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Synthesis and Spectroscopic Characterization of Azo Dyes Derived From 4-(Dimethylamino) Benzaldehyde: Solvatochromic and Antimicrobial Studies

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ABSTRACT

The discharge of synthetic dyes and auxiliary biocides from textile finishing operations constitutes a significant source of aquatic pollution, driving demand for multifunctional colorants that consolidate processing steps and reduce chemical effluent loads. This study reports the aqueous-based synthesis of two azo dyes via diazotisation of 1-aminoanthraquinone and 4-chloroaniline followed by coupling with p-dimethylaminobenzaldehyde, yielding an orange product (Dye 1, 77.0%) and a red product (Dye 2, 87.5%). UV–Visible spectroscopy revealed a positive solvatochromic shift of 11 nm for Dye 1 (λ_max = 469 nm in acetone → 480 nm in DMSO), indicative of intramolecular charge-transfer character with potential utility in environmental polarity sensing, whereas Dye 2 exhibited solvent-independent absorption at 471 nm. FT-IR analysis confirmed the presence of azo, carbonyl, and amino functionalities in both compounds. Antimicrobial evaluation against Staphylococcus aureus, Escherichia coli, and Candida albicans demonstrated that Dye 1 possessed superior activity (MIC = 12.5, 12.5, and 50 mg mL^{-1}, respectively) compared with Dye 2 (MIC = 25, 50, and 100 mg mL^{-1}). The integration of colouring and antimicrobial properties within a single molecular framework offers a viable strategy for eliminating separate biocidal finishing steps, thereby reducing the discharge of persistent and bioaccumulative antimicrobial agents into aquatic ecosystems. Future work should prioritise biodegradability assessment, aquatic ecotoxicity profiling, and life-cycle analysis to establish the full environmental viability of these multifunctional colorants.

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INTRODUCTION

The global textile and leather industries collectively consume an estimated 10,000–15,000 different synthetic dyes and pigments, with annual production exceeding 7 × 10^5 tonnes [1]. Approximately 10–15% of these colorants are lost during dyeing and finishing operations and subsequently discharged into wastewater streams, contributing to severe contamination of surface and groundwater resources in major textile-producing regions [1,2]. The release of synthetic dyes into aquatic ecosystems reduces light penetration, disrupts photosynthetic activity, depletes dissolved oxygen, and introduces recalcitrant organic pollutants that resist conventional biological degradation [1,3]. In developing economies, where wastewater treatment infrastructure is often inadequate, the environmental and public health consequences of dye-laden effluents are particularly acute.

Among the various classes of synthetic colorants, azo dyes characterised by one or more azo linkages (–N=N–) connecting aromatic or heteroaromatic moieties account for

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approximately 60–70% of all commercial dyes. While their structural versatility, synthetic accessibility, and broad colour gamut make them indispensable to the textile, leather, paper, and food industries, their environmental persistence and the potential release of carcinogenic aromatic amines upon reductive cleavage have raised significant ecological and regulatory concerns [1,4]. Consequently, there is a pressing need to develop azo colorants that combine desirable tinctorial properties with reduced environmental impact, enhanced biodegradability, and multifunctional performance characteristics that minimise the overall chemical load of industrial processing.

A promising approach to reducing the environmental footprint of textile and leather finishing lies in the design of multifunctional dyes single molecular entities that simultaneously provide coloration and additional performance attributes such as antimicrobial, UV-protective, or flame-retardant properties [2,5]. Conventional finishing protocols typically require separate application steps for dyeing and biocidal treatment, each involving substantial quantities of water, energy, and auxiliary chemicals. By integrating antimicrobial functionality directly into the dye chromophore, it becomes possible to eliminate or reduce the need for separate biocidal finishing baths, thereby decreasing water consumption, energy expenditure, and the volume of hazardous chemical effluent generated during processing [2,5].

