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SYNTHESIS AND CHARACTERIZATION OF NEW AZO-SCHIFF BASES AND THEIR BIOLOGICAL ACTIVITY

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ABSTRACT

This work focused on the synthesis of new azo Schiff bases which were prepared by condensation of 3-formyl-4-hydroxy phenylazo benzene with both 1,3-diamino xylylene, 2,6-diamino Pyridine, 1,5-diamino-2-methyl pentane respectively. The Schiff bases were checked by different spectral technique (LC-MS, GC-MS, 1H-NMR, IR). The new azo Schiff Bases were studied for antibacterial activities against (Bacillus subtilis and Staphylococcus aureus) are Gram positive and (Salmonella typhi and Escherichia coli) is Gram negative. The azo compound ligand were exhibited a vari-able activity of inhibition on the growth of the bacteria.

Keywords: *Azo Schiff base, 3-Formyl-4-Hydroxy Phenylazo Benzene, Spectral Technique, Anti-Microbial Activity.*

1. INTRODUCTION

Schiff bases are compounds which prepared by the condensation of aldehyde or ketone with primary amines [1-3]. Schiff bases are used in the synthesis of some industrial, bioactive and inorganic compounds [4-5]. The compounds include good fungicdal activity [6], Anticancer [7-9], antibacterial [10-12], antifungal [13,14], antiinflammatory [15-17] and herbicidal activities[18]. Azo compounds are widely used as dyes and pigments. Another application is analytical chemistry. On the other hand azo compounds show biological activities [18]. Schiff bases and azo compounds are important structures in the medicinal and pharmaceutical fields [19]. Azo compounds, with two phenyl rings separated by an azo (-N=N-) bond, are versatile molecules and have received much attention in research areas both fundamental and application [20]. The strong electronic absorption maximum can be tailored by ring substitution to fall anywhere from the ultraviolet to red-visible regions, allowing chemical fine-tuning of colour [20]. This, combined with the fact that these azo groups are relatively robust and chemically stable, has prompted extensive study of azo benzene-based structures as dyes and colorants. Furthermore, the light-induced inter conversion allows systems incorporating azo group to be used as photo switches, effecting rapid and reversible control over a variety of chemical, mechanical, electronic and optical properties [21].

Recently heterocyclic azo compounds have been used in the Mitsunobu reaction [22]. Usually, azo compounds were synthesized by diazoti-zation of the amine in mineral acid at about 0°C [22-26]. Schiff bases are used as substrates in the preparation of a large amount of bioactive and industrial compounds [27-31]. Azo compounds have a

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great biological activity as well as industrial importance [32,33]. The interaction of metal complexes with biological systems, which is the field of bio coordination chemistry, is receiving an increasing interest [34]. In this study new Schiff base of 3-formyl-4-hydroxy phenylazo benzene with 1,5-diamino -2 - methyl pentane,1,3-diamino xylene and 2,6-diamino Pyridine were prepared and subjected to antimicrobial activity against different gram-positive and gram-negative bacteria.

2. EXPERIMENTAL

2.1. MATERIALS AND METHODS

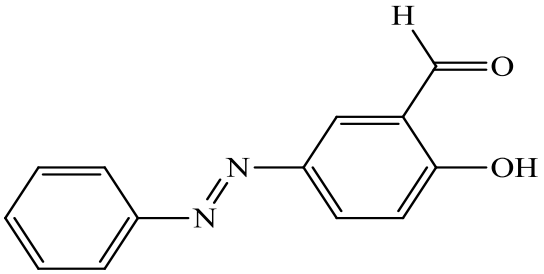
Ethanol, dimethyl formamide, salicyl-aldehyde, aniline, 1,3-diamino xylylene, 2,6-diamino Pyridine, 1,5-diamino-2-methyl pentane, Nutrient gar, Filter paper discs (Whatman No.1 filter paper, 5mm diameter). All materials obtained from Sigma Aldrich Company. IR spectra were recorded on Jasco 300 FT-IR spectrometer using compressed KBr discs. Mass spectra of the compounds were measured on a micro mass Quattro LC-MS/MS, GC-MS/MS Spectrometer. 1H-NMR spectra were recorded at ambient Bruker DT-400 Spectrometer using $CDCl_3$ with DMSO- DMF as the internal standard, M.P Apparatus Digital (32-300 ^{0}C).

2.2.1 Synthesis of 3-formyl-4-hydroxy phenylazo benzene

Azoaldehyde was prepared from aniline and 2-hydroxy benzaldehyde in aqueous medium at 0-5 ^{0}C according to a reported method and was purified by recrystallization from warm 95% ethanol [35].

2.2.2 Synthesis of Bis-N-(5-phenylazo-2-hydroxy-1-benzylidine)-1,3-diamino xylylene (I)

A hot ethanolic solution (40ml) of 3-formyl - 4-hydroxy phenylazo benzene (2.26g - 0.01 mol) was added to a hot ethanolic solution (40ml) of 1,3-diamino xylylene. (0.68g - 0.005 mol) and the reaction mixture was refluxed for 3 hour. A solid mass (Scheme 2) seprated out on cooling [36, 37]. It was suction filtered, washed with ethanol, diethyl ether and recrystallized from suitable solvent, then dried in vacuum.



Scheme 1 3-formyl-4-hydroxy phenylazo benzene

Orange; Yield: (80 %), m.p=189.3-189.6 ^{0}C, Empirical formula: ($C_{34}H_{28}N_6O_2$), M.Wt: 552.

Mass spectrum (m/z):552. (Fig.1.)

UV-Vis: λmax = 355 nm (Fig.2.)

IR (KBr disk): 3417.86-3468.01 cm^{-1} (O-H), 3008.95-3062.96 cm^{-1} (C-H, aromatic), 2881.65 cm^{-1} (C-H, aliphatic), 1631.78 cm^{-1} (C=N), 1585.49 cm^{-1} (C=C, aromatic), 1489.05 cm^{-1} (-N=N-), 1284.59 cm^{-1} (C-O, phenolic) (Fig.3.)

1H-NMR ($CDCl_3$-400MHz) δ=14 (s, 2H, OH), 8.584 (s, 2H, CH=N), 7.085 - 8.023 (m, 20 H, Ar), 4.899 (s, 4H,-CH_2) (Fig.4).

Elemental analysis: $C_{34}H_{26}N_8O_4$, Calculated (%) C: 73.90; H: 5.11; N: 15.21 Found (%) C: 73.97, H: 5.17, N: 15.18.

