

Antimicrobial Activity of Chemically Modified Anionic Azo Dyes and Their Potential for Treating Infectious Diseases

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ABSTRACT: Antimicrobial evaluation of quaternary ammonium salts (QAS) and silver nanoparticles (AgNPs) incorporated with some food dyes—Sunset Yellow, Tartrazine, and Allura Red is studied. FTIR spectroscopic analysis was used to confirm the chemical interaction of the key functional groups. Functional groups interactions, such as -N=N , C=C , and -OH stretching vibrations indicate successful interaction between the dye molecules and the carrier materials. The antimicrobial activities of these compounds were tested against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella spp.* Results showed enhanced inhibition zones, particularly for the dye incorporated with silver nanoparticles (dye-AgNPs). A combination of tartrazine dye with the nanoparticles showed the highest inhibition against *S. aureus*. On the other hand, the quaternary ammonium salt showed an improved activity in combination with the azo dyes, especially against Gram-positive strains. The impressive antimicrobial performance could be attributed to the increased lipophilicity of the samples that allows a better penetration into the bacterial membranes. Generally, the research provides the potential use of azo dye-functionalized QAS and AgNPs as effective antimicrobial agents, especially in targeting resistant Gram-positive pathogens.

KEYWORDS: Azo dyes; antimicrobial; bacteria; quaternary ammonium salt; silver nanoparticles.

1. INTRODUCTION

Azo dyes are among the most widely used dyes in the textile and food industries. The commercially produced dyes represent around 70% of total production. Furthermore, the dyes are also used in printing, pharmaceutical, and cosmetics industries (Jerca, 2022). The significant segment of azo dyes structure is azobenzene, and it is a well-known class of stimuli-responsive molecules (Joshi, 2012). Azobenzene commonly exists in cis or trans isomers. The isomerism of the compound follows two major mechanisms: rotation and inversion. The rotation process involves the cleavage of the -N=N- double bond to allow a free rotation around the single bond. The rotation results in the -C-N-C- dihedral angle changing, while an -N-N-C- angle remains fixed at 120° . On the other hand, in the inversion mechanism, the -N=N-C- angle increases to 180° , while the -C-N=N-C- dihedral angle remains fixed; this results in a transition state in which a nitrogen atom is hybridized sp (Banaszak-Leonard, 2021).

The structural phenomenon of azobenzene to exist as cis and trans isomers has made the compound to have versatile applications in the production of many materials. For example, its derivatives are used in the construction of optical switches, optical waveguides, and memory elements (Joshi, 2012). Moreover, incorporating azobenzene onto polymer matrices could be used to produce photo responsive materials (Acierno, 2004). Another important application of azobenzene and its derivatives is in the area of biomedicine. Production of antioxidant, antiviral, antimicrobial, anticancer, and antidiabetic compounds are also reported (Concilio, 2005).

Application of colorants for antimicrobial purposes has been reported. The colorants extracted from plants showed impressive dual properties: textile coloration and antimicrobial property (Singh, 2016). Similarly, colorants from natural or synthetic sources are reported to have multiple applications, such as textile coloration, insecticidal action, and UV protectors (Dantas, 2023).

Drug-resistant microbes is a continuously increasing problem in the world today. Deadly pathogens such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis* pose a serious concern. The drug resistance usually results from the rapidly evolving bacterial species that are capable of inhibiting the pharmacological action of drug molecules through different types of mechanisms, such as enzymatic inactivation, modification of drug targets, or biofilm formation. Moreover, the low affinity of the drug molecules for the bacterial cell wall requires high concentrations and long-term treatments to achieve the desired benefits and performance. These drawbacks could lead to adverse effects (Ball, 2004).

This research involves a two-step approach to produce some efficient, non-toxic antimicrobial compounds derived from the combination of azo compounds with quaternary ammonium salts and silver nanoparticles. The presence of the colourants in both the ammonium salts and silver nanoparticles showed a significant antimicrobial inhibition.



2. EXPERIMENTAL SECTION

2.1. Materials

All reagents used in the experiment were of analytical grade and were used without further purification. The glasswares were thoroughly washed with detergent, rinsed with water, and dried in an oven at 100 °C before use. The weight of the samples was carried out on Metler balance, model H30AR. The FTIR spectra of the dye samples and the salts were obtained using an FTIR spectrophotometer (Cary 630, Agilent Technology) in the range of 400-4000 cm.

