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PII: S0022-2860(18)30900-1

DOI: 10.1016/j.molstruc.2018.07.081

Reference: MOLSTR 25486

To appear in: Journal of Molecular Structure

Received Date: 19 May 2018

Revised Date: 24 July 2018

Accepted Date: 24 July 2018

Please cite this article as: S.A. Khan, S. Shahid, S. Kanwal, K. Rizwan, T. Mahmood, K. Ayub, Synthesis of novel metal complexes of 2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid formazan dyes: Characterization, antimicrobial and optical properties studies on leather, *Journal of Molecular Structure* (2018), doi: 10.1016/j.molstruc.2018.07.081.

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Synthesis of Novel Metal Complexes of 2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid Formazan Dyes: Characterization, Antimicrobial and Optical Properties Studies on Leather

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Abstract: We have developed novel formazan dyes (18-23) of enhanced fastness properties with cost-effectiveness in an aqueous system, without employing any Buffers and organic solvents. Their development was entailed of the synthesis of 2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid followed by the diazotization of 2-aminobenzoic acid that further reacted with the 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid. The multi-chromic (1:1 and 2:1) metal complexes of 2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid had been developed with the salts of Cr, Fe, Co, Cu and Ni; and they were characterized by elemental analysis, powder X-ray crystallography, Ultraviolet-visible, Fourier transforms infrared, Proton nuclear magnetic resonance and C¹³-nuclear magnetic resonance spectroscopic techniques. Density functional theory (DFT) studies of all dyes (18-23) were performed to evaluate the most stable geometries and structural parameters as well. The synthesized formazan dyes were evaluated for their different fastness (light, wash, perspiration), exhaustion and fixation properties on goat leather fabric and were revealed to have virtuous fastness properties (3-5, 4-5, 3-5, and 4-5) with high percentage value of exhaustion and fixation ranged from 91-97 % and 90-98 % respectively. The synthesized formazan dyes (18-23) were developed different colors such as Red, Brown, Green, Black, and Blue on leather. Synthesized formazan dyes were also evaluated for their antibacterial propensity on leather substrate and in solution by agar well diffusion method. The metal complex formazan dye (21) was demonstrated the significant bactericidal propensity against E. coli, S. aureus, Klebsiella and B. subtilis in solution and on leather by exhibiting maximum ZOIs (19±0.05 mm, 25±0.07 mm, 23±0.09 mm, 27±0.03 mm)

and percentage reduction in bacterial growth ($60\pm0.03\%$, $69\pm0.07\%$, $80\pm0.05\%$ and $86\pm0.08\%$) respectively. Hence, newly synthesized formazan dyes (**18-23**) have efficient optical and antibacterial properties that would be proved valuable for the development of new industrial products to be commercialize.

Keywords: Synthesis; Formazan dyes; Characterization; Optical properties; Antimicrobial propensity

1. Introduction

Both the formazan and azo dyes have formal resemblance with each other, because of the presence of azo group in their structural molecule; however, formazan dyes molecules have enough dissimilarities in their structures than azo dyes to be considered as a separate class [1]. Because of the π - π * transitions of π electrons in formazan skeleton, the formazan/tetrazolium and their derived metal complexes dyes i.e. Cr, Fe, Cu, Ni, Co etc. impart color and they have (-N=N-C=N-NH-) formazan skeleton in their molecules [2]. Moreover, the formazans dyes have propensity to form metal complexes upon metallization process with the salts of different metals such as FeSO₄.7H₂O to form Fe-complex, CrCl₃.6H₂O to form Cr-complex and CuSO₄.5H₂O to from Cu-complex etc. This is because of fact that the formazan dyes are comprise of the characteristics features of multidentate ligands with donor atoms. The non-metal complex as well as metal complex formazan dyes of various kinds of metals involve in producing the intense color of different shades i.e. Red, Blue, and Brown etc., which are directly depend on the structure either what type of auxochromes as well as chromospheres are attached to color imparting molecule [3]. Ciba was the pioneer who used the first molecule of tetradentate formazan metal complexes as a colorant material for the dyeing purposes of wool in 1947 [4]. Subsequently, numerous tetradentate formazans metal complexes had been invented and they were become significant class of dye, specifically in the sectors of acid and reactive dyes even though additional applications have also been reported for them [5]. The formazan dyes have become important reactive dyes for cotton as well as for leather fabric [6]. In respect of investigation on their use as an end-product, research studies were focused to improve their synthetic routes and more deeper studies were done to understand the relationships of chemical constitutions of the formazan dyes to the color properties [4]. The plentiful literature related to formazans dyes is available which displays their synthesis, structural features, photochromic transitions, tautomer formation, redox potentials [7,8] as well as the synthesis of crown formazans [9,10]. Beside of their use as a colorant material, the formazan and tetrazolium salts were also employed for the identification of the existence of Brucella species in Brucella ring test in milk [11,12]. The Brucellosis is a bacteriological contamination which blowouts from animals to human beings most frequently through un-pasteurized milk, cheese, and other dairy products [13]. In normal and neoplastic tissues, the formazan systems and blue tetrazolium salts are employed to unveil enzymes activity [14]. The formazan dyes system is also comparatively more beneficial in the identification of efficiency of anti-cancer drugs [15,16]. The un-metallized and metal complex formazan dyes are also employed in analytical chemistry because of the development of more intense color of different shades upon combining with the substrate to be analyzed [17]. Because of their profligate and intense color reaction, non-metal and metal complex formazans dyes can be employed for the spectroscopic identification of variety of metal ions through fluorescence mechanism [18]. The reported literature are also disclosed that unmetalized and metal complex formazan dyes also have potential to act as antimicrobial to inhibit the growth of microbial agents. Moreover, they also have capacity to perform their action as an antioxidant agent by inhibiting the growth of free radicals that are produced due to oxidative stress and hydrolysis. The presence of different functionality such as (-OH, -SO₃H, -NO₂, -CH₃) in their molecules empower them to spectacle improve biological applications. Because of their extraordinary and substantial features towards colorant material, antimicrobial and antioxidants, they must be have commercial worth but in actual practice, it is not like that because of their high production cost. Various literature reported their synthesis through different methods and strategies with the use of different type of Buffers and organic based solvents, which make their cost high, environmentally un-friendly and eventually non-commercializable. The aims of the present research study were involved to (i) develop novel un-metallized and metal complex formazan dyes of enhanced fastness properties with cost-effectiveness in an aqueous system, without employing any Buffers and organic solvents (ii) evaluation of their antimicrobial activities.

2. Experimental Work

The current research work was performed at the R&D Lab, Shafi Reso-Chem, Lahore Pakistan and research laboratory, Department of Chemistry, University of Management and Technology, Lahore Pakistan. The used chemicals and reagents in present research were of analytical grade as well as all of them were purchased from BASF (Germany) and Sigma Aldrich, USA.

2.1. Physical measurement

The functional group recognition of the synthesized formazan dyes (18-23) was accomplished by using Agilent Cary 630, Agilent Technologies, USA, FTIR spectrophotometer in the range of 450-4000 cm⁻¹. By employing UV-visible spectrophotometer = Spectra Flash SF 550, Data color Inc., USA, the electronic absorption spectral studies of the synthesized formazan dyes (18-23) were performed in the water at the range of 200-800 nm. Through NMR Bruker DPX 400-Operating at 300/75-13C MHz spectrophotometer, the H1-NMR spectral investigations were recorded in a CDCl₃ solvent for the synthesized formazan dyes (18-23). By employing the PANalytical X'Pert diffractometer PRO instrument with Cu-Ka radiation (wavelength 0.154 nm) operating at 40 kV and 30 mA, the powder X-ray di raction analysis was executed to explore the crystallinity of the synthesized formazan dyes (18-23). The measurements were scanned for di \Box raction angles (2 θ) ranging from 20° to 90° with a step size of 0.02° and a time per step of 1 s. By using Flash EA 1112 elemental analyzer, the elemental analysis (C, H, N, S) of the synthesized formazan dyes (18-23) was performed to compare the %age composition theoretically vs. practically. The melting points determination of the synthesized formazan dyes (18-23) was performed employing the Melting Point apparatus, Gallenkamp, UK. The Portable pH Meter Model PHB4pH was used to monitor the pH of the reaction and synthesized formazan dyes. The Leather Dying Drum Machine = Jiangsu Lianyungang Leather Machinery Factory China, Model# R-350-6 was employed for the application of dyes on goat leather fabric. The Xenon Fad-o-meter Model# XF-15N, Shimudzu Corporation Kyoto Japan Light was used for the assessment of fastness properties of synthesized formazan dyes (18-23) on leather fabric. The progress of the complete synthesis of formazan dyes (18-23) was monitored through thin layer chromatography (TLC) in this research work and TLC was executed on aluminum sheets of $2 \times$ 5 cm dimensions, which were pre-loaded with silica gel 60F254 of the thickness of 0.25 mm (Sigma Aldrich). The visualization of the developed chromatograms were implemented under the ultraviolet light at the wavelength range of 254-366 nm or with the iodine vapors.

2.3. General methodology for the preparation of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5)

The preparation of coupler such as 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) was entailed of the transformation of 4-aminobenzenesulphonic acid (1) into diazonium salt (2) which was yielded 4-hydrazinylbenzenesulfonic acid (3) on reduction in the presence of sodium

sulfite and HCl at 0-5 °C temperature and lastly, required product (5) was obtained through the condensation reaction of benzaldehyde (4) with compound (3) [19].



