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Synthesis and antimicrobial activity of some compounds derived from diazo dyes

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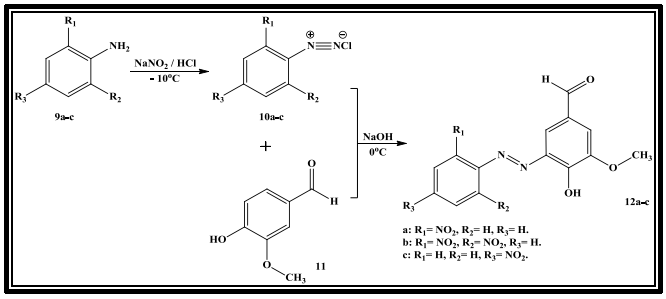
Abstract
Three of diazo dye derivatives 12a-c were synthesized *via* the reaction of diazonium salts 10a-c with vanillin 11 in good yields. The prepared compounds were assigned by FTIR spectra, UV–Vis spectra and melting points determination. Compounds 12a-c were evaluated for antimicrobial activities employing three bacteria strains *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. Interesting compounds 12a and 12b showed selective significant activities to *P. mirabilis* bacteria. Moreover, 12b showed considerable activity at concentration 0.0625 mM (++ = IZ 13–19 mm). Finally, compound 12b showed antibacterial activity against *S. aureus* at concentration 0.25 mM and *E. coli* at concentrations of 0.25 mM and 0.125 mM.

Keywords: synthesis, diazo dyes, diazonium salts, nitroanilines, FTIR, UV–VIS, antimicrobial

1. Introduction
Synthetic dyes are considered one of the important chemical compounds which are containing preliminary chemical materials of many fields in our life such as foods, fabrics, pharmaceuticals, plastics, cosmetics, papers, printing, textiles, drugs, rubber and leather industries. Annually worldwide, more than 10,000 different dyes and pigments are manufactured over 7 x 10^5 tons [1]. Synthetic dyes are easy to prepare low-cost, firmness, a high stability to light, temperature, detergent, microbial attack, and variety in color compared with natural dyes [2, 3]. It is believed that many azo dyes are toxic, carcinogenic and mutagenic to humans, such as benzidine or other aromatic compounds [4] which have mainly –N=N– bonds [5].
Recently, many researchers used chemical processes to remove diazo dyes containing hydroxyl groups from aqueous solution by using graphene oxide-doped [6] or electrolyte degradation or oxidation process by using boron-doped diamond anode and stainless steel cathode from wastewater [7]. Other researchers used biological processes to remove diazo dyes by modified celery *Apium graveolens* [8], by used local agriculture waste shell's seeds of *Ziziphus spina-christi* in Hadhramout, Yemen [9] and by immobilized fungi [10].
Qiu *et al.* have been synthesized azo dyes having aromatic hydroxyl group for dyeing polyester fabric [11], while Ozkinali and Karayel have been synthesized azocalix[4]arenes in all their conformational structures by diazo-coupling reaction [12]. In addition, five-member heterocyclic rings were synthesized *via* diazo dye compounds in a one-pot three component system [13-15].
Based on previous information, we were interested to synthesize three of diazo dye derivatives 12a-c *via* diazonium salts 10a-c which were coupled with vanillin 11, Scheme 1. The synthesized diazo dye derivatives 12a-c were assigned using melting points, FTIR and UV–Vis spectroscopy. However, the microbial inhibitory of these derivatives against some pathogenic bacteria such as *Staphylococcus aureus* as gram +ve, *Escherichia coli* and *Proteus mirabilis* as gram –ve were tested.



Scheme 1: The coupling reaction between diazonium salts 10a-c with vanillin 11 to produce diazo dye derivatives 12a-c.

