Global Journal of Medical and Pharmaceutical Sciences
Website: https://grpublishing.org/
ISSN (*online*): 2957-3629
ISSN (*print*): 2957-3610

OPEN ACCESS
Volume-1 | Issue-4 | Nov-Dec-2022

Original Research Article

Preparation, Biological and Analytical studies of novel pharmaceutical azo dye

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Abstract:
The new azo dye (A_1), that named (2S,5R,6R)-6-((R)-2-((3-carboxy-4-hydroxyphenyl)diazenyl)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid was prepared. And then characterized using m.p., IR, UV-visible and elemental analysis CHN. Analytical studies of (A_1) were showed that the best solubility was in methanol, without straying from the linear connection, owing to suggest The major factor controlling the shift in absorption beaks is the influence of the dielectric constant. However, the pH effect of (A_1) in a range of buffer solution was revealed that the appropriate pH values were discovered to be in the pH12 range, and that three isopiestic points were achieved We determined the ionization (pKa) and protonation (pKp) constants. due to designate the ionization strategy in acidic and basic media. The synthetic azo dye remained harmless, nontoxic and do not has any heamolysis effect in the human blood cells. Therefore, the biological activity of (A_1) against different bacteria, (*Staphylococcus Aureus, Escherichia Coli, Bacillus Cereus* and *Pseudomonas Aeruginosa*) and two different fungi (*Aspergillus Albicans* and *Candida Albicans*) were studied. The results were indicated that the dye can affect the fungi more than bacteria. Owing to recommend the new azo dye as novel drug or chemical sanitizer for these microorganisms.

Keywords: *Azo dyes, Elemental analysis, Ionization constants, Protonation constants, Haemolysis effects, Biological activity*

INTRODUCTION

Azo dyes have gotten a great Scientific research is given focus.," as well as an excessive critical role in chemical analysis. The structural features of azo compounds, which typically produce a C=C, N=O, N=N, aromatic rings, C=O, and NO2 are the colorants.. However, the teams that responsible to make alteration in the The azo (-N=N-) and nitroso (-N=O) colors are used., but the other groups in fact do consequently in some circumstances conditions. Analytical tests on azo dye (1) and diazo dye (2) were conducted (2). (1) and (2) had the best solubility in methanol and ethyl acetate, respectively, with no deviation from the linear relationship in either, which is due to the fact that the effect of the dielectric constant is the most important factor. consider to be the regulatory of the shift, that associated to the absorbent beaks Despite the fact that the pH impacts of (1) and (2) were tested in a range of buffer solutions, the results revealed one and two isopiestic sites, respectively. The ionization (pKa) and protonation (pKp) constants were also validated using the half height approach for (1) and (2). in association with the presence nitrogen atoms with OH-groups. Further, analytical studies of another diazo dyes, were showed that the solvents effect were revealed high ethanol and distillate water solubility. And the pH effect was showed that the appropriate values were at (pH= 12) in each diazo dye. Three isopiestic points were gained and the pKa and pKb constants were calculated for each.[10] These findings supported the proposed ionization technique in acidic and basic media. The azo compounds were also verified to offer a multiple uses in volumetric analysis, especially those that have different colours in the acidic and basic media. A powerfully coloured dyes were actually envisioned for their use in medicine as anti-diabetic, anti-cancer, anti-bacterial, and anti-antineoplastic agents. They are also known to be implicated in the suppression of DNA, RNA, carcinogenesis, and protein production. So, it's possible that the azo dyes' interaction with the protein's active site is caused by the presence of -N=N- in their molecular structure.

Experimental section

Utilizing an aFT-IR-8400S, the IR spectrum was certified. Using a KBr disc in the (600–4000) cm-1 range, the Shimadzu (Japan) Fourier Transform Infrared Spectrophotometer measures the spectrum. Buchi B190K was used to measure the melting points of azo dyes. Using a spectrophotometer, the absorption spectra of ethanol (1×10^{-4} M) was obtained. The Chemistry Department of the Education College of Pure Science at Basrah University in Iraq carried out

the melting point, IR, UV-Visible spectrophotometer, and measurements of the CHN elemental composition. In this investigation, the PH-Meter was used to evaluate the ionization and protonation constants for hydroxyl and nitrogen groups for a series of acetate and universal buffer solutions with varying pH values (2-12). (H. Jurgons Co. Bremen, L. Puls Munchen15).