Recent investigations have demonstrated the feasibility of this approach. Agho et al. [6] reported the synthesis and biological evaluation of isocyano-functionalised azo dyes with notable antimicrobial properties, while Obadahun et al. [7] demonstrated the potential of thiadiazole-containing organic dyes as antifungal agents. These studies underscore the growing recognition that molecular design can be leveraged to develop colorants that serve dual environmental and functional roles, reducing the reliance on heavy-metal-based biocides and formaldehyde-releasing preservatives that pose significant ecological risks.

In this context, *p*-dimethylaminobenzaldehyde represents an attractive building block for the development of environmentally conscious azo colorants. Its molecular framework incorporates a strongly electron-donating dimethylamino group and an aldehyde functionality, both of which can influence the electronic distribution, solvatochromic response, and biological activity of the resulting dye. The selection of 1-aminoanthraquinone and 4-chloroaniline as diazo components was guided by the desire to compare an extended polycyclic aromatic system with a simpler halogenated phenyl framework, thereby generating structural diversity for structure–property and structure–activity investigations.

The aqueous-based synthetic protocol employed in this study aligns with several principles of green chemistry [8], including the use of water as the primary reaction medium, moderate reaction temperatures, and the avoidance of chlorinated organic solvents during the coupling step. While the overall process cannot yet be classified as fully "green" owing to the use of mineral acids and the generation of inorganic salt by-products, it represents a step toward more sustainable dye synthesis compared to protocols that rely extensively on volatile organic solvents.

The optical behaviour of organic dyes is influenced not only by their intrinsic molecular structures but also by the surrounding solvent environment a phenomenon known as solvatochromism [9,10]. Changes in solvent polarity, polarizability, and hydrogen-bonding capacity can stabilise the ground and excited electronic states to different extents, resulting in shifts in the wavelength of maximum absorption (λ_max). From an environmental perspective, solvatochromic dyes are of considerable interest as molecular probes for monitoring solvent polarity changes in industrial effluents, detecting micro-environmental variations in contaminated water systems, and serving as colorimetric indicators in environmental remediation processes [9,10].

Furthermore, the antimicrobial finishing of textiles and leather products conventionally relies on biocidal agents such as triclosan, silver

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nanoparticles, quaternary ammonium compounds, and organotin derivatives many of which are environmentally persistent, bioaccumulative, and toxic to aquatic organisms [11]. The development of dyes with intrinsic antimicrobial activity offers a pathway toward reducing the discharge of these hazardous biocides into the environment, provided that the dyes themselves demonstrate acceptable ecotoxicological profiles.

Accordingly, this study focuses on the synthesis of azo colorants using *p*-dimethylaminobenzaldehyde as the coupling component and 1-aminoanthraquinone and 4-chloroaniline as the respective diazo components, with the following environmentally oriented objectives: (i) to synthesise the target azo dyes using an aqueous-based protocol that minimises the use of hazardous organic solvents; (ii) to characterise the prepared compounds through physical measurements, FT-IR spectroscopy, and UV–Vis spectroscopy; (iii) to investigate the solvatochromic behaviour of the dyes in solvents of differing polarity (acetone and DMSO), with a view to assessing their potential as environmental polarity probes; (iv) to evaluate the antimicrobial activities of the synthesised dyes against selected bacterial and fungal organisms as a preliminary assessment of their potential to replace conventional biocidal finishing agents; and (v) to discuss the environmental implications of the findings and identify directions for future research on the ecotoxicological and life-cycle performance of the prepared compounds.

