Antimicrobial Activities, Absorption Characteristics and Tautomeric Structures of o,o′-Hydroxyazo Dyes Containing an Acryloyloxy Group and Their Chromium Complexes

Akriloil Grup İçeren o,o′-Hidroksiazo Boyar Maddelerinin ve Komplekslerinin Tautomerik Yapıları, Absorbsiyon Karakteristikleri ve Antimikrobiyal Aktiviteleri

Research Article

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ABSTRACT

The UV-VIS behavior of (E)-1-((2-hydroxy-5-methylphenyl)diazenyl)naphthalene-2,7-diol and the acrylate derivatives and their chromium complexes were determined in different solvents, pH and temperature. The dyes were found to exist in azo and hydrazone tautomeric equilibrium in solution. And also, the o,o′-dihydroxyazo dyes and chromium complexes were tested for antimicrobial activity against *Enterococcus faecalis, Staphylococcus aureus*, *Pseudomonas aeroginosa, Escherichia coli* and *Candida albicans*. Dye I generally affected all microorganisms, while compounds II, III and IV were more effective against *P. aeroginosa* and *E. coli*. Because dye I has an antimicrobial effect on both bacteria and fungus it finds good use in various applications such as textile fiber dyeing, and biomedical studies. Significantly, this dye could also lead to the development of new antifungal drugs.

Key Words

o,o′-dihydroxyazo, Antimicrobial activity, Azo-ester dyes, Chromium complexes

ÖZET

E-1-((2-hydroxy-5-methylphenyl)diazenyl)naphthalene-2,7-diol'ün ve akrilat türevlerinin ve onların krom komplekslerinin farklı çözücüler, pH ve sıcaklıklardaki UV-VIS davranışları tespit edildi. Çözücü içinde boyalar azo ve hidroazo tautomeric dengede bulundu. Aynı zamanda o,o′-dihydroksi azo boyaları ve krom komplekleri Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli ve Candida albicans antimikrobiyal aktivite açısından test edildi. Boya I genellikle tüm mikroorganizmalar üzerine etkili iken, II, III ve IV' cü bileşikler P. aeroginosa ve E. coli üzerine daha fazla etkilidir. Boya I hem bakteriler hem de mayalar üzerinde antimikrobiyal etkiye sahip olduğu için, tekstilde lif boyaması ve biyomedikal çalışmalar gibi çeşitli uygulamalarda kullanımı uygun bulunmuştur. Bu boya önemli ölçüde yeni antifungal ilaçların geliştirilmesine yol açabilir.

Anahtar Kelimeler

o,o′-dihidroksiazo, Antimikrobiyal aktivite, Azo-ester boyaları, Krom kompleksleri

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INTRODUCTION

Azo dyes and their metal complexes are well recognized for their wide use in fields such as textile fiber dyeing, biomedical studies, advanced applications in organic synthesis, and high technology fields like lasers, liquid crystalline displays, electro-optical devices and ink-jet printers [1-4]. Azo dyes continue to be respected as a very important class of chemical compounds, receiving much attention in scientific research [5]. Besides, azo dyes and metal complexes play an essential role in the chemistry of living organisms, and their biological importance continues to be studied [6]. The spread of multi drug-resistant strains of fungus and bacteria and the relatively few drugs available, make it almost mandatory to discover new classes of antifungal and antibacterial compounds that inhibit these resistant mechanisms, triggering a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them, used for their empiric antimicrobial properties [7].

The fastness properties and synthesis of these dyes [4, 8] have been reported earlier, though the UV-VIS spectral behavior in different pH and temperatures and biological activities are yet to be described. The tautomeric behavior of several hydroxy azo dyes has been recognized in different solutions. This tautomerization resulting from the intramolecular proton transfers between the azo nitrogen atom and hydroxyl oxygen atoms, has been studied using various techniques, including UV-VIS and NMR spectroscopy [9]. Proton tautomerism plays an important role in many branches of chemistry, particularly biochemistry [10].

In this study, we report the UV-VIS behavior of (E)-1-((2-hydroxy-5-methylphenyl) diazenyl)naphthalene-2,7-diol and acrylate derivatives and their chromium complexes in organic solvents of different polarities, different pH and temperatures. We have also attempted to study the antimicrobial effect of these dyes on Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeroginosa* and *Escherichia coli*) and fungus (*Candida albicans*).

MATERIAL AND METHODS

Reagents and Instruments

All chemicals of analytical grade purity were used without further purification. Figure I illustrates the formula of dyes I-IV.

