Synthesis, Characterization And Antibacterial studies of some Azomethine and Azo-Compound Derivatives of selected Sulfa Drugs

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Abstract

Three series of compounds have been synthesized, the first series included preparation of Schiff-bases (NC1-NC4) by condensation reaction of Salicylaldehyde with a select species of Sulfa drugs like , Sulfanilamide, Sulfadiazine , Sulfamerazine and Sulfathiazole . The second series included preparation of azo compounds (Azo1 , Azo2 , Azo3and Azo4) Which contains aldehyde group via converting Sulfathiazol, Sulfadiazine and Sulfanilamide to dizonium salt followed by coupling reaction with 2-hydroxy benzaldehyde in alkaline medium . The third series includes synthesis of azo Schiff bases (CH1-CH8) in excellent yield via condensation of different sulfa drugs and ethylene diamine . The structures of synthesized compound have been characterized by spectroscopic methods such as infrared , ^{1}H-NMR and mass spectra . The purity of compound and evaluation of R_f value were determined by TLC. The above mentioned compound were studied in vitro for their antimicrobial activity against Staphylococcus aureus , Escherichia coli and fungicidal activity against Candida albicans and Aspergillusniges Also the antioxidant of some compounds have been Calculated .

Keywords: *azo, azo-Schiff base,Schiff bases, sulfa drugs, 2-hydroxybenzaldehyde*

Introduction

Schiffare important organic compounds derived from the condensation reaction of primary or secondary amines and compatible aldehyde or ketone , which named by Hugo Schiff with general structure (RCH=NR`) , where R and R' represent alkyl or aryl substitutes [1,2] .Schiff bases considered as important intermediates for the synthesis of many bioactive compounds like β-lactams [3]. Also Schiff bases are announced to show a variety of exciting biological actions involving antifungal [4,5], antibacterial [6,7] . On the other hand azo compounds constitute one of the largest and most varied groups synthetic organic substances which is still used in abundance today .Azo compounds are highly important an widely used in different application fields , like coloring agent for food , inkjet preinter.[8,9]. Furthermore; azo compounds are well known for many types of biological properties such as , antifungal, antibacterial and anti-inflammatory activities .[10,11]. Also both azo compound and Schiff bases are important structure in pharmaceutical and medicinal fields and it has been suggested that the azomethine linkage might be responsible for the biological effectiveness showed by Schiff bases [12,13]. Sulfonamides especially sulfa drugs are the first viable chemotherapeutic utilized for microorganism disease in human beings . Many sulfa drugs and its derivatives have a wide range of pharmacological application , for example : Oral hypoglycemic ,antiepileptic , against hypertensive , antiprotozoal , antiretroviral and used as diuretic . furthermore many studies have been demonstrated that some sulfa drugs are also read to abstract cancerous cell.[14,15]

EXPERIMENTAL

Materials and reagents

Salicyladehyde was obtained from BDH, Sulfanilamide , Sulfadiazine , Sulfamerazine and Sulfathiazole from Sigma –Aldrich . Sodium carbonate , HAC , HCl , Sodium nitrate ($NaNO_2$) . were obtained from Fluka and used without further purification … The measurements of melting point were done on Bauchi 510 .

Instrumentation

The spectra of Infrared were registered as solid pellets by using KBr on Shimadzu. FT-IR model 8400 S Spectrophotometer at range 4000-400 cm^{-1}. spectra of ^{1}H-NMRwere done in a Brucker spectrophotometer (400MHz) and using DMSO-d_6 as solvent and TMS as internal reference .

Synthesis of Schiff bases (NC1-NC4) :

A mixture of 2-hydroxybenzaldehyde (1.22 gm , 1mmol), ethanol (20ml) and concentrated glacial acetic acid (2ml) was mixed with strring until a clear solution was obtained to this solution{ (1mmol) , 0.172 g , 0.250 g , 0.264g , 0.255 g } of (Sulfanilamide , Sulfadiazine , Sulfamerazine and

Sulfathiazole) respectively which dissolved in (30ml) of absolute ethanol has been added with reflexed for (2-3h) until an orange precipitate was obtained . The originating precipitate filtered , washes several time with ethanol and recrystallized from methanol .The purity Schiff bases were evaluated by thin layer chromatography by using chloroform / ethyl acetate (3:1) .