2.2.3. SYNTHESIS OF BIS-N-(5-PHENYLAZO-2-HYDROXY-1-BENZYLIDINE) - 2,6-DIAMINO PYRIDINE. (II) SCHEME (3)

Azo-coupled precursors were prepared as described previously [35] was added (0.01 mol) of 3-formyl-4-hydroxy phenylazo benzene in 30 ml from absolute ethanol to 2,6-diamino Pyridine, (0.005 mol) in 60 ml ethanol. The reaction mixture was refluxed

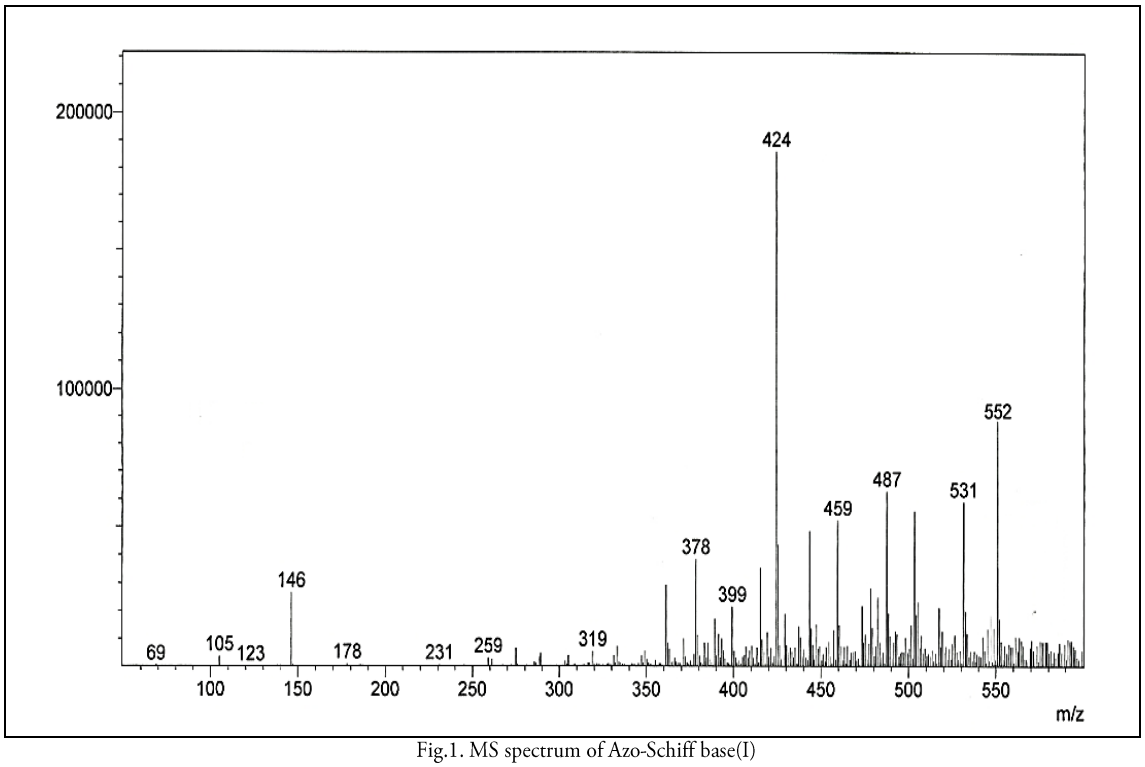


Fig.1. MS spectrum of Azo-Schiff base(I)

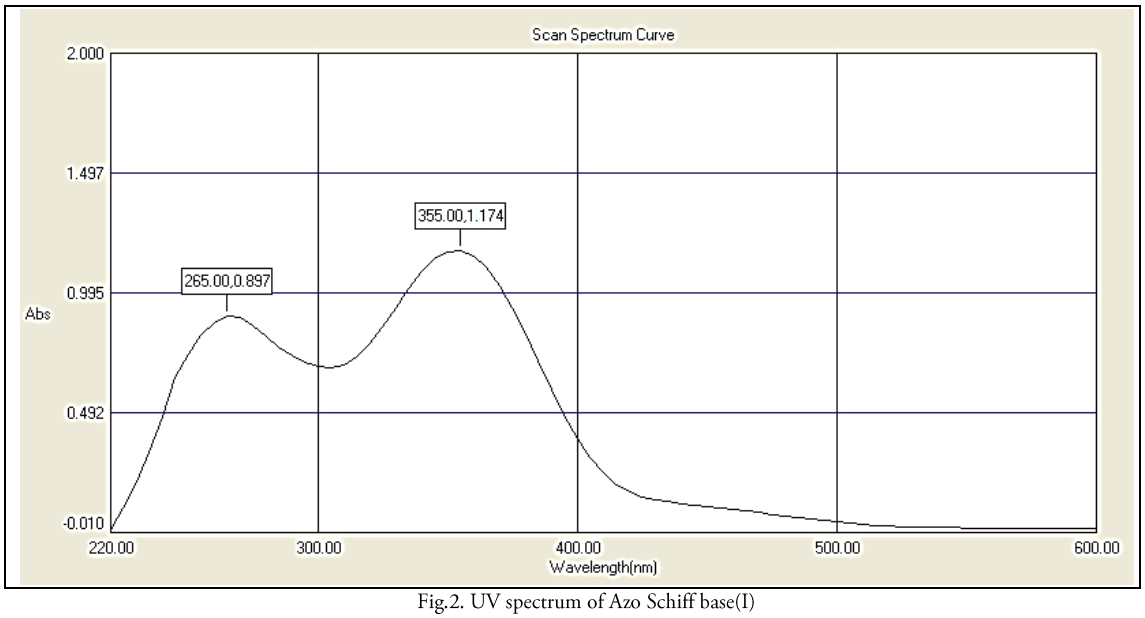


Fig.2. UV spectrum of Azo Schiff base(I)

