Synthesis, Characterization and Antibacterial Activity of Two Novel Azo Compounds

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Abstract

In this study, two novel azo compounds were synthesized by diazonation of 4-aminoantipyran followed by coupling with 4-(dimethylamino)benzaldehyde and *N*-(4-hydroxyphenyl)acetamide. The synthesis azo compounds were characterized by IR, CHN, UV-Vis., TLC and melting points. Also, was carried out to determine the antibacterial activity of these compounds against burn infection isolated bacteria. Thirty samples were collected from patients related to Samawa-teaching hospital burn unit. Sixty five isolate from both sex males (53.33%) and females (46.66%). *Staphylococcus aureus* was found to be must common isolated (43.07%), then *Pseudomonas aeuroginosa* (21.53%), *Klebsiella* spp. (12.30%), *E. coli* (9.23%), *Proteus mirabilis* (7.69%), *Streptococcus pyogenes* (4.61%) and *Aeromonas hydrophila* (1.53%) . The synthesis azo compounds showed a good antibacterial activity against the isolated bacteria by using disk diffusion method. MICs values were determined for all types in broth modulation assay.

Introduction

Azo compounds contain one or more azo groups (-N=N-) which are linked to SP^2 hybridized carbon atoms [1]. The compound known as monoazo compound have only one (-N=N-) group while diazo and triazo compounds contain two and three (-N=N-) group, respectively. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocycles or aliphatic groups [2]. Synthesis of most azo compounds involves diazotization of a primary aromatic amine, followed by coupling with one or more nucleophiles [3].

Azo compounds are highly colored and have been used as dyes and pigments for a long time. They have been receiving much attention and have been widely used in many practical applications such as coloring fibers [4,5], photoelectronic applications [6], printing systems [7,8], optical storage technology [9], textile dyes [10,11] as well as in biological field, where these compounds have antimicrobial, anti fungal and antitumor [12,13,14], and in analytical chemistry [15,16].

Burn is a thermal injury of the skin, although electrical and chemical injuries may also result[17]. In patient with burn over than 40% of the total body surface area, 75% of all deaths following thermal injuries are related to infection [18]. The antibacterial activity of azo compounds were studied, such as manufacturing clothes products with antimicrobial properties, there fore, this will reduce odor-generating microorganisms (bacteria) carried by the products [19,20]. If that occurred can lead to dermal infection product deterioration and allergic response [21].

The recent study has focused on the effects of two azo compounds against wound infection isolated bacteria.

Materials and Methods

1- Materials and Instruments

All chemicals used in the present investigation were supplied from BDH and Fluka. The purity of the synthesis azo compounds were checked by TLC. The IR spectra of azo compounds were recorded on Shimadzu FT-IR spectrophotometer. The UV-Vis spectra of azo compounds were recorded on Spectro Scan 80D spectrophotometer. The CHN data of azo compounds were recorded on Euro vector EA 3000A Element analyzer. The melting points were determined by classical method.

2- Synthesis of azo compounds

Synthesis Azo compound I

1. Prepare 30 ml of 10% aqueous sodium hydroxide solution by dissolving 3 g of sodium hydroxide in 30 ml of water in an 150-ml conical flask.
2. Dissolve (0.01 mol) of 4-(dimethylamino)benzaldehyde into the sodium hydroxide solution. Stir the mixture until complete dissolution. Cool the solution with an ice-water bath.

*We use *N*-(4-hydroxyphenyl)acetamide substitute 4-(dimethylamino) benzaldehyde to synthesis compound II.