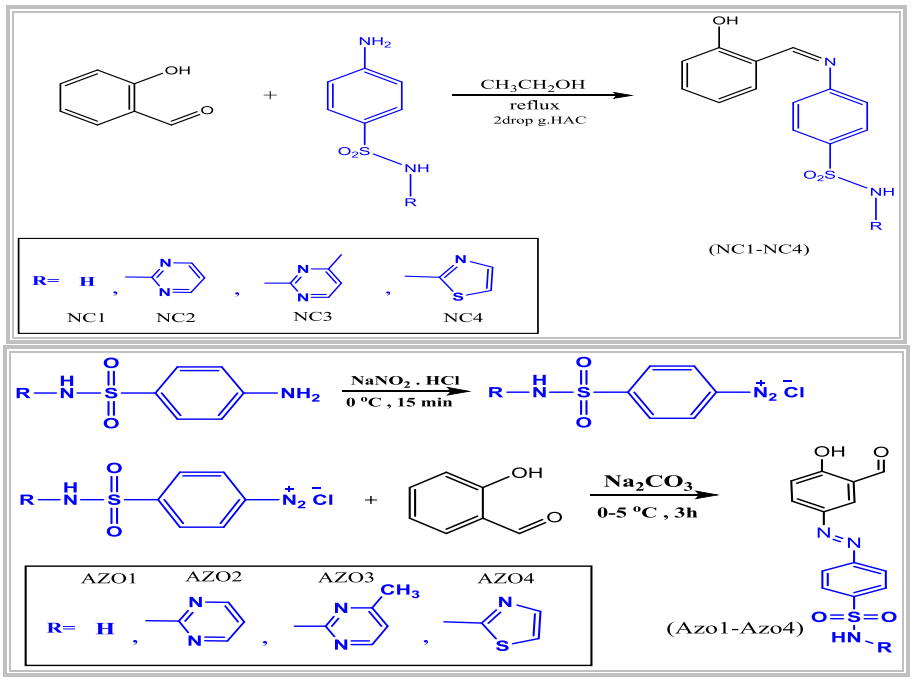
Synthesis of azo aldehyde (Azo1-Azo4) Compounds:

The compounds was synthesized as described by Erdem [16] . 2mmol (0.5106 g , 0.5g , 0.344g , 0.528) of sulfa drugs derivative (Sulfanilamide , Sulfadiazine , Sulfathiazole and Sulfamerazine) respectively have been dissolved in aqueous acidic solution (2ml of concentrated HCl and 10ml of water), the solution was stirred and cooled to 0-5 C^o , then aqueous solution of sodium nitrate (3mmol , o.25g) was added dropwise with stirring for period 15 minutes , maintaining temperature below 0-5 C^o , diazonium salt of sulfa drugs derivatives have been configured . In other flask 2-hydroxybenzaldehyde (3mmol.0.25g) dissolved in 20ml aqueous solution containing (10mmol , 1.06g) of $Na_2CO_3$and the mixture cooled to 0-5 C^o in an ice bath , slowly and gradually added to the solution of the cold diazonim salt of mentioned previously sulfa drugs . The addition process at 0-5 C^0 and keeping pH about 6-7. The precipitate that arose out was filtered and washed several times with water, then recrystallized from absolute ethanol to afford the appropriate azo compounds . the purity of azo compounds were estimated by thin layer chromatography by using ethyl acetate : hexane (3:7) as showing in Table (1). Scheme 1

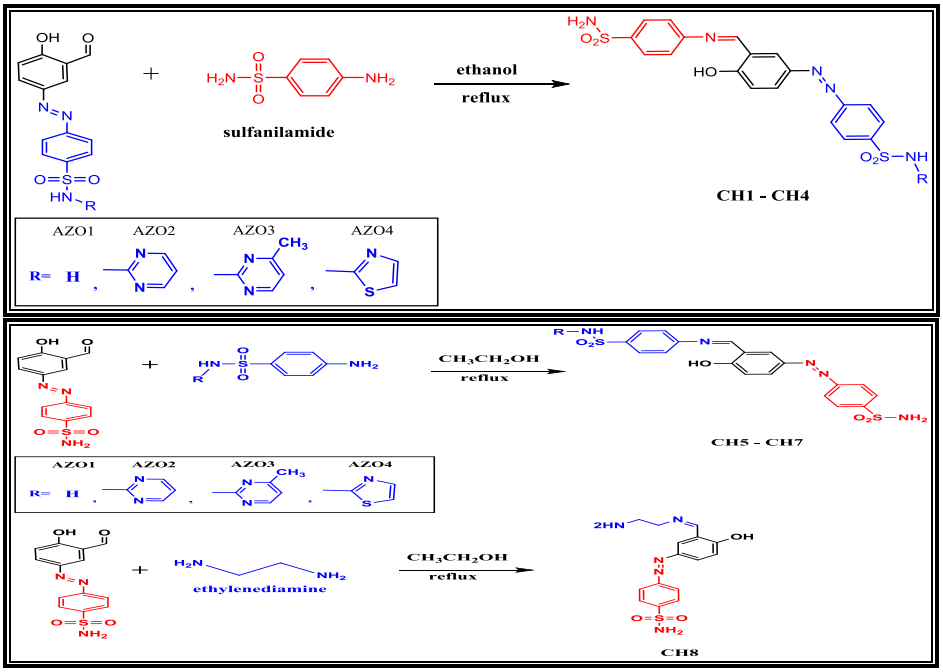
Synthesis of azo-azomethine (CH1-CH8):