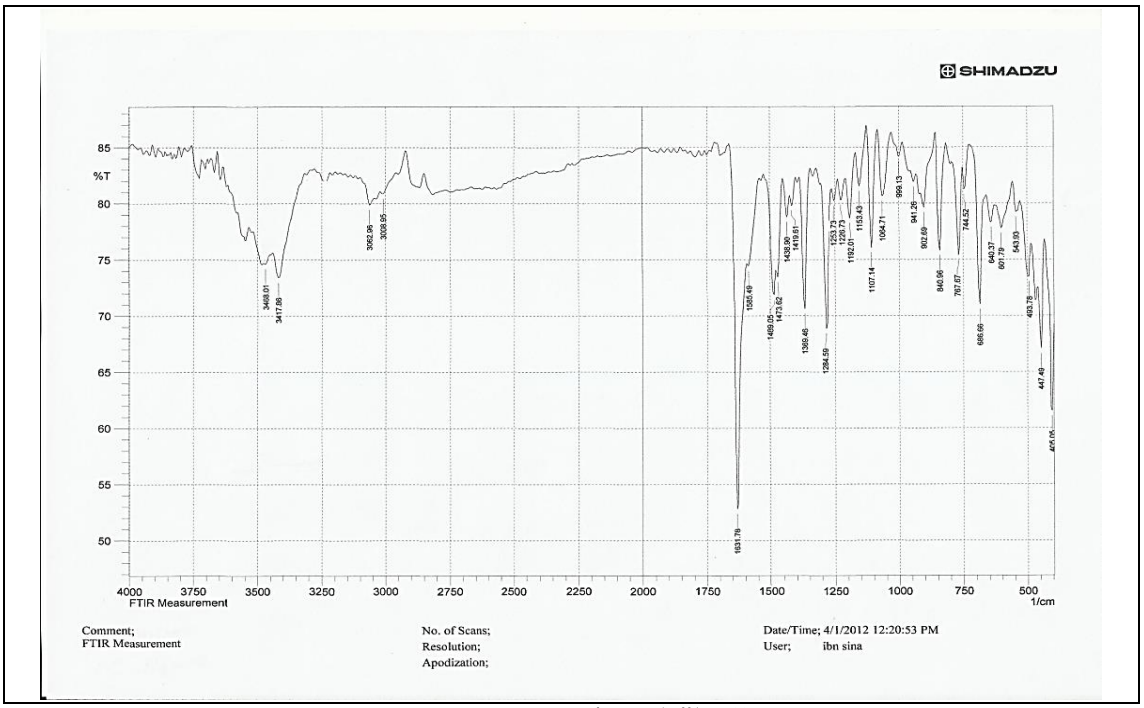


Fig.3. IR spectrum of Azo-Schiff base(I)

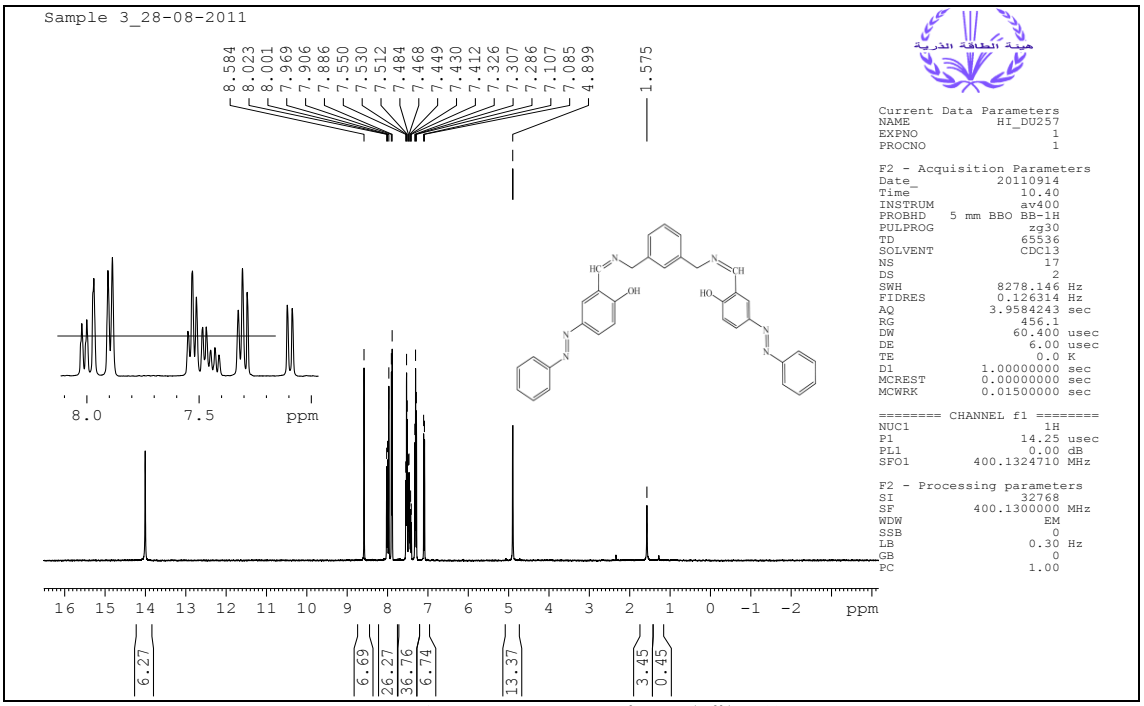


Fig.4. 1-HNMR spectrum of Azo-Schiff base(I)

for 3 hour with continue stirrer. A solid mass formatted on hot. The reaction mixture was allowed to cool room temperature, it was suction filtered and recrystallized from suitable solvent, then dried in vacuum [36, 37].

Dark red; Yield: (75 %), m.p=206 - 209 ^{0}C, Empirical formula: ($C_{31}H_{23}N_7O_2$), M.Wt: 525

Mass spectrum (m/z):525 (Fig.5.).

UV-Vis: λmax = 345 nm (Fig.6.).

IR (KBr disk): 3414.86-3475.73 cm^{-1}(O-H), 3005.10-3059.10 cm^{-1} (C-H), aromatic), 1612.49 cm^{-1} (C=N), 1566.20 cm^{-1} (C=C, aromatic), 1485.19 cm^{-1} (N=N), 1280.73 cm^{-1} (C-O), phenolic) (Fig.7.).

1H-NMR ($CDCl_3$-400MHz) δ=13.7(s, 2H, OH), 8.089 -6.995 (m, 19 H, Ar), 8.809 (s, 2H, CH=N. (Fig.8.).

Elemental analysis: $C_{31}H_{23}N_7O_2$, Calculated (%): C: 70.84; H: 4.41; N: 18.66 Found(%) C: 70.80, H:4.38, N:18.6.

2.2.4. Synthesis of Bis-N-(5-phenylazo-2-hydroxy-1-benzylidine) - 1, 5-diamino-2-methyl pentane (III)

Azo-coupled precursors were prepared as described previously [35] was added (0.01 mol) of 3-formyl-4-hydroxy phenylazo benzene in 30 ml from absolute ethanol to 1,5-diamino-2-methyl pentane, (0.005 mol) in 60 ml ethanol, The reaction mixture was refluxed for 3 hour with continue stirrer. A solid mass formatted on hot. The reaction mixture was allowed to cool room temperature; it was suction filtered Scheme (4) and recrystallized from suitable solvent, then dried in vacuum [36, 37].

Orange; Yield: (78 %), m.p=140 - 143 ^{0}C, Empirical formula: ($C_{32}H_{32}N_6O_2$).M.Wt: 532.