Preparation of azo dye (A_2)

The process of (2S,5R,6R)-6-((R)-2-((3-carboxy-4-hydroxyphenyl)diazenyl)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid *(A1). In a 1 s beaker, Ampicillin (0.006 mol., 2.096 g) was combined with conc. HCl (1.75 mL) before being added to with 10 mL of distilled water. In the second beaker, 0.468 g of NaNO2 was dissolved in 5 mL of distilled water to create the solution. The first beaker was then filled with the $NaNO_2$ solution. After that, the resultant diazonium salt was added to 2-hydroxybenzoic acid salicylic acid (0.006 mol, 0.828 g) in sodium hydroxide solution at a concentration of 25%. The azo dye was produced by recrystallizing the resultant crude in ethanol and hexane (yield: 63%); m.p.* (58-60) ^{o}C to purify the dye in ethanol and hexane. Elemental Analyses calculated for $C_{23}H_{22}N_4O_7S$: 55.41; 4.42; 11.24; found; 56.35; 4.55; 11.36.

The pH effect

Weights (0.025 g) in (50 mL) of ethanol were dissolved to create the stock solution, which was then used to create the solution of azo dye (1 x 10^{-3} M). Then, using a variety of buffer solutions (2–12), (0.5 mL) of each dye were taken from their stock solutions, each containing (1 x 10^{-3} M), and diluted with (5 mL) to produce each buffer solution (1x10^{-4} M) concentration.

The Solution of the azo dye in ethanol

To create the stock solution of azo dye, weight (0.025 g) was dissolved in (50 mL) of ethanol, yielding a dye concentration of (1 x 10^{-3} M). Following that, (0.5 mL) of each dye was taken from its stock solution, which was (1 x 10^{-3} M), and diluted with (5 mL) of ethanol to create (1 x 10^{-4} M) concentration.

The solvent effect

The stock solution of the azo dye was made by dissolving weight (0.025 g) in (50 mL) of solvents, including ethanol, methanol, water, DMSO, and chloroform, to get the dye's concentration (1 x 10^{-3} M) in each solvent. Next, (0.5 mL) of the dye was taken from their stock solution, which contained (1 x 10^{-3} M), and diluted with (5 mL) of each solvent to produce (1 x 10^{-4} M) concentration.

Cellular toxicity

The following was done using haemolytic red blood cells to determine the toxicity of the azo and diazo dyes under study: Following the creation of a stock solution of 200 mg/mL, many diluted solutions containing 1000, 500, 250, 100, and 50 "g"/mL were created.

The Eppendorf tubes received 0.8 mL of each diluted solution. Each tube received 0.2 mL of red blood cells as well. Additional equipment included two Eppendorf tubes. A positive control was supplied to the second tube, tap water, while 0.8 mL of Ringer solution was put to the first tube as a negative control. Red blood cells were then diluted to 0.2 mL in each tube. Following this, the tubes were incubated for 3 hours at 370C in a specialized incubator, and the findings were recorded.