2. Materials and methods
2.1 General
FTIR spectra were recorded with JASCO FTIR 4600, Single beam, Path Laser, by KBr disk technique in the frequency range of 4000–400 cm^{-1}. UV–Vis spectra were recorded with JASCO V–750 Spectrophotometer in ethanol at the wavelength range of 900–200 nm. All experiments and instrumental analyses were done at PCB Research Laboratory, Faculty of Science, Hadhramout University, Mukalla, Hadhramout, Yemen. All microbial experiments were done at Dar Alshifa Medical Specialized Center, Mukalla, Hadhramout, Yemen.

2.2 General synthesis
2.2.1 Nitroanilines 9a-c
All nitroanilines compounds 9a-c were prepared from aniline 1 with modification as described previously [16, 17].

2.2.2 Diazo dye derivatives 12a-c

Diazo dye derivatives 12a-c were prepared with modification as described previously [13-15]. Nitroaniline compound 9 (6.91 g, 0.05 mol) was dissolved by heating gently in 40 mL of 3 M HCl and then was cooled in an ice bath to 0ºC. A freshly prepared solution of 1 M $NaNO_2$ was added slowly in which the temperature remains –10ºC. The diazonium salt solution 10 was kept in the ice bath and immediately use to the next step. Another solution of vanillin 11 (7.61 g, 0.05 mol) in 100 mL of 1 M NaOH was cooled with stirring in the ice bath. It was added slowly to the diazonium salt solution 10 and the mixture was stirred for 15 min. The solid was filtered by vacuum filtration, and washed several times with water to produce 12a: m.p.: 160.3ºC (B.P.: 542.0±50.0ºC 760 Torr [18, 19], yield 16.0 g, 85.7%, 12b: m.p.: 88.0ºC, yield 8.0 g, 46.2% and 12c: m.p.: 185ºC (142–143ºC [20, 21], yield 12.5 g, 83%.

2.3 The methodology of antimicrobial activity

2.3.1 Bacterial strains

The bacteria strains used in this study are most commonly involved in causing gastroenteritis, wound and burn exudates, urinary tract infection and ear secretions. Three bacteria strains (*Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*) were isolated from different patients attending Dar Alshifa Medical laboratory in Mukalla, Hadhramout – Yemen, and used throughout this study**.** The isolated bacteria were subcultured on Nutrient agar (Difco) and incubated aerobically at 37ºC for 24 hrs. Organisms were maintained in the laboratory on nutrient agar slopes at 4ºC [22].

2.3.2 Determination the minimal inhibitory concentration of 12a-c

The minimum inhibitory concentration MIC of diazo dye derivatives 12a-c was determined by using a different dilution for those derivatives, which diluted with sterile ethanol 75%. It was weighed the amount of 12a-c separately, and dissolved in ethanol 75%. The following concentration was prepared (1: 2, 1: 4, 1: 8, 1: 16 and 1: 32) from the stock of each solution [22, 23].

2.3.3 Antimicrobial activity

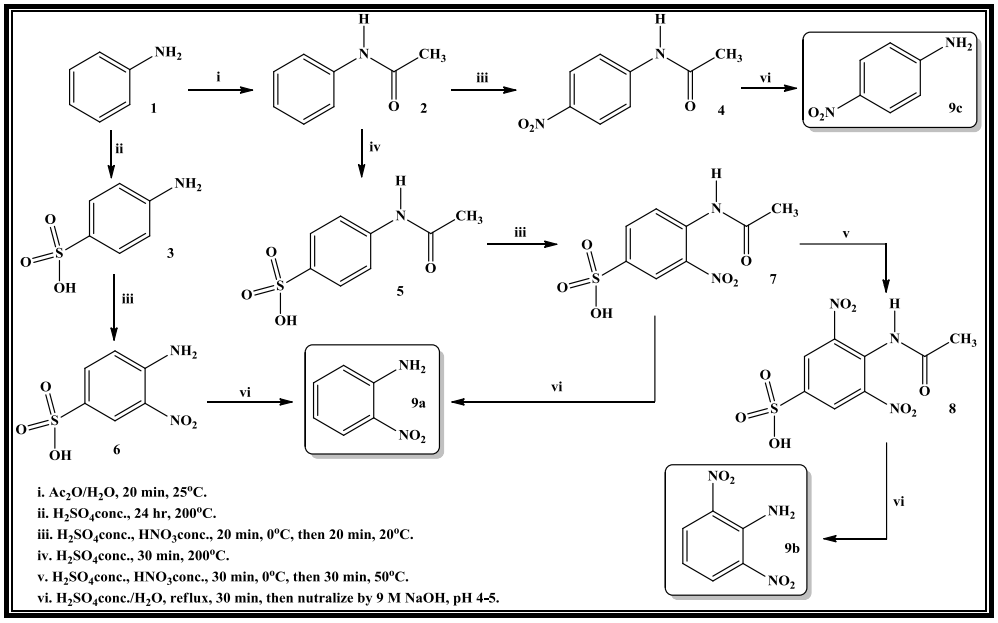
The antibiotic susceptibility tests were carried out by the Kirby–Bauer disk diffusion technique according to the Clinical Laboratory Standard Institute guidelines [24, 25]. About 20 mL of the sterilized medium was poured into each sterile 9 cm diameter of Petri-dish and allowed to solidify. Bacterial suspension for each strain tested was adjusted at 3 x 10^8 CFU/mL by McFarland scale which prepared by mixing 0.1 mL of 1.0% dehydrate barium chloride with 9.9 mL of 1.0% sulfuric acid H_2SO_4 as described by McFarland [26].

