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Analytical Studies and Biological Activity of New Azo Dye and Its Complexation with Zinc

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ABSTRACT

Azo dye (AD), (*Z*)-5-amino-2-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl) phenyl) sulfonyl) diazenyl) phenol) and its complex with zinc were prepared and characterized using FTIR, UV-visible, elemental analysis and mass spectrum. Analytical studies showed that this dye had high solubility in ethanol and gave three isopiestic points 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. Further, the synthetic AD was provided non-toxic effects using different concentrations, and did not show any heamolysis effects in the cells in contrast with another chemical compound. The new AD is considered as novel medicine or chemical sanitizer. Further, the biological activity of AD and its complex was tested against four different bacteria, (*Staphylococcus Aureus, Escherichia Coli, Bacillus Cereus* and *Pseudomonas Aeruginosa*) and two different fungi (*Aspergillus Albicans* and *Candida Albicans*). The results showed that the complexation of AD with zinc was decreased the biological activity of synthetic AD.

1. Introduction

Azo dyes have received a great attention in scientific research due to their association with various human health problem (Kirkan & Gup, 2008; Majeed, 2013; Ali, et al., 2017; Ali, et al., 2018). These compounds are extremely importance in the chemical analysis. 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. However, 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 (Fayadh, et al., 2015). Analytical studies of many azo dyes and diazo dyes were the area of interest (Mutar & Ali, 2021; Issa & Zewail, 1971; Ali, et al., 2019). These compounds were verified to offer a multiple use in volumetric analysis, especially those that have different colours in the acidic and basic media (Fayadh, et al., 2015). A powerfully coloured dyes were actually envisioned for their pharmaceutical status as anti-diabetic, 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 (Otutu, 2013; Eady et al., 2018). Also known to be involved in the inhibition of DNA, RNA, carcinogenesis and protein synthesis (Farghaly & Abdallah, 2008). The presence of -N=N- in the molecular structure of azo dyes may responsible for their interaction with the active site of the protein. In this study, new pharmaceutical azo dye and its complex with zinc were prepared and characterized. Analytical and biological activities were also investigated. The results were variable and seem to be good using AD. However, the biological activity was decreased by complexation with zinc.

2. Materials and Methods

Melting point apparatus of Buchi B190K was used to measure the melting point of AD. IR spectrum carried on a FT-IR-8400S.Fourier Transform Infrared Spectrophotometer

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Shimadzu (Japan) using a KBr disc at a range (600–4000) cm^{-1}. Absorption spectrum in ethanol (1 x 10^{-4} M) was determined on a spectrophotometer.

2.1. Preparation of Synthetic AD and their Complex with Zinc

AD, 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, (Primo & Fröehlich, 2005). In the 1st solution of celecoxib drug (0.006 mol,2.288 g), HCl (1.75 mL) was mixed with 10 mL of distilled water. 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 AD. The synthetic AD was recrystallized using ethanol and hexane to yield (63%) from brown titled azo dye (1).; m.p.: (145-147) ^oC. 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 AD 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 AD (1.00 g, 0.002 mol) in (50) mL of absolute ethanol. the mixture was refluxed with continuous stirring for 1-3 h. Then the mixture was cooled down to form crystals, which were filtered and washed with water, hot ethanol and ether to get red complex (AD-Zn).

2.2. pH Effect

The stock solution (1 x 10^{-3} M) of AD was prepared by dissolving of 0.025 g in ethanol (50 mL). Then, a 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.

2.3 Solvent Effect

A 0.5 mL of stock solution above was 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.