Scheme 1: The synthesis of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5)

2.3.1. The diazotization of *p*-Aminobenzenesulfonic acid (1)

The 173 g (1 mole) of *p*-Aminobenzenesulfonic acid (Sulfanilic acid) (**1**) having 100 per cent purity was added in 1500 ml water in 3 L beaker and addition of 65 g of Na₂CO₃ was made slowly due to the foam formation along with mixing on multiple agitator to complete dissolution. Then the solution was sifted to confiscate un-dissolved particles and scums. Then ice jacket was made around the beaker to accomplish temperature 0-5 °C. Then during constant agitation, the 400 ml HCl was added progressively again because of the foam formation (foam was formed due to the evaluation of CO₂ from the reactant solution). Then the 100 g of ice flakes was added into the solution to sustain temperature 0-5 °C. At that temperature, solution of Sodium nitrite (73 g of NaNO₂ in 150 ml water) was added into the reactant beaker drop-wise in 15 minutes and the congo-iodo paper was checked which was positive by giving blue coloration. After that, the reaction mixture was permitted to complete reaction at continuous stirring for 1:30 hr. Subsequently, the diazonium salt was sifted through the sanction unit linked with the vacuumpump [20]. The preparation of diazonium salt (**2**) of 4-aminobenzenesulphonic acid (**1**) were presented in scheme **1**.

2.3.2. The preparation of 4-hydrazinylbenzenesulfonic acid (3)

For the reduction of diazonium salt, 565 g of crystalline $Na_2SO_3.7H_2O$ was added into a beaker of 3 L containing 830 ml of water and provided the stirring until a clear solution of it was not achieved. The Sodium sulfite solution was cooled in an ice bath to accomplish 0-5 °C temperature and on this temperature, the diazonium salt wet paste (2) was transferred into the beaker of Sodium sulfite solution in 1 hr and orange coloration was started to appear in reaction solution without turbidity. Afterwards, a constant stirring was provided to the reaction solution for 1 hr. Subsequently, the reaction solution was heated to boil-age on constant stirring and 664 ml of concentrated HCl was added in 30 min. The color of reaction solution was become lightens and lastly very light yellow color was seemed at the end of reaction. The reaction mixture was cooled down to room temperature over a period of over-night and after the formation of precipitate formation, reaction mixture was sifted through sanction unit, and cold water was employed to wash the precipitates. The precipitates of 4-hydrazinylbenzenesulfonic acid (3) dried out in an electric-oven at the temperature of 100 °C. Further purification of the 4-hydrazinylbenzenesulfonic acid (3) was performed following the method [19]. The percentage yield of the product was 96 % of the calculated amount. The preparation of 4-hydrazinylbenzenesulfonic acid (3) was denoted in scheme 1.

2.3.3. The preparation of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5)

The 198.75 g (1 mole) quantity (considering 95% pure) of 4-hydrazinylbenzenesulfonic acid (3) was measured accurately on analytical-balance and was added into a beaker of 3 L having 2508 ml H₂O and 99.99 ml sodium hydroxide was transferred to accomplish pH~10. The reaction mixture was heated on constant stirring at the temperature of 45 °C for 30 min to complete dissolution. Subsequently, the reaction mixture was cooled down to room temperature and was sifted to confiscate contamination and un-dissolved materials. Then, the filtered reaction solution was transferred into the reactor of 3 L capacity and was heated up to the temperature of 55-60 °C. At that temperature, the condensation reaction was performed by the addition of 106.2 g (1mole) of benzaldehyde (4) dropwise in 30 min on constant stirring and heating (55-60 °C) with constant mixing was provided to the reaction mixture until the reaction was completed. The reaction was completed in 3 hr and the precipitation of the required product was accomplished through salting out and acidification process at pH~2.5 at room temperature (30 °C). The 20 % of NaCl of the total volume of the solution and 25 ml HCl were employed for precipitation. Afterwards, the precipitates was filtrated off at ambient temperature and the filtered cake of the product was dried out in an electric-oven at the temperature of 60 °C until constant weight obtained. The percentage yield was 97 % of the theoretical amount and the product was 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) which was coupler reagent used for the prepartion of formazan dyes (18-23) through the azo-coupling reaction. This product (5) was purified through the procedure of [20,21] and the prepartion of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) was denoted in Scheme 1.

2.4. General methodology for the preparation of formazan dyes (18-23)

The 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5) which were acted as coupler reagent were prepared in first step (Scheme 1). The 2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid (18) were prepared in the second step and was non-metal complex formazan dye which was transformed into metal complexes with the salts of Iron (19), Copper (20), Chromium (21), Nickel (22) and Cobalt (23) and were characterized them through different spectroscopic-techniques. Scheme 2 were presented the synthesis of un-metallized and metal complex formazan dyes (18-23).



Scheme 2: The preparation of non-metal complex formazan dye (18) and metal complex formazan dyes of Iron (19), Copper (20), Chromium (21), Nickel (22) and Cobalt (23)
2.4.1. Synthesis of un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18)

The 13.714 g (0.1 mole) on 100 % pure bases of anthranilic acid (2-aminobenzoic acid) (10.1) was added into 500 ml beaker containing 150 ml water and the constant stirring was provided through multiple agitators. Afterward, the 40 ml of HCl was transferred into the reaction mixture and permitted for 15 min to complete dissolution of the reaction mixture. Then the reaction solution was sifted off through the suction unit to eliminate impure and un-dissolved substances. The filtered reaction solution was ice-jacketed in beaker and 30 g of ice flakes were transferred into the beaker to accomplish the zero centigrade temperature. Subsequently, the solution of NaNO₂ /water (7.3 g/l5 ml) was transferred into the reaction beaker in four portions. The positivity of Congo red and Iodo paper was tested for hydrochloric acid and sodium nitrite respectively and the reaction solution was agitated at the temperature of 0-5 °C for 1 hr. The Congo-iodo= +ve was patterned after every fifteen min throughout the stirring for 1 hr. After that, excess amount of Iodo was made negative through the addition of 5 g of Sulfamic acid (H₃NSO₃) which finally yielded the diazonium-salt (11.1) and its purification was performed through filtration-process. The diazonium-salt (11.1) was ready for azo coupler reaction. In which diazonium salt (11.1) of anthranilic acid (2-aminobenzoic acid) (10.1) was coupled with 28.48 g (0.1 mole) (97 % percent pure basis) of 276.31 g/mole of 4-[(2Z)-2benzylidenehydrazinyl] benzene sulfonic acid (5) that was accomplished by the dissolution of (5) into 400 ml H₂O at highly alkaline pH~10-11 at the temperature of 0-5 °C. The alkaline-pH attained with the transfer of 35 g Na₂CO₃ and the diazonium-salt (11.1) transfer to coupler reagent (5) was completed within 1 hr. Azo-coupling reaction was completed in 3 hr and yielded the un-metallized formazan dye (18) which cooled down to room temperature. After that, the unmetallized formazan dye (18) was isolated by 35 ml HCl (pH~4.75) and 22 % NaCl of the total volume of dye. The synthesized formazan dye (18) was sifted off and dried out at the temperature of 60-70 °C until the constant weight obtained. The obtained yield of the product (18) was 86 % over theoretical calculation and scheme 2 were presented the synthesis of formazan dye (18). Reddish Brown (Solid). Yield (86%). Melting Point (380°C). λ_{max} in nm (log ε): λ_{max1} 550 (0.490), λ_{max2} 360 (0.698), λ_{max3} 230 (0.25). FTIR (KBr, Cm⁻¹) ^vmax: 1535 (Ar-C=N, str), 1365 (Ar-N=N, str), 1600 (aromatic C=C), 820 (-CN=NC-, str), 3380 (Ar-N-H, str), 1430 (Ar-SO₃H, str), 1690 (Ar-C=O, str), 2930 (Ar-OH carboxylic acid, str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.30, (s 1H, Aromatic-COOH), 1.498 (s 1H, NH), 12.18 (s 1H, SO₃H), 7.60-7.91 (m 13H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ: 155 (C=N), 166.5 (-COOH), 117.6, 123.8, 125.4,

125.6, 126.9, 127.3, 128.7, 128.8, 129.9, 131.0, 133.9, 134.7, 139.2, 146.8, (Aromatic carbons). Anal. Calc. for $C_{20}H_{16}N_4O_5S$ (MW: 424.429 g/mol): C, 56.60; H, 3.80; O, 18.85; N, 13.20; S, 7.55% and Found: C, 56.55; H, 3.75; O, 18.80; N, 13.15; S, 7.51%.

2.4.2. Detailed procedure for the synthesis of metal complex formazan dyes (19-23)

For the preparation of metal complex formazan dyes (19-23), the un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) which was tridentate-ligand had been synthesized following the procedure ascribed in scheme 2. After that, 1:1 metal complex formazan dyes (19, 20, 22 and 23) were synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) with the equimolar concentration (0.1 mole) of FeSO₄.7H₂O (27.80 g), CuSO₄.5H₂O (24.97 g), NiSO₄.7H₂O (28.09 g) and Co(CH□CO□)□·4 H□O (24.91 g) at pH~6.40-6.50 and at temperature 65-70 °C respectively. While, 2:1 metal complex formazan dye (21) were synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) with 0.05 mole (13.32 g) concentration of CrCl₃.6H₂O (26.64 g, 0.1 mole) at pH~6.70 at elevated temperature 90-100 °C. The metallization reaction was proceeded in 2-liter glass reactor with heating mantle and slightly acidic pH of the metallization reaction for each metal complex formazan dye (19-23) was adjusted with HCl (35-40 ml). In detail, 0.1 mole unmetallized formazan dye (18) (ligand) was dissolved in 150 ml H₂O in glass reactor and pH was adjusted with HCl accordingly to the synthesis of above stated metal complex formazan dyes. At required pH level, the heating such as (65-70 °C for 1:1 and 90-100 °C for 2:1) was provided to the ligand dye solution through heating mantle. At required temperature level as stated above, the metal salts was transferred into the ligand dye solution in 30 min. Subsequently, heating was provided continuously to the reaction mixture of ligand and metal salts until completion of metallization according to the synthesis of required metal complex formazan dye, as each of them required different time and temperature for the completion of metallization reaction. The metallization reaction time for the synthesized metal complex formazan dyes (19-23) was 3.5 hr, 4.5 hr, 5hr, 2.5 hr and 3 hr respectively and the resulted metal complex formazan dyes solution was cool down to room temperature for their isolation process. The each metal complex formazan dye (19-23) was isolated through acidification and salting out process for which HCl with different amounts was added for pH (4.0, 1.75, 0.80, 1.30 and 1.10) and the NaCl (15 %, 23

%, 18 %, 25 % and 19 %) of the total volume of the respective dye solution was added respectively. After that, respective metal complex dyes solution was stirred for 2-3 hr for complete isolation from the solution and then they were filtered off employing filtration unit linked with vacuum pump and dried out them in an electric oven at the temperature 60-70 $^{\circ}$ C until the constant weight of the dye. Over the theoretical calculation, the obtained yield for the synthesized metal complex formazan dyes (**19-23**) were 91 %, 91 %, 93 %, 84 %, and 84 % respectively. The scheme **2** is denoted the reaction for the synthesis of metal complex formazan dyes (**19-23**).