I; (E)-1-((2-hydroxy-5-methylphenyl)diazenyl) naphthalene-2,7-diol

II; (E)-1-((2-hydroxy-5-methylphenyl)diazenyl) naphthalene-2-hydroxy-7-acrylate

III; Triaqua((E)-1-((2-hydroxy-5-methylphenyl) diazenyl)naphthalene-2,7-diol) chromat (III) chloride

IV; Sodium Bis((E)-1-((2-hydroxy-5-methylphenyl) diazenyl)naphthalene-2-hydroxy-7-acrylate) chromate (III). 2.5 Aqua

These dyes were synthesized according to the earlier described method [4, 8]. UV-VIS spectra were obtained on a Lambda 25 UV/VIS spectrometer.

Antimicrobial activity of dyes

Microorganism growth

This study was investigated the antibacterial and antifungal effects to against Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 6538), gram-negative bacteria (*Pseudomonas aeroginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) and fungus (*Candida albicans* ATCC 10231) of the synthesized dyes I-IV. In this study, nutrient broth and agar (Diffco) were used for *E. coli* cultures; Tryptone-Yeast extract-Cystine (TYC) broth and agar for *E. fecalis* and *S. aureus* cultures; Eosin Methylene Blue (EMB, Merck) broth and agar for *P. aeroginosa* cultures; and Sabouraud Dextrose broth and agar (SD, Merck) for *C. albicans* cultures. Strains were cultured overnight at 37 ± 1°C and stored at -86°C in a broth containing 10% glycerol as the cryoprotective agent.

Antimicrobial and antifungal effects of dyes I, II, III, and IV

The antimicrobial and antifungal effects of the synthesized dyes I-IV were determined by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