2.2. Methodology

2.2.1 Synthesis of Silver Nanoparticles

The procedure reported by Rajesh Pandiyan, 2023 was used with little modification. The leaves of *Azadirachta indica* were collected from Umaru Musa Yar'adua University and washed with distilled water to remove dirt and dust, the fresh neem leaves were chopped into small pieces and air dried. The chopped and dried leaves (10 g) were boiled in water (100 mL) at 60 °C for 30 minutes. The leaf extract was obtained by filtration. The broth was then stored at 5 °C for further analysis. 1mM of AgNO₃ (20 mL) was added to 10 mL of the leaf extract. The mixture was incubated at room temperature for 20 min (Marzullo, 2024).

2.2.2 Synthesis of Quaternary Ammonium Salt

The synthesis was carried out in an inert atmosphere where 10 mL of triethylamine was added in a 250 mL round-bottom flask. Benzyl chloride (10 mL) was slowly added to the reaction mixture in a drop wise, the reaction mixture was continuously stirred at 30 °C under reflux for 40 min and monitored using TLC. HCl (2 mmol) was added to quench the reaction mixture. The organic layer was extracted with diethyl ether. The layer was washed with water to remove impurities and dried over anhydrous magnesium sulphate. The solvent was removed using a rotary evaporator to obtain the crude quaternary ammonium salt. Crude quaternary ammonium salt was purified by recrystallization (Marzullo, 2024).

2.2.3 Incorporation of Silver Nanoparticles and Quaternary Ammonium Salts onto Azo Dyes

Silver nanoparticle (0.5 g) was dissolved in ethanol (5 mL), and the azo dyes were separately mixed with stabilized silver nanoparticles under gentle stirring. The interaction of silver nanoparticles with the dye molecules may lead to the formation of a composite material (Atlaskina, 2021).

On the other hand, quaternary ammonium salt (0.5 g) was dissolved in ethanol (5 mL), and the azo dyes were separately mixed with stabilized quaternary ammonium salts under gentle stirring. There may be an ion pair formation since quaternary ammonium salts are cationic, and the dyes used are anionic azo dyes. The ionic interaction is important during the coupling of azo dyes and quaternary ammonium salts.

2.3. Antibacterial Activity

Different test concentrations of 1000, 500, 250, and 125 mg/mL were prepared to test the fractions on agar plates to detect the presence of antibacterial properties. All the plates were inoculated with the test bacteria by streaking the surfaces of the agar plates over the entire sterile agar, and the plates were allowed to dry for 5 min. The plates were placed in an incubator for 48 h at 38 °C, and the test concentrations were dispersed in each well after inoculation of the bacteria, and the inhibition zone was observed. Amoxicillin was used as a control in the experiment.

3. RESULTS AND DISCUSSION

3.1 Sample A (quaternary ammonium salt)

Figure 1 shows the FTIR spectrum of a quaternary ammonium salt. The absorption peak at 1476 cm⁻¹ is due to C=C stretching vibration, and at 1013 cm⁻¹ due to C=N vibration band. The absorption band at 3369 cm⁻¹ is due to -OH stretching vibration, probably from absorbed moisture. The appearance of the absorption at 1403 cm⁻¹ is due to the azo group N=N from the dye molecule.