2.4.2.1. Synthesis of [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] Fe II complex (1:1) (19)

For the preparation of metal complex formazan dye (19), the un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) which was tridentate-ligand had been synthesized following the procedure ascribed in scheme 2. After that, 1:1 metal complex formazan dye (19) was synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) with the equimolar concentration (0.1 mole) of FeSO₄.7H₂O (27.80 g) at pH~6.40-6.50 and at temperature 65-70 °C respectively according to the above mentioned procedure (Scheme 2). Dark Brown (Solid). Yield (91%). Melting Point (418°C). λ_{max} in nm (log ε): λ_{max1} 490 (0.860), λ_{max2} 350 (1.10), λ_{max3} 225 (0.30). FTIR (KBr, Cm⁻¹) ^vmax: 1530 (Ar-C=N, str), 1420 (Ar-N=N, str), 1590 (aromatic C=C), 840 (-CN=NC-, str), 1390 (Ar-SO₃H, str), 1670 (Ar-C=O str), 675 (Metal-O, str), 3420 (O-H, str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.15, (s 1H, SO₃H), 8.26-8.70 (m 13H Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ: 155.2 (C=N), 169.4 (-COOH), 117.6, 125.3, 125.4, 127.3, 128.6, 128.8, 129.7, 130.3, 131.0, 134.7, 136.3, 139.2, 145.5, 150.5, (Aromatic carbons). Anal. Calc. for C₂₀H₂₀FeN₄O₈S (MW: 532.305 g/mol): C, 45.13; H, 3.79, O, 24.05; N, 10.53; S, 6.02; Fe, 10.49%. Found: C, 45.09; H, 3.76; O, 24.01; N, 10.49; S, 6.01; Fe, 10.45%.

2.4.2.2. Synthesis of [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] Cu II complex (1:1) (20)

For the preparation of metal complex formazan dye (20), the un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) which was tridentate-ligand had been synthesized following the procedure ascribed in scheme 2. After that,

1:1 metal complex formazan dye (**20**) was synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (**18**) with the equimolar concentration (0.1 mole) of CuSO₄.5H₂O (24.97 g), at pH~6.40-6.50 and at temperature 65-70 °C respectively according to the above mentioned procedure (Scheme 2). Reddish Brown (Solid), Yield (91%), Melting Point (408°C). λ_{max} in nm (log ε): λ_{max1} 460 (0.985), λ_{max2} 345 (0.89), λ_{max3} 225 (0.30). FTIR (KBr, Cm⁻¹) °max: 1520 (Ar-C=N, str), 1475 (Ar-N=N, str), 1590 (aromatic C=C), 850 (-CN=NC-, str), 1470 (Ar-SO₃H, str), 1610 (Ar-C=O, str), 690 (Metal-O, str), 3460 (O-H, str). ¹H-NMR (CDCl₃, 400 MHz) δ : 11.70 (s 1H, SO₃H), 8.30-8.80 (m 13H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ : 155.9 (C=N), 168.9 (-COOH), 117.8, 125.2, 125.5, 127.7, 128.3, 128.9, 129.7, 130.2, 131.1, 134.8, 136.6, 139.5, 145.1, 150.7, (Aromatic carbons). Anal. Calc. for C₂₀H₂₀CuN₄O₈S (MW: 540.006 g/mol): C, 44.48; H, 3.73; O, 23.70; N, 10.38; S, 5.49; Cu, 11.77% and Found: C, 44.44; H, 3.69; O, 23.65, N, 10.35; S, 5.44; Cu, 11.74%.

2.4.2.3. Synthesis of Bis-[2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] Cr III complex (2:1) (21)

The **2:1** metal complex formazan dye (**21**) were synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (**18**) with 0.05 mole (13.32 g) concentration of CrCl₃.6H₂O (26.64 g, 0.1 mole) at pH~6.70 at elevated temperature 90-100 °C according to the above mentioned procedure (Scheme 2). Chocolate Brown (Solid), Yield (93%), Melting Point (479°C). λ_{max} in nm (log ε): λ_{max1} 430 (1.05), λ_{max2} 340 (0.89), λ_{max3} 225 (0.35). FTIR (KBr, Cm⁻¹) °**max:** 1540 (Ar-C=N, str), 1465 (N=N, str), 1600 (aromatic C=C), 860 (-CN=NC-, str), 1430 (Ar-SO₃H, str), 1715 (Ar-C=O, str), 650 (Metal-O, str). ¹H-NMR (CDCl₃, 400 MHz) δ : 11.45 (s 2H, SO₃H), 8.25-8.75 (m 26H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ : 155.4 (C=N), 169.6 (-COOH), 117.2, 125.6, 125.8, 127.5, 128.1, 128.7, 129.8, 130.9, 131.5, 134.3, 136.8, 139.7, 145.3, 150.9, (Aromatic carbons). Anal. Calc. for C₄₀H₂₈CrN₈O₁₀S₂ (MW: 896.824 g/mol): C, 53.57; H, 3.15; O, 17.48; N, 12.49; S, 7.15; Cr, 5.80% and Found: C, 53.56; H, 3.14; O, 17.45; N, 12.45; S, 7.11; Cr, 5.76%.

2.4.2.4. Synthesis of [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] Ni II complex (1:1) (22)

For the preparation of metal complex formazan dye (22), the un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) which was tridentate-ligand had been synthesized following the procedure ascribed in scheme 2. After that, 1:1 metal complex formazan dye (22) was synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) with the equimolar concentration (0.1 mole) of NiSO₄.7H₂O (28.09 g) at pH~6.40-6.50 and at temperature 65-70 °C respectively according to the above mentioned procedure (Scheme 2). Greyish Black (Solid), Yield (84%), Melting Point (436°C). λ_{max} in nm $(\log \varepsilon): \lambda_{max1} 420 (1.30), \lambda_{max2} 335 (1.15), \lambda_{max3} 215 (0.25).$ FTIR (KBr, Cm⁻¹) ***max:** 1530 (Ar-C=N, str), 1450 (Ar-N=N, str), 1570 (aromatic C=C), 835 (-CN=NC-, str), 1295 (Ar-SO₃H, str), 1615 (Ar-C=O str), 680 (Metal-O, str), 3445 (O-H, str). ¹H-NMR (CDCl₃, 400 MHz) δ: 11.85 (s 1H, SO₃H), 8.15-8.65 (m 13H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ: 155.5 (C=N), 169.8 (-COOH), 117.1, 125.1, 125.7, 127.9, 128.5, 128.6, 129.3, 130.5, 131.6, 134.6, 136.5, 139.8, 145.4, 150.8, (Aromatic carbons). C₂₀H₂₀NiN₄O₈S (MW: 535.153 g/mol): C, 44.89; H, 3.77; O, 23.92; N, 10.47; S, 5.99; Ni, 10.97% and Found: C, 44.85; H, 3.74; O, 23.89; N, 10.45; S, 5.96; Ni, 10.95%.

2.4.2.5. Synthesis of [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] Co III complex (1:1) (23)

For the preparation of metal complex formazan dye (23), the un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) which was tridentate-ligand had been synthesized following the procedure ascribed in scheme 2. After that, 1:1 metal complex formazan dye (23) was synthesized by the metallization reaction of 0.1 mole (42.44 g) un-metallized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) with the equimolar concentration (0.1 mole) of Co(CH \Box CO \Box) \Box ·4 H \Box O (24.91 g) at pH~6.40-6.50 and at temperature 65-70 °C respectively according to the above mentioned procedure (Scheme 2). Dark Reddish Brown (Solid), Yield (84%), Melting Point (441°C). λ_{max} in nm (log ε): λ_{max1} 410 (1.10), λ_{max2} 330 (1.29), λ_{max3} 210 (0.45). FTIR (KBr, Cm⁻¹) ^vmax: 1560 (Ar-C=N, str), 1460 (Ar-N=N, str), 1615 (aromatic C=C), 855 (-CN=NC-, str), 1320 (Ar-SO₃H, str), 1680 (Ar-C=O, str), 660 (Metal-O, str), 3410 (O-H, str). ¹H-NMR (CDCl₃, 400 MHz) δ : 11.95 (s 1H, SO₃H), 8.30-8.75 (m 13H, Ar-H). ¹³C-NMR (CDCl₃, 75 MHz) δ : 156 (C=N), 169.0 (-COOH), 116.9, 124.9, 125.9, 126.9, 128.9, 129.0, 129.5, 130.4,

131.3, 134.9, 136.9, 139.4, 145.9, 160.1, (Aromatic carbons). Anal. Calc. for C₂₀H₂₀CoN₄O₈S (MW: 535.393 g/mol): C, 44.87; H, 3.77; O, 23.91; N, 10.46; S, 5.99; Co, 11.02% and Found: C, 44.86; H, 3.75; O, 23.90; N, 10.44; S, 5.96; Co, 11.01%.