3. The diazonium salt solution can be prepared as below:

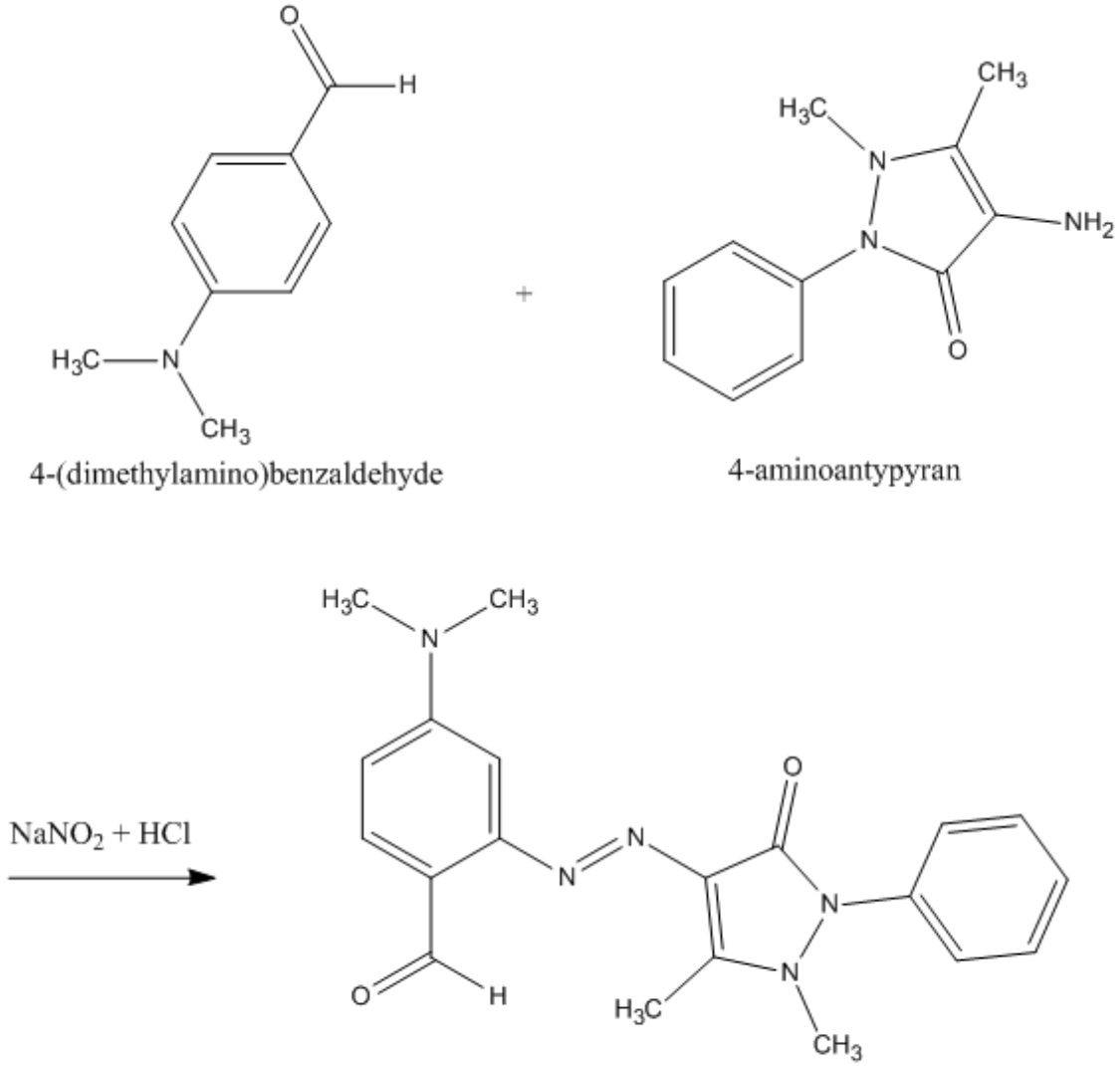
(a) Dissolve (0.01 mol) of $NaNO_2$ in 5 ml of water.

(b) Put (0.01 mol) of 4-aminoantipyran into 45 ml of water. Add slowly 12 ml of concentrated hydrochloric acid and stir the mixture until the 4-bromoaniline is dissolved completely.

(c) Cool the 4-aminoantipyran solution in an ice-bath. While keeping the solution at 0 °C add the sodium nitrate solution slowly with a dropper. The mixture should be stirred during addition. When the addition is completed, stir the mixture for another 2-3 minutes.