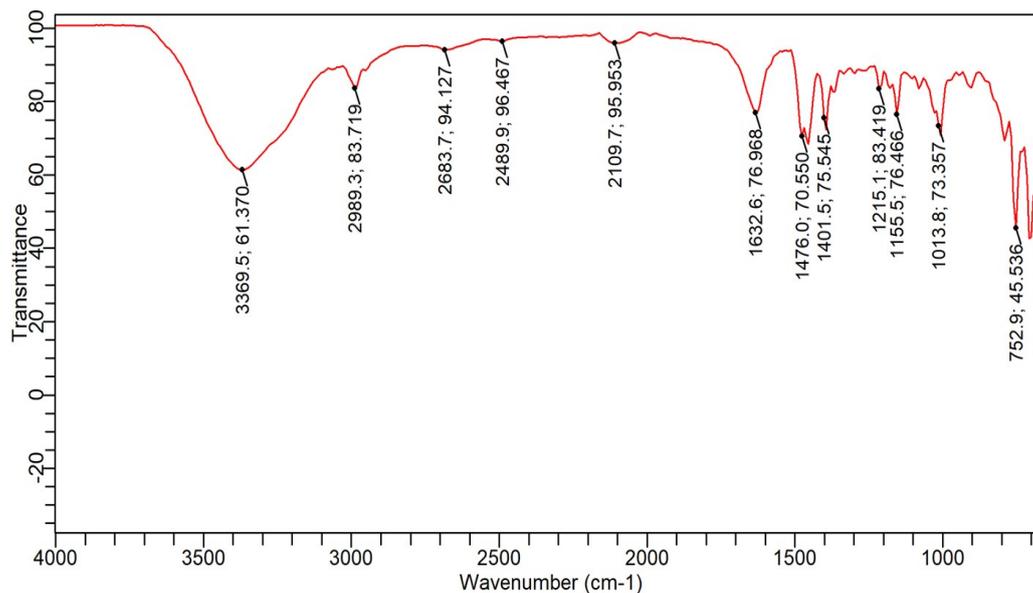


Figure 1. FTIR spectrum of quaternary ammonium salt

In **Figure 2**, the FTIR spectrum of sunset yellow + quaternary ammonium salt (A1) shows an increase in absorption band $\nu(\text{C}=\text{C})$ to 1669 cm^{-1} , suggesting an enhanced conjugation with the azo dye. The absorption band at 1408 cm^{-1} $\nu(\text{N}=\text{N})$ confirms the presence of azo functional group incorporation (Bandara, 2012). The stretching band at 3384 cm^{-1} $\nu(\text{OH})$ reflects strong hydrogen bonding with the dye molecule.

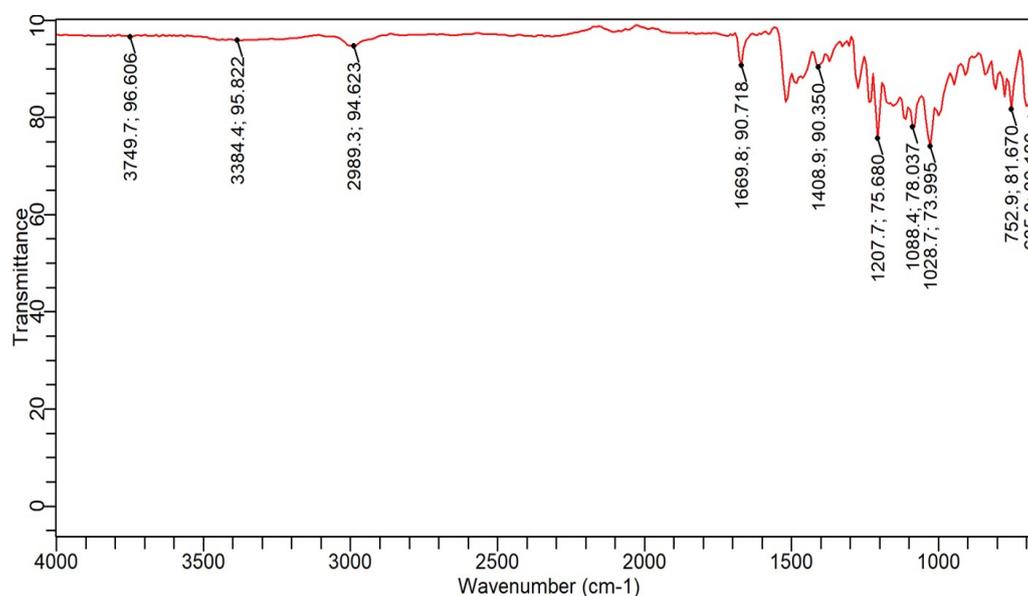


Figure 2. FTIR spectrum of sunset yellow + quaternary ammonium salt (A1)

Figure 3 shows the FTIR spectrum of tartrazine with QAS (A2). The absorption band at 1476 cm^{-1} $\nu(\text{C}=\text{C})$ is attributed to the quaternary salt (Joshi, 2012). A minor shift in the absorption band of $\nu(\text{C}-\text{N})$ to 1021 cm^{-1} suggests the interactions between the salt and the dye molecule. Similar bands observed at 1401 cm^{-1} $\nu(\text{N}=\text{N})$ and at 3384 cm^{-1} $\nu(\text{OH})$ frequencies indicate consistent structural integration.