About 0.1 mL of the prepared bacterial suspension was spread evenly onto the agar surface using a cotton swab and kept in a refrigerator for 2 hrs. The compound diffuses into the agar resulting in a concentration gradient that is inversely proportional to the distance from the disc. The test medium was Mueller–Hinton agar [27]. Thereafter, all prepared plates were incubated at 37ºC for 24 hrs [28]. After that, the size of the inhibition zone around the disc is a measure in mm of the degree of inhibition [24, 25].

Seven of different antibiotics disks were used as a reference for evaluation of antibacterial: Ciprofloxacin, Amikaci, Clarithromycin, Cefaclor, Cefepime, Azithromycin and Co-trimoxazole [29]. The antibiotics were compared to the effect of synthesized compounds to the antimicrobial activity of these pathogenic bacteria.

3. Results and discussion

Many of nitroaniline derivatives are one of the common compounds that early prepared [16, 17]. In this work, we prepared three of nitroaniline compounds 9a-c *via* aniline 1 as starting material by the classical methods, Scheme 2. All obtained compounds 2–8 from that reactions were investigated by FTIR.



Scheme 2: Preparation route towards of nitroanilines 9a-c from aniline 1.

2.4 FTIR Spectra

The FTIR spectra of 9a-c are depicted in Fig. 1, while the selected FTIR data are listed in Table 1. The IR spectra of three nitro compounds 9a-c showed the absence of the amide group absorption with the presence of two spikes at the range of 3482–3200 cm^{-1} corresponding to a primary

amino group, and two strong bands at the range 1497–1322 cm^{-1} which corresponding to nitro group stretching absorption. The IR spectra also showed characteristic absorption of the =C–H stretch at 3106–3059 cm^{-1} and the unsaturated C=C stretch around 1598 cm^{-1}. The spectrum of 9a showed absorption at 742 cm^{-1}, while the spectrum of 9c showed absorption at 839 cm^{-1}, which is indicated the *ortho* and *para*-substitutions in benzene ring respectively [30].

The sharp stretching band in 9a-c appear at 3478, 3295 and 3482 cm^{-1}, respectively, for the free ν_{as} $–NH_2$ as a strong feature for asymmetrical stretching bands, while other symmetrical stretching bands of the same functional group ν_s $–NH_2$ appear at 3349, 3260 and 3361 cm^{-1}, respectively. The weak intensity bands observed of benzene rings at the respective 3175, 3194 and 3220 cm^{-1} that are ascribed to the stretching of aromatic ν C=C–H. The δ_s $–NH_2$ bending (scissoring) vibration of primary amine shown strong absorption at 1569, 1599 and 1587 cm^{-1}, respectively. However, the asymmetrical bending vibration of ν_{as} $–NO_2$ occurred at the respective 1498, 1488 and 1471 cm^{-1}, while the symmetrical bending vibration of ν_s $–NO_2$ appeared at 1345, 1322 and 1300 cm^{-1}, respectively [31].

