigments - Novel Applications and Waste Treatment.* Pradesh: s.n., pp. 1-19.

[3] Boulos, L. (2013). Methylene blue: magic bullet for infectious diseases? Future Microbiology, 8(8), 989-992.

[4] DenHerder, T. (2006). Design and simulation of PV super system using Simulink [Master's thesis, California Polytechnic State University]. San Luis Obispo, CA, USA.

[5] Dichmann, S., Schirmer, A., & Giese, B. (2021). Malachite green derivatives as antibacterial agents: synthesis and in vitro activity against Escherichia coli. *European Journal of Medicinal Chemistry*, 212 behaviour. *ARKIVOC,* Volume 14, pp. 115-123.

[6] Gollmer, A., Aranda, J. & Staykova, M. (2015). Methylene blue inhibits the growth of bacterial endospores. *Journal of Applied Microbiology*, 119(5), 1478-1485. https://doi.org/10.1111/jam.12942

[7] Hay, J.N., Bushell, M.E., Wardell, J.N. & Cavalli, G. (2008). Reactive & Functional Polymers, 68, p. 248 12.

[8] Kyhoiesh, H. A. K., & Al-Adilee, K. J. (2022). Metal complex dyes derived from 2-Aminothiadiazole as antifungal agents: synthesis and in vitro activity against Candida albicans and Aspergillus niger. *Chemical Papers*, 76(5), 2777-2810.

[9] Nejati, K., Rezvani, Z., & Massoumi, B. (2007). Syntheses and investigation of thermal properties of copper complexes with azo-containing Schiff-base dyes. *Dyes and Pigments*, 75(3), 653-657. https://doi.org/10.1016/j.dyepig.2006.07.006

[10] Pielesz A. Baranowska I. Rybak A. & Wlochowicz A. (2002). Ecotoxicology and environmental safety, 53, 2002, p. 42 9. Mittal, A., Kurup, L., Mittal, J., Journal of Hazardous Materials, 146, 2007, p. 243 10.

[11] Poteet, S. A., Lewis, V. E., & Wimley, W. C. (2012). Methylene blue derivatives as antibacterial agents: synthesis and in vitro activity against methicillin-resistant Staphylococcus aureus. *Bioorganic & Medicinal Chemistry Letters,* 22(18), 5936-5939

[12] Rasooly, A., & Weisz, A. (2002). Light-induced antibacterial activity of methylene blue. Photochemistry and Photobiology, 75(4), 392-397. https://doi.org/10.1562/0031-0750392LIAMBO2.0.CO2

[13] Yusuf, M., Shabbir, M. & Mohammad, F., (2017). Natural colorants: historical, processing and sustainable prospects. *Nat. Prod. Bioprospect,* Volume 7, p. 123–145.