The azo-azomethine compounds have been produced by general method which includes condensation reaction of azo compounds (Azo1-Azo4) with selected sulfa drugs as compounds containing amine groups . The first categories (CH1-CH4) have been prepared by mixing 1mmol of (Azo1,Azo2,Azo3,) respectively and 1mmol (0.172g) of sulfanilamide that dissolved in 40ml of absolute ethanol and refluxed for 3 hours, while the second categories (CH5- CH8) was prepared in which equimolar 1mmol of (Sulfanilamide , Sulfadiazine and ethylene diamine) respectively and (1mmol , 0.0305g) of azo compound (Azo1) in 40ml of absolute ethanol have been reflexed for 3hours , previous mix solution leaves to cool down to room temperature . After leaving it to cool down , the azo-azomethine compounds (CH5-CH8) were obtained as colored precipitate , that range in color from pink to brown ,which filtered off , washed with cold absolute methanol and dried it [17].

The reaction pathway and purity was continuously monitored by using thin layer chromatography in which the eluent that used was ethyl acetate : hexane (3:7) . The various preparation of all these new compounds are outline in Scheme 1and 2 and Table 1.

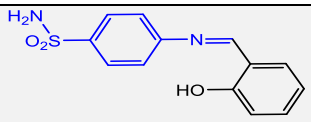
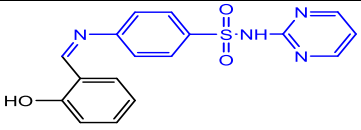
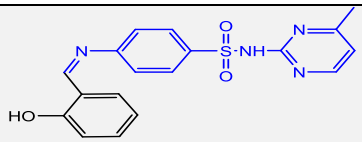
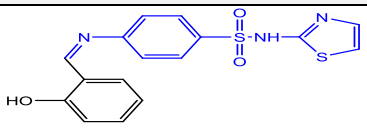


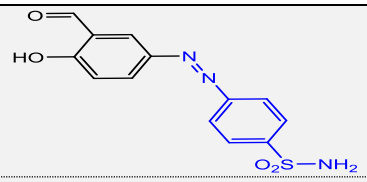
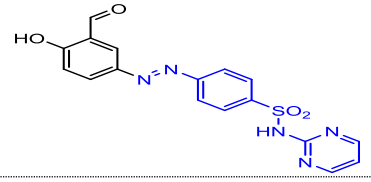
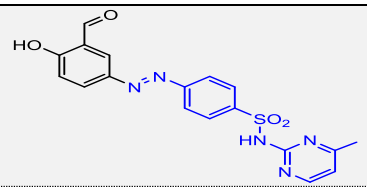
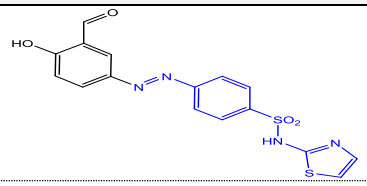
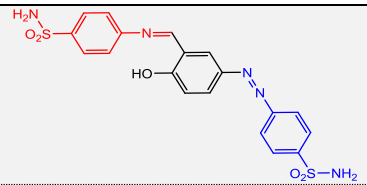
Scheme -1- Synthesis of azo sulfa drugs (NC1,NC4) and (Azo1-Azo4)

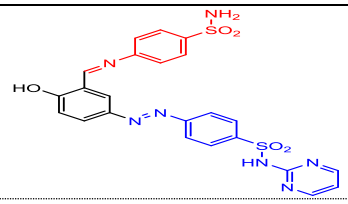
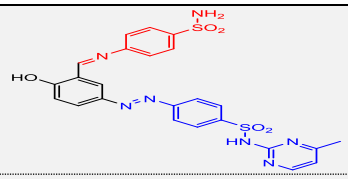
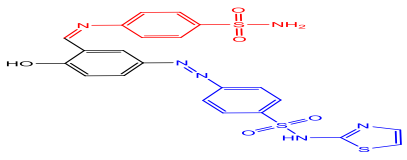
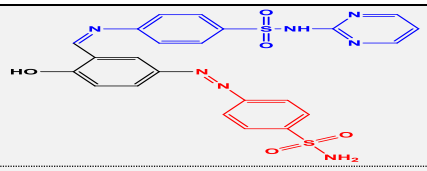
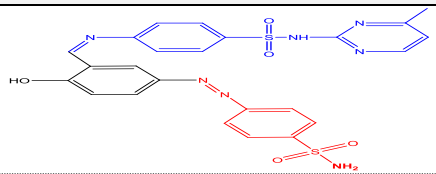


