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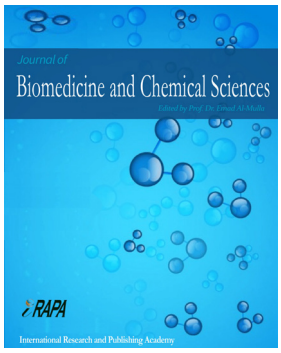

Analytical Studies of New Azo dye and the Diminished in their Biological Activity by Complexation with Zinc

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ABSTRACT

Azo dye (1), that characterized (Z)-5-amino-2-(((4-(5-(p-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl) phenyl) sulfonyl) diazenyl) phenol) was prepared. Then characterized using m.p., IR, UV-visible, mass spectrum and elemental analysis (CHN). Analytical studies approved on this dye; which showed high solubility in ethanol and gave three isopiestic points, that when studying the pH effect in a range of buffer solutions. The protonation constants pKb1 and pKb2 of nitrogen atom and the ionization constant pKa of OH-group were equal to 3.4, 7.26 and 10.5 respectively. These results indicated the suggested ionization scheme in acidic and basic media. However, due to recommend the new azo dye as novel medicine or chemical sanitizer especially in dual infection. the complex of azo dye (1) with Zn was decreased the biological activity of dye.

INTRODUCTION

Azo dyes have received a great attention in scientific research, these compounds extremely importance in the chemical analysis (Kirkan & Gup, 2008; Majeed, 2013). The structural features of azo compounds, which characteristically produce a colour are C=C, N=O, N=N, aromatic rings, C=O and NO_2. Though, the groups that responsible to make variation in the colour are the azo (–N=N-) and nitroso (– N=O), but the other groups really do accordingly under certain conditions. Analytical studies of many azo dyes and diazo dyes were the area of interest (Ali et al., 2017; Ali et al., 2018; Fayadh et al., 2015). These compounds were verified to offer multiple uses in volumetric analysis, especially those that have different colours in the acidic and basic media (Majeed, 2013). A powerfully coloured dyes were envisioned for their pharmaceutical status as antidiabetic, antineoplastic, antibacterial (Mutar & Ali, 2021) and anticancer agent such as their effect in human breast MDA-MB231 cancer cells through its ability to destroy the DNA of the cancer cells (Majeed, 2013; Ali et al., 2017) and known to be involved in the inhibition of DNA, RNA, carcinogenesis, and protein synthesis. The presence of -N=N- in the molecular structure of azo dyes may responsible for their interaction with the active site of the protein.

Experimental Section

Melting point of azo dye attended using Buchi B190K and the IR spectrum carried on aFT-IR-8400S. Fourier Transform Infrared Spectrophotometer Shimadzu (Japan) by using a KBr disc at a range (600 – 4000) cm^{-1}. Absorption spectrum in ethanol, (1 x 10^{-4} M) determined on a spectrophotometer. These measurements were done in Chemistry Department–Education College of pure science at Basrah University, Iraq. But, the accurate mass spectrum and the C.H.N

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were measured in Tehran University in Iran.

Preparation of Synthetic Azo dye (1) and their Complex with Zinc

Azo dye (1), that named (*Z*)-5-amino-2-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl) phenyl) sulfonyl) diazenyl) phenol was prepared using celecoxib drug, (0.006 mol.,2.288 g), which mixed with HCl (1.75 mL) and 10 mL of distilled water, (1st solution) (Farghaly & Abdallah, 2008; Primo & Fröhlich, 2005). Then, the 2nd solution was prepared by mixing of $NaNO_2$ (0.468 g) with water (5 mL). the later was then added to the 1st solution to give diazonium salt, (4-(2-(tert-butylamino)-1-hydroxyethyl)-2-(hydroxymethyl)), which added to 3-amino phenol, (0.006 mol., 0.655 g) in 25% sodium hydroxide to give azo dye (1). The synthetic azo dye recrystallized using ethanol and hexane to yield (63%) from brown titled azo dye (1).; m.p.: (145-147) °C. CHN of $C_{23}H_{18}F_3N_5O_3S$: calculated 55.04; 3.59; 13.96; found; 55.36; 3.67; 14.09.