The biological effects of azo dye (A_1) on several bacterial species

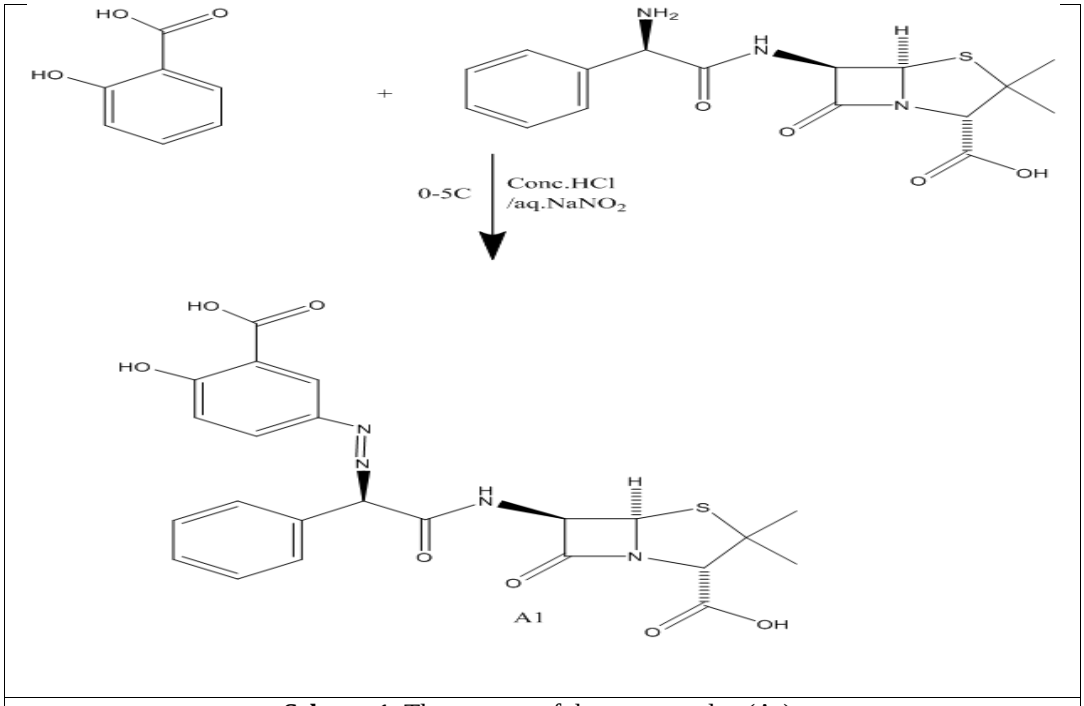
The azo dye (A_1)biological 's action was investigated. against different types of gram-negative and gram-positive pathogenic bacteria (a dye treated bacteria, which displays respond to the dye is considered positive and which do not respond is considered negative). The types of bacteria are (*Escherichia coli, Staphylococcus aurous, Pseudomonas aeruginosa,* and *Bacillus cereus*), using a nutrient medium (Maller Hinton Agar) (MHA) for bacteria was used to culture and multiply different bacteria. These cultures were incubated at a temperature of (1 ±36oC), which is the appropriate degree for the growth of bacteria for a period of (24) hours. The Well-Variant Agar Diffusion technique [14] was used to grow bacterial isolates by plotting method on the nutrient agar medium for the purpose of obtaining colonies. It is left to dry and after it cools, a semi-solid gelatinous layer is formed from the agar, and the bacteria or fungi used for the study grow on the sterile nutrient medium of the acre. The grown mushrooms amounted to (0.1 ml) and then added to it (0.1 ml) of each prepared dye solution (A_1) at a concentration of (ml/mg 5) of dimethyl sulfoxide (DMSO), then the cultivated dishes were kept in the incubator for twenty-four hours at a temperature of (1 ±36^{o}C) under anaerobic conditions and by using a gas releasing kit, then the biological activity was measured by measuring the diameter of the bacterial growth inhibition zone around the holes measured in millimeters (the area free of bacterial growth).

Biological effects of the azo dye (A_1) on two types of fungi

The azo dye (A_1) was verified against two types of fungi (*Aspergillus niger* and *Candida albicans*), using (Plate-Hole Diffusion Method) technique, ' then the effect of antifungal through fungal test (PDA), by distribution technique on the growth media (*Aspergillus niger* and *Candida albicans*) in sterile saline. Then, standardized the innate suspension system (100 µL), (0.85%) (10^6 conidia/ mL) from each innate suspension system, and after spreading it on the surface of the dishes and leave it for (10 min.), then small holes with a diameter of (7 mm) were drilled and put (100 µL) from prepared samples. Then the cultivated dishes were placed in the incubator at (28^oC) from (DMSO), then the biological activity was measured after (72 h.) by measuring fungal growth diameter inhibition zone around these holes measured in millimeters (the area free of fungal growth).

Result and Discussion

The azo dye, that characterized (2S,5R,6R)-6-((R)-2-((3-carboxy-4-hydroxyphenyl)diazenyl)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid was prepared, (Scheme 1).



Scheme 1. The prepare of the new azo dye (A_1).