MATERIALS AND METHODS

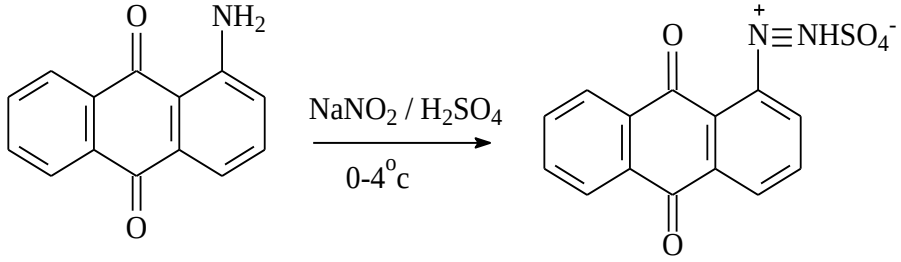
Materials

4-chloroaline, p-aminoanthraquinone, P-dimethylaminobenzaldehyde, Ethanol, distilled water, sodium nitrate, Acetone, hydrochloric acid, Acetic acid, Methanol, Sodium chloride, textile material (Nylon.6,6),Thermometer, pH meter, heating mantle, magnetic stirrer, electronic balance, Gallenkamp Melting point apparatus, Agilent CARRY 300 UV -visible spectrometer, Agilent CARY 630, FT-IR, Electric oven, beakers, round bottom flask, volumetric flask, Buchner flask, hot plate, Steam bath, sample bottle.

Methods

Procedure for Diazotization

In a 250, the amine (10.50 g,0.05 mole) was dissolved in hot water (100 cm^3) containing anhydrous sodium carbonate (2.65 g, 0.025 mole). The solution was filtered and concentrated hydrochloric acid (10.5 cm^3) was added to the solution, followed by ice (60 g). The mixture was stirred at (0-5 ^oC). A solution of sodium nitrite (53.7 g) and water (10.0 cm^3) was added dropwise for over 15 minutes. The mixture was thereafter stirred for further 15 minutes to give a suspension of diazonium salt. The same procedure was repeated using 4-chloroaniline.



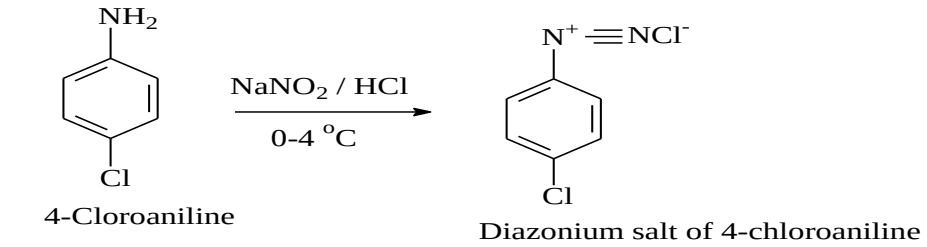
1-aminoanthraquinone

Diazonium salt of 1-aminoanthraquinone

Scheme 3.1: Diazotization of 1-amino anthraquinone

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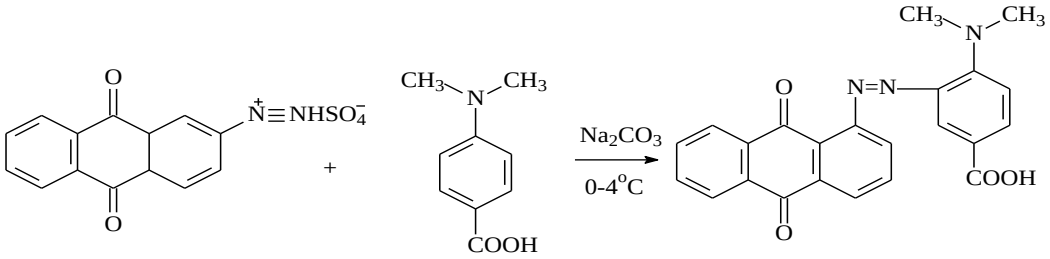
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Scheme 3.2: Diazotization of 4-chloroaniline