Then, the complex of azo dye (1) with Zn was prepared with a molar ratio of 1:2 (metal: ligand). An aqueous zinc (II) sulfate (0.29 g, 0.001 mol.) was dissolved and mixed with azo dye (1.00 g, 0.002 mol.) in (50) mL of absolute ethanol. the mixture was refluxed with continuous stirring for (1-3) hours. Then the mixture was cooled down to form crystals, which were filtered and washed with water, hot ethanol and ether to get red complex (1-Zn).

pH Effect

The stock solution (1 x 10^{-3} M) of azo dye (1) was prepared by dissolving (0.025 g) in ethanol (50 mL). Then, the 0.5 mL was taking from stock and diluted with (5 mL) buffer solution, ranging (2-12), to give ($1x10^{-4}$ M) concentration for each solution.

Solvent Effect

Take 0.5 mL from prepared Stock solution above, (1 x 10-3 M) and mixed with 5 mL of each of ethanol (1), methanol (2), water (3), DMSO (4) and chloroform (5), to prepare ($1x10^{-4}$ M) concentration of (1) in each solvent.

Cellular Toxicity

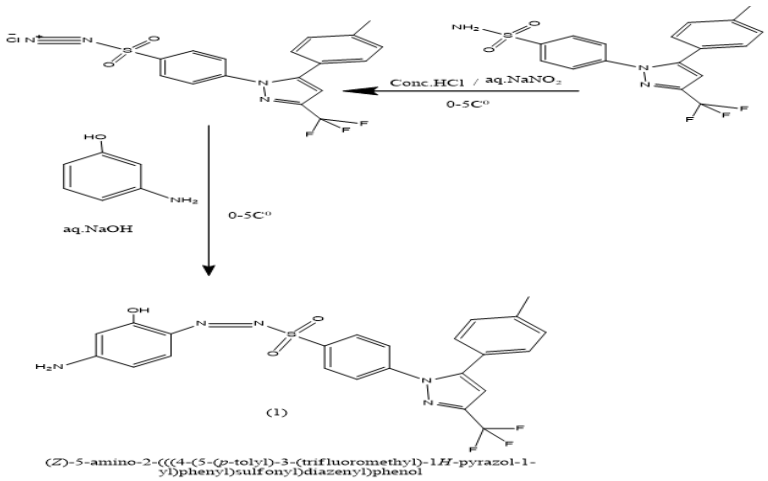
The Xian-guo and Ursola method (Xian-Guo & Ursula, 1994; Thangavelu et al., 2013) was applied to measure the toxity of azo dye (1), using haemolytic red blood cells as following: A stock solution of 200 mg / mL was prepared and followed by ıg a series of diluted (1000, 500, 250, 100,50 $\mu g/mL$) solutions. Then 0.8 mL of each diluted solution was added to Eppendorf tubes. Followed by adding 0.2 mL of red blood cells to each tube and equipped. Thus, In the first tube, 0.8 mL of Ringer solution was added as a negative control, but the tap water was added to second tube as a positive control. Then 0.2 mL of red blood cells was added to each tube. The results were recorded after the incubation for 3 h. in a special incubator and the variations in these solutions were checked.

Biological Action of (1) Against Bacteria and Fungi

Reactivity of prepared azo dye against different. types of bacteria, (*Escherichia coli*, *Staphylococcus aurous*, *Pseudomonas aeruginosa* and *Bacillus cereus*) and two different fungi, (*Aspergillus nigra* and *Candida albicans*) was confirmed, using a nutrient medium (Maller Hinton Agar) (MHA) and the Well-Variant Agar Diffusion technique (Ali et al., 2009; Fayadh et al., 2015).

RESULT & DISCUSSION

The azo dye, (*Z*)-5-amino-2-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-*yl*) phenyl) sulfonyl) iazinyl) phenol was prepared as shown in (Scheme 1).