2.5. Computational methods

Energy minima structures were obtained by using the Gaussian 09 software [22], and geometries and structural parameters were extracted with the help of GaussView05 [23]. Optimization was performed by using the B3LYP/6-311G(d,p) level of theory. Frequency analysis was performed at the same level to confirm the true optimization (no imaginary value for any frequency).

2.6. Dyeing method for valuation of color shade development on leather

The synthesized un-metallized and metal complex formazan dyes (18-23) had been evaluated for color shade development features by their application on goat leather fabric at 2 % dyeing rate through standard procedure stated by [21,24]. Accordingly, the dye solution (250 ml, 1.0 g dye equivalent to 2 % of goat crust weight) was transferred into the dyeing-drum. The dye bath pH~5.5 had been accustomed through the transfer of (5.0 ml. 10% w/v) of acetic acid solution. Afterwards, the dye bath volume had been attuned to 500 ml through the addition of required water amount and then, on constant agitation at 24 rpm, 50 g leather pieces were transferred into the dye bath. The dye-bath contents had been agitated at room temperature (30 °C) for 1 hr and its temperature 50-55 °C was up-raised progressively over a period of 35 min and continued stirring for 1 hr at that temperature. While on the rotation of dyeing drum, the addition of 5.0 ml of formic acid was accomplished to attain the pH~3.6 and again rotation of 30 min more was provided to the dyeing drum until the complete fixation of the applied formazan dye molecules (18-23) onto the leather. Afterwards, the dye-liquor was transferred in volumetric flask (500 ml and the leather fabric pieces was washed through the cold-water and combined solution of the dye liquor and washings was then further diluted to 500 ml with water. From this diluted solution, 2.0 ml was further diluted to 100 ml with water and the absorbance of this solution was recorded to estimate the exhaustion of the applied dyes (18-23) on goat leather fabric. The dyed leather piece was piled up over-night to dry; provided the chukrum and toggle and lastly, mounted on shade card. A weighed amount of leather piece was stirred in boiling acidified

pyridine which dissolves the unfixed dye from fabric and from the absorbance of this solution percentage fixation was determined.

2.7. Assessment of fastness properties on leather

The synthesized un-metallized and metal complex formazan dyes (**18-23**) were assessed for their fastness properties like color change, staining on adjacent fiber, light, wash and perspiration on goat leather fabric at 2 % dyeing rate following the standard procedure ascribed by [25].

2.8. Antibacterial potential determination

The synthesized formazan dyes (**18-23**) was scrutinized for their antibacterial potential against different pathological strains such as *Bacillus subtilis* (ATCC 9637), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Klebsiella* (ATCC 10031) on substrate such as goat leather fabric and in solution [26]. The synthesized formazan dyes (**18-23**) for their antibacterial propensity on agar plates were examined through well-known agar well-diffusion procedure [27,28] and the standard antibiotic drug such as "Cefradine" was cast off as a positive control for antibacterial comparative studies. The bacteriological strains were attained from Pakistan Chemical Scientific Industrial Research (PCSIR) laboratories complex, Lahore, Pakistan.

2.8.1. Antibacterial potential of synthesized formazan dyes (18-23) in solution

In the first step, by using distilled water, the solutions of 1 M strength of standard drug and the synthesized formazan dyes (**18-23**) in 1L volumetric flask were prepared separately [29]. In the second step, by dissolving 28 g of nutrient agar into 1000 ml distilled water in 1 L volumetric flask, the solution of nutrient agar was prepared and for complete and even dissolution of dissolved nutrient agar, the constant stirring was provided for 15 min. After that, flask mouth had been sealed off through cotton plug for the preservation of cultured media. Then, cultured media was pasteurized in an autoclave at 120-121 °C temperature by maintaining pressure such as 15 LD/ sq inch for 20-25 min. After pasteurization, cultured media was cooled down to room temperature and was cast off for the development of inoculum of bacteriological strains [30].

In the third step, already cultured slants of each frozen bacteria had been taken and a loop full of cultured microbes were transferred into the test-tube containing pasteurized nutrient agar and preserved for future use. Afterwards, from stock slants, *E. coli, B. subtilis, S. aureus, and Klebsiella* were transferred into four conical flasks containing 25 ml of nutrient agar and they

were hatched in a shaker at 37 °C temperature for 24 hr; and these cultures were cast off as a inoculum [31].

In the fourth step, as a basal layer, the newly prepared 200 ml of molten nutrient agar was shifted into twenty-eight pasteurized petri-dishes. Then, 1 ml inoculum of microbial strains such as *E. coli, B. subtilis, S. aureus, and Klebsiella* were transferred into twenty-eight petri-dishes and covered them through their lids and permitted them for a while to solidify and cool down to room temperature. After that, at three peripheral positions, the 2 mm diameter of solidify agar core was bored through sanitized hollow iron rod. Then the hovels were packed through the standard drug and already prepared samples and the petri-dishes were permitted for 1 hr and then in order to complete the reaction for bio-activity, the petri-dishes was hatched at 37 °C temperature for 24 hr in an incubator. Finally, clear zones diameter were calculated through the Vernier calipers [32].

2.8.2. Antibacterial potential of synthesized formazan dyes (18-23) on substrate (Leather fiber)

The synthesized formazan dyes (**18-23**) and standard drug were examined for their antibacterial potential on the goat leather fabric, which was dyed by them accordingly the procedure stated in dyeing section. The leather fabric specimens of 1 inch² dyed with synthesized formazan dyes (**18-23**) and standard drug were individually transferred into the nutrient agar of 200 ml inoculated with *E. coli*, *B. subtilis*, *S. aureus*, *and Klebsiella*. After that, they were hatched for the time-period of overnight such as 16 hr at the temperate of 37 °C. The similar experiment as performed with the dyed leather fabric was also accomplished for the non-dyed leather fabric [33-35]. The percentage-reductions in bacterial growth by the standard drug (Cefradine) and the synthesized formazan dyes (**18-23**) were expressed as follows:

$R = B - A/A \times 100$

Here, R = % reduction in bacterial population; B = absorbance (660 nm) of the media inoculated with microbial strains and undyed fabric; A = absorbance (660 nm) of the media inoculated with microbial strains and dyed fabric.

2.9. Antifungal potential evaluation of synthesized formazan dyes (18-23)

The synthesized formazan dyes (**18-23**) were assessed for their antifungal potential following the standard procedure stated by [36-39]. The fungal strains such as *Trichoderma harzianum* (ATCC 20846), *Aspergillus chevalieri*, (ATCC 9908), *Aspergillus candidus* (ATCC 34330), *Aspergillus flavus* (ATCC 9643), *Penicillium stipitatum* (ATCC 10500) and *Aspergillus niger* (ATCC 16404) were cast off for the determination of their antifungal potential. The fungous microbes were obtained from the microbiology laboratory of University of Veterinary and Animal Science Lahore, Pakistan. The standard drugs "Fluconazole and Terbinafine" were employed as a positive control for the comparative study of antifungal potential of the synthesized formazan dyes (**18-23**) against different fungal strains.

3. Results and discussion

In present research studies, the un-metallized formazan dye (18) and metal complex formazan dyes were manufactured by using metal salts of Iron (Fe) (19), Copper (Cu) (20), Chromium (Cr) (21), Nickle (Ni) (22), and Cobalt (Co) (23). The synthesized formazan dyes (18-23) were characterized by using elemental analysis, ¹H-NMR, ¹³C-NMR, powder XRD, FT-IR and UV-visible spectroscopy. The assessment of their performance as a colorant material was carried out through leather-processing drums on the leather fabric of goat.

3.1. Theoretical structural studies

Since last decade quantum chemical methods have attained the attention of scientific community because of their wide range applications in different fields [40-42]. Among quantum chemical methods, DFT is more popular due to its accuracy with low computational cost [43-45]. Nowadays chemists are widely using the DFT methods for studying the energy minima conformations and their geometrical parameters as well [46-49]. The most stable structures of all synthesized dyes (**18-23**) are optimized at B3LYP/6-311G(d,p) level of DFT and shown in Fig. 1. The important structural parameters (bond lengths and bond angles) of all dyes are given in the Table 1.

The important bond lengths in free ligand i.e. un-metallized formazan dye (**18**), such as N3-N5, N5-C7, N2-N6, N6-C7, N2-C8, O4-C9 and O5-C9 are 1.31, 1.31, 1.26, 1.39, 1.41, 1.35 and 1.20Å, respectively. The important bond angles such as N3-N5-C7, N5-C7-N6 and C7-N6-N2 are 121.6, 128.1 and 116.9°, respectively.