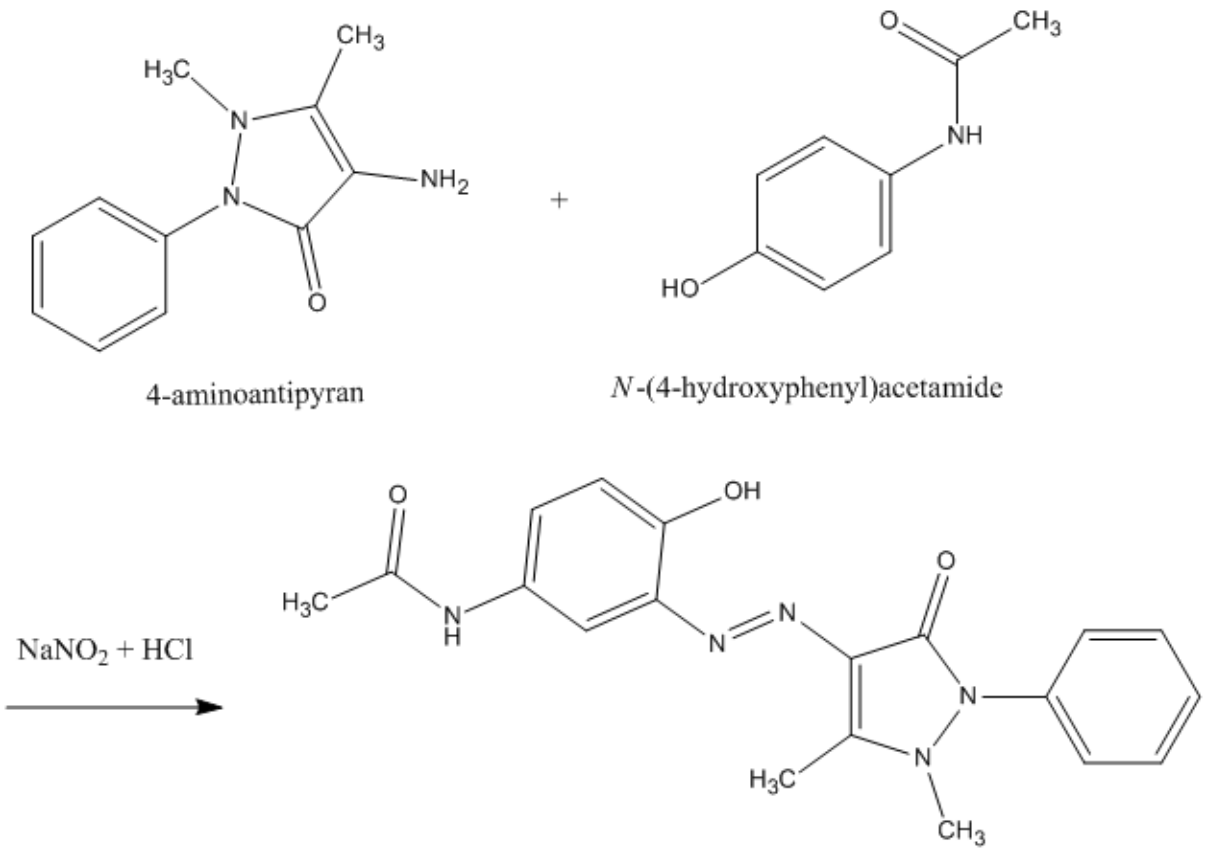
4. To the alkaline 4-(dimethylamino)benzaldehyde solution add the diazonium salt solution slowly. The reaction mixture should be stirred efficiently and cooled in an ice-water bath during the addition.
5. The resulting precipitate was filtered, washed several times with cold water and recrystallized from hot chloroform.

Azo compounds were synthesized according to following scheme 1 and scheme 2. The physical and analytical data obtained for these compounds are shown in table 1.



(*E*)-2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)-4-(dimethylamino)benzaldehyde

scheme 1: Azo compound I



scheme 2: Azo compound II

Table 1: The physical and analytical data for synthesized azo compounds

Compound	Molecular formula	Molecular weight	Color	Physical state	M.P. °C	λ_{max} (nm)	R_f
I	$C_{20}H_{21}N_5O_2$	363.41	Red	Powder	242-244 °C	492	0.79
II	$C_{19}H_{19}N_5O_3$	365.39	Red	Powder	251 253 °C	513	0.86

3- Sample collections

Thirty samples were collected from patients related to Samawa-teaching hospital burn unit during December to March 2013, from both sex. The specimens were to flood in sterile tubes containing Brain heart infection broth and incubated over night at 37 °C, next cultured on different media routine and selective , and

incubated aerobically for 24 hrs at 37 °C. The identifications based on Morphology and Gram staining, also by using the conventional biochemical methods [22].