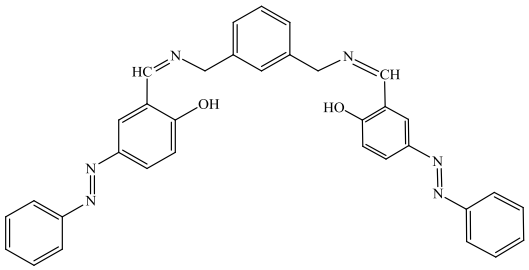
Mass spectrum (m/z):532. (Figure 9)

UV-Vis: λmax = 370 nm (Figure 10)

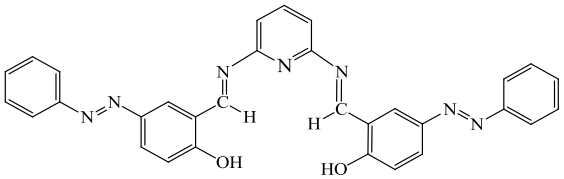
IR (KBr disk): 3417.86-3475.73 cm^{-1}(O-H), 3059.10 cm-1 (C-H, aromatic), 2812.21-2935.66 cm^{-1} (C-H, aliphatic), 1635.64 cm^{-1} (C=N), 1585.49 cm^{-1} (C=C, aromatic), 1496.76 cm^{-1} (-N=N-), 1284.59 cm^{-1} (C-O, phenolic) (Fig.11.).

1H-NMR ($CDCl_3$-400MHz) δ=14.318 (d, 2H, OH), 8.438-8.458 (d, 2H,CH=N), 7.080 -8.012 (m,16 H, Ar), 3.510-3.685 (t,4H-CH2-N=),1.375-1.974 (m,4H,-CH_2-), 1.059-1.076 (t,3H,-CH_3) (Fig.12.).

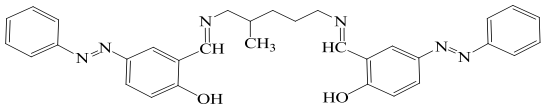
Elemental analysis: $C_{31}H_{23}N_7O_2$, Calculated (%) C: 72.16; H: 6.01; N: 15.78. Found (%) C: 72.17, H: 5.91, N: 15.63.



Scheme 2 Azo Schiff base(I)



Scheme 3 Azo Schiff base(II)



Scheme 4 Azo Schiff base(III)

2.3. BIOLOGICAL Activity

The prepared compounds were tested for their antimicrobial activity against four species of bacteria (Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella typhi) using filter paper disc method [38]. The screened compounds were dissolved individually in DMSO (dimethyl sulfoxide) in order to make up a solution of 50, 100, and 200 μg/ml concentration for each of these

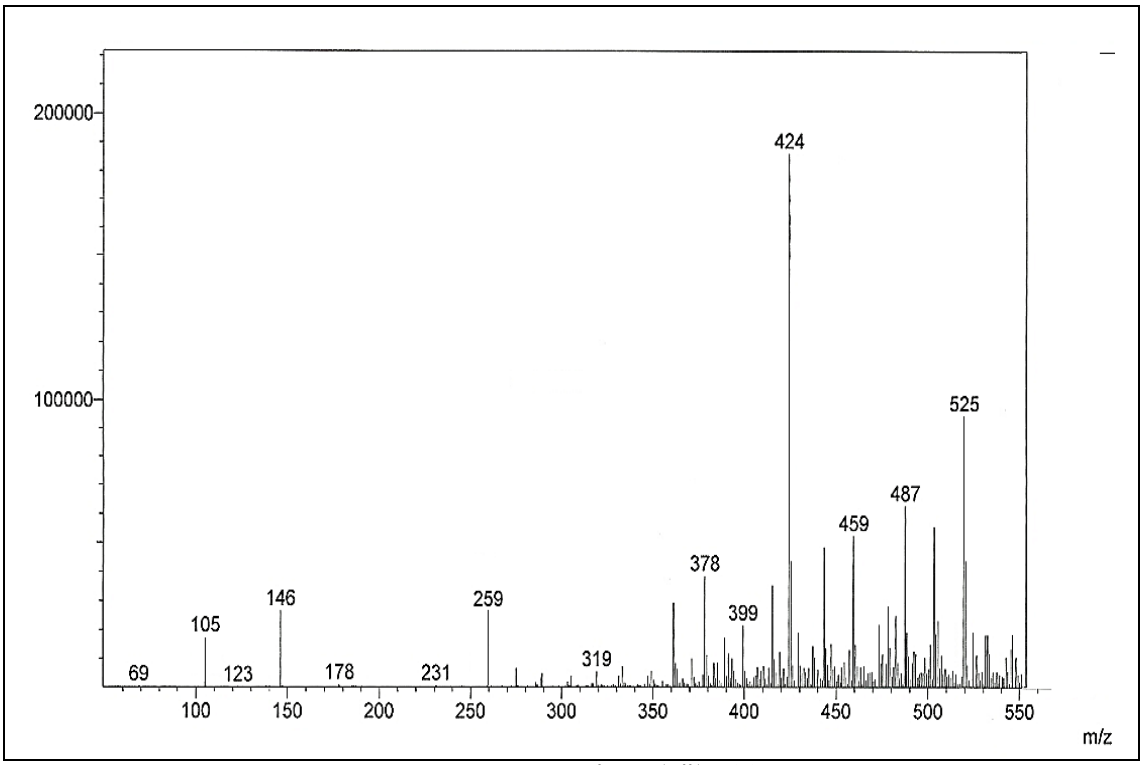


Fig.5. MS spectrum of Azo-Schiff base(II)