All metal complex formazan dyes (18, 19, 20, 22, 23) except (21) have distorted octahedron geometry mainly due to the presence of a tridentate ligand. The tridentate ligands, due to its

geometric constraints, forces the other three ligands (water molecules) to adopts position where the inter-ligand angles deviate from 90°. The Fe-metal complex formazan dye (**19**) has N-M-N bond angle of 87.81 degrees, which, in turn, results in N-M-O bond angles 93.18 and 100.40 degrees. Three water ligands make angles of 99.50, 168.75 and 86.76 degree with N3. Some other important bond angles in (**19**), such as N2-Fe1-N3, O4-Fe1-N2, N3-N5-C7, Fe1-N3-N5, Fe1-N2-N6, N5-C7-N6, C7-N6-N2, Fe1-O4-C9 and Fe1-N2-C8 are 87.8, 91.1, 121.1, 121.7, 121.7, 126.4, 119.7, 214.8 and 117.9°, respectively. The important bond lengths such as Fe1-N2, Fe1-N3, Fe1-O4, N3-N5, N5-C7, N2-N6, N6-C7, N2-C8, O4-C9 and O5-C9 (atomic labels are in accordance with Fig. 1) in (**19**) are 1.86, 1.93, 1.91, 1.30, 1.33, 1.28, 1.36, 1.42, 1.30 and 1.21Å, respectively. After complexation with Fe, there is change in geometric parameters of ligand. N2-N6 bond length is changed form 1.26 to 1.28Å, N6-C7 is changed from 1.39 to 1.36Å and O4-C9 is changed from 1.35 to 1.30Å. These all changes are due to reorientation and involvement of ligand in the complex formation with metal.

In Cu-metal complex formazan dye (20), the geometry is very much comparable to that of (19). For example, the important bond lengths around Cu, such as Cu1-N2, Cu1-N3, Cu1-O4 are 1.96, 1.93 and 1.90Å, respectively. These bond lengths are slightly lower than those of (19) which may be attributed to smaller van der Waals radius of Cu compared to Fe. The rest of bond lengths are in the similar range as are observed in the Fe-metal complex formazan dye (19). The important bond angles involving the Cu, such are N2-Cu1-N3, O4-Cu1-N2, Cu1-N3-N5, Cu1-N2-N6, Cu1-O4-C9, Cu1-N2-C8 and O4-Cu1-N2 are 89.4, 163.5, 122.7, 124.1, 122.8, 122.8 and 93.7° , respectively. The Cr-metal complex formazan dye (21) is different from the others because in this, the central Cr atom is surrounded by two molecules of un-metallized formazan dye (18). This structural change results in geometry very similar to a regular octahedron, which angles are close to 90 degrees. The important bond lengths around Cr, such as Cr1-N2, Cr1-N3, Cr1-O4, O10-Cr1 and Cr1-N11 in 21 are 1.91, 1.98, 1.88, 1.88 and 1.91Å, respectively. The rest of important bond lengths are in the similar as are observed in the previous two metallized dyes (19) and (20). The important bond angles in (21), as N2-Cr1-N3, O4-Cr1-N2, Cr1-N3-N5, Cr1-N2-N6, Cr1-O4-C9, Cr1-N2-C8, O10-Cr1-O4 and O10-Cr1-N11 are 86.1, 91.3, 120.6, 124.3, 127.4, 120.2, 89.7 and 91.2°, respectively. The important bond lengths and bond angles in Nickle (22) and Cobalt (23) metal complex formazan dyes are comparable to those of (19) and (20) (for detailed values see Table 1).



Dye 18

Dye 19





Dye 21



Fig. 1. The optimized geometries of all dyes (18-23), at B3LYP/6-311G(d,p) level of DFT

Table 1. The theoretical structural parameters (Bond lengths and bond angles) of all dyes (**18-23**) (atomic labelling is in accordance with the labels shown in the Fig. 1)

	Dye 18				Dye 19				
Bond length	(Å)	Bond angle	(⁰)	Bond length	(Å)	Bond angle	(°)		
				Fe1-N2	1.86	N2-Fe1-N3	87.8		
				Fe1-N3	1.93	O4-Fe1-N2	91.1		
		N3-N5-C7	121.6	Fe1-O4	1.91	N3-N5-C7	121.1		
N3-N5	1.31			N3-N5	1.30	Fe1-N3-N5	121.7		
N5-C7	1.31			N5-C7	1.33	Fe1-N2-N6	121.7		
N2-N6	1.26	N5-C7-N6	128.1	N2-N6	1.28	N5-C7-N6	126.4		
N6-C7	1.39	C7-N6-N2	116.9	N6-C7	1.36	C7-N6-N2	119.7		

N2-C8	1.41			N2-C8	1.42	Fe1-O4-C9	124.8	
O4-C9	1.35			O4-C9	1.30	Fe1-N2-C8	117.9	
O5-C9	1.20			O5-C9	1.21			
	Ι	Dye 20		Dye 21				
Cu1-N2	1.96	N2-Cu1-N3	89.4	Cr1-N2	1.91	N2-Cr1-N3	86.1	
Cu2-N3	1.93	O4-Cu1-N2	163.5	Cr1-N3	1.98	O4-Cr1-N2	91.3	
Cu1-O4	1.90	N3-N5-C7	122.2	Cr1-O4	1.88	N3-N5-C7	122.6	
N3-N5	1.29	Cu1-N3-N5	122.7	N3-N5	1.28	Cr1-N3-N5	120.6	
N5-C7	1.33	Cu1-N2-N6	124.1	N5-C7	1.34	Cr1-N2-N6	124.3	
N2-N6	1.28	N5-C7-N6	127.7	N2-N6	1.27	N5-C7-N6	129.2	
N6-C7	1.35	C7-N6-N2	123.8	N6-C7	1.35	C7-N6-N2	121.4	
N2-C8	1.42	Cu1-O4-C9	122.8	N2-C8	1.41	Cr1-O4-C9	127.4	
O4-C9	1.28	Cu1-N2-C8	122.8	O4-C9	1.30	Cr1-N2-C8	120.2	
O5-C9	1.23	O4-Cu1-N2	93.7	O5-C9	1.21	O10-Cr1-O4	89.7	
				O10-Cr1	1.88	O10-Cr1-N11	91.2	
				Cr1-N11	1.91			
	Ι	Dye 22		Dye 23				
Ni1-N2	1.84	N2-Ni1-N3	91.2	Co1-N2	1.85	N2-Co1-N3	91.1	
Ni1-N3	1.85	04-Ni1-N2	94.5	Co1-N3	1.88	O4-Co1-N2	95.7	
Ni1-O4	1.83	N3-N5-C7	120.7	Co1-O4	1.85	N3-N5-C7	120.7	
N3-N5	1.28	Ni1-N3-N5	126.1	N3-N5	1.30	Co1-N3-N5	126.0	
N5-C7	1.33	Ni1-N2-N6	123.7	N5-C7	1.33	Co1-N2-N6	124.8	
N2-N6	1.28	N5-C7-N6	125.9	N2-N6	1.28	N5-C7-N6	127.0	
N6-C7	1.34	Ni1-O4-C9	129.5	N6-C7	1.35	C7-N6-N2	123.5	
N2-C8	1.43	Ni1-N2-C8	123.1	N2-C8	1.43	Co1-O4-C9	125.3	
O4-C9	1.28	C8-N2-N6	112.8	O4-C9	1.29	Co1-N2-C8	122.9	

O5-C9	1.23		C9-O5	1.23	C8-N2-N6	112.1

3.2. FTIR spectral analysis

The fourier-transform infrared spectroscopy (FT-IR) considered as a valuable basis for accomplishing the structural information of organic nature based compounds, therefore the recognition and identification of different functional groups of the synthesized formazan dyes (18-23) was carried out by employing this analytical technique. The FT-IR data of the synthesized formazan dyes (18-23) is presented in Fig. 2. The results of FT-IR spectra were demonstrated that the sharp peaks at 1570-1615 cm⁻¹ were exhibited by the synthesized formazan dyes (18-23) which were indicted the manifestation of C=C (Aromatic stretching). The small peaks at 1520-1560 cm⁻¹ in the spectra of the synthesized formazan dyes (18-23) were designated the presence of C=N (Stretching) which affirm the formation of formazan dye as shown in Fig. 2 (a). Further, the small peaks were perceived at 1365-1475 cm⁻¹ in their spectra, that were demonstrating the existence of N=N (Stretching). The broad peak in the spectra of synthesized un-metallized formazan dye (18) at 3380 cm⁻¹ revealed the existence of N—H stretching. The sharp peaks experimental at 820-860 cm⁻¹ in their spectra illustrated the presence of C-N=N-C skeleton, which were also acknowledged and confirmed the formation of desired formazan dyes (18-23) successfully. The peaks that were perceived at 1230-1470 cm⁻¹, 1610-1715 cm⁻¹, 2930 cm⁻¹ in their spectra were found to acknowledge the existence of -S₃OH, Ar-C=O, and OH (Carboxylic acid stretching) respectively. A broad and sharp peak detected at 3410-3460 cm⁻¹ that was attributed to OH functional group. The IR peaks for OH (Carboxylic acid stretching) and N-H were only detected before metallization in the spectra of dye (18) that performances as ligand as presented in Fig. 2 and both IR peaks vanish after the metallization as in the synthesized formazan dyes (19-23). The IR peaks of M-O were found to perceived only after the metallization of dye (18) with metal like Fe, Cu, Cr, Ni, and Co in their respective metal complex formazan dyes (19-23). The formation and manifestation of metal-oxygen bond in terms of metal ligand bond to form metal complex formazan dyes (19-23) was confirmed from the small peaks observed at 650-690 cm⁻¹ in their spectra as presented in Fig. 2 (b). Furthermore, the IR peaks of OH (stretching) were only experimental in metal complex formazan dyes (19, 20, 22 and 23) as these are (1:1) metal complexes of Fe, Cu, Ni and Co respectively. Whereas that IR peak of OH (stretching) were not observed the in spectra of synthesized formazan dye (21) as,



this dye was (2:1) metal complex of Cr. Hence, FT-IR spectra outcomes were magnificently confirmed the synthesis of formazan dyes (18-23) and were founded in close agreement with the [3].