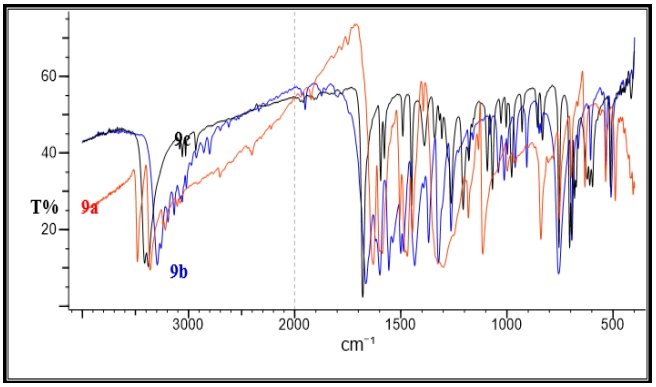


Fig 1: FTIR spectra of prepared nitroanilines 9a-c.

The FTIR spectra of 12a-c are depicted in Fig. 2 and selected FTIR data are listed in Table 1. Compound 12a was obtained as an orange solid compound with m.p. 160.3ºC (b.p. 542.0±50.0ºC 760 Torr [18, 19]. It showed in its IR spectrum a broad O–H absorption around 3372 cm^{-1}, also, intense carbonyl absorption at 1693 cm^{-1} with two peaks at 2724 and 2844 cm^{-1} characteristic of the aldehyde C–H stretch. There are unsaturated =C–H stretch at 3175 cm^{-1}, and characteristic C=C stretching frequency for aromatic rings around 1598 cm^{-1}[32, 33].

As a new compound 12b, it was obtained as brown solid dye with m.p. 88.0ºC. The IR spectrum of 12b exhibited absorption band of the hydroxyl group around 3277 cm^{-1}. In addition, to the strong C=O stretching absorption at 1666 cm^{-1} with an aldehyde C–H stretching frequencies around 2750 cm^{-1}. The C=C stretch at 1619 cm^{-1} indicated an aromatic ring, confirmed by the unsaturated C–H absorption at 3020 cm^{-1}. Compound 12c was obtained as a yellow-brown solid compound with m.p. 185ºC, which it was obtained with Amaravathi *et al.*[20] and Kumar *et al.*[21] at 142–143ºC.

The IR spectrum of 12c showed a broad ν –OH band around 3466 cm^{-1}. Also, the spectrum showed the absorption at 1694 cm^{-1} which together with the two peaks at 2740 and 2845 cm^{-1} indicated the presence of aldehyde group. The presence of both unsaturated C=C stretching at 1602 cm^{-1} and unsaturated C–H stretching at 3067 cm^{-1} confirmed the presence of the aromatic rings. The broad band in 12a-c appear at 3372, 3277 and 3466 cm^{-1}, respectively, for the ν –OH frequency, while the bands with weak intensity observed of benzene rings at 3175, 3161 and 3110 cm^{-1} are ascribed to the stretching of aromatic ν C=C–H. The strong stretching bands of ν C=O of aldehyde were observed at 1693, 1666 and 1694 cm^{-1}, respectively [32, 33].

Indeed, the symmetrical stretching band of –N=N– in *trans*-azo compounds is forbidden in IR, while it absorbs in 1576 cm^{-1} in Raman spectrum. Additionally, the unsymmetrical stretching band of the same functional group with *para*-substituted of an electron-donating group showed at 1429 cm^{-1} [31]. The bands with strong intensity observed of azo bonds at the respective 1574, 1589 and 1536 cm^{-1} are ascribed to the symmetrical stretching of ν_s –N=N–, which they are observed near 1597 cm^{-1}[34]. While the bands observed at 1411, 1430 and 1427 cm^{-1} assigned to the asymmetrical stretching band of the same functional group, respectively.