2.4 Cellular Toxicity

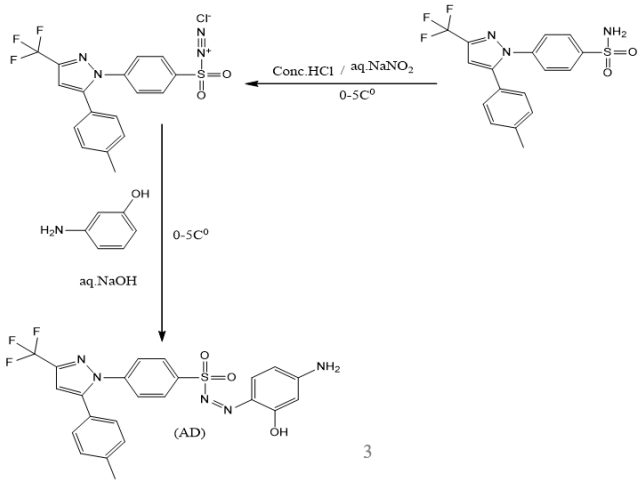
The Xian-guo and Ursola method, (Xian-guo & Ursula, 1994) was applied to measure the toxity of AD using haemolytic red blood cells as following: A stock solution of 200 mg/mL was prepared and followed by preparing a series of diluted (50,100, 250, 500 and 1000 $\mu g/mL$) solutions. Then 0.8 mL of each 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.

2.5 Biological Action of (1) Against Bacteria and Fungi

Reactivity of prepared AD 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), (Thangavelu & Gopi , 2013; Balch & Smith, 1994) and the Well-Variant Agar Diffusion technique.

3. Results and Discussion

The AD was prepared as shown in Scheme 1.



Scheme. 1. The synthetic AD

Scheme 1 shows that the AD was prepared as a derivative of drug using Fox method, (Fox, 1910) with optimize the stoichiometry and the conditions of the reaction. This AD 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 appeared in the region 2922.16 cm^{-1}. But, the (O=S=O) band appeared in the region 1340.53 cm^{-1}.

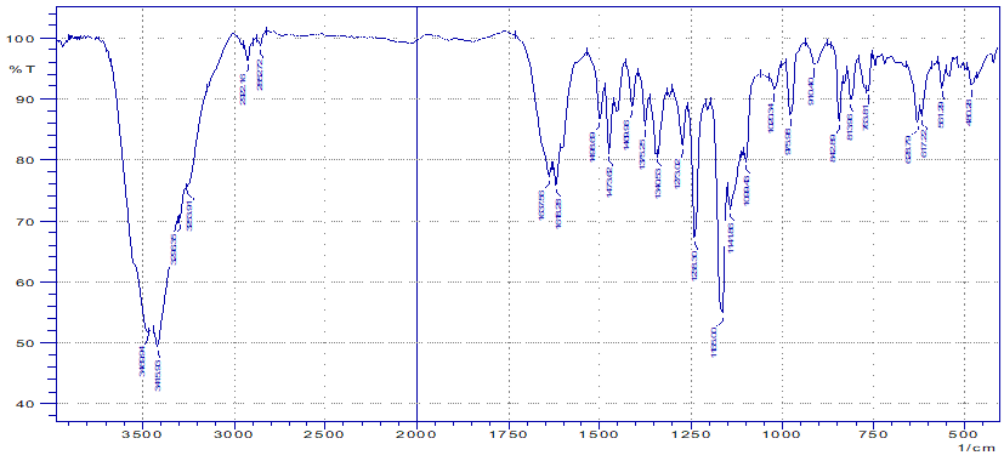


Fig. 1. The FT-IR spectrum of the AD.

Elemental analysis was 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 AD at m/z were equal to 501 as shown in Figure 1, which identical wit molecular weight 501.11.