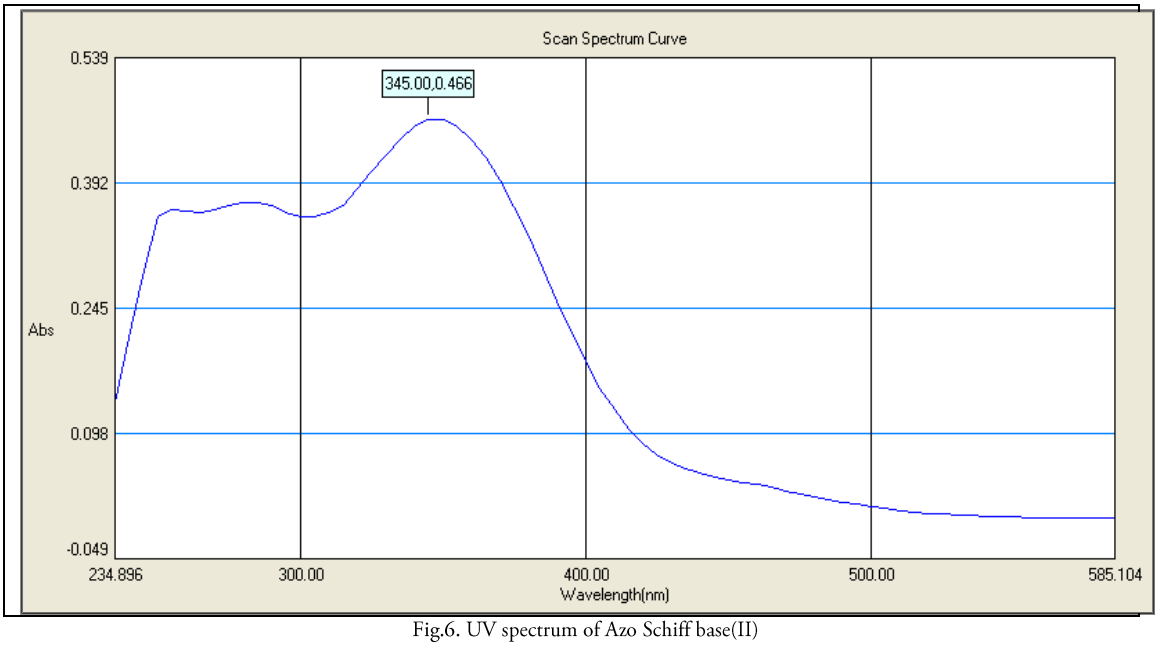


Fig.6. UV spectrum of Azo Schiff base(II)

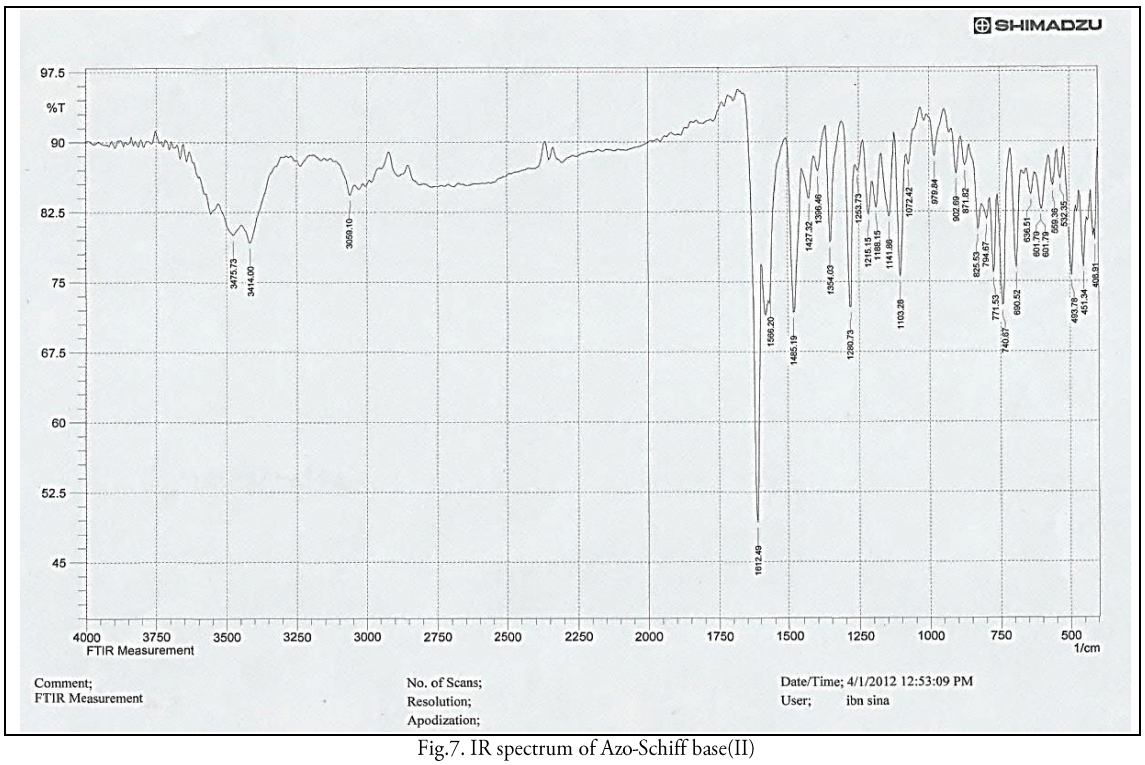


Fig.7. IR spectrum of Azo-Schiff base(II)

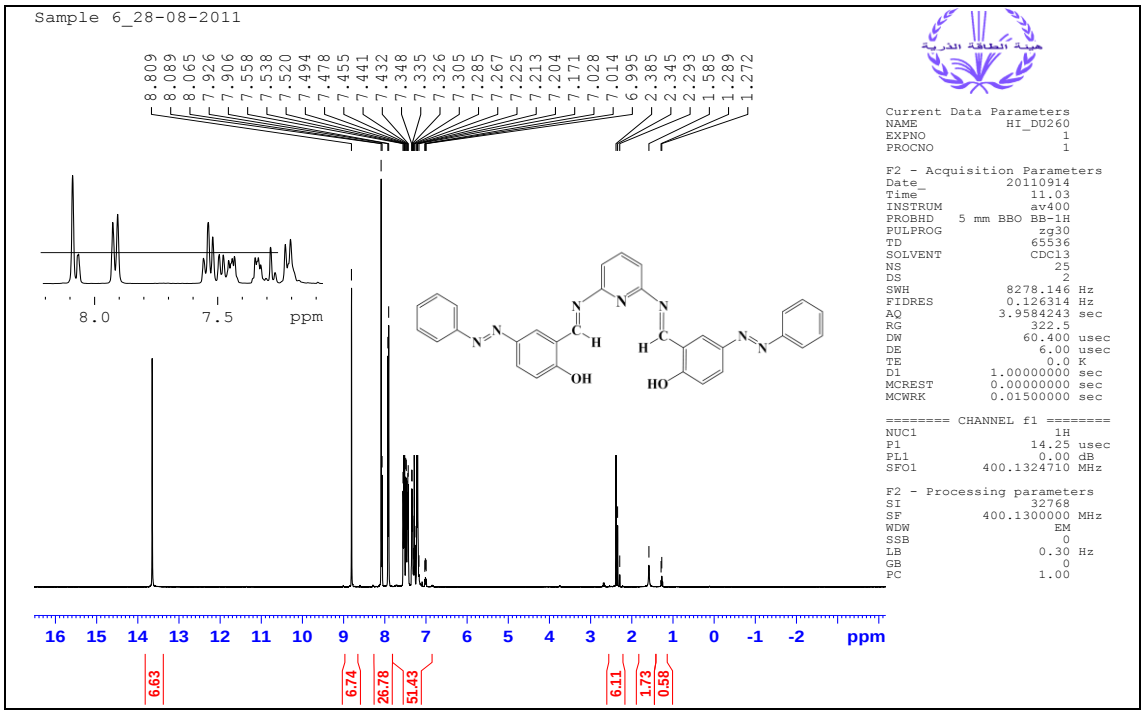


Fig.8. 1-HNMR spectrum of Azo-Schiff base(II)