Fig. 2. The FT-IR spectra of the synthesized un-metallized formazan dye (18) (a) and metal complex formazan dye (19) (b)

3.3. ¹H-NMR spectral analysis

The ¹H-NMR spectroscopy is most expedient and viable analytical tool for the identification and structure elucidation of a molecule, with respect to hydrogen-1 nuclei inside the molecule of a substance to be analyzed; therefore, we further employed the ¹H-NMR spectroscopy to get more-insight structural information and confirmation of our desired synthesized formazan dyes (**18-23**). The results of ¹H-NMR spectral studies of the synthesized formazan dyes (**18-23**) were

demonstrated that the chemical shift values (δ : 7.60-7.91) were attributed for the (m 13H, Ar-H) of the synthesized un-metalized formazan dye (18) as presented in Fig. 3 (a). While, the chemical shift values (δ : 8.26-8.70), (δ : 8.30-8.80), (δ : 8.15-8.65), (δ : 8.30-8.75) for the (m 13H, Ar-H) and (δ : 8.25-8.75) for the (m 26H, Ar-H) were designated for the synthesized metal complex formazan dyes Fe (19), Cu (20), Ni (22), Co (23) and Cr (21) 2:1 respectively (Fig. 3 b). The results were displayed that the chemical shift values of multiplet of aromatic hydrogens (m 13H, 26H, Ar-H) in the metal complex formazan dyes (19-23) were shifted towards lower field than the synthesized un-metallized formazan dyes (18). This is because of the fact that an electrondonating group amine (N-H) was substituted to the aromatic ring of the synthesized formazan dye (18) that was responsible for shifting the chemical shift (δ) value to higher field. The literature studies indicate that the electron-withdrawing group (-SO₃H, -COOH, -NO₂) are promising for shifting the chemical shift values to lower field while the electron-donating groups (-NH₂, CH₃, -OH) are liable for shifting the chemical shift (δ) value to higher field. Conversely, amine (N-H) group were absent in all other synthesized metal complex formazan dyes (19-23). Moreover, the chemical shift peaks value for (s 1H, SO₃H) was designated at (11.15-12.18) in all synthesized formazan dyes (18-23). The ¹H-NMR spectral investigations were also illustrated and inveterate the formation of metal complexes with the un-metallized formazan dye (18), were acting as a ligand. As two functional groups such as amine (N-H) and carboxylic acid attached to the aromatic ring (Ar-COOH) were presenting the chemical shift (δ) values for their hydrogen nuclei at (1.498 and 11.30) only in synthesized un-metallized formazan dye (18) respectively but were not showing their chemical shift peaks in the synthesized metal complex formazan dyes (19-23). This is because of the fact that the amine (N-H) and carboxylic acid attached to the aromatic ring (Ar-COOH) were involved in the formation of metal-oxygen and metal nitride coordinate covalent bond with the metals (Fe, Cu, Cr, Ni and Co) with the elimination of their hydrogen to form their respective metal complex. This observation evidently and admissibly has inveterate the formation of coordinate covalent bond among non-metal complex formazan dye ligand (18) and metals (Fe, Cu, Cr, Ni, and Co) in metal complex formazan dyes 19, 20, 21, 22, and 23 respectively. Hence, ¹H-NMR and FT-IR spectral studies were successfully avowed the synthesis of our desired formazan dyes and current research outcomes were found in agreement with the earlier reported studies [3].



Fig. 3. The ¹H-NMR spectra of synthesized un-metallized (**18**) and metal complex (**19**) formazan dyes (a and b) and ¹³C-NMR spectrum of synthesized un-metallized (**18**) (c) recorded in CDCl₃

at 25 °C

3.4. UV-visible spectral investigation

The absorption maxima for the synthesized un-metallized formazan dye (18) and metal complex formazan dyes (19, 20, 21, 22 and 23) was observed in distill H₂O for their UV-visible spectral investigation. Results were demonstrated that the distinctive three peaks of absorption maxima were perceived in the UV-vis spectrum of synthesized formazan dyes (18-23). It has been observed from the spectrum pattern that value of λ_{max1} was assigned and specific for the framework (-C-N=N-C-) of formazan dyes (18-23) and because of that fact, current research studies relates to the λ_{max1} value. The formazan peak value of λ_{max1} is perceived typically at the wavelength range of 410-500 nm. Sometimes these peaks could be redirected probably at the wavelength range of 350-600 nm, based on the molecular structure of the formazan and their peak absorption is resulted due to two different types of electronic transition in the framework of the formazan such as $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition. The values of λ_{max2} are generally give their impression at the wavelength range of 300 nm to 350 nm, which are assigned for the electronic transitions of azo group (-N=N-), is present in the molecule of formazan dyes. Furthermore, the values of λ_{max3} are typically observed at the wavelength range of 270 nm to 300 nm that are resulted because of $n \rightarrow \pi^*$ transition of azomethine (-C=N-) functional group in the molecules of formazan dyes. Results of UV-visible spectral investigation were disclosed that the synthesized formazan dyes (18-23) consisted of stated identical chromophoric functionalities. However, the large change in the value of λ_{max} were perceived in the form of their bathochromic and hypochromic shift when un-metallized complex formazan dye (18) were converted to metal complex formazan dyes (19-23) through metallization process. The results were exhibited that the absorption maxima values of λ_{max1} for the synthesized formazan dyes (18-23) were detected at 550 nm, 490 nm, 460 nm, 430 nm, 420 nm and 410 nm respectively. While the absorption maxima values of λ_{max2} and λ_{max3} for the synthesized formazan dyes (18-23) observed at the wavelength of (360 nm, 350 nm, 345 nm, 340 nm, 335 nm and 330 nm) and (230 nm, 225 nm, 220 nm, 225 nm, 215 nm and 210 nm) respectively and these observations were designated that there was found a shift of longer wavelength to shorter wavelength (Hypsochromic effect) as presented in Fig. 4. The reason behind the blue shift mechanism of metal complex formazan dyes

(19-23) than non-metal complex formazan dye (18), the metal behave as Lewis acid and pull out the electron density towards itself from the ligands that ultimately lead the transformation of longer wavelength to shorter wavelength. Results were revealed that for the metal complex formazan dye (19), a blue shift of 60 nm was experimental with the color transformation from yellowish brown to dark reddish brown from its non-metal complex formazan dye (18). Similar hypsochromic effect was also observed in all synthesized metal complex formazan dyes i.e. Cu (20), Cr (21), Ni (22) and Co (23) with the blue shift of 90 nm, 130 nm, 160 nm and 205 nm respectively with color transformation from yellowish brown to cyan, greyish beige, indigo and violet respectively.



Fig. 4. The UV-Visible spectra for the synthesized un-metallized and metal complex formazan dyes (18-23)

3.5. Elemental analysis

The elemental analysis technique implicates to determine mass of different fractions associate with Carbon, Hydrogen, Nitrogen, heteroatoms such as (F, Cl, Br, I, At), Sulfur and metal atoms of the analyzed compound. The elemental analysis technique also known as CHNS analysis technique, which provide valuable and significant knowledge in structure elucidation of an unknown molecule. Moreover, by employing CHNS analysis technique, purity level of newly synthesized compound can also be verified [3]. Therefore, we employed elemental analysis technique to get more deep knowledge and confirmation about the structure of our synthesized formazan dyes (18-23); their outcomes presented in spectral studies section evidently revealed the correlation between the calculated and experimentally founded mass of different fractions associate with the synthesized formazan dyes (18-23). Henceforth, elemental analysis data and

spectral investigates were successfully corroborated the structures of our synthesized formazan dyes (18-23) demonstrated in scheme 2.

3.6. Powder-XRD investigations

The synthesized formazan dye [2-((phenyl (2-(4-sulfophenyl) hydrazono) methyl) diazenyl) benzoic acid] (18) and its respective synthesized metal complex formazan dyes (19-23) were assessed for single crystal growth in different kinds of solvents such as dimethyl formamide (DMF), ethyl alcohol and chloroform but remained fruitless. Therefore, powder X-ray diffraction was employed to find out more they are either amorphous or crystalline in nature. The nature of lattice parameters, crystal system and cell volume of the synthesized formazan dyes (18-23) was determined through X-ray powder di a raction analysis. The results of XRD pattern i.e. sharp and intense peaks with no broadening were evidently exhibited that the synthesized formazan dyes (18-23) were crystalline in nature (Fig. 5). Further, a High Score Plus software was employed to execute their di araction indexing patterns and unit cell parameters were determined from the indexed data enlisted in Table 2. Moreover, it has been observed from the powder XRD investigations that XRD patterns of synthesized metal complex formazan dyes (19-23) and unmetallized formazan dye (18) were entirely di erent from each other, which were authenticating the formation of coordination compounds successfully. From the results, it has been found that the monoclinic structure was demonstrated by synthesized formazan dyes (18, 19, 20, 22 and 23) however, orthorhombic structure was presented by dye (21); the monoclinic and orthorhombic crystal structures were reported for similar type of compounds by [50-52,33]. Furthermore, the mean crystallite sizes "D" of the synthesized formazan dyes (18-23) were calculated from di raction data. According to the Scherrer equation (D = $0.9\lambda/(\beta \cos\theta)$, where λ is X-ray wavelength (1.5406 °A), θ is Bragg di \Box raction angle, and β is the full width at half maximum of the di raction peak) [53,54]. The average crystallite size 39 nm to 80 nm were observed for all the synthesized formazan dyes (18-23) (Table 2).



Fig. 5. The XRD-spectra of synthesized un-metallized and metal complex formazan dyes (18-23)Table 2. The XRD data of the synthesized formazan dyes (18-23).