4- Antibacterial Activity

The structured azo compounds were screened for the presence of antibacterial effects against the isolated bacteria from burned infections. i.e. *S.aureus*, *S.pyogenes* a G+ve and *E.coli*, *P. aeuroginosa*, *Klebsiella spp*., *P.mirabilis*, and *A.hydrophila* a G-ve bacteria by disc diffusion method [23]. All the test bacteria were cultured on nutrient agar for inoculums source. Two azo compounds were prepared , azo (I) used with final concentration 250 µg\ml and azo (II) with 125 µg\ml, 6mm diameter filter paper discs were soaked in that concentrations. After streaking the test bacteria on Muller Hinton agar the discs placed on the previously seeded plates and incubated at 37 °C for (18-24) hrs. Different antibiotics were used as comparative source for antibacterial activity, Ciprofloxacin (CIP 5 µg\disc), Amikacin (AK 30 µg\disc), Tetracycline (TE 30 µg\disc), Amoxillin (AX 25 µg\ disc) . The inhibition zone of three replicate measured after incubation period summarized in table 2.

5- Minimum Inhibitory Concentration (MIC)

In this step, MICs were determined by broth micro dilution method [24]. All the isolated bacteria were prepared by using fresh culture to turbidity equivalent to 0.5 McFarland standard tube. Inoculums diluted in phosphate buffer saline (PBS) (8 gm NaCl, 0.2 gm KCl, 0.2gm KH_2PO_4, 1.15gm Na_2HPO_4 to give approximately $1x10^6$ cfu\ml. Two fold serial dilution of azo compounds were done in Muller Hinton broth in 96 wells plate starting from a stock solution of azo (I) 250 µg\ml and azo (II) 125 µg\ml. An equal volume of bacterial inoculums was added to each well in the micro titer plate. The latest wells are broth only for control. The inoculated plates were then incubated at 37 °C for 24 hrs, and the result was readed spectrophotometrically at 620 nm using microplate reader. The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control (wells broth only without inoculum, which ~ 45.0). The effects are reported in table 3.

Results and Discussion

1-Isolated bacteria

In the present study, thirty swabs were collected for both sex males 53.33% and females 46.66% from patients with burn infections reached Al-Samawa teaching hospital burn unite. Sixty five isolates yielded, contain seven bacterial types were diagnosed. The most common *S.aureus* (43.07%) followed *P.aeuroginosa* (21.53%), *Klebsiella* spp. (12.30%), *E.coli* (9.23%), *P.mirabilis* (7.69%),

S.pyogenes (4.61%) and *A.hydrophila* (1.53%). Burn infection conceder one of the major health problems in the world wide [25]. however, the burn become infected due to the environment at the site of wound is ideal for the multiplication of infecting organisms [26].
S.aureus the most common pathogen in burn units, that agreement with [27], while *P.aeuroginosa* has less percent [28-29]. But this results disagreement with [30] and [31] whose found that *P.aeuroginosa and Klebsiella spp*. the most common pathogens in burn infection.

2-Antimicrobial activity:-
The bioassay results for *invitro* antibacterial activity of two azo compounds (I and II) against seven bacterial types of G. positive and G. negative by measuring the inhibition zone on agar plates after incubation time. Both azo compounds exhibited varying degrees of antibacterial effects. In azo (I) the high effect was against *S.pyogenes* (17mm) and less in other types. while in azo (II) showed more effects against all species specially in *E.coli* (28 mm). This results more useful when compared with tested antibiotics as cleared in table 2.
The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of microbes cell walls or on differences in ribosomes of organisms [32]. Although the exact mechanism is not understood biochemically, mode of action of antimicrobials may involve [33]. Interference with cell wall synthesis, damage as a result of which cell permeability may be altered or they may disorganize the lipoprotein leading to cell death, deactivate various cellular enzyme, denaturation of one or more proteins of the cells and formation of a hydrogen through the azo group with the active center of cell constituents, resulting in interference with normal cell process.