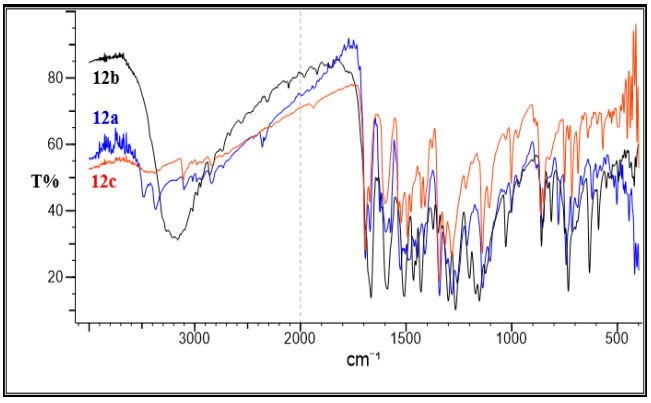


Fig 2: FTIR spectra of prepared diazo dye derivatives 12a-c.

Table 1: FTIR spectral data of each 9a-c and 12a-c (cm^{-1}) in KBr

Cpd.	OH Arom.	NH_2 Arom.	C–H Arom.	CH_3 Aliph.	N=N	NH_2	CH_3	NO_2	C–O–C aliph.
9a	–	ν_{as} 3478 ν_s 3349	3175	–	–	1569	–	ν_{as} 1498 ν_s 1345	–
9b	–	ν_{as} 3295 ν_s 3260	3194	–	–	1599	–	ν_{as} 1488 ν_s 1322	–
9c	–	ν_{as} 3482 ν_s 3361	3220	–	–	1587	–	ν_{as} 1471 ν_s 1300	–
12a	3372	–	3175 3108	*as* 2981 *sy* 2836	*sy* 1574 *as* 1411	–	*as* 1446 *sy* 1390	*as* 1485 *sy* 1342	*as* 1283 *sy* 1138
12b	3277	–	3161 3020	*as* 2976 *sy* 2858	*sy* 1589 *as* 1430	–	*as* 1454 *sy* 1372	*as* 1508 *sy* 1301	*as* 1266 *sy* 1153
12c	3466	–	3110 3067	*as* 2981 *sy* 2865	*sy* 1536 *as* 1427	–	*as* 1440 *sy* 1376	*as* 1477 *sy* 1345	*as* 1285 *sy* 1140

Hence, it is believed the lower symmetrical stretching of both nitro groups at *ortho* position in 12b due to the intramolecular hydrogen bonding and the dipole interaction between them with phenolic and aldehydic hydrogen's atoms, Fig. 3. These overlaps leave these bonds to have a little electron density, a little less stiff, and as a result, vibrate a little more slowly. The O–H stretching frequency in 12b reflects the stronger intramolecular hydrogen bonding showing a very broad absorption around 3277 cm^{-1}, Fig. 3. On the other hand, the IR spectra of both 12a and 12c display slightly lower asymmetrical stretching frequencies of $–NO_2$ and O–H that of intramolecular and intermolecular hydrogens bonding, respectively, Fig. 3 and 4.

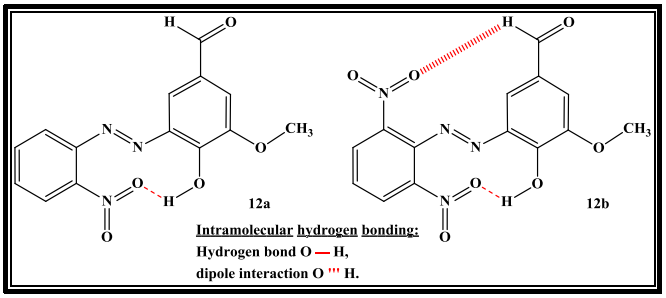


Fig 3: The possibility of dipole interaction and intramolecular hydrogen bonding in 12a and 12b.