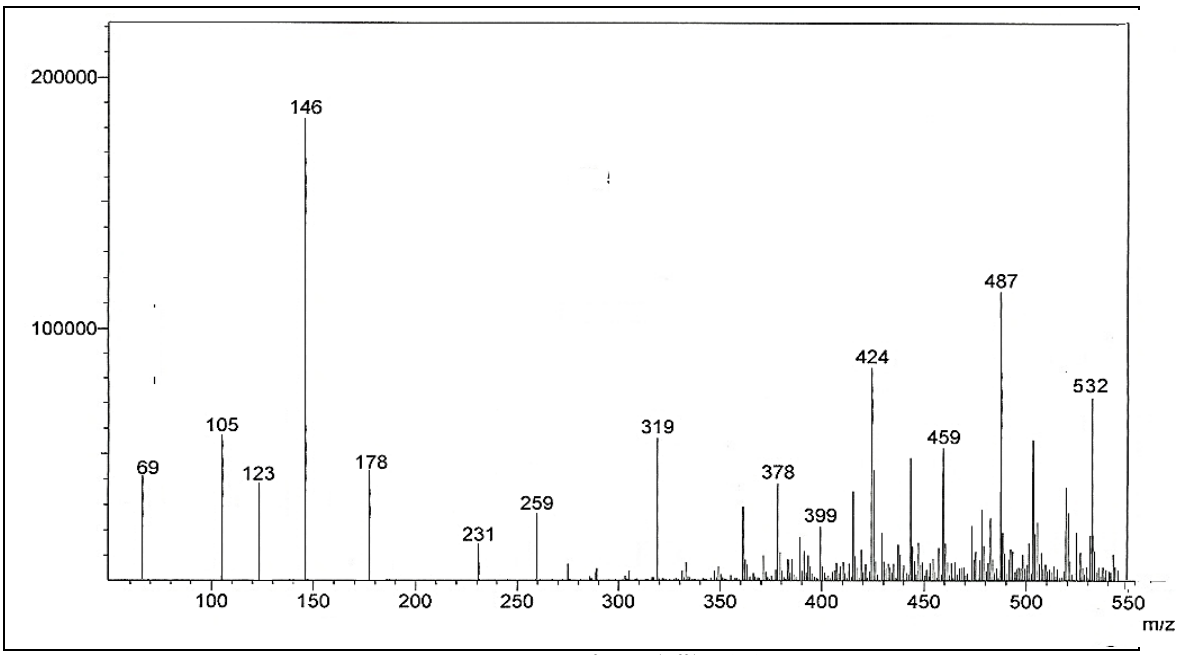


Fig.9. MS spectrum of Azo-Schiff base(III)

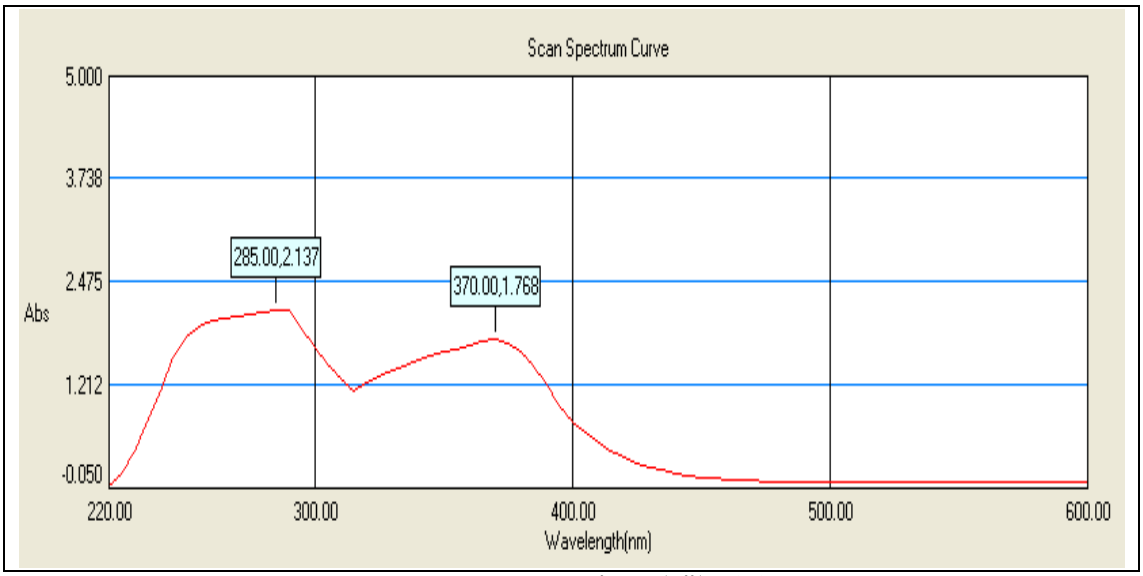


Fig.10. UV spectrum of Azo Schiff base(III)