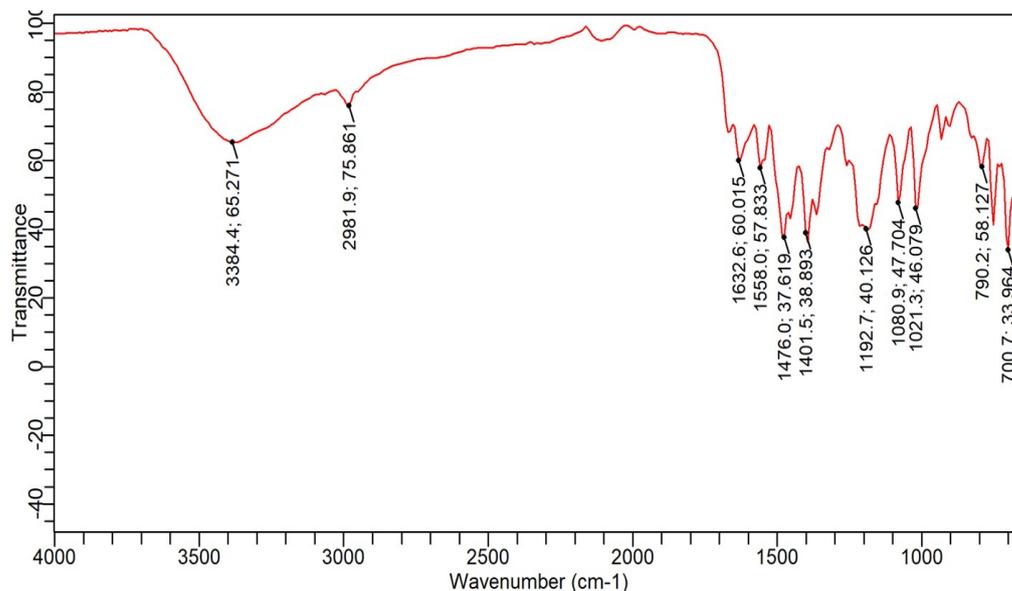


Figure 3. FTIR spectrum of tartrazine + quaternary ammonium salt (A2)

Figure 4 shows the FTIR spectrum of allura red dye with the ammonium salt (A3). An absorption band at 1461 cm^{-1} $\nu(\text{C}=\text{C})$ reflects interaction of QAS with the dye (with possible alteration of π -conjugation). Similarly, absorption bands at 1401 cm^{-1} $\nu(\text{N}=\text{N})$ and $\nu(\text{OH})$ stretching vibrations at 3384 cm^{-1} frequencies show the azo dye interactions.

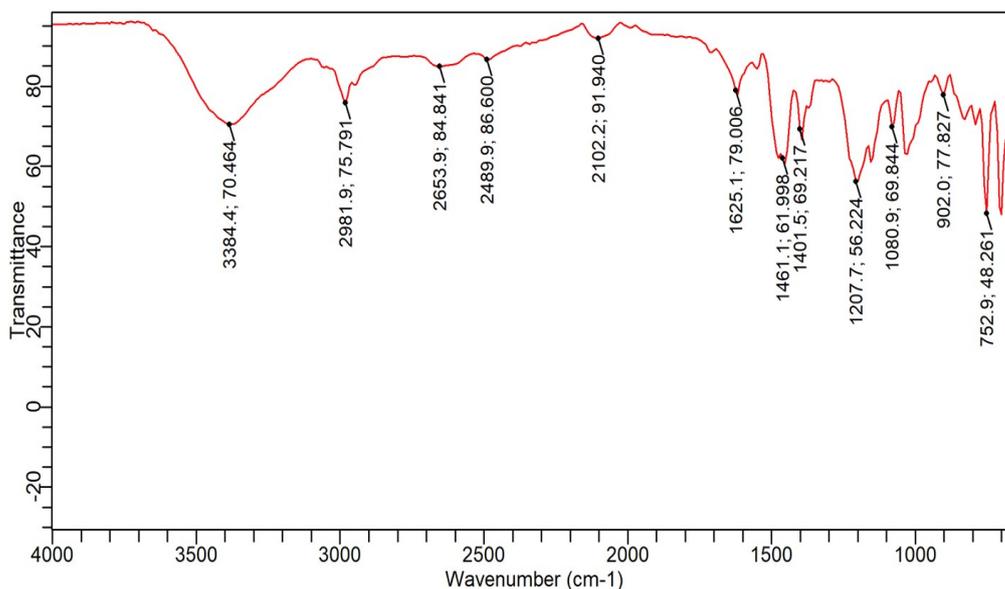


Figure 4. FTIR Spectrum of allura red + quaternary ammonium salt (A3)

The FTIR of sunset yellow dye with silver nanoparticles (B1) is shown in **Figure 5**. There is an observed shift in absorption bands at 632 cm^{-1} $\nu(\text{C}=\text{C})$ and $\nu(\text{N}=\text{N})$ at 1543 cm^{-1} . These changes confirm azo dye integration onto the nanoparticles. The $\nu(\text{OH})$ stretching band at 3548 cm^{-1} indicates hydrogen bond formation with the sunset yellow dye molecule, and this is consistent with the earlier reports on dye-nanoparticles interactions (Joshi, 2012).