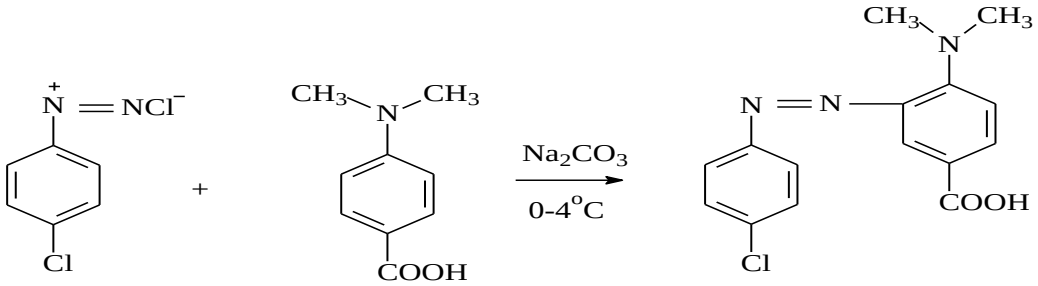
Procedure for Coupling

In a 250cm3 beaker, the coupling compound (7.2 g, 0.05 moles) was dissolved in 10% cold solution of sodium hydroxide (40 cm^3). The solution was cooled to 5 °C in an ice bath and then poured slowly with stirring into the diazonium salt solution. The solution was stirred very well for 10 minutes and the mixture was heated until all the solid dissolved. Sodium chloride (20 g) was added to decrease the solubility further. The solution was warmed until the sodium chloride dissolved completely. The solution was thereafter allowed to stand for 1 hour in the air and cooled in an ice-bath until crystallization is completed. The product was collected in a Buchner and washed with little saturated salt solution and dried at 60 °C.



Dye 1

Scheme 3.3: Coupling reaction of 1-aminoanthraquinone with P-dimethylaminobenzaldehyde



Dye 2

Scheme 3.4: Coupling Reaction of the Diazonium salt of 4-Chloroaniline with P-dimethylaminobenzaldehyde

Purification of the Dye

The crude dyes were purified by recrystallisation from ethanol (30–35 cm^3). The dye was dissolved in hot ethanol, filtered while hot to remove insoluble impurities, and the filtrate was allowed to cool slowly in an ice bath. The resulting crystals were collected by filtration, washed with cold ethanol, and dried in an oven at 50 °C. The

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use of ethanol a low-toxicity, biodegradable solvent for purification aligns with green chemistry principles favouring safer solvents and auxiliaries [8].

Characterization of the Dyes

Melting point Determination

The melting point of the dyes was determined using Gallenkamp melting apparatus.

Determination of Ultra violet-visible spectroscopy of the dyes

The visible absorption spectra of the dyes were recorded on an Agilent CARY 300UV-Visible spectrometer from dye solutions in DMSO and ethanol at a concentration of 1.5×10^{-5} mol/l as described by [12].

Infrared Spectroscopy

FT-IR spectra were recorded on an Agilent Cary 630 FT-IR spectrophotometer in the range 4000–650 cm^{-1} using the attenuated total reflectance (ATR) technique. Spectral assignments were made by comparison with literature values for analogous azo dye structures [1,4,6].

Evaluation of the Antimicrobial Activities of the Synthesised Dyes

Test Organisms

The antimicrobial activities of the synthesised dyes were evaluated against selected clinical isolates of bacteria and fungi obtained from the Department of Microbiology, Ahmadu Bello University, Samaru, Zaria, Nigeria. The test organisms included *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

Culture Media

The culture media used for the antimicrobial investigation included Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), Nutrient Agar (NA), and Mueller Hinton Broth (MHB). The media were prepared according to the manufacturers' instructions and sterilised by autoclaving at 121 °C for 15 minutes.

Determination of Zones of Inhibition Using the Agar Well Diffusion Method

The antimicrobial activities of the synthesised dyes were initially evaluated using the agar well diffusion method. Standardised inocula of the bacterial and fungal isolates were separately spread on the appropriate agar plates using sterile swab sticks. Mueller Hinton Agar was used for the bacterial isolates, while Potato Dextrose Agar was used for the fungal isolate. Four wells were aseptically punched into each inoculated agar plate using a sterile cork borer. The wells were labelled according to the concentrations of the dye solutions, which were 200, 100, 50, and 25 mg/mL, respectively. Each well was filled with 0.2 mL of the appropriate dye solution.