Scheme. 1. the synthetic azo dye (1)

The scheme shows that the azo dye was prepared as a derivative of drug using Fox method, with optimize the stoichiometry and the conditions of the reaction (Otutu, 2013; Eady et al., 2018). This azo dye was characterized using different techniques. The FTIR spectrum, (Figure 1) shows that the stretching vibration of the ν (O-H) group in the region 3369.94 cm^{-1}. But, the ν (N=N) stretching vibration band was appeared in the region 1447.01 cm^{-1}. Other bands with this region can be considered as skeletal vibrations, the (C=C) stretching vibration of the aromatic ring shows a strong band in the region 1618.28 cm^{-1}. The aromatic CH bands were appeared in the region 2922.16 cm^{-1}. But, the (O=S=O) band was appeared in the region 1340.53 cm^{-1}.

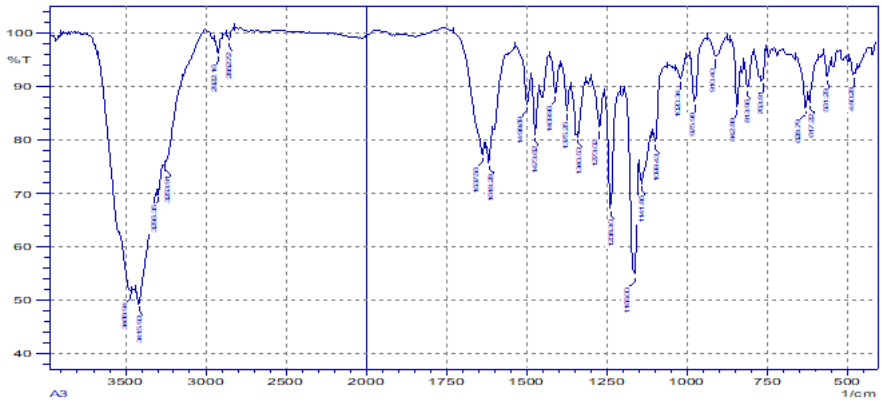


Fig. 1. The FT-IR spectrum of (1)

Elemental analysis also gained for $C_{23}H_{18}F_3N_5O_3S$, (55.36; 3.67; 14.09), which matching theoretical results, (55.04; 3.59; 13.96). Add to which, the mass spectrum was displayed that the peak of (1) at m/z were equal to 105 as seen in figure 2 below, which identical wit molecular weight 501.11.

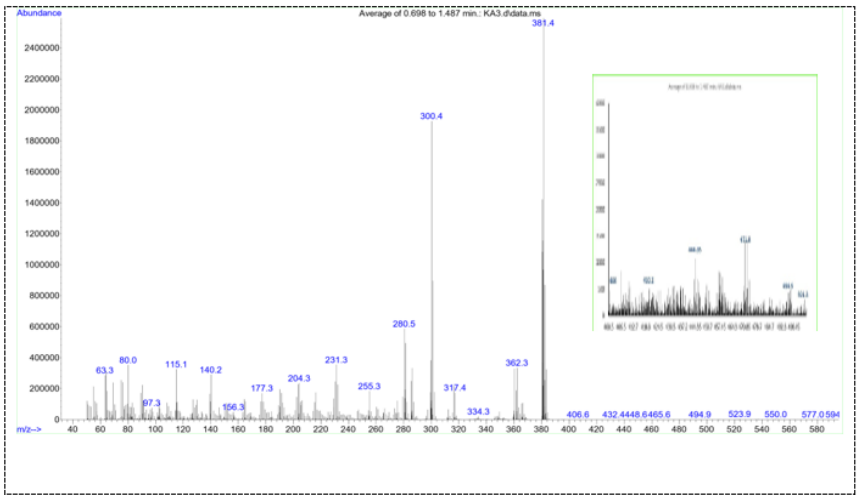


Fig. 2. The mass spectrum of the azo dye (1)

The UV-visible spectrum of (1) was documented at the range (250-500) nm in ethanol, (Figure 3). The absorption spectrum of synthetic azo dye (1) showed bands at (280 nm) and (410 nm) related to (π -π*) and (n-π*) respectively.