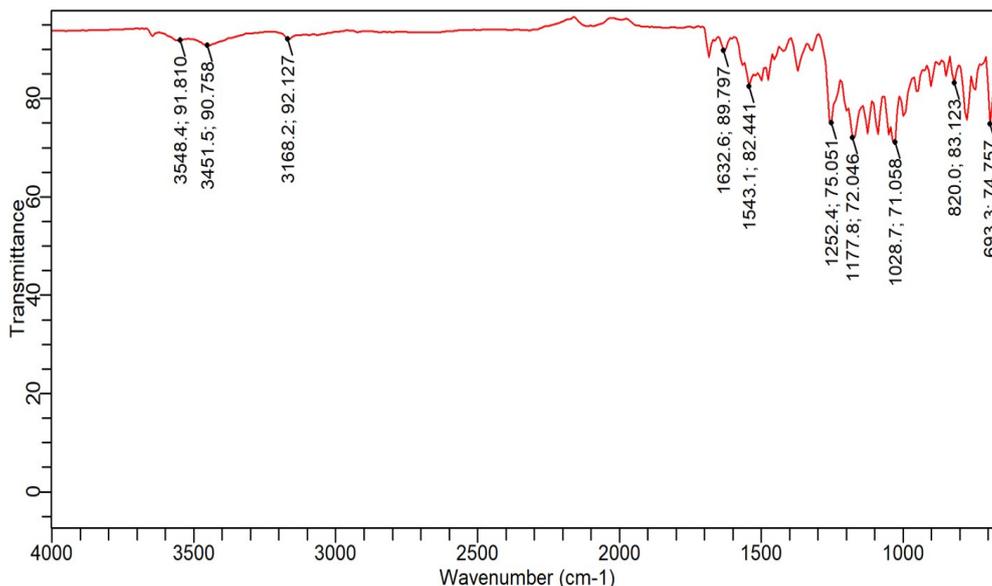


Figure 5. FTIR spectrum of sunset yellow + silver nanoparticles (B1)

The FTIR of the tartrazine dye sample with AgNPs (B2) is shown in **Figure 6**. The absorption band at 1476 cm^{-1} assigned for $\nu(\text{C}=\text{C})$, is the same as in A3. The $\nu(\text{N}=\text{N})$ at 1535 cm^{-1} confirms successful incorporation of the two components, while the absence of any stretching band for the group $\nu(\text{OH})$ shows reduced hydroxyl interactions.

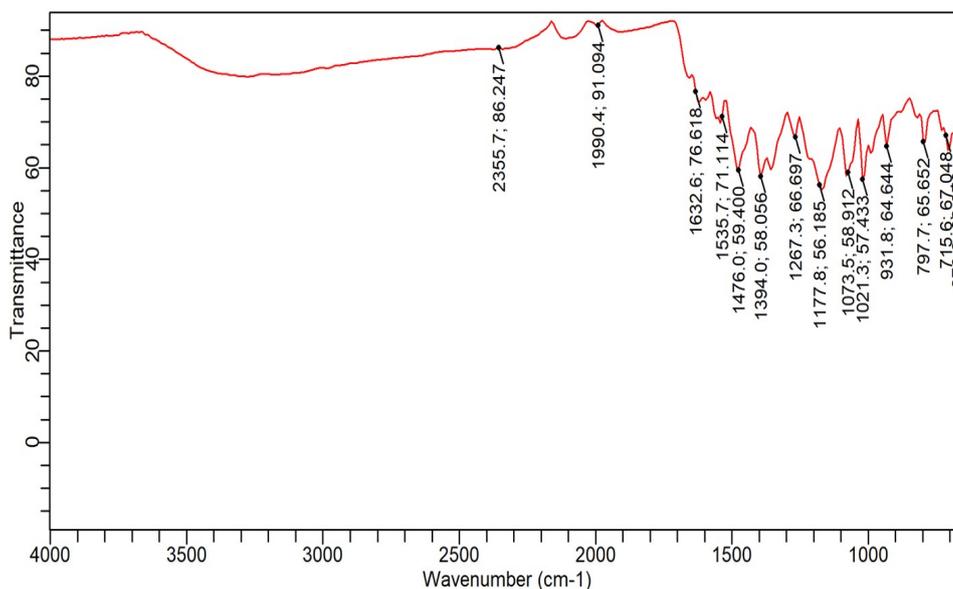


Figure 6. FTIR Spectrum of Tartrazine + Silver nanoparticles (B2)