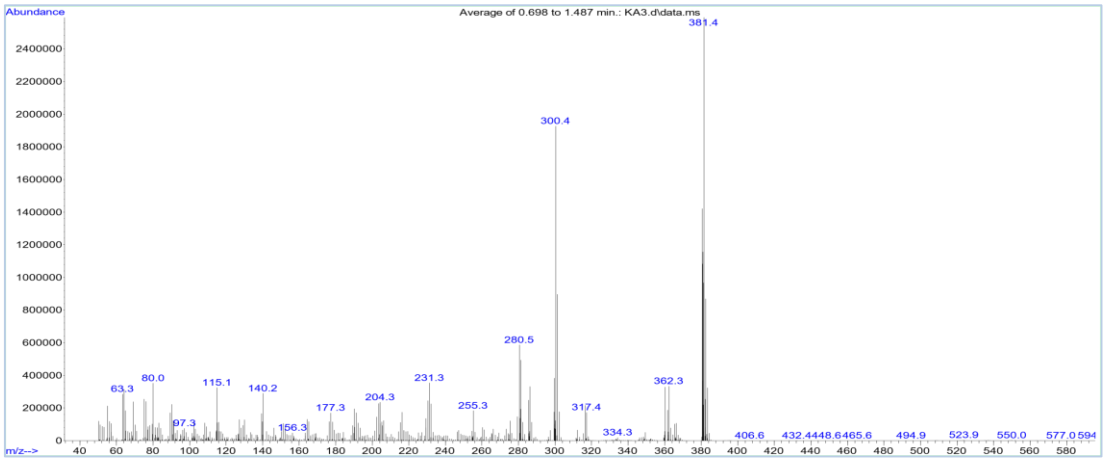


Fig. 2. The mass spectrum of the AD.

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

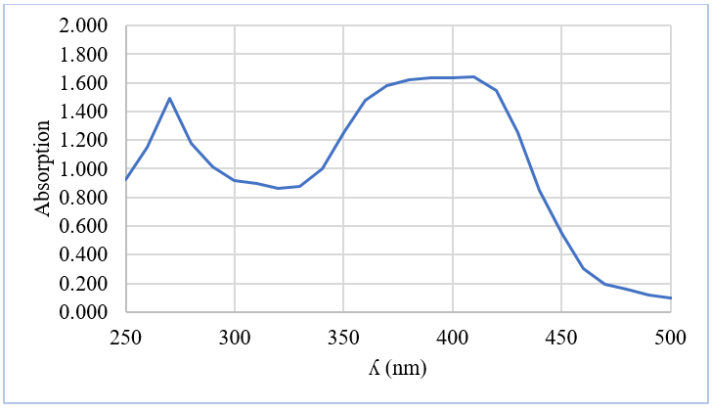


Fig. 3. UV-vis spectrum of AD in ethanol.

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

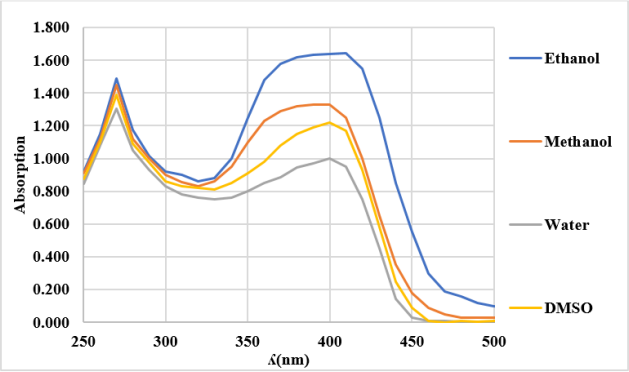


Fig. 4. The UV-vis spectrum AD in a set of solvents

Figure 4 shows that the best solubility of AD 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
The UV-visible spectrum of AD in different solvents

Solvent	(AD)	
	max(nm)λ	εmax(× 10^{-4})
Ethanol	410	1.64
Methanol	400	1.33
Water	400	1.00
DMSO	400	1.22

The results from in Table 1 indicate that the synthetic dye was affected by the solubility and dielectric constant (D), which can be expressed in relation to Gati and Szalay (Ali, et al., 2020) (Table 2) as shown in equation 1.

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

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 were designating the linear relationship as shown in Figure 5.

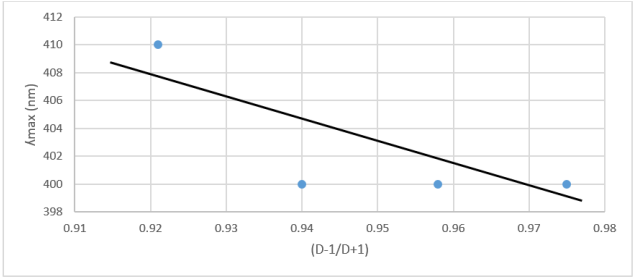


Fig. 5. Relation between (D) and λmax (nm) in diverse solvents.

Further, the pH effect in the range of λ (250-500) nm was also studied for AD in a range of buffer solution at pH (1-12) using $1*10^{-4}$ M concentration as seen in figure (6) below.