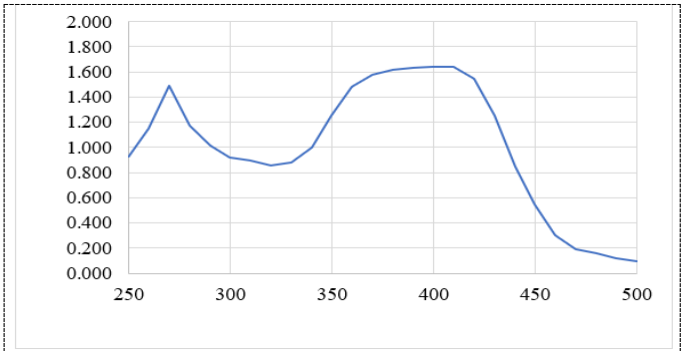


Fig. 3. UV-vis spectrum of (A3) in ethanol

Analytical studies of (1) was focused in many ways, first of all, the solvent effect was studied, (Figure 4) using different solvents, (ethanol, methanol, water and DMSO).

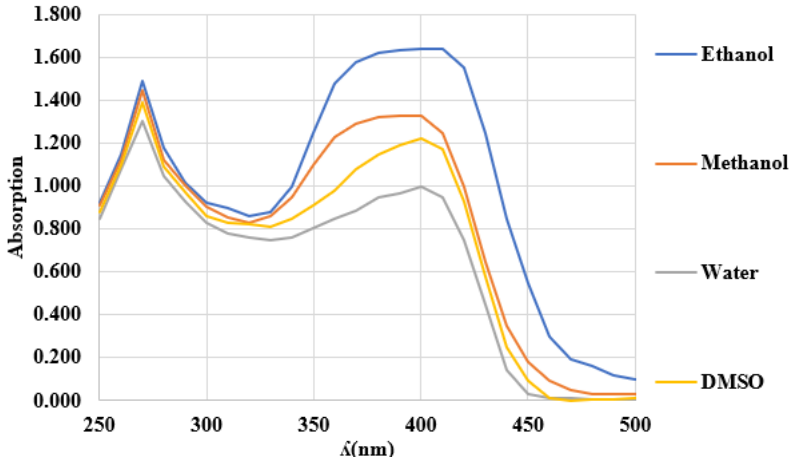


Fig. 4. The UV-vis spectrum (1) in a set of solvents

Figure 4 above was showed that the best solubility of azo dye (1) was in ethanol, and the results showed diverse values of λ_{max}, (Table 1) that attributed to n -π* transition of the azo group and indicates the absence of the hydrazone formula within the studied region.

Table 1
UV-visible spectrum of (1) in different solvents

Solvent	(1)	
	$_{max}$(nm)λ	ε_{max}(×10^{-4})
Ethanol	410	1.64
Methanol	400	1.33
Water	400	1.00
DMSO	400	1.22

The results from table above were indicated that the synthetic dye was affected by the solubility and dielectric constant (D), which can be expressed in relation to Gati and Szalay (Majeed et al., 2011; Ali et al., 2020) (Table 2) as below:

$$\Delta\tilde{V}=\left[(a\text{-}b)\left(n^2\text{-}1\,/\,2n^2\text{+}1\right)\right]+b(D\text{-}1\,/\,D\text{+}1).^{18}$$

Table 2
Dielectric constant of solvents

Id.	D	(D-1)/(D+1)
Ethanol (1)	24.30	0.921
Methanol (2)	32.70	0.940
Water (3)	78.40	0.975
DMSO (4)	47.00	0.958

Therefore, the results of Figure 4 above were designated the linear relationship as shown in Figure 5 below.