Figure 7 shows the FTIR spectrum of allura red dye with the silver nanoparticles (B3). Absorption band at 1490 cm^{-1} $\nu(\text{C}=\text{C})$ and at 1550 cm^{-1} $\nu(\text{N}=\text{N})$ points a stronger interaction of the nanoparticles with the dye. The $\nu(\text{OH})$ stretching band at 3414 cm^{-1} and bending mode at 1326 cm^{-1} indicate vigorous hydroxyl group interaction.

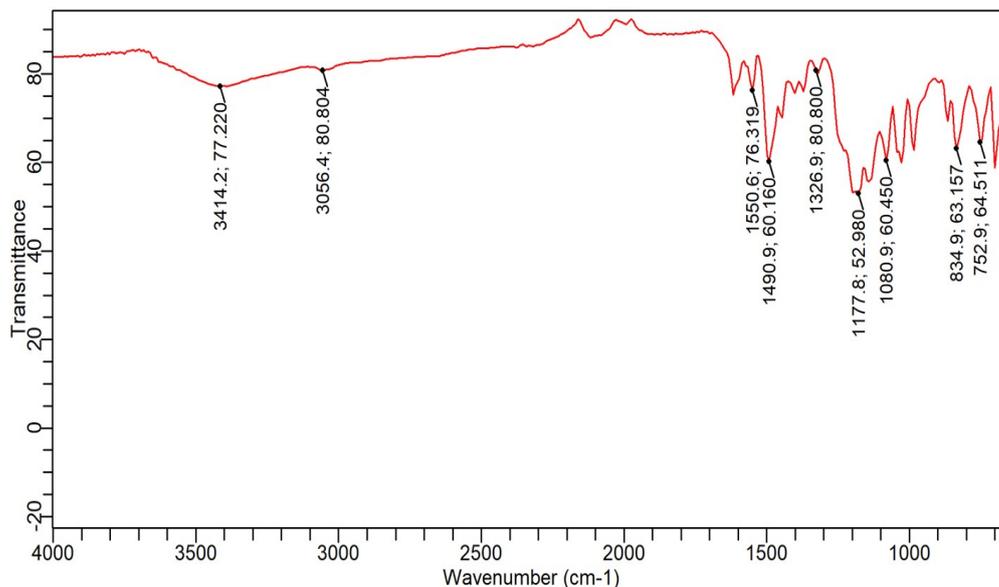


Figure 7. FTIR spectrum of tartrazine + silver nanoparticles (B3)

3.2 Antimicrobial activity of chemically modified azo dyes

Tables 1 and 2 provide data on the antimicrobial effects of different compounds and combinations on four bacterial strains: *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. These results explore the efficacy of combining quaternary ammonium salts, some selected azo dyes, and silver nanoparticles. The control group demonstrated the highest zones of inhibition across all bacterial strains and concentrations in both tables. This confirms its efficacy as a standard antimicrobial agent. The consistent trend across all strains highlights the reliability of the experimental setup and validates the comparison of other compounds (Rutala, 2004).

Sample A exhibited moderate antimicrobial activity against all bacteria; zones of inhibition reduced as the concentration decreased, indicating dose-dependent efficacy. *Staphylococcus epidermidis* showed slightly higher sensitivity compared to the other strains. Quaternary ammonium salts act by disrupting microbial membranes, leading to cell death (Maillard, 2002).

Table 1. Antimicrobial activity of the modified azo dyes with silver nanoparticles and quaternary ammonium salts.