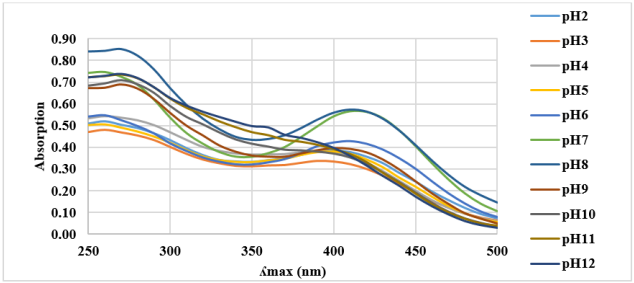


Fig. 6. The pH effect in AD using a range of buffer solution.

The results showed that the suitable pH values were found to be in the pH12, and three isopiestic points were gained in Figure 7. Therefore, the pKa of hydroxyl group and the pKb of the nitrogen atom in the synthetic AD were calculated by applying the half height method. From this method the pK values were attended using equations 2 and 3. 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) ..... (2)

$$A1/2 = \frac{A_l + A_{min}}{2} \quad \ldots\ldots\ (3)$$

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

Table 3
Ionization and protonation constants of AD

Id.	λmax (nm)	A1/2	pKp1	A1/2	pKp2	A1/2	pKa1
(AD)F	410	0.340	3.4	0.381	7.26	0.360	10.5

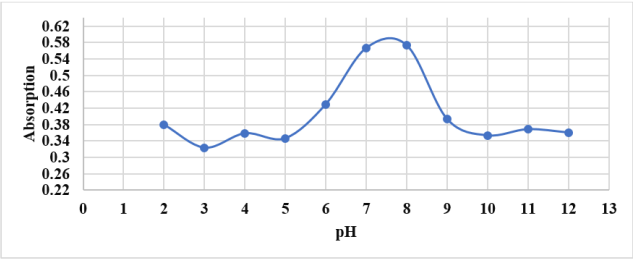
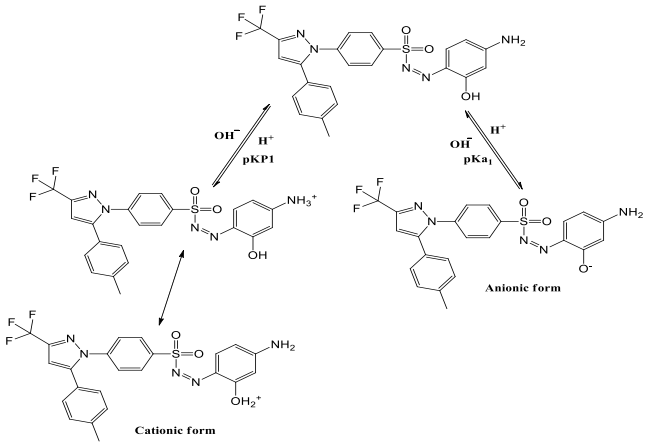


Fig. 7. Absorbance-pH curve of AD at λ_{max} 410 nm.

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



Scheme. 2. Suggested ionization of AD in the acidic and basic media.

Furthermore, the biological activity of AD 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. Nevertheless, the best reactivity was observed towered *Pseudomonas aeruginosa* and *Bacillus cereus* (Figures 8).