The new azo dye (A_1), which derived from the two drugs *Ampicillin* and *salicylic acid* was characterized by IR spectrum, CHN as well as the UV-visible spectrum The stretching vibration of the v (O-H) bands in the IR spectra of the unbound ligand are responsible for the medium band around (A_1, Figure 1) 3468.01 cm^{-1}. Although the azo (-N=N-) group is visible at 1444.68 cm^{-1} and, the band around 1612.49 cm^{-1} back to C=C stretching and the C-H band was appeared in the regions 2918.30 cm^{-1}

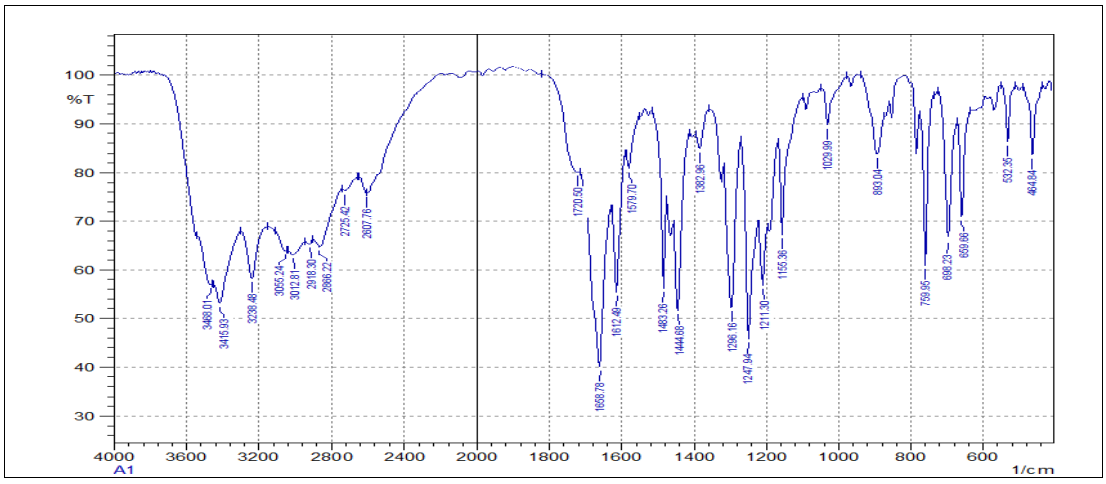


Figure 1. FT-IR spectrum (A_1)

Elemental analysis was also calculated for $C_{23}H_{22}N_4O_7S$ as shown in Table (1) below.

Table (1): Analytical chemistry of azo dye (A_1)

CHN	C%		H%		N%	
	Calculated	Found	Calculated	Found	Calculated	Found
	55.41	56.35	4.42	4.55	11.24	11.36

For the synthetic dye, the UV-visible spectrum was seen at wavelengths between (250 and 550 nm) in ethanol. At 360 nm and 400 nm, which correspond to (π -π*) and (n-π*), respectively, the absorption spectra of (A_1) was presented. Analytical studies were also curried on (A_1), So the solvent effect of was studied, (Figure 2) using a variety of solvents The outcomes were showed which has the highest solubility of the azo dye (A_1) was in methanol.

Figure 2. The UV-vis spectra of the innovative azo dyes (A1) in a variety of solvents.

Figure (2) above shows various values of λ_{max}, (Table 2) attributed to π -π* transition of the azo group and denotes the lack of the hydrazone formula inside the investigated area.

Table (2): The UV-visible spectrum of the azo dye (A_1)

Id.	(A_2)	
Solvent	$_{max}$(nm)ʎ	()ε_{max}
Methanol	380	1.55
Ethanol	400	1.72
Water	380	1.51
DMSO	390	1.50
Chloroform	390	1.70

The table revealed the ג$_{max}$(nm) of (A_1) in the absorption spectrum due to indicate, that the synthetic azo dye was influenced by solubility and dielectric constant (D), which may be described in connection to the Gati and Szalay equations, as following:

The F(D) and were also computed (Table 3), demonstrating a linear relationship where the dielectric constant is the only influence determining the shift beak.

Table (3): The values of the dipole moment constants in the solvents with the maximal wavelengths of the synthetic dye

The Solvent	D	(D-1)/(D+1)
Ethanol	24.30	0.921
Methanol	32.70	0.940
Water	78.40	0.975

DMSO	47.00	0.958
Chloroform	9.10	0.802

The results, (Figure 3) also indicated, that there is no departure from the linear relationship, which is due to the fact that the influence of the dielectric constant is the key component and may govern the shift of the absorption beaks.

- 1- Chloroform, 2- Ethanol, 3 DMSO - , 4- Water , 5- Methanol.

Figure 3. The relationship between dipole moment constants and maximum wavelengths in various solvents.

The absorption spectra of a 1 $x10^{-4}$ M solution of (A_1) across a range of wavelengths (250-560 nm) and pH values (2-12) were graphically depicted. in Figure (4) below.