Compounds	Concentration and zones of inhibition							
	<i>Staphylococcus aureus</i>				<i>Staphylococcus epidermidis</i>			
	Concentration (µg/mL)				Concentration (µg/mL)			
	1000	500	250	125	1000	500	250	125
Sample A	12.6	10.2	9.1	7.3	13.4	10.5	9.8	8.2
A1	10.9	9.4	8.3	7.1	12.6	11.2	7.8	ND
B1	15.1	12.3	10.7	8.9	12.9	10.4	9.8	8.1
A2	10.2	8.6	7.6	ND	10.8	9.3	8.6	ND
B2	16.1	12.6	8.7	8.3	13.6	11.5	8.8	7.3
A3	10.2	8.4	8.8	7.2	14.7	12.2	10.7	8.6
B3	11.8	10.4	9.7	8.1	10.3	8.4	7.9	ND
Control	29.6	18.3	12.9	10.8	27.9	24.1	21.7	17.2

On the other hand, incorporating the azo dyes to quaternary ammonium salts (A1, A2, and A3) showed varying levels of activity. A1 showed a moderate efficacy against *E. coli*, *Salmonella spp.*, and *Staphylococcus epidermidis*. A2 showed a similar trend as A1, with marginally reduced inhibition zones. A3 showed a higher efficacy against *S. epidermidis*, suggesting synergistic effects with certain Azo dyes. These results indicate that the chemical properties of the dyes may influence the overall antimicrobial activity.

Table 2. Effects of modified dyes on other samples of microbes

Compounds	<i>Escherichia coli</i>				<i>Salmonella spp</i>			
	Concentration				Concentration			
	($\mu\text{g/mL}$)				($\mu\text{g/mL}$)			
	1000	500	250	125	1000	500	250	125
Sample A	14.6	12.4	11.9	9.9	13.4	10.2	8.7	7.2
A1	10.9	9.4	7.8	ND	15.3	12.4	10.6	9.7
B1	9.9	8.3	ND	ND	10.7	9.5	7.4	ND
A2	11.3	9.6	8.1	7.1	10.3	9.1	7.8	ND
B2	12.3	10.7	9.4	7.6	12.6	10.8	9.2	7.5
A3	14.9	10.3	7.8	ND	13.7	11.3	9.9	8.1
B3	13.6	10.1	9.2	ND	10.8	8.2	ND	ND
Control	29.4	21.6	18.3	16.9	26.1	22.4	20.1	17.3

Sample A : Quaternary ammonium salt
 Control : Amoxicillin
 A1 : Sunset yellow + Quaternary ammonium salt
 A2 : Tartrazine + Quaternary ammonium salt
 A3 : Allura red + Quaternary ammonium salt
 B1 : Sunset yellow + Silver nanoparticles
 B2 : Tartrazine + Silver nanoparticles
 B3 : Allura red + Silver nanoparticles salt
 ND : Not detected

3.3 Azo dyes with silver nanoparticles (B samples)

Silver nanoparticles exhibited significant antimicrobial activity, particularly when combined with Azo dyes (B1, B2, and B3). Among the samples, B1 (sunset yellow + silver nanoparticles) shows higher activity against *E. coli* and *S. aureus*, likely due to the broad-spectrum properties of silver nanoparticles. B2 (tartrazine + silver nanoparticles) outperformed other combinations in its activity against *Staphylococcus aureus*. B3 (allura red + silver nanoparticles): showed moderate efficacy against *S. epidermidis* and reduced inhibition at lower concentrations. Silver nanoparticles are known to induce oxidative stress in bacteria, disrupt DNA synthesis, and damage cell membranes, thereby enhancing antimicrobial activity when used in combinations (Rai, 2009).

3.4 Bacterial strain susceptibility

There were variations in susceptibility between bacterial strains: The gram-negative bacteria (*E. coli* and *Salmonella spp.*) are slightly more resistant to the combination of the dye samples with the nanoparticles compared to silver nanoparticles only. While the gram-positive bacteria (*S. aureus* and *S. epidermidis*): Showed greater sensitivity to all treatments, particularly silver nanoparticle combinations (B1 and B2). This difference could be attributed to the structural differences in gram-negative and gram-positive cell walls (Rai, 2009).

Allura Red combinations (A3, B3) showed notable activity against *Staphylococcus epidermidis*. This indicates the potential application for dye-based formulations in targeting specific gram-positive pathogens, particularly in skin or healthcare-associated infections (Galdiero, 2015).

These antimicrobial materials present an interesting tool in healthcare environments. Moreover, it can be ventured that due to multiple mechanisms of antimicrobial actions associated with each component in the samples, bacterial or antimicrobial resistance is highly unlikely. Future build-up research in the area, such as antifungal properties and pH control, could confirm the versatile potential of the samples in treating infectious diseases.