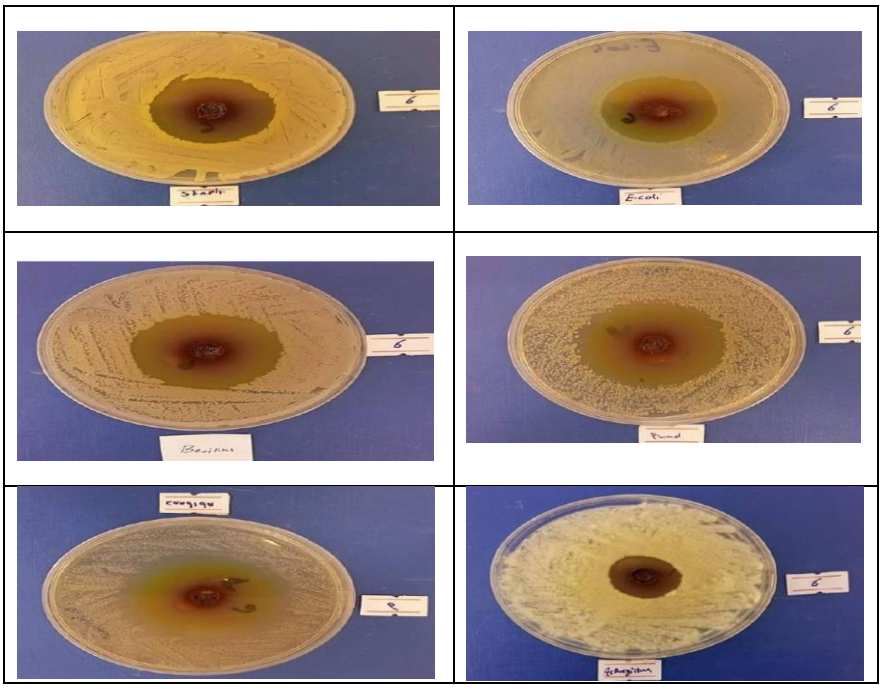


Fig. 8. The biological activity of AD with 4 types of bacteria and 2 types of fungi

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The inhibition zones demonstrate very good result of AD in inhibiting the growth of *Pseudomonas aeruginosa* more than *Candida albicans* more than other types, which seems to be perfect (Table 4).

Table 4
The biological activity of (AD).

Id.	Inhibition Zones (mm)					
	Escherichia coli	Staphylococcus aureus	Bacillus cereus	Pseudomonas aeruginosa	Candida albicans	Aspergillus niger
(AD)	37	37	40	45	46	22

Table 4 illustrates that the AD 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.* However, the effect of AD against *Aspergillus niger* was low.

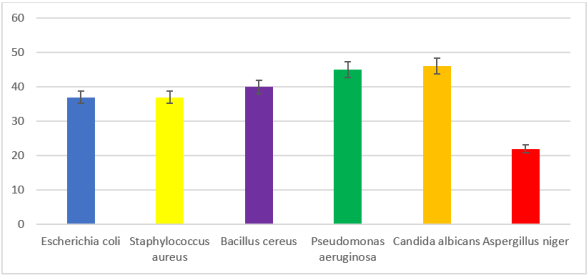

Fig. 9. The biological activity of AD.

The synthetic AD was also 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 AD in contrast with chemicals.

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

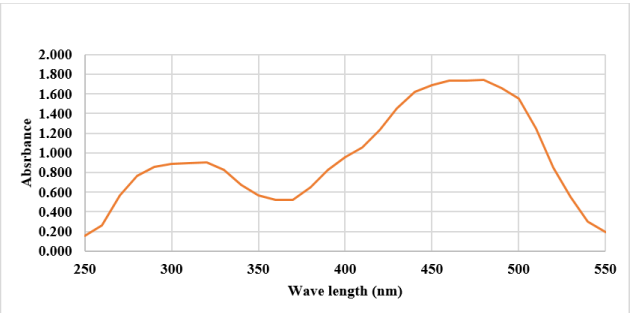

Fig. 11. The absorption spectra of the AD and its complex with zinc: [Dye] =[Metal]= 1×10-4M.

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

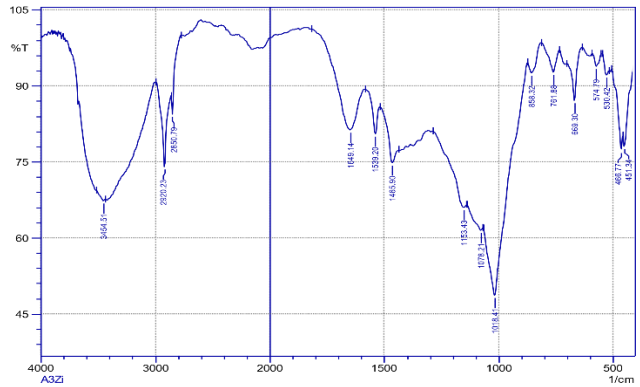

Fig. 12. IR spectrum of the complex (AD-Zn).