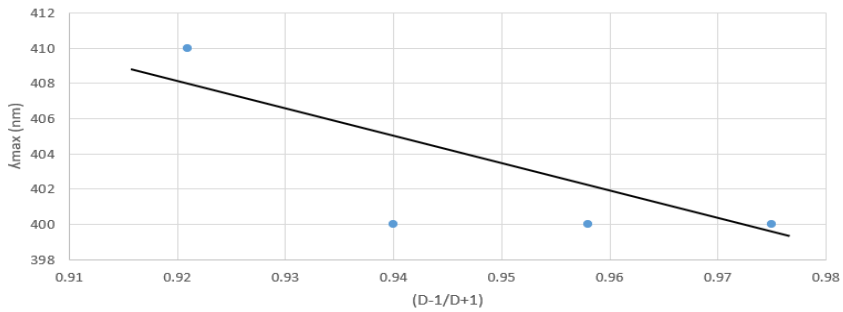


Fig. 5. Relation between (D) and λ max(nm) in diverse solvents

Further, the pH effect in the range of λ (250-500) nm was also studied for (1) in a range of buffer solution at pH (1-12) using $1*10^{-4}$ M concentration as seen in figure (6) below. The results were showed that the suitable pH values were found to be in the pH12, and three isopiestic points were gained in Figure (7) below. Therefore, the pKa of hydroxyl group and the pKb of the nitrogen atom in the synthetic azo (1) were calculated by applying the half height method.[7] From this method the pK values were attended using equations (1) and (2) below. This method was depending on the fact that the limiting absorption (Al) represents complete conversion of one form to other. Since pK is equal to pH at which the two forms exist in equivalent amount, then the pH corresponding to half the height of the absorbance, the pH curve is equal to pK.

pK= pH (at A1/2) ..... (1)

$$A1/2=\frac{A_l+A_{min}}{2} \quad \text{..... (2)}$$

The pK at (A1/2) of (1) was envisioned from the absorbance-pH curve as realized in Figures (7) below.

Table 3
Ionization and protonation constants of (1)

Id.	$\lambda_{max\,(nm)}$	$A_{1/2}$	pK_{p1}	$A_{1/2}$	pK_{p2}	$A_{1/2}$	pK_{a1}
(A_3)	410	0.340	3.4	0.381	7.26	0.360	10.5

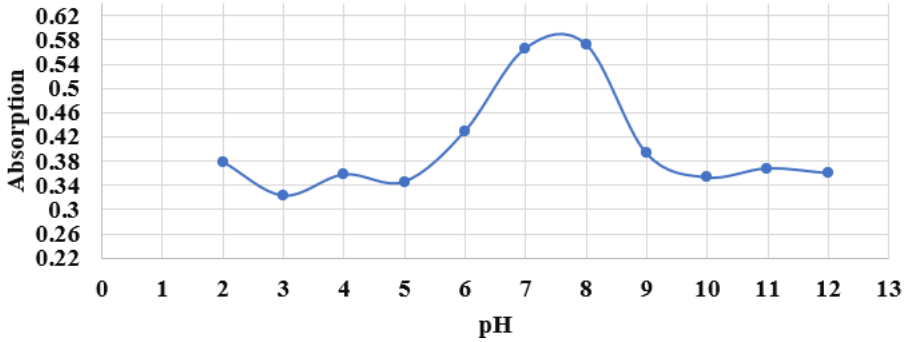
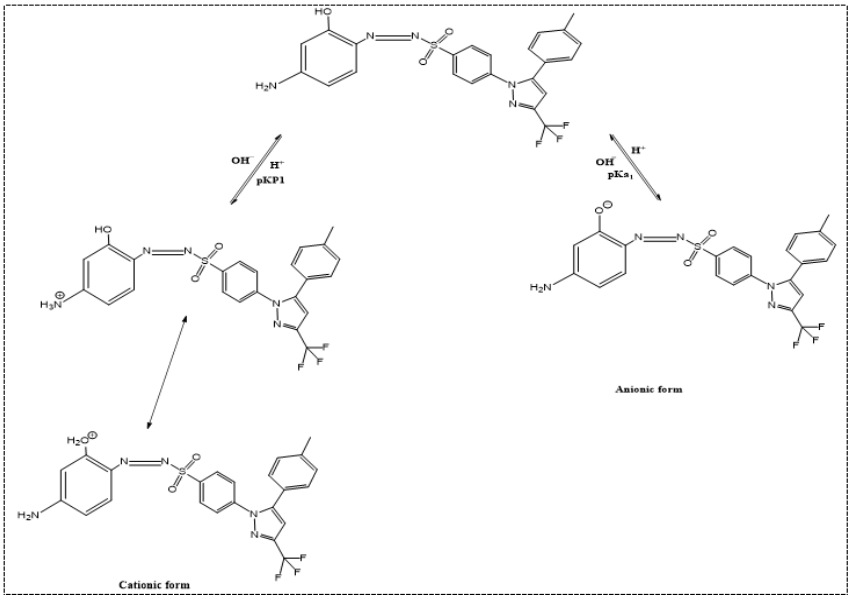


Fig. 7. Absorbance-pH curve of (1) at λmax 410 nm

The absorption spectra, (Figure 6) of (1) in the varying pH values are explained in the Scheme (2) below. The results were indicated the existence of the following equilibrium scheme of which displays the suggested ionization of azo dye in acidic and basic media.