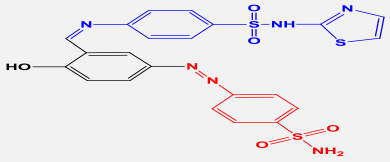
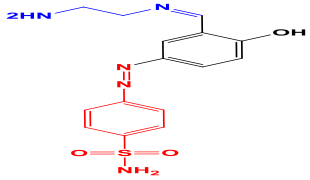
Scheme -2- Synthesis of azo-azomethine compounds (CH1-CH8)

Table -1- the symbol , synthetic formula , IUPAC name and physical data of synthetic compounds (NC1-NC4) , (Azo1-Azo4) and (CH1-CH8)

Sym.	*Chemical Formula* *IUPAC name*	*MOl.formula* *Molecular Weight*	*Color And Form Physical*	*Yield %*	*m.p (C^o)*	*R_f*
NC1	 4-((2-hydroxybenzylidene)amino)benzenesulfonamide	$C_{13}H_{12}N_2O_3S$ 276.31	Light yellow crystals	72	198-201	0.41
NC2	 (Z)-4-((2-hydroxybenzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide	$C_{17}H_{14}N_4O_3S$ 354.38	Light yellow powder	70	148-152	0.7
NC3	 (Z)-4-((2-hydroxybenzylidene)amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide	$C_{18}H1_6N_4O_3S$ 368.41	Light yellow powder	60	140-143	0.53
NC4		$C_{16}H_{13}N_3O_3S_2$ 359.42	Light yellow	75	114-111	0.6

Sym.	*Chemical Formula* *IUPAC name*	*MOl.formula* *Molecular Weight*	*Color And Form Physical* crystals	*Yield %*	*m.p (C^O)*	R_f
	(Z)-4-((2-hydroxybenzylidene)amino)-N-(thiazol-2-yl)benzenesulfonamide					
Azo1	 4-((3-formyl-4-hydroxyphenyl)diazenyl)benzenesulfonamide	$C_{13}H_{11}N_3O_4S$ 305.31	Dark Brown	80	207-210	0.41
Azo2	 (E)-4-((3-formyl-4-hydroxyphenyl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide	$C_{17}H_{13}N_5O_4S$ 383.38	Yellow	40	decompose at 300C^0<	0.7
Azo3	 (E)-4-((3-formyl-4-hydroxyphenyl)diazenyl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide	$C_{18}H_{15}N_5O_4S$ 397.41	Brown	33	100-105	0.64
Azo4	 (E)-4-((3-formyl-4-hydroxyphenyl)diazenyl)-N-(thiazol-2-yl)benzenesulfonamide	$C_{16}H_{12}N_4O_4S_2$ 388.42	Brown	50	decompose at 300C^0<	0.6
CH1	 4-((4-hydroxy-3-(((4-sulfamoylphenyl)imino)methyl) phenyl)diazenyl) benzenesulfonamide	$C_{19}H_{17}N_5O_5S_2$ 459.50	Dark Red	50	230-235	0.56

Sym.	Chemical Formula IUPAC name	MOl.formula Molecular Weight	Color And Form Physical	Yield %	m.p (C^O)	R_f
CH2	 4-((1E)-(4-hydroxy-3-(((4-sulfamoylphenyl)imino)methyl) phenyl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide	$C_{23}H_{19}N_7O_5S_2$ 537.57	Light Brown	38	245-249	0.62
CH3	 4-((1E)-(4-hydroxy-3-(((4-sulfamoylphenyl)imino)methyl) phenyl)diazenyl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide	$C_{24}H_{21}N_7O_5S_2$ 551.60	Red	33	100-105	0.53
CH4	 4-((4-hydroxy-3-((Z)-((4-sulfamoylphenyl)imino)methyl)phenyl)diazenyl)-N-(thiazol-2-yl)benzenesulfonamide	$C_{22}H_{18}N_6O_5S_3$ 542.60	Dark Red	40	200-203	0.65
CH5	 4-(((Z)-2-hydroxy-5-((4-sulfamoylphenyl)diazenyl) benzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide	$C_{23}H_{19}N_7O_5S_2$ 537.57	Brown	65	238-240	0.74
CH6	 4-(((Z)-2-hydroxy-5-((4-sulfamoylphenyl)diazenyl)benzylidene)amino)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide	$C_{24}H_{21}N_7O_5S_2$ 551.10	Orange	63	232-234	0.7