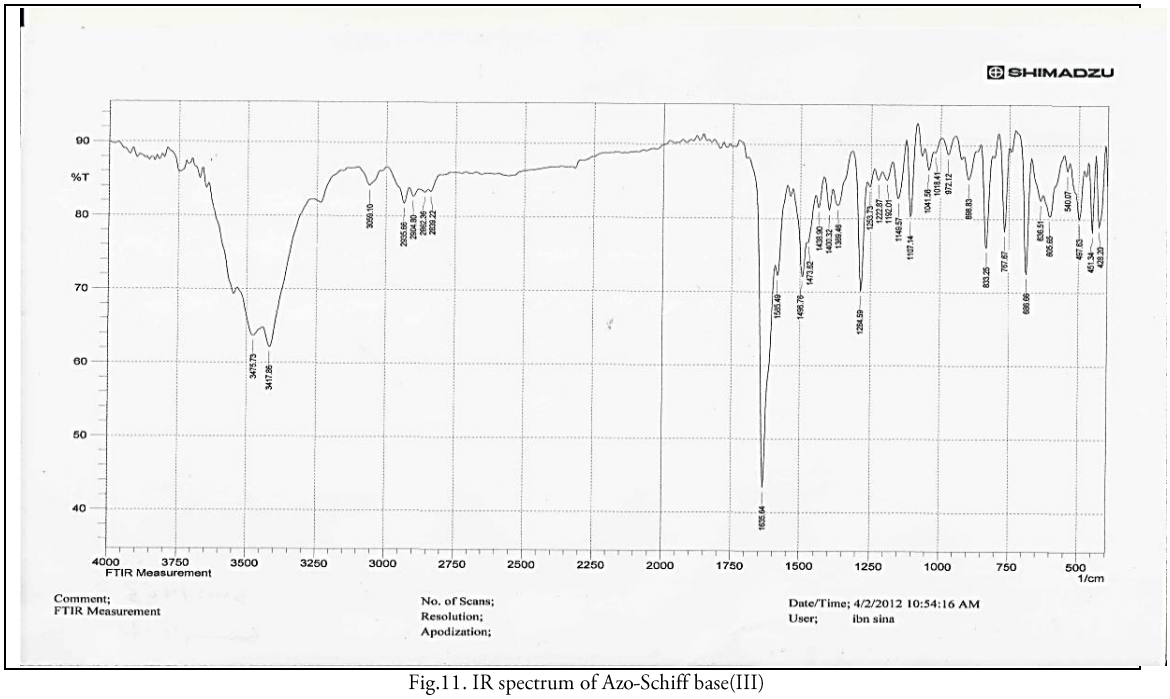


Fig.11. IR spectrum of Azo-Schiff base(III)

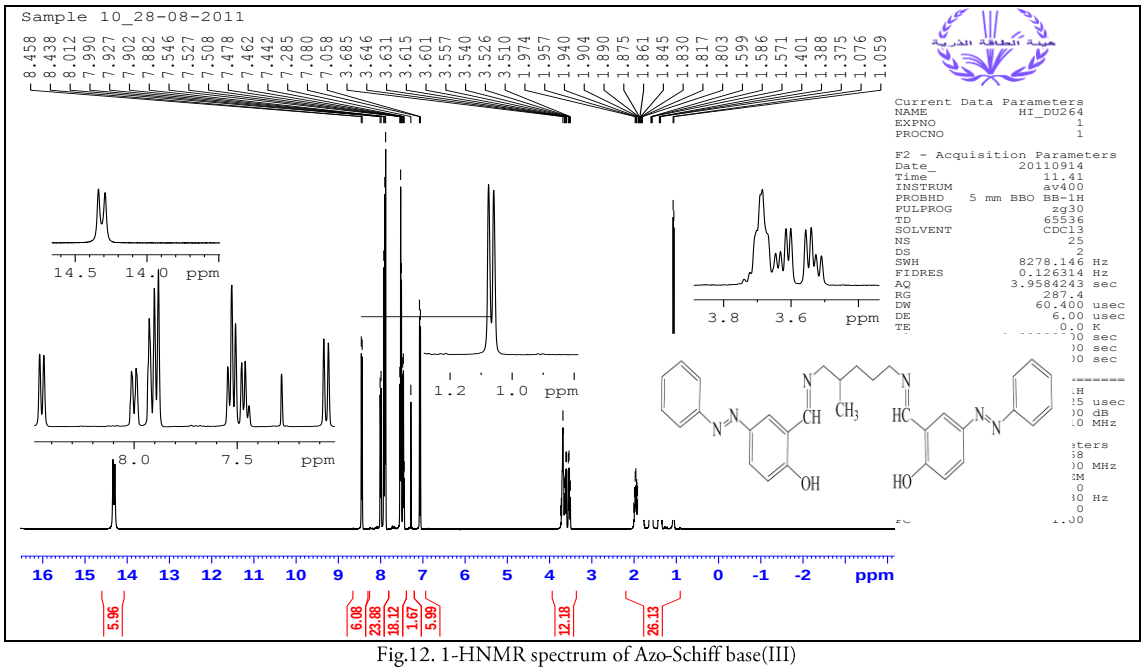


Fig.12. 1-HNMR spectrum of Azo-Schiff base(III)

TABLE 1: COLOUR, MOLECULAR WEIGHT, MELTING POINT OF (I, II, III).

Azo Shiff base	Color	M.Wt	Melting point ^{0}C	λ_{max} nm	Yield %	Crystallization Solvent
I	Orange	552	189.3-189.6	355	80	Toluene or DMF
II	Dark red	525	206-209	345	75	Toluene or p-Xylene
III	Yellow	532	140-143	370	78	Dioxan

TABLE 2: ELEMENTAL ANALYSIS DATA OF AZO SCHIFF BASES (I, II, III)

Azo Shiff base	Elemental analysis Calculated (Found %)		
	C	H	N
I	73.97 (73.90)	5.17 (5.11)	15.18 (15.21)
II	70.80 (70.84)	4.38 (4.41)	18.67 (18.66)
III	72.17 (72.16)	5.91 (6.01)	15.63 (15.78)

compounds. Filter paper discs (Whitman No.1 filter paper, 5mm diameter) were saturated with the solution of these compounds. The discs were placed on the surface of solidified Nutrient agar dishes seeded by the tested bacteria. The diameters of inhibition zones (mm) were measured at the end of an incubation period, which was 24 h at 37°C for bacteria. Discs saturated with DMSO are used as solvent control. Ciprofloxacin 100 µg/ml was used as reference substance for bacteria [38].

3. RESULTS AND DISCUSSION

3.1. SYNTHESIS

The reactions proceeded smoothly, producing the corresponding Azo Schiff bases in good yield. The Schiff bases were checked by different spectral technique (LC-MS, GC-MS, 1H-NMR, IR) which helps in elucidating their empirical formula (Table 1).

3.2. ELEMENTAL ANALYSES OF AZO SCHIFF BASES

The results of elemental analyses of Azo Schiff bases are shown in Table 2.

3.3. IR SPECTRA

The IR Spectral data are shown in Table 3 of the prepared azo schiff bases. The three bands at 1631.78, 1612.49 and 1635.64 cm^{-1} are attributed to imine group (-HC=N-) for (I, II, III) (Figures 3, 7, 11), respectively. The bands in the spectra at 1585.49, 1566.20 and 1585.49 cm^{-1} are due to (C=C) of aromatic rings. The v(OH) stretching frequencies bands are observed at 3417.86 - 3468.01, 3414.86 - 3475.73 and 3417.86 - 3475.7 cm^{-1} for (I,II,III), respectively. The IR spectra of (I, II, III) shows characteristic absorption bands at 1473.62 -1489.05, 1485.19 and 1496.76 cm^{-1} due to ν(N=N) stretching vibrations respectively. The bands which observed for all compounds at 1284.59, 1280.73 and 1284.5 cm^{-1} are due to ν(C-O) vibration.