Scheme. 2. Suggested ionization of (1) in the acidic and basic media

Furthermore, the biological activity of (1) against different bacteria, (*Staphylococcus Aureus, Escherichia Coli*, *Bacillus Cereus* and *Pseudomonas Aeruginosa*) and two different fungi (*Aspergillus Albicans* and *Candida Albicans*) were tested. the dye shows high activity against each microorganism. But the best reactivity was observed towered *Pseudomonas aeruginosa* and *Bacillus cereus.* (Figures 8).

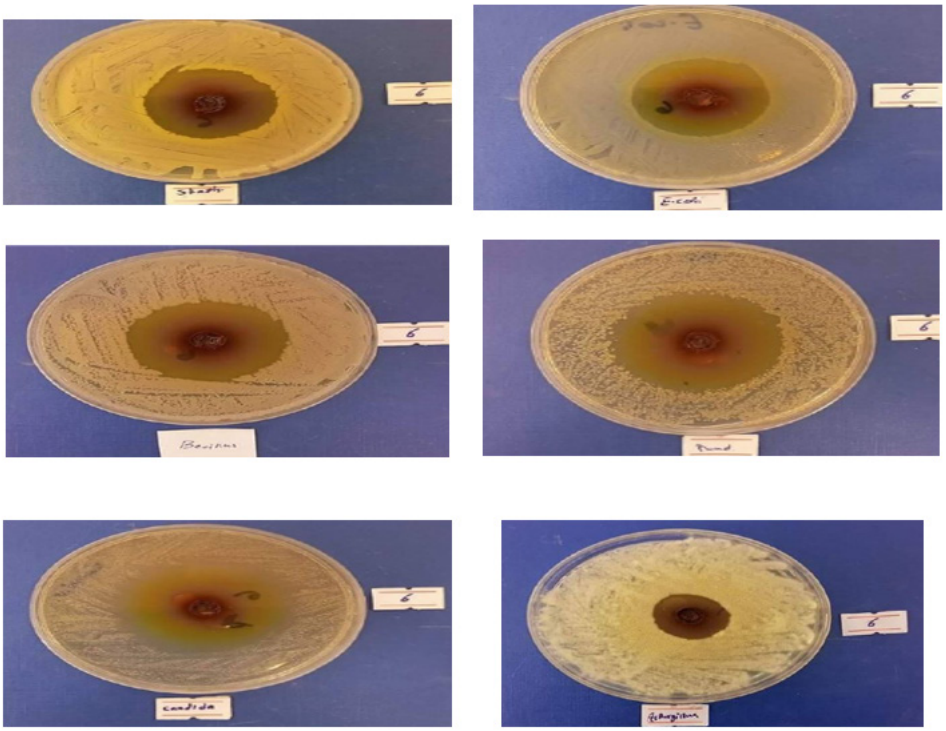


Fig. 8. The biological activity of azo dye (1) with 4 types of bacteria and 2 types of fungi

The inhibition zones demonstrate very good result of (1) in inhibiting the growth of *Pseudomonas aeruginosa* more than *Candida albicans* more than other types, which seems to be perfect.

Table 4
Inhibition Zones (mm)

ID	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
(1)	37	37	40	45	46	22

The table illustrates, that the azo dye (1) can effect *Staphylococcus Aureus, Escherichia Coli, Bacillus Cereus* and *Pseudomonas Aeruginosa, Aspergillus Albicans* and *Candida Albicans* very well with variable results, (Figure 9). The best effect was against *Candida albicans* and *Pseudomonas aeruginosa.* But the effect of (1) against *Aspergillus niger* was low.