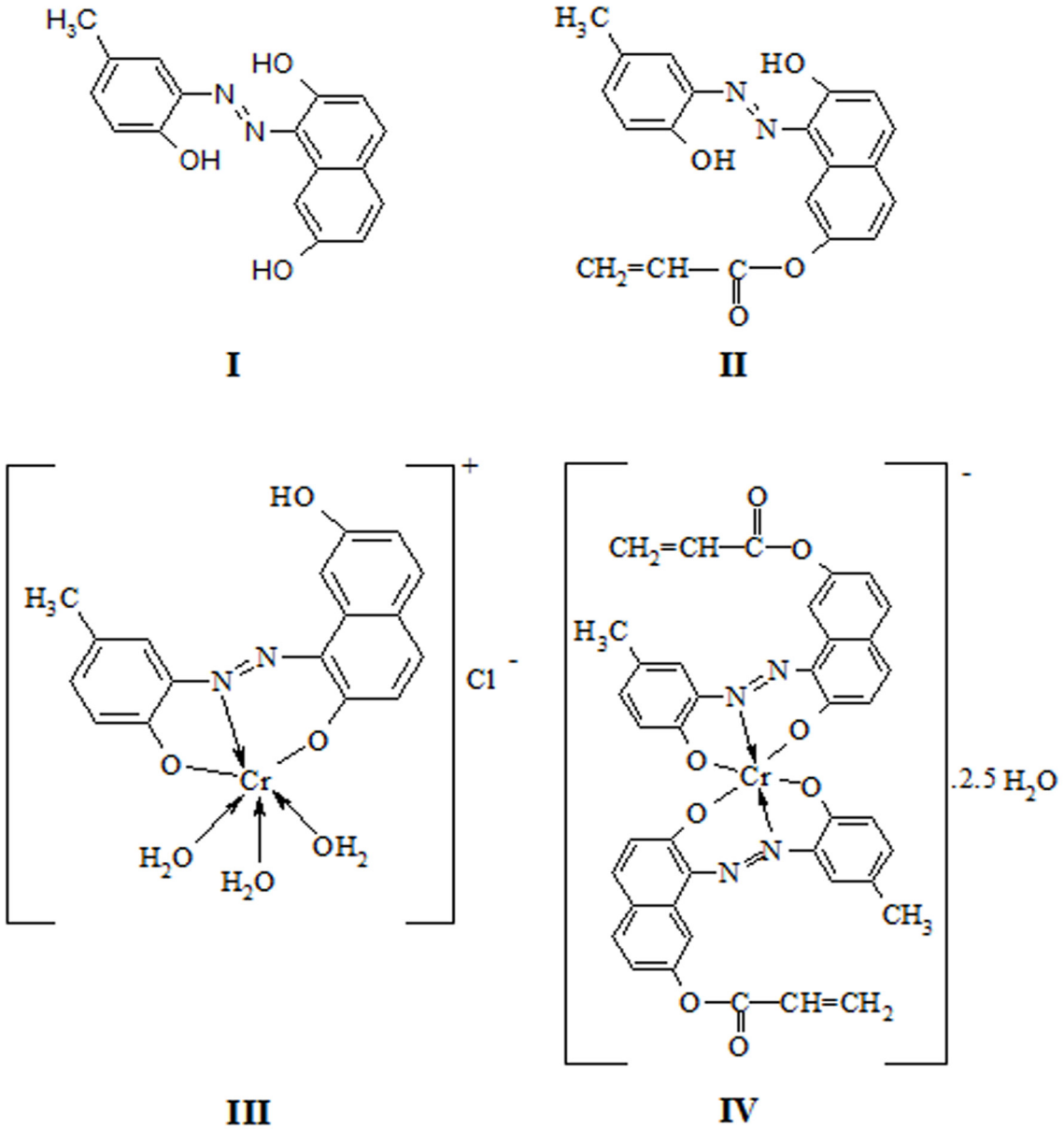

Figure 1. The structure of the 7-hydroxy-o,o'-dihydroxyazo dyes and their chromium complexes.

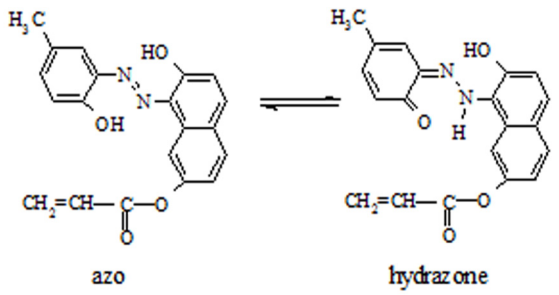

Figure 2. Azo and hydrazo tautomeric forms of dye II

First, 0.01 g of each of the dyes was dissolved in 1 ml dimethylsulfoxide (DMSO). The microorganisms were grown in a suitable broth at 37 ± 1 °C for 24 h. All the agar plates were prepared with a final depth of 4 mm. Next 0.1 ml suspension of tested microorganisms (10^8 cells/mL^{-1}; turbidity = McFarland barium sulfate standard 0.5) was spread on the agar plates. After, sterile 6-mm in diameter filter paper discs (Whatman, no. 4) were placed on the agar plates and impregnated with 5 μL, 10 μL, and 25 μL of dyes in DMSO.

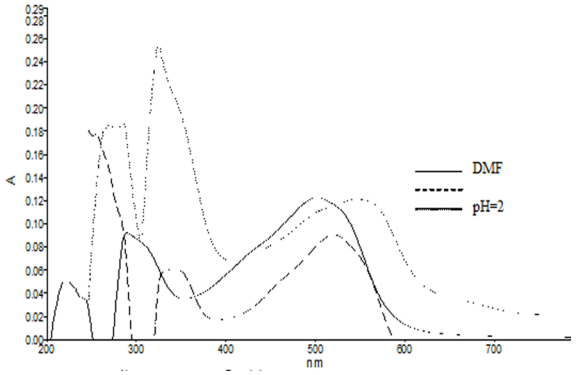

Figure 3. Absorption spectra of dye I (1x10-5 M), pH 2 and pH 12 in DMF

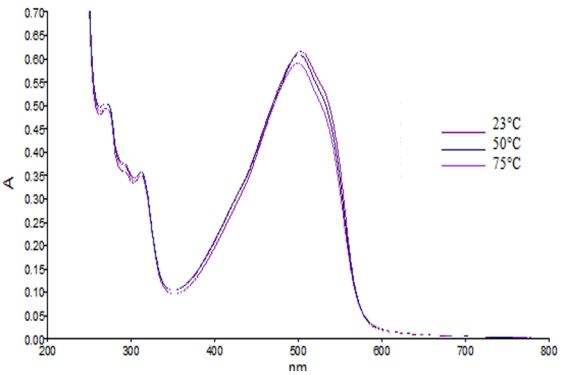

Figure 5. Absorption spectra of dye I (5x10-5 M) at different temperatures in ethyl alcohol.