Table 2: biological activity of two azo compounds [zone of inhibition (mm) of three replicate]

Test organisms	Azo	Antibiotics

	compounds					
	Inhibition zone diameter (mm)		Inhibition zone diameter (mm)			
	I	II	Ciprofloxacin CIP (5)	Amikacin AK (30)	Tetracycline TE (30)	Amoxillin AX (25)
S.aureus	13	11				
S.pyogenes	17	19				
E.coli	11	28				
P.aeuroginosa	11	16				
Klebsiella spp.	10	14				
P.mirabilis	12	25				
A.hydrophila	10	22				

3- Minimal inhibitory concentration (MIC):-

MIC value of two azo compounds were measured against all the bacterial types by micro dilution assay. There are a variable MICs vales as show in table 3 which indicates that active groups of the synthesized azo compounds N=N, N–H and O–H specially in azo (II**)** can cause remarkable effects on the tested organisms.

Table 3: shows MIC in µg\ml of azo compounds

Azo compounds	Bacterial types						
	Minimal inhibitory concentration (µg/ml)						
	S.aureus	*S.pyogenes*	*E.coli*	*P.aeuroginosa*	*Klebsiella spp.*	*P.mirabilis*	*A.hydrophila*
I	66.0	68.0	70.0	65.0	57.0	81.0	80.0
II	72.0	67.0	46.0	56.0	46.0	67.0	57.0

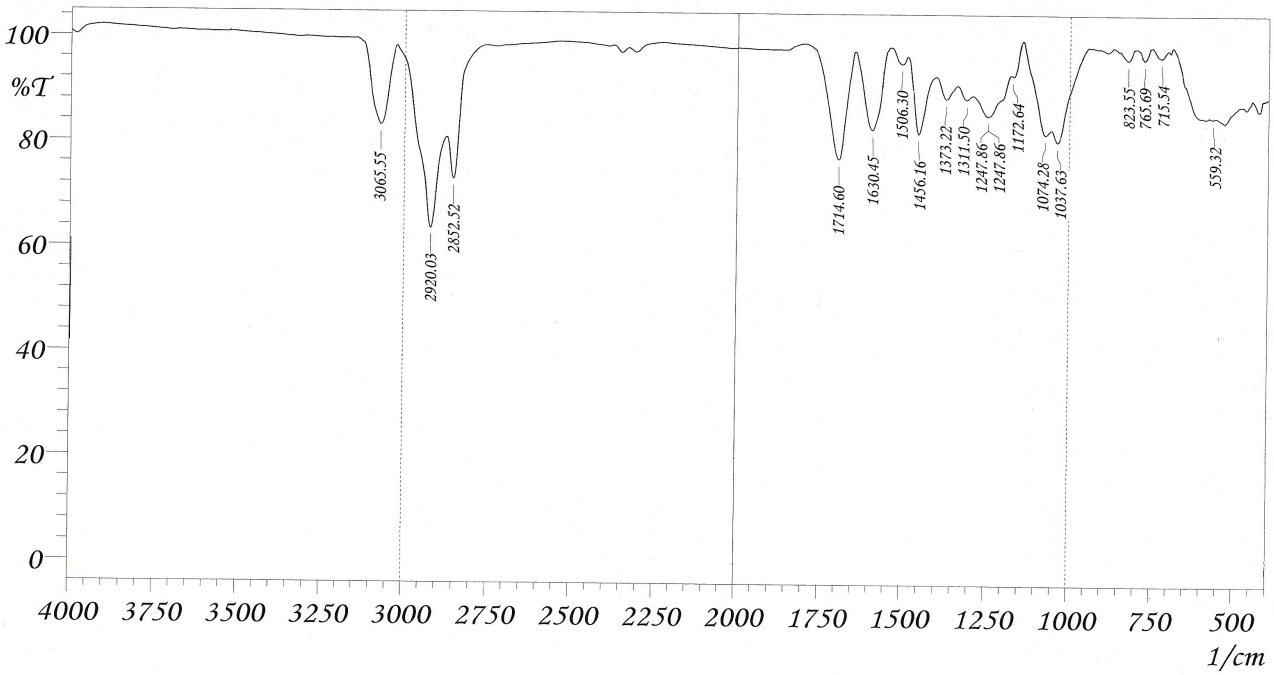
*Negative control absorbency (45.0)