The inoculated plates were allowed to stand for approximately one hour to facilitate diffusion of the dye solutions into the agar medium. The bacterial plates were incubated at 37 °C for 24 hours, while the fungal plates were incubated at approximately 26–27 °C for about three days. After incubation, the plates were examined for clear zones around the wells, indicating inhibition of microbial growth. The diameters of the inhibition zones were measured in millimetres using a calibrated ruler. Sparfloxacin at 5 µg/mL was used as the positive control for the bacterial isolates, while fluconazole at 5 µg/mL was used as the positive control for the fungal isolate.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the synthesised dyes were determined using the broth dilution method. Mueller Hinton Broth was used as the diluent for the preparation of the dye concentrations. Serial dilutions were prepared to obtain concentrations of 100, 50, 25, and 12.5 mg/mL. The test organisms were introduced into the tubes containing the different dye concentrations and broth. The inoculated tubes were incubated under the specified conditions. Following incubation, the tubes were examined for visible growth using turbidity as the indicator. The MIC was defined as the lowest concentration of

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the dye at which no visible microbial growth was observed. The MIC results were recorded for each dye against the respective test organisms.

Determination of Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC)

The minimum bactericidal and minimum fungicidal concentrations were determined by subculturing samples from the tubes that showed no visible growth during the MIC investigation. A sterile wire loop was used to transfer a portion of the contents of the clear MIC tubes onto appropriate sterile agar plates. The bacterial plates were incubated at 37 °C for 24 hours, while the fungal plates were incubated under the conditions used for the fungal organism. After incubation, the plates were examined for the presence or absence of microbial growth. The lowest concentration that prevented subsequent growth on the agar medium was recorded as the MBC for the bacterial isolates or the MFC for the fungal isolate.

RESULTS AND DISCUSSION

Physical Properties and Synthetic Yield

The physical characteristics of the synthesised dyes are summarised in Table 1.

Table 1. Physical properties of the synthesised azo dyes.

S/N	Samples	Melting Point (°C)	Percentage Yield	Colour
1	Dye 1	163-178	77.0	Orange
2	Dye 2	158-162	87.5	Red

The synthesised dyes exhibited distinct colours orange for Dye 1 and red for Dye 2 consistent with the differing extents of π-conjugation provided by the anthraquinone and chlorophenyl frameworks, respectively. Dye 2 afforded a higher percentage yield (87.5%) compared with Dye 1 (77.0%), indicating more efficient product recovery under the reported aqueous conditions. From a sustainability perspective, higher synthetic yields translate directly into reduced raw material consumption and lower waste generation per unit of product, aligning with the green chemistry principle of atom economy [8]. The broader melting point range of Dye 1 (15 °C span) may suggest the presence of residual impurities or incomplete purification, which could influence the dye's environmental fate and biodegradation behaviour. Further purification optimisation potentially employing column chromatography with bio-derived eluents may be warranted to improve product consistency and reduce the environmental burden of reprocessing.

UV–Visible Absorption

The electronic absorption data for the synthesised dyes in solvents of differing polarity are presented in Table 2.

Table 2: Wavelengths of maximum absorption of the dyes

Dye	λmax in DMSO (nm)	λmax in acetone (nm)	Difference (nm)
Dye 1	480	469	+11
Dye 2	471	471	0

Dye 1 exhibited an 11 nm bathochromic (red) shift in DMSO relative to acetone, indicating that the more polar and strongly hydrogen-bond-accepting DMSO preferentially stabilised the excited electronic state of this dye. This positive solvatochromic response is consistent with intramolecular charge-transfer (ICT) character in the electronic transition, where the excited state possesses a larger dipole moment than the ground state and is therefore more effectively stabilised by polar solvents [9,10]. The dimethylamino donor and the azo–carbonyl acceptor system in Dye **1** create a push–pull chromophore that is particularly responsive to solvent polarity changes.