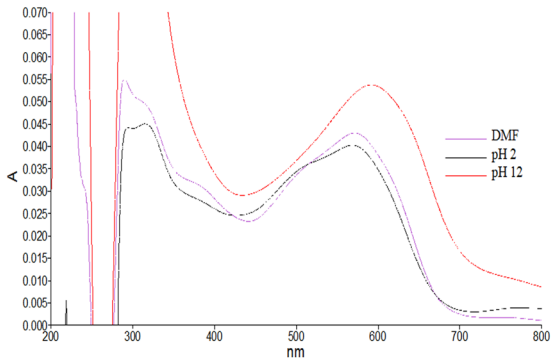

Figure 4. Absorption spectra of dye III (1 x 10^{-5} M), pH 2 and pH 12 in DMF

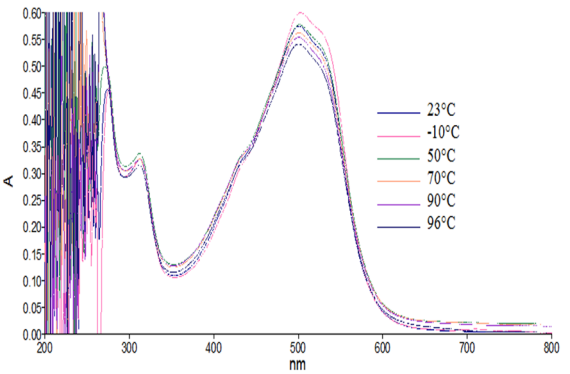

Figure 6. Absorption spectra of dye II (5 x 10^{-4} M) at different temperatures DMF.

These plates were incubated at 37 ± 1 °C for 24-48 h. DMSO without dyes was used as control. Amoxicillin (20 μg) + Clavulanic acid (10μg), Amikacin (30 μg), Ampicillin (10 μg) and Nystatin (25 μg) were screened under similar conditions as the standard antibiotic discs. At the end of the incubation period the inhibition zones around the discs were measured as millimeters.

RESULTS AND DISCUSSION

In the light of our prior studies, the synthesis and characterization of o,o'-dihydroxyazo dyes and chromium complexes were described by IR, UV-VIS, ^{1}H-NMR spectroscopic techniques, magnetic susceptibility, thin layer chromatography, and elemental analysis. The synthesized dyes and complexes were applied on wool and nylon fabric. Fastnesses to light, washing and rubbing of the dyed materials were measured [4,8]. Therefore, this work mainly focused on investigating the tautomeric behavior of the dyes (Figure 1) in different solvents, pH and temperature to determine their antimicrobial effects.

We investigated the UV-VIS spectral behavior of the azo and azo-ester compounds possessing a polymerizable acryloyloxy group, and their chromium complexes in acetone, ethyl alcohol, and DMF (dimethylformamide) of pH 2 and pH 12 in DMF. We earlier on determined the maximum wavelength and absorption in DMF and acetone, but could not compare dye (I) and the ester derivative (II) with each other. In this study, we also investigated the UV-VIS spectral behavior of compounds in varying pH and temperatures. The working solutions (1 x 10^{-4} -1 x 10^{-5} mol.L^{-1}) were prepared by diluting the stock solution of the compounds (1 x 10^{-3} mol.L^{-1}). The absorption data of the dyes and their complexes are listed in Table I. The UV-VIS spectra of the azo I and azo-ester II compound exhibited one band at 482-486 nm and a shoulder at 420-426 nm in acetone. These bands appeared as absorption maxima with high extinction coefficient and represented the azo and hydrozone forms (Figure 2) [11-13]. The shoulder peak in 420-426 nm represented the azo form, and the peak at 482-486 nm corresponded to the hydrazone form. In acetone and ethyl alcohol,

Table 1 The absorption data of compounds

Dyes	Wavelength (λ_{max} (nm))(logε (L.mol^{-1}.cm^{-1}))				
	Acetone	EtOH	DMF	pH 1-2 (DMF)	pH 12 (DMF)
I	420º (3.70) 486 (5.31)	436º (4.51) 502 (4.78) 521º (4.74)	433º (3.89) 501 (4.08) 523º (4.02)	443º (3.95) 496 (4.11) 525º (4.01)	489º (4.08) 524 (4.11) 656º(3.52)
II	426º (4.47) 482 (5.30)	432º (4.58) 494 (4.82)	441º (4.53) 501 (4.76) 519º (4.73)	443º (4.57) 497 (4.77) 519º (4.71)	446º (4.54) 483 (4.61) 527 (4.65) 541 (4.66) 651º (4.02)
III	510º (3.41) 570 (4.21)	503º (3.88) 595 (3.79) 606º (3.74)	534º (3.57) 571 (3.60) 755 (3.22)	534º (3.57) 568 (3.60) 380 (2.57)	592 (3.69)
IV	511º (4.04) 570 (4.14) 619º (4.00)	521º (4.37) 559 (4.39) 602º (4.26)	524º (4.10) 572 (4.17) 609º (4.08)	530º (4.11) 569 (4.14) 606º (4.05)	535º (4.14) 592 (4.25) 634º (4.17)