Sym.	Chemical Formula IUPAC name	MOl.formula Molecular Weight	Color And Form Physical	Yield %	m.p (C^O)	R_f
CH7	 4-(((Z)-2-hydroxy-5-((4-sulfamoylphenyl)diazenyl) benzylidene)amino)-N-(thiazol-2-yl)benzenesulfonamide	$C_{22}H_{18}N_6O_5S_3$ 542.60	Orange	54	255-260	0.53
CH8	 4-((3-((Z)-((2-aminoethyl)imino)methyl)-4-hydroxyphenyl)diazenyl)benzenesulfonamide	$C_{15}H_{17}N_5O_3S$ 347.39	Brown	42	206-209	0.65

Antimicrobial Activity

The antimicrobial activity of synthesized compounds assayed in vitro against two kind of bacteria , *Staphylococcus aureus* as Gram - positive bacterium and *Escherichia coli*as Gram - negative bacterium , also the antimicrobial properties includes other types of pathogens which was fungal species , *Candida albicans* and *Aspergillusniger* . All the tests against these microorganism was performed by Mueller – Hinton agar (MHA) method .

The process includes taking 0.2 ml of bacteria inocula and fungal inocula were placed on the surface ofsabouraud dextrose agar (S.D.A.) medium and Natrient agar (N.A.) medium respectively . The reference drugs (Sulfadiazine , Sulfisoxazole , Sulfanilamide and Nystatin and the tested compound were dissolved in DMSO with concentration of 0.03g/ml for every tested compound . The synthesized compound were placed in central pore in bacterial plates and incubated at (37± 2 C^0) , while fungal plates have been incubated at (28 ± 2C^0) for 72 hours . Zone of inhibition of each isolate were measured in millimeter [18,19].

Antioxidant assay using the β – carotene bleaching method

The anti-oxidant ability of prepared compounds were determined by using β – carotene bleaching method [20] . The method is done on foundation basis of the oxidative losses of β – carotene / linoleic acid emulsion to assess the anti-oxidation capability of the prepared compounds . The method includes taking (0.02ml) of linoleic acid and (0.2ml) of Tween 20 that placed in a round flask and (1ml) β – carotene (prepared by dissolving 0.2mg/ml in chloroform) has been added to the mixture . Then (3.8ml) of the mixture was dosed with (0.2ml) of tested sample and reference (BHT) compound .

Finally the absorbance has been read at 470 nm , then the samples were submittedto thermal autoxidation at 45C^0 by using water bath for 2 hours. At last the absorbance was measured every 15 minute . The anti-oxidant activity (AA) has been count up using the following
equation .

$$AA = \left[1 - \left(\frac{Aj - At}{Aj^* - At^*}\right)\right] \times 100$$

Where :-
Aj : the measured absorbance value of sample at Zero time
At : the measured absorbance value of sample after incubation (105) minute at 45C^0
Aj* : the measured absorbance value of control at zero time .

At* : the measured absorbance value of control after incubation (105) minute at 45 C^0 . [20].

Result and Discussion

All the synthesized compounds were prepared from different sulfa drugs and for all three series mentioned earlies in this paper , whether they are Schiff bases compounds , azo compounds and azo-azomethine compounds . The synthesized compounds are solid , stable in air and non-hygroscopic compounds . The Schiff base compounds (NC1- NC4) were prepared via reaction of 2-hydroxybenzaldehyde with sulfa drugs derivatives in 1:1 mole ratio gave corresponding compounds as yellow solids in appropriate yield . The azo compounds (Azo1- Azo4) were prepared by coupling reaction of diazotized of Sulfanilamide , Sulfadiazine , Sulfathiazole and Sulfamerazine respectively with 2-hydroxybenzaldehyde (Scheme -1-) . We obtained solid, colored compounds, gradations of colour from dark red to light red .

The reaction of these azo-azomethine compounds with some sulfa drugs derivatives which contain amino group in 1:1 mole ratio gave the expected compounds (CH1- CH8) in appropriate yields as solid colored compounds (Scheme -2-) . The melting point , colors and Retention Value(R_f) for these compounds have been mentioned .

Spectroscopic Analysis

Infrared spectra FT-IR

In order to illustrate the system of bonding , the IR spectrum of synthesized compounds (NC1-NC4) , (Azo1- Azo4) and (CH1-CH8) were studied on carful . Disappearance of stretching vibration of NH_2 group belonging to sulfa drugs and instead of it a strong bands observed in the range (1593-1631)cm^{-1} which assigned to the υ (HC=N-) [21]. On other hand the total absence of υ (C=O) absorption in IR spectra of compounds (Azo2) and (Azo3) and (CH1-CH8) compounds which clearly indicated that new Schiff bases had been formed . In addition , the stretching vibration of phenolic (OH) appear at the range (3466-3284) cm^{-1} in all synthesized compounds . In the IR spectra of (Azo1) and (Azo3), appearance of absorption band at (1490-1506) cm^{-1} of υ(N=N) and (1084-1250) $cm^{-1}$of υ(C-O) are in agreement with the structures proposed. Also all the synthesized compounds which containing (SO_2) moiety showed strong bands of Asymmetric stretching vibration at the range (1330-1369) cm^{-1} and (1145-1161) cm^{-1} to symmetric stretching vibration .[22]. The spectral data are given in **Table2** and the **Figures** (**1**)