Figure 4. The pH effect in the novel azo dyes (A_1)

The results indicated that the appropriate pH values for each dye were determined to be in the pH12 range. In the figure above, three isopiestic points were obtained. As a result, the pKa of the hydroxyl group and the pKb of the nitrogen atom in the synthetic azo dye (A_1) were computed using the half height technique.[7] Equations (1) and (2) below were used to calculate the pK values using the aforementioned procedure. This approach was predicated on the idea that the limiting absorption (Al) signifies a whole transformation from one form to another. Since pK is equal to the pH when both forms are present in equal amounts, the pH corresponding to half the absorbance height corresponds to pK.

(1)

(2)

The pK at (A1/2) of (1)) based on the absorbance-pH curve shown in Figure (5) below, which was anticipated.

Figure 5. A_2 absorbance-pH relationship at maximum 470 nm

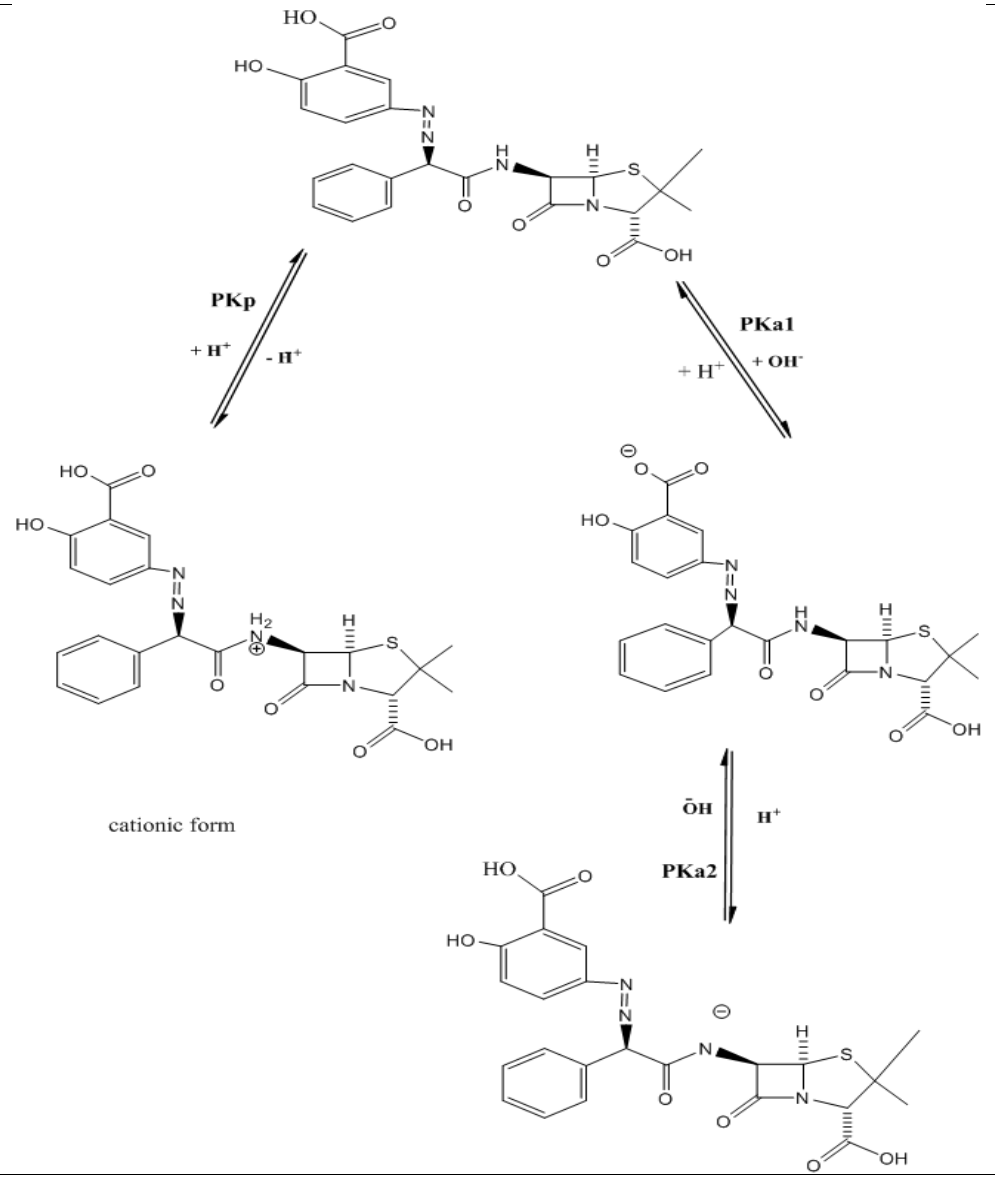
The results obtained from the absorbance-pH curve in figure above are given in table (4) below.