º: shoulder peak

the absorption maxima of dye II, demonstrated a hypsochromic shift ca. (486 nm to 482 nm) 4 nm and (502 nm to 494 nm) 8 nm in comparison with I (14). This occurred by decreasing the order of activation of the acryloyloxy moiety instead of the hydroxyl group. Further, dye II was hydrolyzed with aqueous sodium hydroxide at pH 12 and procedure the sodium salts of dye II and acrylic acid. In DMF, dyes I and II revealed spectra very similar to each other and showed a strong absorption band at 501 nm and two shoulder strong absorption bands at 433-441 nm and 519-523 nm, respectively. These

Table 2. Antimicrobial activity of the tested dyes (I, II, III and IV) and different amount of the dyes.

Agents	*E. fecalis*	*S. aurues*	*P. aeroginosa*	*E. coli*	*C. albicans*
Ia	+	+	+	+	-
Ib	++	++	++	+++	+
Ic	+++	+++	+++	++++	++
IIa	-	+	++	++	-
IIb	+	+	+++	+++	-
IIc	++	++	++++	++++	+
IIIa	-	+	+	+	-
IIIb	-	+	++	++	-
IIIc	-	++	+++	++++	-
Iva	-	-	+	+	-
IVb	-	+	++	++	-
IVc	-	++	+++	+++	-
AMC	-	-	-	-	-
AK	+++	+++	+++	+++	-
AMP	++	++	++	++	-
NYS	-	-	-	-	+++
DMSO	-	-	-	-	-

Amount of dyes; Ia, 5µl; Ib, 10µl; Ic, 25µl; IIa, 5µl;IIb, 10µl; IIc, 25µl; IIIa, 5µl;IIIb, 10µl;IIIc, 25µl; IVa, 5µl;IVb, 10µl;IVc, 25µl
AMC; Amoxicillin + Clavulanic acid, AK; Amikacin, AMP; Ampicillin, NYS; Nystatin
Diameter of the inhibition zone (+), till 10mm; (++) till 15 mm; (+++), till 20 mm.

bands appeared in the hydrazone and azo forms, with the shoulder peak in 433-441 nm representing the azo form, and the other corresponding to the hydrazone form. These results revealed that in DMF, the compounds are in equilibrium between the azo-hydrazone tautomeric forms, although the hydrazone form is dominant. DMF is a more polar solvent and it is generally recognized that more polar solvents favor the hydrazone form, whereas less polar solvents favor the azo form [9]. The absorption maxima of all the compounds demonstrated a hypsochromic shift ca. 5-3 nm in pH 2 and a bathochromic shift between 40 nm and 20 nm in pH 12 in comparison with DMF (Figure 3-4).

The absorption maximum of the complexes III and IV in all the solvents demonstrated a bathochromic shift between 93 nm and 64 nm compared with dyes (I-II), consistent with the literature values [15, 16]. The spectral data (Table 1) lead to the conclusion that the absorption band at 482-595 nm generally shows a bathochromic shift (positive solvatochromisim) as the solvent polarity increased. The influence of solvents on the compounds increases in the order DMF > EtOH > Acetone. This observed behavior is accounted for as the prepared azo dyes in the ground state and in the excitation state indicate different polarities [17].

The UV-VIS spectra of compound I were studied at different temperatures in EtOH. Figure 5 shows the UV spectra of dye I at 23°C, 50°C and 75°C. It was observed that the absorbance of the bands decreases by raising the temperature. The spectra of this compound determined three absorption bands at all temperatures. One is with a shoulder at ca. 426 nm, characteristic for the azo tautomer form, whereas the other is ca. 502 nm with a shoulder at ca. 521 nm for the hydrazone form. The absorption band at 502 nm demonstrated a hypochromic shift with rise in temperature. Similar results were obtained for dye II in DMF at different temperatures, as well as illustrated in Figure 6. The spectra of dye II in DMF at different temperatures determined three absorption bands. One is with a shoulder at ca. 441 nm for the azo form while the other is ca. 501 nm with a shoulder at 519 nm for the hydrazone form. The absorption band at 501 nm demonstrated a hypochromic shift with rise in temperature. However, when dye II solution was cooled at -10°C, the absorption band (519 nm) for the hydrazone form was strengthened.