Dye#		Lattice P	arameters		Volume	Crystallite	Crystal
					(A ³)	size D	system
	a (A)	b (A)	c (A)	β (°)		(nm)	
18	11.0421	17.9426	9.2121	101.2034	1189.727	63	Monoclinic
19	13.2111	6.1211	10.0991	100.0103	490.901	70	Monoclinic
20	14.8888	11.8583	9.8097	90.098	1401.872	52	Monoclinic
21	15.9812	13.1231	10.0821	110.0873	1503.087	39	Orthorhombic
22	17.1212	8.2332	14.0987	112.3120	1531.312	80	Monoclinic
23	18.1101	8.8981	16.2003	114.0863	1940.051	49	Monoclinic

Values are mean \pm SD triplicate assays.

3.7. Assessment of color properties of synthesized formazan dyes (18-23)

3.7.1. Fastness properties

The synthesized formazan dyes (18-23) were evaluated for their color shade development along with the assessmet of different kinds of fastness properties such as color change, staining on adjacent fibre, light, wash and perspiration on leather fabric of goat. The results of their fastness properties are presented in Table 3. Results shows that the synthesized formazan dyes (18, 19, 20, 21, 22 and 23) developed different kinds of color shades such as Red, Brown, Green, Black, and Blue with different types of color tones like Bright brick, Reddish, Bluish, Greyish and Greenish on leather respectively (Fig. 6). Results were demonstrated that average wash fastness (3-4) were accounted for the synthesized un-metallized formazan dye (18) while good wash

fastness (4-5, 4-5, 5, 4 and 4-5) were experimented for metal complex formazan dyes (19-23) respectively. The wash fastness is the oppressive potency put forth by the dyed fabric to keep its color on the course of its washing with either soap or detergent. The synthesized metal complex formazan dye (21) unveiled the significant and improved wash fastness features. Furthermore, good to excellent fastness properties such as color change, staining on adjacent fiber, light and perspiration were demonstrated by synthesized un-metalized formazan dye (18) and metal complex formazan dyes of Fe (19), Cu (20), Cr (21), Ni (22) and Cobalt (23) on leather fabric (Table 3). The fastness characteristics of the dye molecules are directly influence by the physical and chemical interaction of dyes to the applied substrate (leather). A direct relationship exist between the fastness properties of color change, staining on adjacent fibre, wash, light, and penetration with the physical and chemical interactions of dyes to the applied substrate [55,56]. As the more are, the electrostatic interactions then rapid diffusion of dyes takes place to the substrate underneath depth; more will be the penetration level and all dyes (18-23) were shown the excellent penetration level [55,56]. Hence, the synthesized formazan dyes (18-23) possess all significant to be good enough at all tested level. Figure 6 exhibited the color shades developed on goat leather fabric by the application of synthesized formazan dyes (18-23).



Fig. 6. Application of synthesized formazan dyes (18-23) on leather at 2% depth Table 3. The fastness properties of synthesized formazan dyes (18-23).

	Staining on	Color	Penetration	Wash	Light	Perspiration
Dye #	adjacent Fiber	Change		Fastness	Fastness	Fastness
18	4-5	4-5	4-5	3-4	3-4	4

19	4-5	5	5	4-5	4-5	4-5
20	5	4-5	4-5	4-5	4	5
21	5	4-5	5	5	4-5	5
22	4-5	4-5	4-5	4	4	4-5
23	5	4-5	5	4-5	4-5	5

Values are mean \pm *SD triplicate assays.*

3.7.2. Fixation and exhaustion properties

The fixation and exhaustion properties of the synthesized formazan dyes (18-23) were evaluated by their application at the strength of 2% with the reference of dry crust weight of leather; and their results are showed in Table 4. The physical interactions (hydrogen bonding) and chemical interactions (ionic bond) were the driving forces, which perform their role for the fixation of synthesized formazan dyes (18-23) to the substrate of goat leather fabric, however the concentration of the applied dye molecules perform significant role for the exhaustion properties [55,56]. Results were demonstrated that percentage of exhaustion (92-97%) and fixation (90-98%) were perceived on leather fabric dyed by 2% of the synthesized formazan dyes (18-23) respectively. It has been observed that synthesized formazan dye (21) unveiled the maximum exhaustion and fixation properties (97 and 98 %) but the minimum (92 and 90%) were exhibited by synthesized formazan dye (18) respectively (Table 4). Results shows that extraordinary and significant fixation and exhaustion properties were revealed by synthesized formazan dyes (18-23) which were demonstrating their rapid diffusion onto the leather fabric under the dying condition and parameters. In addition, a factor of strong physical and chemical interactions were also persistent between them, which enable the dye molecules to show enhanced fixation and exhaustion properties (Fig. 7). The strong physical phenomenon was exhibited by synthesized formazan dyes (18-23) because of the existence of strong polar groups such as (-SO₃H, -OH and -COOH) (Scheme 2) which were made strong hydrogen bonding with the functionalities of leather as chemical nature of leather is a protein, polyamide and nylon [20,55,56]. Previously reported literature shows that the physical instruction between dye molecule and leather fiber become stronger with the increasing number of polar group's substitution to the dye molecules [33,55]. Whereas, during the dyeing process, the strong chemical interaction was developed with the formation of ionic bond between the synthesized formazan dyes (**18-23**) acting as negative (anionic) charge and leather fabric behaving as positive (cationic) charge [55]. Hence, because of these two factors, the exhaustion and fixation properties of the synthesized formazan dyes were increased. This was concluded from application results, the synthesized metal complex formazan dyes (**19-23**) have extraordinary fixation and exhaustion properties than un-metallized formazan dye (**18**); and chromium metal complex formazan dye (**21**) were shown the best properties than metal complexes formazan dyes of Fe (**19**), Cu (**20**), Ni (**22**) and Co (**23**). A close agreement was observed among the results of fastness, fixation and exhaustion properties. As between these properties, there is found a direct relationship that depends upon the electrostatic interactions that is the factor by which rapid diffusion of dyes takes place to the substrate underneath depth [33,55,56]. More is the fixation and exhaustion, more will be the fastness properties and poor is the fixation and exhaustion, lower will be the fastness properties [55].

	Shade on	$\lambda_{\max}(\mathbf{nm})$	Fixation	Exhaustion
Dye #	Leather	in H ₂ O	%(C)	%(C)
18	Bright Red	551	90	92
19	Reddish Brown	505	92	91
20	Bluish Green	475	94	94
21	Greyish Black	456	98	97
22	Bluish Red	439	97	96
23	Reddish Blue	421	96	95

Table 4. The exhaustion and fixation properties of the synthesized formazing	an dyes	(18-23)
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Values are mean \pm *SD triplicate assays.*



Fig. 7. Interaction phenomenon between synthesized formazan dyes (18-23) and leather fabric

3.8. Antibacterial potential evolution

3.8.1. Antibacterial potential of synthesized formazan dyes (18-23) in solution

The antibacterial potential of synthesized formazan dyes (18-23) was determined against different bacterial strains such as of gram-positive and gram-negative bacteria in solution; and their outcomes were showed that growth of all bacterial strains were effectively and strongly inhibited by them. Results were demonstrated that the effective antibacterial propensity against all bacterial strains was displayed by the synthesized un-metallized formazan dye (18). Moreover it has been observed that the un-metallized formazan dye (18) were manifested the comparable antibacterial potential results to the employed standard drug in terms of their ZOIs values against all employed bacterial strains (Fig. 8). While, the synthesized metal complex formazan dyes (19-23) were found to forbid the development of bacteriological strains significantly than that of standard drug (Cephradine) and formazan dye (18). Among synthesized metal complex formazan dyes such as Fe (19), Cu (20), Ni (22) and Co (23), substantial antibacterial propensity was revealed by chromium complex formazan dye (21) which was exhibited the maximum ZOIs values (19 \pm 0.13 mm, 25 \pm 0.10 mm, 23 \pm 0.11 mm and 27 \pm 0.10 mm) against of all tested bacterial strains (E. coli, S. aureus, Klebsiella and B. subtilis) respectively (Fig. 8 and 9). Furthermore, against each bacteriological strain, the value of minimum inhibitory concentration (MICs) for each synthesized formazan dyes were also determined in (mg/mL) and synthesized formazan dye (21) was demonstrated the 0.24, 0.08, 0.09 and 0.07 mg/ml of MICs against E. coli, S. aureus, Klebsiella and B. subtilis respectively (Table 5). It has been observed that MICs

results of all the dyes were analogous with the standard drug. Hence, it was apparent and concluded from the results of ZOIs and MICs that the nature of synthesized formazan dyes is bactericidal and bacteriostatic that enable them to exhibit significant antibacterial potential in solution.

 Table 5. The minimum inhibitory concentration (MICs) of synthesized formazan dyes (18-23)

 against each bacteriological strain

Bacterial	Minimum inhibition concentration (mg/mL)						
Strains	Standard	Dye 18	Dye 19	Dye 20	Dye 21	Dye 22	Dye 23
E. coli	0.30	0.35	0.27	0.28	0.24	0.29	0.27
S. aureus	0.10	0.12	0.09	0.10	0.08	0.08	0.11
Klebsiella	0.14	0.16	0.13	0.12	0.09	0.11	0.13
B. subtilis	0.08	0.09	0.07	0.06	0.07	0.07	0.08

Values are mean \pm SD triplicate assays.