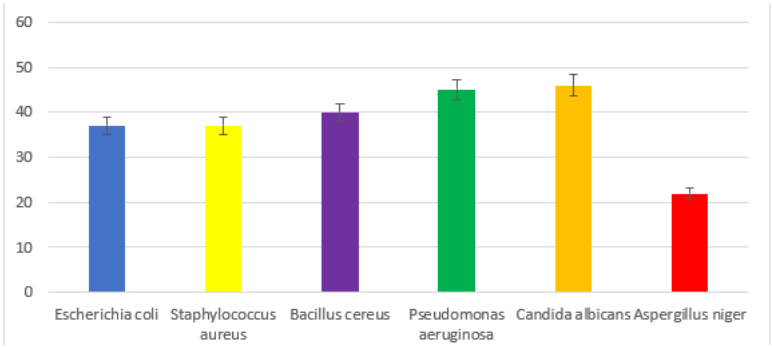


Fig. 9. The biological activity of (1)

The synthetic azo dye (1) also was provided a nontoxic effect using different concentrations, (Figure 10) and didn't show any heamolysis effect in contrast with other chemicals.

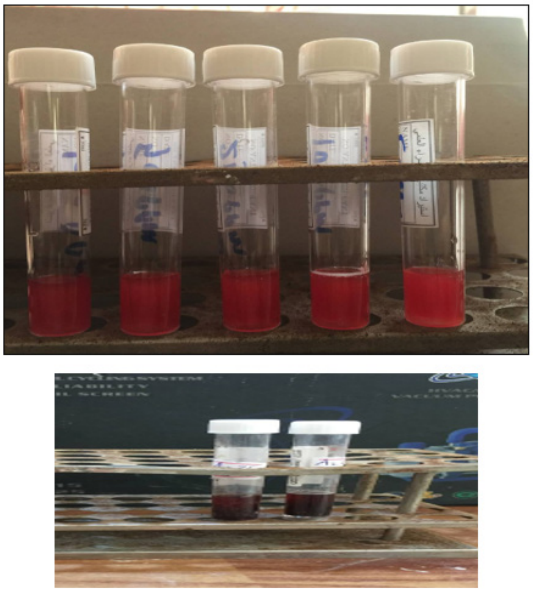


Fig. 10. Nontoxic effect of (1) and chemicals

These results approved, the possibility of using (1) as a new medicine or sanitizer because of it is harmless, cheap and non-toxic. Further, the complex (1-Zn) was prepared at optimum conditions. Then, the UV-visible spectrum of complex was recognized at the range (250-550) nm in ethanol, (Figure 11).

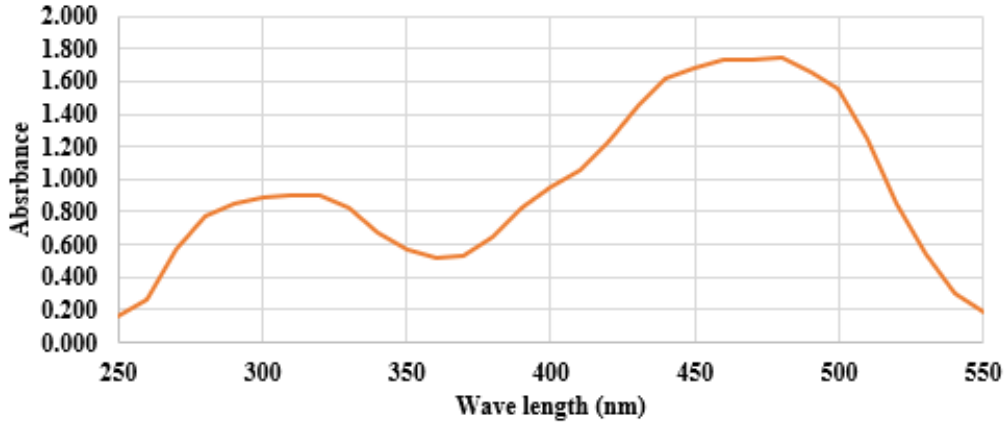


Fig. 11. below shows the absorption spectra of the dye and its complex with zinc. [Dye] =[Metal]= 1×10^{-4} M

Figure (11) above shows that the λ_{max} of the complex (1-Zn) is equal to the (320 and 480 nm) in compared with λ_{max} of azo dye (1), which equal to (280 and 410 nm) using ethanol as a reference. IR spectrum of complex (1-Zn) was also attended, (Figure 12).