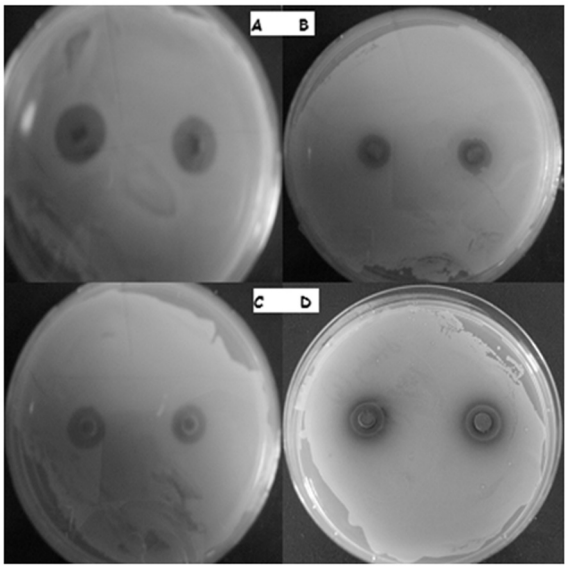

Figure 7. The inhibition zones of the dyes on microorganisms (A, Pseudomonas aeroginosa-IIc; B, Escherichia coli-IIIc; C, Enterococcus fecalis-Ic; D, Staphylococcus aureus-Ic).

The *o,o'*-dihydroxyazo dyes and their metal complexes are principally used as chromium and cobalt complexes for their properties of dyeing protein and polyamide fibers with excellent light and washing fastness, as well as in electrophotographic applications for photoconductors. In our prior studies, we measured the fastness values of the dyed fabrics to light, washing, and rubbing [7]. The antimicrobial activity of some of the azo dyes, schiff bases and their metal complexes has been reported earlier [5, 6, 18, 19], although the antimicrobial activity of these dyes is yet to be reported. Therefore, different microorganisms have been chosen, and the antimicrobial effect of dyes on these microorganisms was determined according to the disc diffusion method. The data for the antimicrobial tests are summarized in Table 2.

Antimicrobial activity of DMSO against the test organisms was investigated, but no antimicrobial activity was observed against any of the organisms. As evident from Table 2, all the dyes and metal complexes exhibited different antimicrobial activity against the microorganisms tested. Further, the inhibition zones of some microorganisms are shown in Figure 7. In the present study, it was observed that dyes and metal complexes were generally

very active against Gram-negative bacteria like *P. aeroginosa* and *E. coli*. High amount of dyes II and III particularly, showed more microbial activity than the other dyes and different amount of the dyes against these strains. Dye I generally affected all microorganisms, while compounds II, III and IV were more effective against Gram-negative bacteria (*P. aeroginosa* and *E. coli*). While dyes I and II revealed less effective antimicrobial activity against *E. fecalis*, other dyes did not. As clearly shown in Table 2, dye I shows strong antimicrobial activities against all microorganisms tested. Therefore, in classifying the antibacterial activity as Gram-positive or Gram-negative, dye I would be active more against Gram-positive than Gram-negative bacteria.

However, all the dyes also exhibited an activity on Gram-negative bacteria that could be responsible for many infections. However, in this study, all the dyes were active against Gram-negative bacteria. On comparing the results with other microorganisms, *E. coli* was found to be the bacterium most influenced against dyes, whereas *C. albicans* and *E. fecalis* were the most resistant bacterium. Dye I alone revealed a toxic effect on *C. albicans*, which is due to the fact that its cell wall structure and the yeast cell wall are quite complex.

In conclusion, both solvent and pH affect the azo-hydrazone equilibrium. A good understanding of the reported phenomena becomes important with respect to characteristics such as color control and electrical properties for this important class of dyes. As dye I exerts an antimicrobial effect on both bacteria and fungus it has wide used applications in textile fiber dyeing and biomedical studies. This dye could lead to the development of new antifungal drugs. It is therefore important to study their antimicrobial activity on various dye substrates.

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