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From an environmental monitoring perspective, the solvatochromic sensitivity of Dye 1 is noteworthy. Compounds that exhibit measurable spectral shifts in response to changes in solvent polarity have potential applications as colorimetric probes for detecting organic pollutant gradients in wastewater, monitoring solvent composition in industrial effluents, and assessing the polarity of micro-environments in contaminated soil and sediment matrices [9,10].

The 11 nm shift observed for Dye 1, while modest, is readily detectable with standard UV–Vis instrumentation and could be amplified through structural optimisation in future studies for example, by introducing stronger electron-withdrawing groups to enhance the ICT character. In contrast, Dye 2 showed no observable solvatochromic shift (λ_max = 471 nm in both solvents), suggesting that its electronic transition is less sensitive to the surrounding solvent environment. This solvent-independent absorption behaviour may be advantageous in applications requiring colour stability across varying environmental conditions, such as outdoor textile applications exposed to diverse climatic and moisture regimes, or in dye formulations intended for use in regions with variable water quality.

FT-IR Spectral Analysis

The FT-IR spectral data for the synthesised dyes are summarised in Table 3.

Table 3. Characteristic FT-IR vibrational frequencies (cm^{-1}) of the synthesised azo dyes.

Dye	O–H/N–H str.	C–H str.	C=C str.	C=O str.	–N=N– str.
1	3410.5	3019	1658.4	1625	1580.4
2	3347.1	3194, 2944	1621.4	1664	1558.0

The FT-IR spectra confirmed the presence of functional groups consistent with the proposed azo dye structures. The broad absorption bands in the 3347–3411 cm^{-1} region are attributable to O–H and/or N–H stretching vibrations, while the bands near 1558–1580 cm^{-1} are tentatively assigned to the azo (–N=N–) stretching mode. The carbonyl stretching vibrations observed at 1625 and 1664 cm^{-1} are consistent with the aldehyde and/or anthraquinone carbonyl functionalities present in the molecular frameworks. The C–H stretching bands in the 2944–3194 cm^{-1} region confirm the presence of both aromatic and aliphatic C–H bonds.

From an environmental degradation standpoint, the identification of these functional groups is significant because the susceptibility of azo dyes to biodegradation is strongly influenced by the nature and accessibility of their functional groups [1]. The presence of electron-donating dimethylamino substituents and carbonyl groups may influence the redox potential of the azo bond and, consequently, the dye's susceptibility to reductive cleavage by anaerobic microorganisms in wastewater treatment systems and contaminated sediments. However, definitive conclusions regarding biodegradability cannot be drawn from FT-IR data alone, and dedicated biodegradation studies (e.g., OECD 301 series) are strongly recommended. It should be emphasised that individual band assignments are tentative and should not be treated as conclusive structural proof without complementary analytical techniques such as ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry (HRMS).

Antimicrobial Activity

Agar Well Diffusion Assay

The zones of inhibition produced by the synthesised dyes and the standard antimicrobial agents are presented in Table 4.

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Table 4: Zone of Inhibition (mm) of the Synthesized Dyes Against the Test Organisms

Test Organism	Dye G	Dye H	Sparflo	Fluco
Staphylococcus aureus	33	24	37	-
Escherichia coli	28	21	35	-
Candida albicans	22	13	-	32

Key: Sparflo: Sparfloxacin = 5µg/ml (Positive control drug for bacteria), Fluco: Fluconazole = 5µg/ml (positive control drug for fungi)

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the synthesized dyes was determined to establish the lowest concentration capable of inhibiting visible microbial growth. The MIC values obtained against the test organisms are presented in Table 6.