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

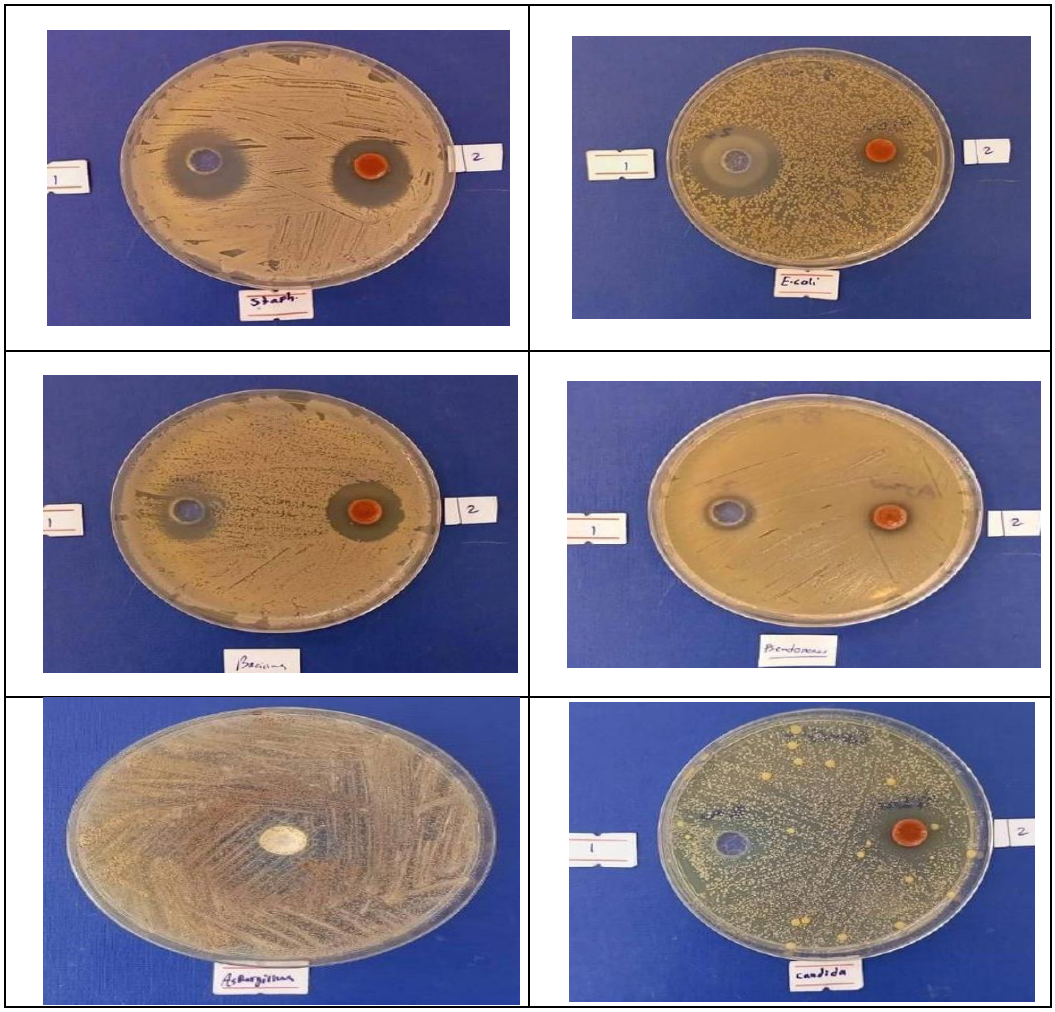

Fig. 13. The biological activity of (AD-Zn) with 4 types of bacteria and 2 types of fungi.

The inhibition zones of complex against *Escherichia coli, Staphylococcus aureus, Bacillus cereus and Pseudomonas aeruginosa* demonstrate reasonable results (Table 5).

Table 5
The biological activity of the complex AD-Zn.

Id.	Inhibition Zones (mm)					
	Escherichia coli	Staphylococcus aureus	Bacillus cereus	Pseudomonas aeruginosa	Candida albicans	Aspergillus niger
(AD-Zn)	14	22	22	11	....	....

These results seem to be lower than that received from AD. Furthermore, the complex did not show any biological activity against *Candida albicans* and *Aspergillus niger*.

4. Conclusion

Azo dye is a compound can be prepared inexpensively because of their starting materials are obtainable and most of the chemicals are completed at or below room temperature. In this work, the synthetic azo dye has good colour, carried non-toxic influence in blood cells and did not

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 Interests

The authors have declared that no competing interests exist.

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