4- Identification of prepared Azo compounds

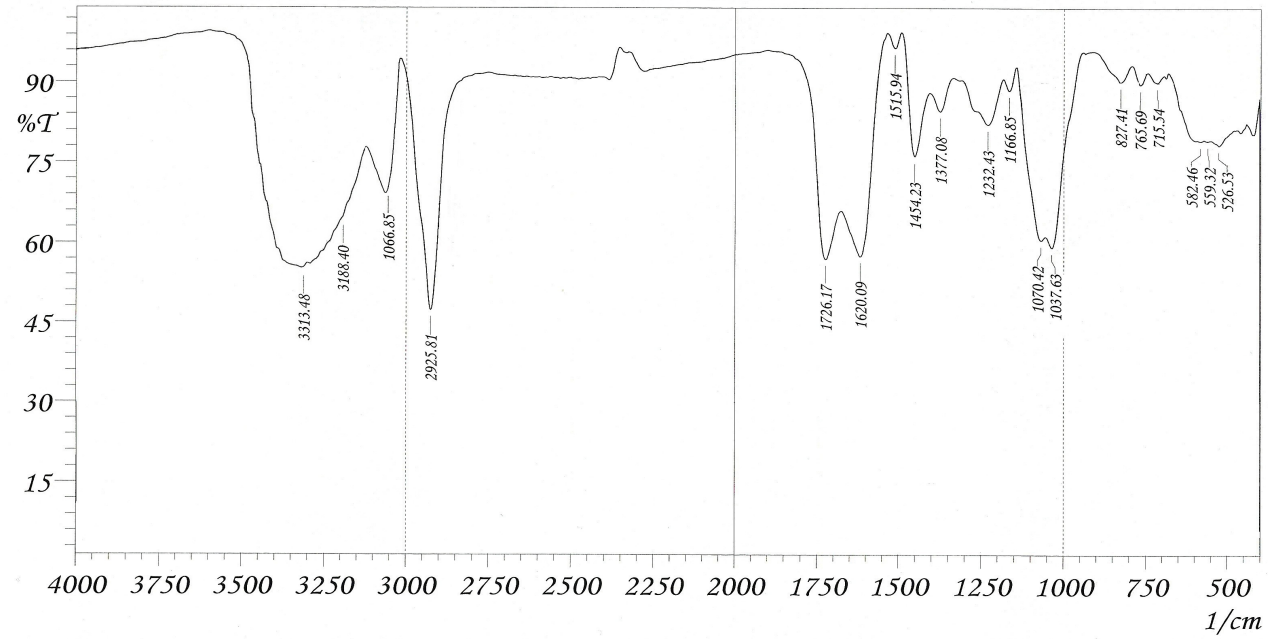
The TLC results showed that only a single spot was observed for synthesized azo compounds.

The azo compounds under study were identified by many techniques: infrared spectra (IR), elemental analysis (CHN) and ultra violet-visible spectra (UV-Vis.).

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. The IR spectra of as shown in Scheme 3, Scheme 4 and Table 5:



Scheme 3: IR Spectra of Azo compound I



Scheme 4: IR Spectra of Azo compound II

Table 5. IR spectral data of synthesized compounds

Compounds	IR Bands	
	Frequency (cm^{-1})	Characteristics
I	3065.55	Aromatic C–H
	2920.03	Aliphatic C–H
	2852.52	Aldehydic C–H
	1714.60	Aldehydic C=O
	1630.45	C=O
	1506.30	N=N
	1456.16	C=C
	1247.86 - 1172.64	C–N
	1074.28 - 1037.63	C–O
II	3313.48	Phenolic O–H
	3188.40	N–H
	1066.85	Aromatic C–H
	2925.81	Aliphatic C–H
	1726.17	C=O
	1620.09	Amidic C=O*
	1515.94	N=N
	1454.23	C=C
	1232.43 - 1166.85	C–N
	1070.42 - 1037.63	C–O