Table 6: Minimum Inhibitory Concentration (MIC) in mg/ml

TEST MICROBES	CONCENTRATION OF DYE (In mg/ml)							
	Dye 1				Dye 2			
	100	50	25	12.5	100	50	25	12.5
Staphylococcus aureus	-	-	-	MIC	-	-	MIC	
Escherichia coli	-	-	-	MIC	-	MIC	-	-
Candida albicans	-	MIC	-	-	MIC	-	-	-

Keys: - =Absence of turbidity, + = presence of turbidity; MIC: Minimum Inhibitory Concentration

Both synthesised dyes exhibited appreciable antimicrobial activity against all three test organisms. Dye **1** produced the largest inhibition zones (33, 28, and 22 mm against *S. aureus*, *E. coli*, and *C. albicans*, respectively), while Dye **2** showed moderate activity (24, 21, and 13 mm). Although the inhibition zones were smaller than those produced by the standard antimicrobial agents sparfloxacin and fluconazole, the observed activities are significant in the context of multifunctional dye development.

The environmental relevance of these findings lies in the potential of antimicrobial dyes to reduce the chemical load of textile and leather finishing processes. Conventional antimicrobial finishing typically involves the application of separate biocidal agents—many of which are environmentally persistent, bioaccumulative, and toxic to aquatic life—after the dyeing step [11]. If the dye itself provides adequate antimicrobial protection, the need for these auxiliary biocides can be reduced or eliminated, resulting in lower concentrations of hazardous substances in finishing effluents. The superior activity of Dye **1** against all three organisms suggests that the anthraquinone-based structural framework may confer enhanced biological activity, possibly through increased lipophilicity and membrane interaction, although this hypothesis requires further investigation.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MBC and MFC values are presented in Table 6.

Table 6. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) in mg/ml

TEST MICROBES	CONCENTRATION OF DYE (In mg/ml)							
	Dye 1				Dye 2			
	100	50	25	12.5	100	50	25	12.5

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TEST MICROBES	CONCENTRATION OF DYE (In mg/ml)							
	Dye 1				Dye 2			
Staphylococcus aureus	-	-	-	MBC	-	MBC	+	+
Escherichia coli	-	-	MBC	+	-	MBC	+	+
Candida albicans	-	MFC	+	+	MFC	+	+	+

Keys: - = Absent of Growth; + = Present of Growth; MBC = Minimum Bactericidal Concentration, MBF = Minimum Fungicidal Concentration

The MIC and MBC/MFC data corroborate the agar diffusion results, confirming that Dye 1 exhibits stronger antimicrobial potency than Dye 2 across all test organisms. The relatively high MIC values (12.5–100 mg mL^{-1}) indicate that the dyes are moderately active rather than highly potent antimicrobial agents. From an environmental standpoint, this moderate activity level may be advantageous: excessively potent antimicrobial agents are more likely to exert selective pressure on environmental microbial communities and contribute to the development of antimicrobial resistance when released into wastewater [11]. A dye with moderate, broad-spectrum antimicrobial activity may provide sufficient protection against biodeterioration of finished products without posing an undue ecological risk, provided that its environmental degradation products are non-toxic.

CONCLUSIONS

Two azo dyes were synthesised from *p*-dimethylaminobenzaldehyde via an aqueous-based protocol, achieving yields of 77.0% (Dye 1, orange) and 87.5% (Dye 2, red). Spectroscopic characterisation by UV–Vis and FT-IR confirmed the structural features of the prepared compounds. Dye 1 exhibited an 11 nm positive solvatochromic shift between acetone and DMSO, indicating potential utility as an environmental polarity probe, while Dye 2 displayed solvent-independent absorption. Antimicrobial evaluation revealed that Dye 1 possessed superior inhibitory activity against *S. aureus* (MIC = 12.5 mg mL^{-1}), *E. coli* (MIC = 12.5 mg mL^{-1}), and *C. albicans* (MIC = 50 mg mL^{-1}) compared with Dye 2.

The integration of colouring and antimicrobial functionalities within a single molecular framework offers a promising strategy for reducing the chemical burden of textile and leather finishing processes, thereby contributing to the broader goal of sustainable industrial manufacturing. However, the environmental viability of these compounds can only be fully established through rigorous biodegradability testing, ecotoxicological evaluation, and life-cycle analysis. Future research should prioritise these assessments alongside structural optimisation to enhance both the functional performance and the environmental compatibility of azo-based multifunctional colorants.

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