Table -2- : FT-IR data of synthetic compounds (cm^{-1}, KBr disc)
(s: strong, vs: very strong, m: medium, w: weak, br: broad)

Com.	υ (OH) cm^{-1}	υ (N-H) Sulfa cm^{-1}	υ (NH_2) Sulfa cm^{-1}	υ (C-H)$_{Aro.}$ Stretching cm^{-1}	υ (C=O) cm^{-1}	υ (CH=N) cm^{-1}	υ (C=C) Aromatic	υ (SO_2) Asym. Sym. cm^{-1}	υ (C-O) Phenolic cm^{-1}	υ (C-H)$_{Aro.}$ Subs. cm^{-1}	υ (Others) cm^{-1}
NC1	3500s	.....	3344-3244 s	3063 w	.....	1620s	1489 w 1454 w	1315 s 1157 s	1095 m	840 m 763 m	2870 w υ (C-H)$_{ali}$ 975 m υ (S-N)$_{str.}$
NC2	3500 br	3078 br	.....	3036 w	.....	1620m	1489 m 1442w	1338 m 1161 s	1091 w	852 m 756 m	2874 υ (C-H)$_{ali}$ 945 m υ (S-N)$_{str.}$, 1581 s υ (C=N)$_{Sulfa}$
NC3	3483 w	3383 w	.....	3047 w	.....	1593s	1496 m	1338 m 1161 s	1091 m	895 m 756 m	972 w υ (S-N)$_{str.}$, 1570 s υ (C=N)$_{Sulfa}$ 2920 w υ (C-H)Asym 2854 w υ (C-H) sym
NC4	3400 br	3147w	.....	3113 w	.....	1620m	1531 s	1315m 1145m	1087 m	852 m 709 s	2870 w υ_{as}(C-H)$_{ali}$ 937 w υ (S-N)$_{str.}$, 1531 s υ (C=N)$_{Sulfa}$
NC5	3421 br	.....	3055 – 3009 s ethyl diamine	3095 w	.....	1631 s	1496 m 1458 m	.....	1149 m	856 m 748 m	2901 w υ (C-H)all

Com.	υ (OH) cm^{-1}	υ (N-H) Sulfa cm^{-1}	υ (NH_2) Sulfa cm^{-1}	υ (C-H)Aro. Stretching cm^{-1}	υ (C=O) cm^{-1}	υ (CH=N) cm^{-1}	υ (N=N) cm^{-1}	υ (SO_2) Asym. Sym. cm^{-1}	υ (C-O) Phenolic -1	υ (C-H)Aro. Subs. cm^{-1}	υ (Others) cm^{-1}
AZO1	3568 br	.....	3333-3248m	3097 w	1662 s	.....	1500 m	1334m 1165 s	1085 m	844 m 744 m	2858 w υ $(C-H)_{ali}$ 906 m υ $(S-N)_{str}$
AZO2	3379w	3271m	.....	3043 m	1658 s	.....	1581 s	1365 w 1145m	1091 w	844m 798 w	2854 w $υ_{as}(C-H)_{ali}$ 952 w υ $(S-N)_{str.}$, 1535 s υ $(C=N)_{Sulfa}$
AZO3	3566 – 3525 br	3151 w	.....	3090 w	1630 s	.....	1492 m	.....	1192 s	823 m	.....
AZO4	3525 br	3151 w	.....	3101 w	1658 m	.....	1531 m	1369 w 1138m	1080 s	844 m 705 m	933 w υ $(S-N)_{str.}$ 1585 s $υ(C=N)_{Sulfa}$

Com.	υ (OH) cm^{-1}	υ (N-H) Sulfa cm^{-1}	υ (NH_2) Sulfa cm^{-1}	υ (C-H)Aro. Stretching cm^{-1}	υ (C=O) cm^{-1}	υ (CH=N) cm^{-1}	υ (N=N) cm^{-1}	υ (SO_2) Asym. Sym. cm^{-1}	υ (C-O) Phenolic -1	υ (C-H)Aro. Subs. cm^{-1}	υ (Others) cm^{-1}
CH 1	3568 br	.....	3321-3259m	3093 w	.....	1624 s	1585 m	1330 s 1161 s	1099 m	837 m 771 m	895 m υ $(S-N)_{str}$
CH 2	3509 br	3263w	3263 w	3090 m	.....	1620 m	1581 s	1342 m 1161s	1091 w	844m 802 w	952 w υ $(S-N)_{str.}$, 1527 w υ $(C=N)_{Sulfa}$
CH 3	3448 br	3375 w	3375 w	3090 w	.....	1654 s	1458 m	1357 m 1145 s	1091m	844 m 771 s	1041 m υ $(S-N)_{str.}$, 1589 w υ $(C=N)_{Sulfa}$
CH 4	3568 w	3271 w	3271 w	3097 w	.....	1627 m	1531 w	1330 m 1145 s	1084m	837 m 732w	2850 w $υ_{as}(C-H)_{ali}$ 933 m υ $(S-N)_{str.}$ 1531 s $υ(C=N)_{Sulfa}$