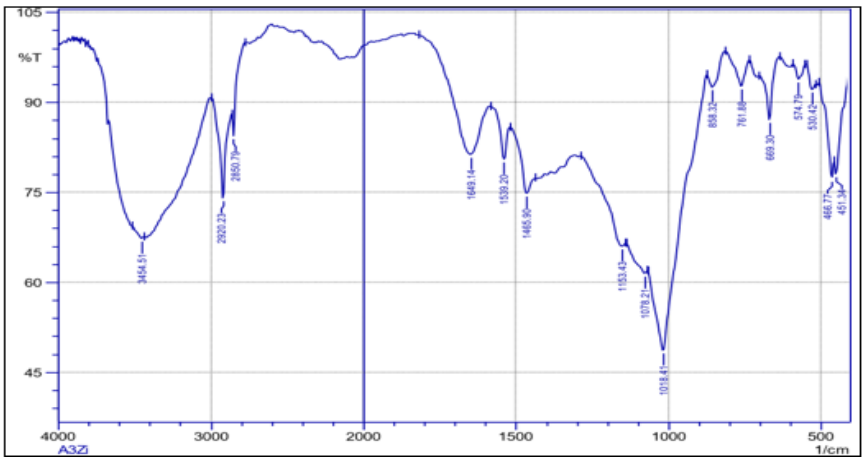


Fig. 12. IR spectrum of the complex (1-Zn)

.Then, the biological activities of the prepared complex (1-Zn) against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* were studied, (Figure 13).

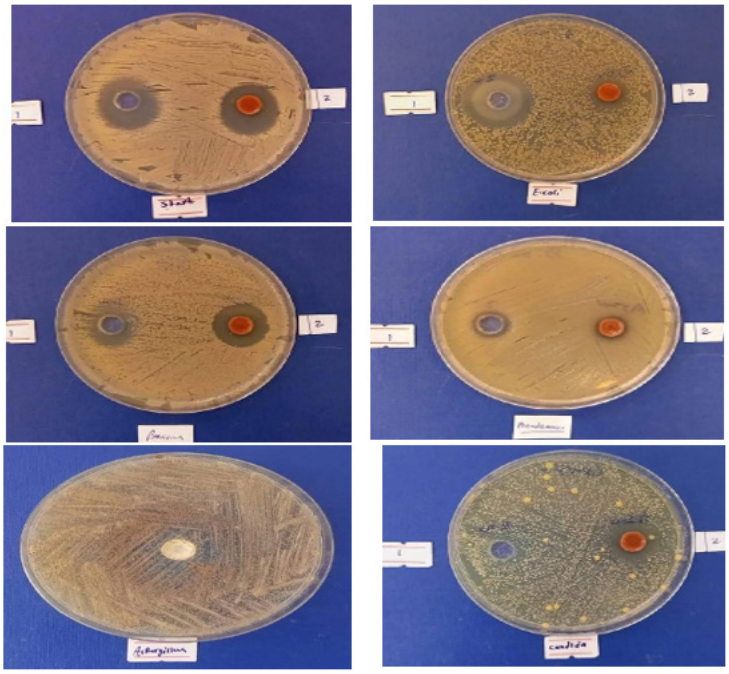


Fig. 13. The biological activity of (1-Zn) with 4 types of bacteria and 2 types of fungi

The inhibition zones of complex against *Escherichia coli, Staphylococcus aureus, Bacillus cereus and Pseudomonas aeruginosa* demonstrate reasonable results.

Table 5
Inhibition Zones (mm)

Id.	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
(A3-Zn)	14	22	22	11	....	....

These results were seeming to be lower than that received from azo dye (1). Furthermore, the complex did not show any biological activity against *Candida albicans* and *Aspergillus niger*.

CONCLUSION

Azo dye is a compound can prepare inexpensively, because their starting materials are obtainable and most of the chemicals are completed at or below room temperature. Add to which, the synthetic azo dye has good colour, carried non-toxic influence in blood cells and didn't display any haemolytic effect in the cells. Azo dye obligated good ability to well activity towered *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. Due to recommend the new azo dye as novel drug or new chemical sanitizer for these microorganisms. Further, the complex of azo dye with Zn can affect their biological activity negatively.

Competing Interest

The authors had no competing interests.

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