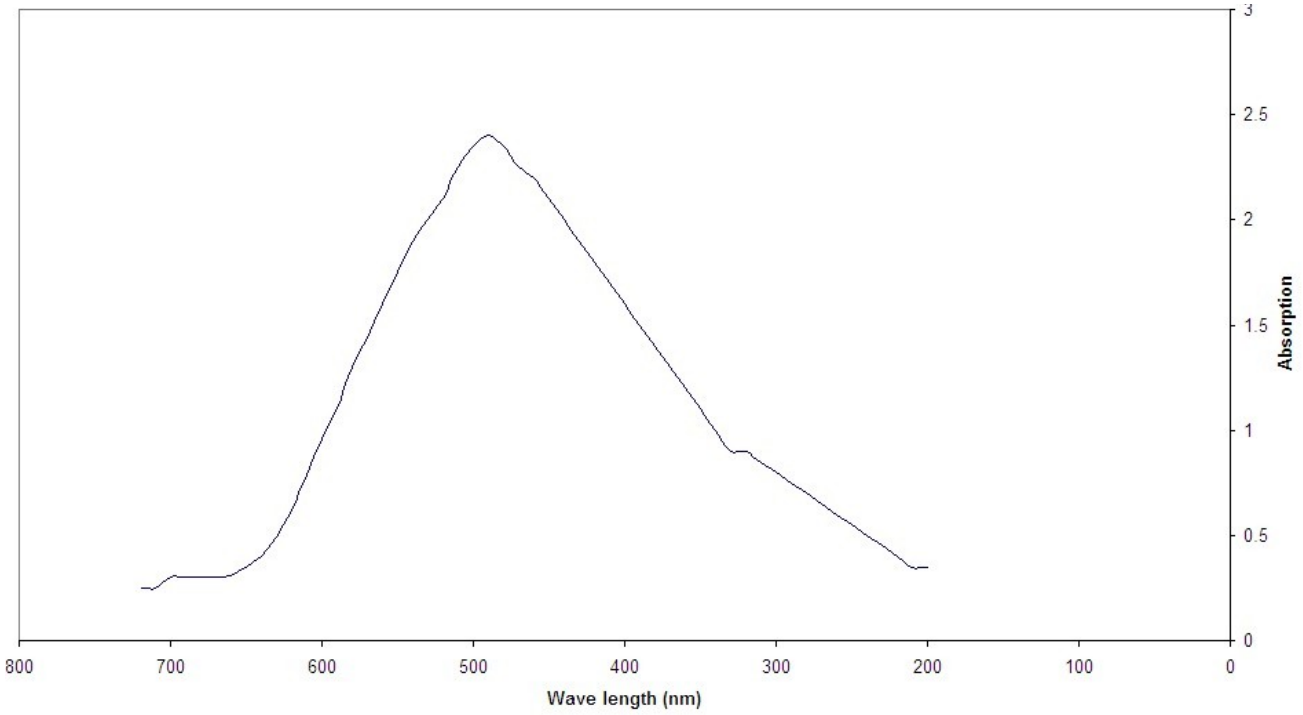
* Because the hydrogen bonding in Amidic C=O, its has lower frequency with comparison another C=O.

The results of elemental analysis CHN of each synthesized azo compounds are shown in Table 6:

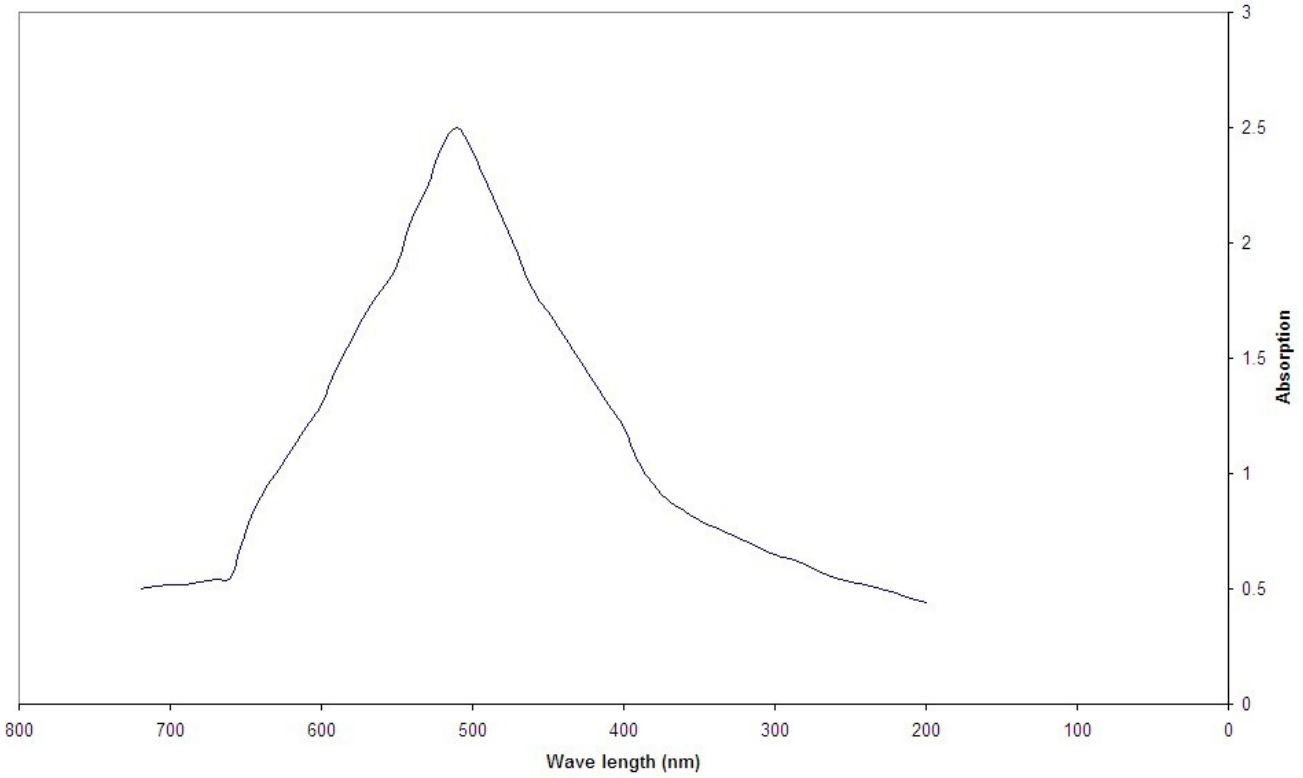
Table 6. elemental analysis CHN of synthesized compounds