Table (4): The ionization and protonation constants of the synthetic azo dye (A1) were determined spectrophotometrically.

The synthetic dye	$\lambda_{max\ (nm)}$	$A_{1/2}$	pK_{p1}	$A_{1/2}$	pK_{p2}	$A_{1/2}$	pK_{a1}
Azo dye (A_2)	470	0.49	2.5	0.59	7.4	0.75	9.5

pK_{p1}= Protonation of the nitrogen atom., pK_{p2}= Protonation of the nitrogen atom pK_{a1}= Ionization of the OH-group., pK_{a2}= Ionization of the OH-group**.**

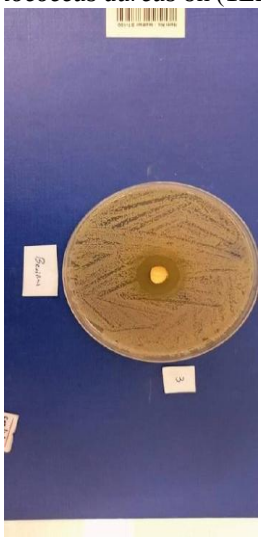
The absorption spectra of (A_1) in various pH levels are discussed in Schemes (2) below. The results revealed the presence of the following equilibrium scheme, which depicts the proposed ionization of azo dye in acidic and basic media.



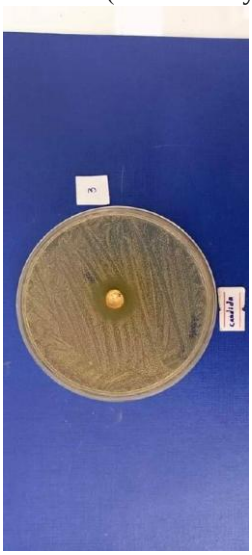
Scheme 2. The predicted ionization of (A1) in acidic and basic environments.

The biological activity of (A1) against, (Figure 6 a-f) different bacteria (Bacillus Cereus, Staphylococcus Aureus, Escherichia Coli, and Pseudomonas Aeruginosa)and two different fungi (*Aspergillus Albicans* and *Candida Albicans*).

.*Staphylococcus aureus* on (1Effect dye (A :(a)



.*Bacillus cereus* on (1Effect of dye (A :(c)



(e): Effect of dye (A1) on the *Candida albicans*

.*Escherichia coli* on (1Effect of dyes (A :(b)

.*Pseudomonas aeruginosa* on (1d): Effect of dye (A)

Aspergillus nigra on (=1Effect of dye (A :(f)

6 Figure with 4 types of bacteria and 2 types of fungi (1The biological activity of azo dye (A

Though, the values of the inhibition zones display the reasonable effect of the dye (A1) in inhibiting the growth of *Pseudomonas aeruginosa* more than *Staphylococcus aureus* and did not affect other types of bacteria as shown in Table (5) below.

Id.	Inhibition Zones (mm)					
	Escherichia coli	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
(A_1)	----	16	---	20	18	23

Table (5) above was clearly shows the effect of (A_1) in the fungi, which seems to be higher than their effect in the bacteria, (Figure 7).

Figure 5. The variation of the biological activity of azo dye (A1) against 4 types of bacteria and 2 types of fungi .

The results were confirming the possibility of using this azo dye as a new drug or as a chemical sanitizer against *Candida albicans, Pseudomonas aeruginosa, Aspergillus niger, and Staphylococcus aureus*, as it is safe, inexpensive and non-toxic.

CONCLUSION

Azo dye is a compounds can prepare inexpensively, because their the majority of the chemistry may be done with readily available materialsis completed lower than or at 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 new chemical sanitizer for these microorganisms.

Acknowledgment

Chemistry Department of the University of Basrah, as part of the prerequisites for a master's degree in Iraq.

Conflicts of interest

No conflict of interest exists, according to the authors.

REFERENCES

No references