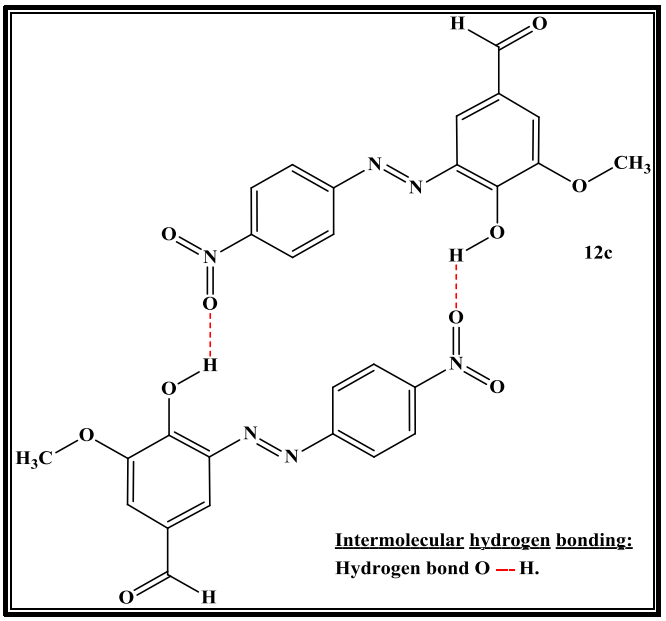


Fig 4: The possibility of intermolecular hydrogen bonding in 12c.

2.5 UV–Vis Spectra

The λ_{max} of vanillin **11** was measured by Rind *et al.* [35] at 280 nm in ethanol from natural and synthetically sources, which it is found at 282 nm by used Woodward–Fieser rules[36, 37]. In 1991, Mustroph was investigated and studied the electronic absorption effects of various azobenzene derivatives, including the effecting of donor substituted of azobenzenes. The λ_{max} of azo –N=N– functional group shows in *n*-hexane at 390–477 nm due to $\pi \rightarrow \pi^*$ [38, 39]. Islik *et al.* were reported the chromophore of azo –N=N– functional group within two or more aromatic rings. The λ_{max} of that compound in $CHCl_3$ were 333.74, 242.7 and 215.7 nm, respectively [40]. While the λ_{max} of some other compounds were absorbed in 251, 280 and 330 nm of nitrobenzene, at 211 and 270 nm of phenol, and of methoxybenzene at the respective 220 and 272 nm [36, 37].

By the way, the UV-Vis spectra of the 12a-c in ethanol show three bands. The first band around 200.2 nm results from $\pi \rightarrow \pi^*$ of the benzene ring, while the second band between 254 and 282 nm results from $\pi \rightarrow \pi^*$ electronic transitions of nitroaniline and vanillin aromatic rings. The third band between 391 and 495 nm that is due to the intramolecular charge-transfer and resulted from $\pi \rightarrow \pi^*$ electronic transitions between nitroaniline and vanillin through the azo group –N = N– as a bridge group. On the other hand, there are very weak band absorptions in the UV-Vis spectra that are probably resulting from $n \rightarrow \pi^*$, and it is indicated the transition of the one nonbonding electrons on oxygen atoms to a π^* antibonding orbital.

2.6 Microbiology activity

The results of the antibacterial activity of diazo dye derivatives 12a-c against *S. aureus*, *E. coli*, and *P. mirabilis* were presented in Table 2. The highest compound effective against bacteria tested of diazo dye derivatives was 12b that inhibited growth of all pathogenic bacteria *S. aureus*, *E. coli* and *P. mirabilis* at concentration 0.25 mM (+ = IZ 7–13 mm) and at concentration 0.125 mM were active only of *E. coli* and *P. mirabilis*, while *P. mirabilis* was observed sensitive of both 12a and 12b with all concentrations. However, the new compound 12b shows the most sensitive at concentration 0.0625 mM (++ = IZ 13–19 mm), Table 2.