Com.	υ (OH) cm^{-1}	υ (N-H) Sulfa cm^{-1}	υ (NH_2) Sulfa cm^{-1}	υ (C-H)Aro. Stretching cm^{-1}	υ (C=O) cm^{-1}	υ (CH=N) cm^{-1}	υ (N=N) cm^{-1}	υ (SO_2) Asym. Sym. cm^{-1}	υ (C-O) Phenolic -1	υ (C-H)Aro. Subs. cm^{-1}	υ (Others) cm^{-1}
CH 5	3568 br	3259 w	3425 - 3356 m	3039 w	.....	1624 m	1585 m	1330 s 1157 s	1091 m	840 m 798 m	2935 w υ (C-H)ali 945 m υ (S-N)str 1585 s υ (C=N)Sulfa
CH 6	3500 br	3383w	3487 - 3383 w	3078 w	.....	1627 m	1570s	1330 m 1157s	1091 w	891m 833 w	2870 w υ_{as}(C-H)ali 964 w υ (S-N)str, 1593 s υ (C=N)Sulfa 1570 s υ (C=C)
CH 7	3568 br	3240 w	3321- 3240 w	3101 w	.....	1620 s	1573 m	1350 m 1141 s	1087m	837 m 744 m	2908 w υ_{as}(C-H)ali 945 m υ (S-N)str, 1531 s υ (C=N)Sulfa 1573 s υ (C=C)
CH 8	3500 br	.....	3309 s	3016 w	.....	1610 s	1573 m	1327 s 1161 m	1099w	821 w 794w	2939- 2862 br υ_{as}(C-H)ali 964 w υ (S-N)str. 1597 m υ(C=N)Sulfa

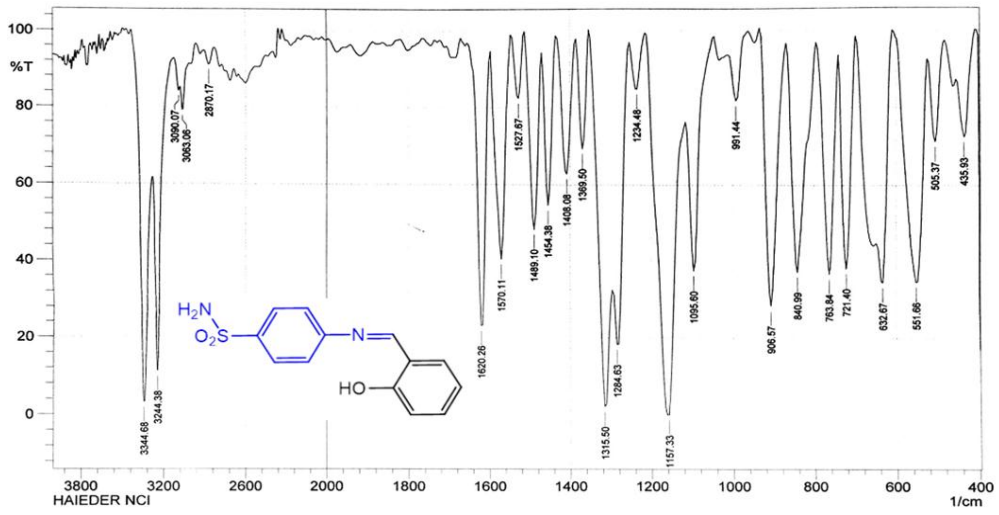


Figure 1:IR Spectrum of the compound (NC1)

^{1}H-NMR Spectra

The ^{1}H-NMR spectral results obtained from all compounds at ambient temperature in DMSO-d_6 .The ^{1}H-NMR analysis appeared to support the reinforce of the synthesis compounds .The (N-H) and (Ar-H) protons appeared in the rang δ (11.09-12.5) ppm and δ (6.39-7.86) ppm respectively [23]. Also all the compounds are characterized by showing singlet signal at δ (12.5-13.33) ppm that attributed to phenolic group (OH), while observed in Schiff bases and azo-azomethine compounds disappearance of the signal of aldehyde group and instead of it a new singlet signal appears at the range δ (8.41- 9.17) ppm which assigned to the proton of azomethine group (HC=N) [24]. Also the compounds which containing (NH_2) group shows signal at δ (6.9-7.13) ppm that attributed to the presence of two protons of this group in Sulfanilamide drug which innervate the desired result. [25] . The ^{1}H-NMR spectral data have been listed in Table (3-4) and showed Figures (2-3).