Compounds	C%		H%		N%	
	Calculated	Found	Calculated	Found	Calculated	Found
I	66.10	66.21	5.82	5.70	19.27	19.42
II	62.46	62.66	5.24	5.20	19.17	19.63

The results of ultra violet-visible spectra (UV-Vis.)of each synthesized azo compounds are shown in Scheme 5 and Scheme 6:



Scheme 5: UV-Vis. Spectra of Azo compound I



Scheme 5: UV-Vis. Spectra of Azo compound II

Conclusions:

S.aureus is the most pathogenesis in burn wound infections. Azo compounds showed good effects against both G positive and negative bacteria specially azo compound (II).

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تحضير وتشخيص مركبات آزو جديدة ودراسة فعاليتها المضادة للبكتيريا

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الخلاصة

في هذا البحث ، تم تحضير مركبين آزو جديدين من خلال اقتران ملح الديازونيوم الناتج من 4-أمينوانتيبايرين مع 4- (ثنائي مثيل أمينو) بنزيلديهايد و *N*-(4-هيدروكسي فينل) اسيتاميد . تم تشخيص مركبات الآزو المحضرة بواسطة طيف الأشعة تحت الحمراء IR و التحليل الدقيق للعناصر CHN وطيف الأشعة فوق البنفسجية-المرئية UV-Vis. وكروماتوغرافيا الطبقة الرقيقة TLC و درجة الانصهار . كذلك تضمن البحث تعيين الفعالية الضد مايكروبيه لمركبات الآزو المحضرة ضد الانواع البكتيريا المعزولة من الحروق. جمعت ثلاثين عينة من المرضى المراجعين لمستشفى السماوة التعليمي وحدة الحروق. تم الحصول على خمسة وستين عزلة بكتيرية من كلا الجنسين حيث كان الرجال بنسبة 53.33% والنساء 46.66%. جاءت المكورات العنقودية الذهبية Stapylococcus aureuse الاكثر عزلا بنسبة 43.07% تليها الزوائف الزنجارية Pseudomonas aueroginosa بنسبة 21.53% وبكتريا الكليبسيلا 12.30% والاشرشيا القولونية E.coli 9.33% وبكتريا Proteus mirabilis بنسبة 7.69% والمكورات المسبحية المقيحة Streptococcus pyogenes بنسبة 4.61% واخيرا بكتريا Aeromonas hydrophila بنسبة 1.53%. اظهرت مركبات الازو المحضرة قدرة تثبيطية عالية لنمو البكتريا المعزولة باستخدام طريقة الانتشار من الحفر. حددت قيم التركيز المثبط الادنى (MICs) لكل الانواع البكتيرية بطريقة سلسلة التخافيف الدقيقة.