Several types of bacteria are susceptible to the antibacterial activity of other compounds regardless of their resistance to antibiotics [41]. The antimicrobial agent groups based on the mechanism of actions are: inhibit cell wall synthesis, depolarize cell membrane, inhibit protein synthesis, inhibit nucleic acid synthesis and inhibit metabolic pathways [42]. Therefore, compound 12c was inactive of all bacteria at whole concentrations that indicated all bacteria tested were the resistance of this compound, Table 2. A similar result was previously reported by Al–Shara′ey *et al* which they have been reported that most bacteria tested were resistant to some synthesized polycyclic compounds [43, 44].

Seven antibiotics used in this study that the highest percentage of antibiotics active were to Ciprofloxacin, Amikaci and Clarithromycin to *S. aureus*, while inactive to both *E. coli* and *P. mirabilis*. Each of Cefaclor, Cefepime, Azithromycin, and Co-trimoxazole were shown more active to *E. coli* and *P. mirabilis* except *S. aureus*. Some studies indicated to better understand virulence and multidrug resistance, revealed that most of the antibiotic resistance genes and many of the virulence genes reside on mobile genetic elements, such as plasmids, transposons, and prophages, indicating substantial horizontal gene transfer from other bacteria [45].

Table 2: Inhibitory growth activity of diazo dye derivatives 12a-c against pathogenic bacteria (diameter of inhibition zone = IZ).

Cpd.	Conc. mM	Microorganisms Mean diameter of inhibition zone IZ (mm)		
		gram +ve	gram –ve	
		S. aureus	*E. coli*	*P. mirabilis*
12a	2.0	–	–	+
	1.0	–	–	+
	0.5	–	–	+
	0.25	–	–	+
	0.125	–	–	+
	0.0625	–	–	+
12b	2.0	–	–	+
	1.0	–	–	+
	0.5	–	–	+
	0.25	+	+	+
	0.125	–	+	+
	0.0625	–	–	++
12c	2.0	–	–	–
	1.0	–	–	–
	0.5	–	–	–
	0.25	–	–	–
	0.125	–	–	–
	0.0625	–	–	–
Cf	5*	+++	–	–
Am	30*	+++	–	–
Ct	15*	+++	–	–
Ca	30*	–	++	+++
Ce	30*	–	++	++
An	15*	–	+	–
Cm	25*	–	+++	+++

Key to symbols: *: µg, Cf: Ciprofloxacin, Am: Amikaci, Ct: Clarithromycin, Ca: Cefaclor, Ce: Cefepime, Az: Azithromycin and Cm: Co-trimoxazole as antibacterial, Disc diameter = 6 mm, Highly active: +++ (IZ > 19 mm), Moderately active: ++ (IZ 13-19 mm), Slightly active: + (IZ 7-13 mm), Inactive: – (IZ < 7 mm).

With Comparing between the antibiotics and synthesized diazo dye compounds 12a-c in antimicrobial activity, it was observed that antimicrobial effect of 12b at concentration 0.25 mM was better than other antibiotics effect against pathogenic bacteria, followed compound 12b at concentration 0.125 mM. We believe that functional groups of these compounds had an increasing effect on the levels of toxicity of bacterial species.

4. Conclusion

In this paper, three nitro aniline compounds 9a-c and their azo dyes 12a-c with vanillin 11 were synthesized and characterized by FTIR and UV-Vis spectroscopy. All compounds are in the solid state, so, their melting points have been determined. IR spectra show the lower symmetrical stretching of nitro groups at 2 and 6 positions in compound 12b, and slightly lower asymmetrical stretching frequencies of $–NO_2$ and O–H in both compounds 12a and 12c that is believed to intramolecular and intermolecular hydrogen's bonding. Antibacterial activity of that three diazo dye derivatives was evaluated. Fortunately, both of the two compounds 12a and 12b exhibit antibacterial activities especially against *P. mirabilis* bacteria. Moreover, compound 12b showed significant antibacterial activity at concentration 0.0625 mM (++ = IZ 13–19 mm), also, it showed antibacterial activity against *S. aureus* at concentration 0.25 mM, and *E. coli* at concentrations 0.25 mM and 0.125 mM.

5. Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

6. Acknowledgments

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