TABLE 3: IR SPECTRAL DATA (cm^{-1}) OF AZO SCHIFF BASES (I, II, III)

Azo Shiff base	ν(C-O) cm^{-1}	ν(N=N) cm^{-1}	ν(C=C) cm^{-1}	ν(C=N) cm^{-1}	ν(C-H) aliph.	ν(C-H)arom. cm^{-1}	ν(OH) cm^{-1}
I	1284.59	1473.62 - 1489.05	1585.49	1631.78	2881.65	3008.95 -3062.96	3417.86 - 3468.01
II	1280.73	1485.19	1566.20	1612.49	--------	3005.10-3059.10	3414.86-3475.73
III	1284.59	1496.76	1585.49	1635.64	2839.2 - 2935.7	3059.10	3417.86-3475.73

TABLE 4: 1H-NMR SPECTRA OF OF AZO SCHIFF BASES (I, II, III)

Azo Shiff base	Chemical Shifts δ ppm		
	OH	CH=N	C-H Aromatic
I	14 (s,2H)	8.584 (s,2H)	8.023 -7.085 (m,20 H)
II	13.7 (s,2H)	8.809 (s,2H)	8.089 -6.995 (m,19 H)
III	14.318 (d,2H)	8.438 – 8.458 (d,2H)	8.023 - 7.085 (m,16 H)

while the bands at 2881.65 and 2839.2-2935.7 cm^{-1} are attributed to (C-H alkanes) for(I, II, III) respectively. Also, the bands at 3417.86 -3468.01, 3414.86-3475.73 and 3417.86 - 3475.73 cm^{-1} are attributed to (C-H Ar) for (I, II,III) respectively. However, in the IR spectra of azo schiff bases this band (C=O) disappeared and a new vibration band for azo methane (-HC=N-) is observed at 1631.78, 1612.49 and 1635.64 cm^{-1} indicating that complete condensation took place[39-40].

3.4. 1H-NMR SPECTRA OF LIGANDS (I, II,III)

The data of ^{1}H-NMR Spectra of prepared azo Schiff bases are shown in Table 4. The ^{1}HNMR spectra of (I, II, III) ligands in d6-DMSO (Figures 4, 8, 12) shows a singlet signal at 14, 13.7 ppm assigned to the protons OH groups of the Ligands (I, II) respectively. The doublet signals 14.318 ppm assigned to the protons OH group of the ligand (III). The multiple signals 8.023 -7.085, 8.089 - 6.995, 8.023 - 7.085 ppm are due to the aromatic protons for (I, II, III) respectively. The doublet signals at 8.438 – 8.458 ppm are due to the 2H, (HC=N) protons of the ligand (III), while the signals at 8.584 , 8.809 ppm are assigned to the 2H, (HC=N) protons of the ligands (I, II), respectively. The other obtained values for ^{1}H-NMR chemical shifts of these compounds are given in the experimental section. These data are in agreement with those previously reported for similar compounds [39]. These results strongly suggest that the proposed compounds have been formed [40].

3.5. BIOLOGICAL ACTIVITY

During the last two or three decades, attention has been increasingly paid to the synthesis of Azo Schiff bases which exhibits

TABLE 5: EFFECT OF NEW AZO SCHIFF BASES ON THE GROWTH OF TESTED BACTERIA (CONC. 200 MG/ML)

Azo Shiff base	Bacteria			
	Gram negative		Gram positive	
	B. subtilis	S. aureus	E.coli	S. typhi
I	14 mm	11 mm	10 mm	12 mm
II	18 mm	15 mm	14 mm	16 mm
IV	10 mm	12 mm	17 mm	16 mm
Control	00 mm	00 mm	00 mm	00 mm
Ciprofloxacin	20 mm	20 mm	20 mm	20 mm

various biological activities including antibacterial, fungicidal, tuberculostatic and plant growth regulative properties [41]. It was judicious to investigate the synthesis of various new types of Azo Schiff bases and studied their antibacterial activity against four strains of bacteria (Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella typhi).

The concentrations used for the screened compounds are 50, 100, and 200 µg/ml. Ciprofloxacin was used as reference standard while DMSO as control and inhibition zones is measured in mm. The new compounds have been tested against a series of bacteria nurs. The test results are presented in Fig.13, where some new compounds were active against tested and other compounds were not active.

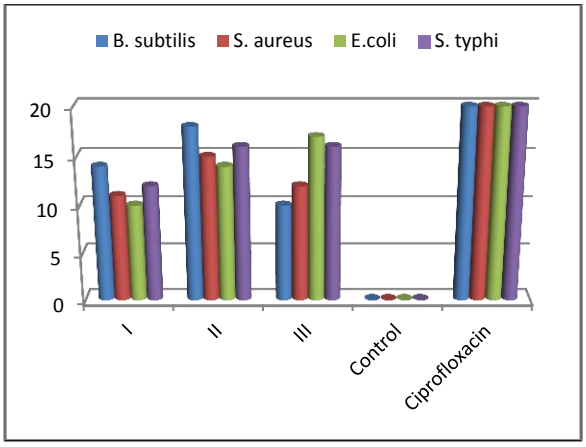


Fig.13. Antibacterial activity (Gram positive, negative) of synthesized compounds

All compounds are not active when using 50, and 100 µg/ml but active in the concentrations of 200 µg/ml as seen in Table 5.

4. CONCLUSION

1- The (I, II, III) compounds are new and were prepared for the first time.

2- The new compounds were identified by ^{1}HNMR, IR, and LC-MS spectral methods.

3-Some of the prepared compounds have been biologically screened i.e. studying their effects against two gram-positive, two gram-negative bacteria. The results show that their activities were found to vary from moderate to very strong.

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تحضير وتمييز قواعد أزوشيف جديدة ودراسة النشاط البيولوجي لها

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الملخص:

في هذا البحث تم التركيزعلى تحضير وتمييز بعض قواعد الأزو شيف والمحضرة من تكثيف ٣ –فورميل ٤-هيدروكسى فينيليزو البنزين مع كلا من ٣,١ دي أمينو زيلين ، ٦,٢ ثنائى أمينو بيريدين ، ٥،١ ثنائى أمينو-٢-ميثيل بنتان على التوالي.

وتمت دراسة نشاط قواعد الأزو شيف كمواد مضاده (LC-MS، GC-MS، 1H- NMR، IR) باستخدام تقنية الأطياف للجراثيم (العضوية الرقيقة والمكورات العنقودية الذهبية) واثبتت الدراسة أنها موجبه الجرام و(السالمونيلا التيفية وكولاي) هي سلبية الجرام . أظهر البحث أن يجند الازو نشاطا متغيرا قادرا على تثبيط نمو البكتيريا.