4. CONCLUSION

The silver nanoparticles derived from *Azadirachta indica* and quaternary ammonium salts with their incorporated azo dyes were synthesized and characterized by FTIR. The antimicrobial activity results indicated that the incorporated azo dyes were found to be active antimicrobial agents. Incorporation of azo dyes into silver nanoparticles and quaternary ammonium salt enhanced their antimicrobial activity due to improved lipophilicity and penetration into the bacterial membrane.

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Conflict of interest: The authors declared no conflict of interest.

Ethical Approval: Not applicable

REFERENCES

- Acierno, D., Amendola, E., Bugatti, V., Concilio, S., Giorgini, L., Iannelli, P., Piotto, SP. Synthesis and characterization of segmented liquid crystalline polymers with the azo group in the main chain. *Macromolecules*. 2004;37:6418–6423.
- Atlaskina, M.E., Atlaskin, A.A., Kazarina, O.V., Petukhov, A.N., Zarubin, D.M., Nyuchev, A.V., Vorotyntsev, A.V., Vorotyntsev, I.V. Synthesis and Comprehensive Study of Quaternary-Ammonium-Based Sorbents for Natural Gas Sweetening. *Environments*. 2021; 8(12):134. <https://doi.org/10.3390/environments8120134>
- Ball, A., Bartlett, J., Craig, W., Drusano, G., Felmingham, D., Garau, J., Klugman, K., Low, D., Mandell, L., Rubinstein, E. Future trends in antimicrobial chemotherapy: Expert opinion on the 43rd ICAAC. *J. Chemother.* 2004;16:419–436.
- Banaszak-Leonard, E., Smith, AB., Johnson, CD., Martinez, RT. Synthesis, Characterization and application of Azo dyes: A review of modern chemical approaches. *J. applied chem*, 2021;15(3):245-267
- Bandara, A., Patel, S., Desai, R. Advances in synthetic dye chemistry. 2012;8(2):112-130.
- Concilio, S., Sessa, L., Petrone, AM., Porta, A. Diana, R., Iannelli, P., Piotto, S. Structure modification of an active azo-compound as a route to new antimicrobial compounds. *Molecules*. 2017;22:875.
- Dantas, D., Ribeiro, AI, Carvalho, F., Gil-Martins, E., Silva, R., Remião, F., Zille, A. Cerqueira, F., Pinto, E., Dias. AM. Redshifted and pH-Responsive Imidazole-based Azo Dyes with Potent Antimicrobial Activity. *Chem. Commun.* 2023;59:2791–2794.
- Galdiero, S., Falanga, A. Vitiello, M., Cantisani, M., Marra, V. Silver nanoparticles as potential antibacterial agents. *Molecules*, 2015;20(5),8856-8874
- Jerca, F.A., Jerca, V.V. Hoogenboom, R. Advances and opportunities in the exciting world of azobenzenes. *Nat. Rev. Chem.* 2022;6:51–69.
- Joshi, D., Mitchell, M., Bruce, D., Lough, A., Yan, H. Synthesis of cyclic azobenzene analogues. *Tetrahedron*. 2012;68 8670-8676. 10.1016/j.tet.2012.06.007.
- Maillard, J-Y. Bacterial target sites for biocide action. *Journal of Applied Microbiology*. 2002;92(S1),16s-27s.
- Marzullo, P., Gruttadauria, M., D'Anna F. Quaternary ammonium salts-based materials: A Review on environmental toxicity, anti-fouling mechanisms and applications in marine and water treatment industries. *Biomolecules*. 2024;7;14(8):957.doi:10.3390/biom14080957.
- Pandiyani, R., Rajagopal, R. Fabrication and characterisation of silver and lead nanoparticles and application upon synthetic azo dye degradation. *Biomass Conv. Bioref.* (2023). <https://doi.org/10.1007/s13399-023-04073-4>
- Rai, M., Yadav, A., Gade, A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv.* 2009;27(1):76-83. doi: 10.1016/j.biotechadv.2008.09.002. Epub 2008 Sep 30. PMID: 18854209.
- Rutala, W.A., Weber, D.J. Disinfection and sterilization in health care facilities: what clinicians need to know. *Clin Infect Dis*. 2004 Sep 1;39(5):702-9. doi: 10.1086/423182.
- Singh, D.K., Luqman, S., Mathur, A.K. Lawsonia inermis L.—a commercially important primaevial dying and medicinal plant with diverse pharmacological activity: A review. *Industrial Crops and Products*. 2015; 65:269-286

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