Fig. 8. The antibacterial potential of synthesized formazan dyes (18-23) against different

bacterial strains in solution

3.8.2. Antibacterial activity of synthesized formazan dyes (18-23) on substrate (Leather fiber)

Because of the substantial and domineering antibacterial potential demonstration by the synthesized formazan dyes (**18-23**) in solution, further, we evaluated them for their antibacterial propensity on dyed leather fabric to check either they are also active on the substrate to prevent

the growth of microbes. For this, we were employed the dyed leather fiber of goat with the synthesized formazan dyes (**18-23**) as a model system and their results in the form of percentage reduction in bacterial growth are presented in Fig. 9. It has been observed from the result that the synthesized metal complex formazan dye (**21**) (**Cr**) exhibited the maximum percentage reduction in bacterial growth on the dyed leather fiber while minimum was demonstrated by the synthesized un-metallized formazan dye (**18**). However, results were revealed that comparable percentage reduction in bacterial growth was shown by all the synthesized formazan dyes (**18-23**) to that of standard drug. The synthesized formazan dye (**21**) were found to demonstrate the percentage reduction in bacterial growth such as $60 \pm 0.03\%$, $69 \pm 0.07\%$, $80 \pm 0.05\%$ and $86\pm0.08\%$ against *E. coli, S, aureus, Klebsiella* and *B. subtilis* while standard drug showed the percentage reduction in bacterial growth such as $55 \pm 0.07\%$, $61 \pm 0.06\%$, $69 \pm 0.09\%$, and $71\pm0.05\%$ respectively. Both the results of antibacterial activities of the synthesized formazan dyes (**18-23**) in solution and on substrate (dyed leather) were found to support each other. Therefore, it has been concluded that nature of the synthesized formazan dyes (**18-23**) is antibacterial and bacteriostatic.





position of functional groups to the benzene ring [55,57]. Literature studies reveal that the electron-withdrawing groups are responsible to enhance the antibacterial potential of the molecule, as they are act as electron deficient while bacterial cell wall as a electron rich center and in addition, *ortho* and *para* positions are considered more promising to exhibit enhanced antibacterial propensities than *meta* position due to the stability factor [57,58]. In our current research work, electron-withdrawing groups such as -SO₃H and -COOH are functionalities substituted on *para* and *ortho* position while metal within the structure of dyes molecules (**18-23**). Because of these factors, our synthesized formazan dyes demonstrated the robust and noteworthy bactericidal potential counter to *E. coli*, *S, aureus*, *Klebsiella* and *B. subtilis*.

3.9. Antifungal potential determination

The outcomes of antifungal propensity of the synthesized formazan dyes (18-23) are presented in Fig. 10 and 11 in the form of their ZOIs and MICs values respectively. Results were indicated that the synthesized un-metallized formazan dye (18) were demonstrated the satisfactory and comparable results of antifungal propensity in counter to the employed standard drugs such as "Fluconazole and Terbinafine" against all fungoid strains (T. harzianum, A. chevalieri, A. candidus, A. flavus, P. stipitatum and A. niger). However, it has been observed from the results that the synthesized metal complex formazan dyes (19-23) exhibited the significant and operative antifungal propensity against all employed mycological strains than that of Fluconazole and Terbinafine (Fig. 10). Moreover, among employed standard antifungal drugs, Terbinafine was found to inhibit the growth of all types of fungal strains powerfully and efficiently than Fluconazole. ZOIs of Terbinafine and Fluconazole were (24 ± 0.03 mm, 28 ± 0.09 mm, $26 \pm$ 0.08 mm, 32 ± 0.07 mm, 30 ± 0.05 mm and 31 ± 0.06 mm) and $(22 \pm 0.08$ mm, 24 ± 0.09 mm, 26 ± 0.07 mm, 28 ± 0.05 mm, 27 ± 0.06 mm, and 29 ± 0.09 mm) against all mycological strains such as T. harzianum, A. chevalieri, A. candidus, A. flavus, P. stipitatum and A. niger respectively. Furthermore, the maximum fungal growth inhibition results among synthesized metal complex formazan dyes (18-23) were demonstrated by the synthesized metal complex formazan dye (21) of chromium based against all mycological strains (T. harzianum, A. chevalieri, A. candidus, A. flavus, P. stipitatum and A. niger) by presenting their maximum ZOIs values such as 28 ± 0.03 mm, 32 ± 0.05 mm, 30 ± 0.09 mm, 36 ± 0.04 mm, 37 ± 0.01 mm and 38 \pm 0.07 mm respectively. The MICs values such as 0.11, 0.08, 0.09, 0.07, 0.08 and 0.06 mg/mL were fashioned by the synthesized formazan dye (21) while 0.13, 0.10, 0.14, 0.08, 0.09, and 0.07 mg/mL MICs values were exhibited by stnadrd drug "Terbinafine" against T. harzianum, A. chevalieri, A. candidus, A. flavus, P. stipitatum and A. niger respectively. Hence, the synthesized formazan dyes (18-23) were found to exhibit very effective broad-spectrum antifungal propensity. The reason behind the demonstration of significant and enhanced broad-spectrum antifungal propensity was the substitution of structural moiety such as electron-withdrawing groups on *para* and *ortho* position on the benzene ring (Scheme 2). Reported literature data describe that the antifungal propensities depend on the substitution and position of functional groups to the benzene ring [55-57]. Moreover, literature studies disclose that the electronwithdrawing groups such as -SO₃H, -COOH, -NO₂, -C≡N, -CF₃, -SO₂CF₃, -CCl₃ and somehow halides than that of electron-donating groups are liable to enhance the fungicidal potential of the molecule, as they act as electron-deficient center while fungi cell wall as a electron-rich center and in addition, *ortho* and *para* positions are considered to be more auspicious to display boosted fungicidal propensities than meta position because of the stsability factor of the molecule [55,57]. As for the electron-withdrawing functional groups, ortho and para positions of the benzene are more stable than that of meta [58,59]. In our current research work, electronwithdrawing groups such as -SO₃H and -COOH are functionalities substituted on *para* and *ortho* position while metal within the structure of dyes molecules (18-23). Henceforth, because of these factors, our synthesized formazan dyes corroborated the significant and remarkable broadspectrum antifungal potential counter to T. harzianum, A. chevalieri, A. candidus, A. flavus, P. stipitatum and A. niger.



Fig. 10. The antifungal potential of synthesized formazan dyes (18-23) against different fungal strains



Fig. 11. The MICs values of synthesized formazan dyes (18-23) against different fungal strains

4. Conclusion

We magnificently and successfully synthesized our desired un-metalized (18) and metal complex formazan dyes (19-23) in water without employing any buffer and organic solvents. Furthermore, theoretical calculations also have been performed to gain structural insight of these synthesized metal complex formazan dyes and the metal complexes (19), (20), (22) and (23) have distorted octahedral geometry mainly due to the geometric constraints posed by one tridentate ligand while the metal complex (21) has regular octahedron geometry because both tridentate ligands have counter balancing effect. Hence, color shades developed on leather fabric by novel formazan dyes (18-23) are intense and had outstanding color properties like color change fastness, staining on adjacent fiber fastness, light fastness, wash fastness, perspiration fastness with their excellent penetration demonstration into the leather fabric. Consequently, these newly synthesized formazan dyes possess remarkable optical properties and have mammoth potential to be commercialize. Another substantial and remarkable finding of this research work was the noteworthy antibacterial and antifungal potential demonstrated by all the synthesized formazan dyes (18-23) in solution and on leather substrate. This research work were also corroborated that the synthesized formazan dyes by their oneself have ability to increase the life-time of leather fabric by precluding them from bacteriological and mycological effects and thus, no need the usage of additional antibiotics and antifungal agents with them. In conclusion, we are reporting that this research work will prove valuable and appreciable for the development of new industrial products.

DECLARATIONS

Acknowledgement

The Department of Chemistry, School of Science, University of Management and Technology, Lahore, Pakistan and Shafi Reso Chemical Lahore, Pakistan were supported this research work (UMT/CHM/RES/2018-002). This research work did not any receive any specific grant from funding agencies in Public, Commercial or not-for-profit sectors.

Conflict of interest

No conflict of interest associated with this work.

Contributions of authors

Shakeel Ahmad Khan, Sammia Shahid and Sadia Kanwal significantly contributed to this research work i.e. experimental work; analysis and manuscript drafting. Komal Rizwan, Tariq Mahmood and Khurshid Ayub were contributed towards computational studies.

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Figure captions

Scheme 1: The synthesis of 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid (5)
Scheme 2: The preparation of non-metal complex formazan dye (18) and metal complex formazan dyes of Iron (19), Copper (20), Chromium (21), Nickel (22) and Cobalt (23)

Fig. 1. The optimized geometries of all dyes (18-23), at B3LYP/6-311G(d,p) level of DFTFig. 2. The FT-IR spectra of the synthesized un-metallized formazan dye (18) (a) and metal complex formazan dye (19) (b)

Fig. 3. The ¹H-NMR spectra of synthesized un-metallized (**18**) and metal complex (**19**) formazan dyes (a and b) and ¹³C-NMR spectrum of synthesized un-metallized (**18**) (c) recorded in CDCl₃ at 25 °C

Fig. 4. The UV-Visible spectra for the synthesized un-metallized and metal complex formazan dyes (18-23)

Fig. 5. The XRD-spectra of synthesized un-metallized and metal complex formazan dyes (18-23)Fig. 6. Application of synthesized formazan dyes (18-23) on leather at 2% depth

Fig. 7. Interaction phenomenon between synthesized formazan dyes (18-23) and leather fabric

Fig. 8. The antibacterial potential of synthesized formazan dyes (18-23) against different bacterial strains in solution

Fig. 9. The percentage reduction in bacterial growth by the synthesized formazan dyes (18-23)

Fig. 10. The antifungal potential of synthesized formazan dyes (18-23) against different fungal strains

Fig. 11. The MICs values of synthesized formazan dyes (18-23) against different fungal strains

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Research Highlights

- ♦ Novel metal complex formazan dyes (18-23) were synthesized in aqueous system.
- * They produced Blue, Violet, Green, Brown and Red coloration on leather.
- ✤ Formazan dyes presented excellent fastness, fixation and exhaustion properties.
- * They poses enormous antimicrobial potential in solution and on leather against microbes.
- ✤ The newly prepared formazan dyes can be used as potential antimicrobial agents.