Table 3: 1H-NMR spectral data of compounds (CN1-CN4)

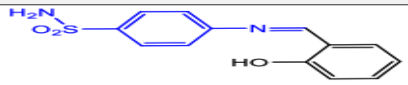
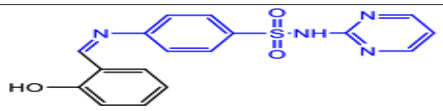
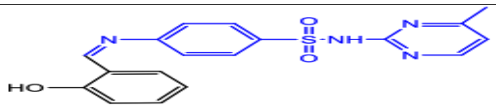
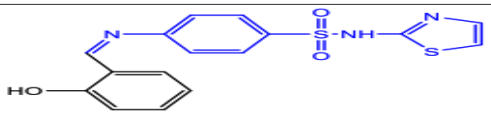
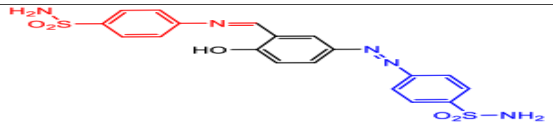
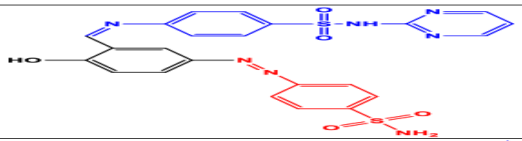
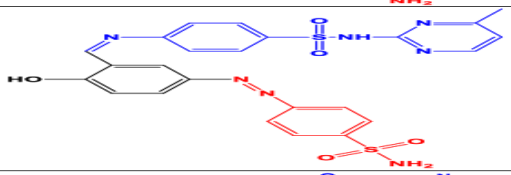
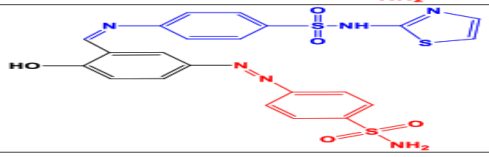
Symbol of com.	Structure	Chemical shift δ(ppm)
NC1		12.63 (1 H, s, OH), 8.99 (1 H, s, HC=N), 7.89 – 7.38 (8 H, m, Ar-H), 6.99 (2 H, s, NH_2), 3.33 (1 H, d, J 1.7,H_2O in DMSO-d_6), 2.50 (1 H, p, J 1.9,DMSO-d_6).
NC2		13.11 (1 H, s,OH), 11.95 (1 H, s,NH), 9.17 (1 H, s,HC=N), 7.23 – 7.20 (3 H, m,Ar-H), 7.20 – 7.15 (4 H, m,Ar-H), 7.05 – 6.93 (4 H, m,Ar-H).
NC3		12.54 (1 H, s,OH), 11.75 (1 H, s,NH), 8.97 (1 H, s,HC=N), 8.35 – 7.71 (4 H,d, Ar-H), 7.70 – 7.40 (4 H, m, Ar-H), 6.99 – 6.92 (2 H, m, Ar-H), 2.33 (3 H, s,CH_3).
NC4		12.50 (1 H, s,OH), 11.82 (1 H, s,NH), 8.96 (1 H, s,HC=N), 7.77 – 6.45 (10 H, m,Ar-H),

Table 4: 1H-NMR spectral data of compounds (CH1 , CH5 , CH6 and CH7)

Symbol of com.	Structure	Chemical shift δ(ppm)
CH1		δ $_H$ (500 MHz, DMSO-d_6) 13.33 (1 H, s), 8.41 (1 H, s), 8.02 – 7.5 (11H, m, Ar-H), 7.28 (4 H, s, NH_2),
CH5		13.21 (1 H, s, OH), 11.23 (1 H, s, NH), 9.14 (1 H, s, HC=N), 7.62 – 6.55 (14 H, m, Ar-H), 7.00 (2 H, s,NH_2),
CH6		13.25 (1 H, s, OH), 11.09 (1 H, s, NH), 9.15 (1 H, s, HC=N), 7.63 – 6.54 (13 H, m, Ar-H), 6.87 (2 H, s, NH_2), 2.34 (3 H, s, CH_3).
CH7		13.32 (1 H, s, OH), 10.38 (1 H, s, NH), 9.14 (1 H, s, HC=N), 7.58 – 7.18 (10 H, m , Ar-H), 6.86 (2 H, s, NH_2), 6.65 (3 H, d, J 88.9,Ar-H), .

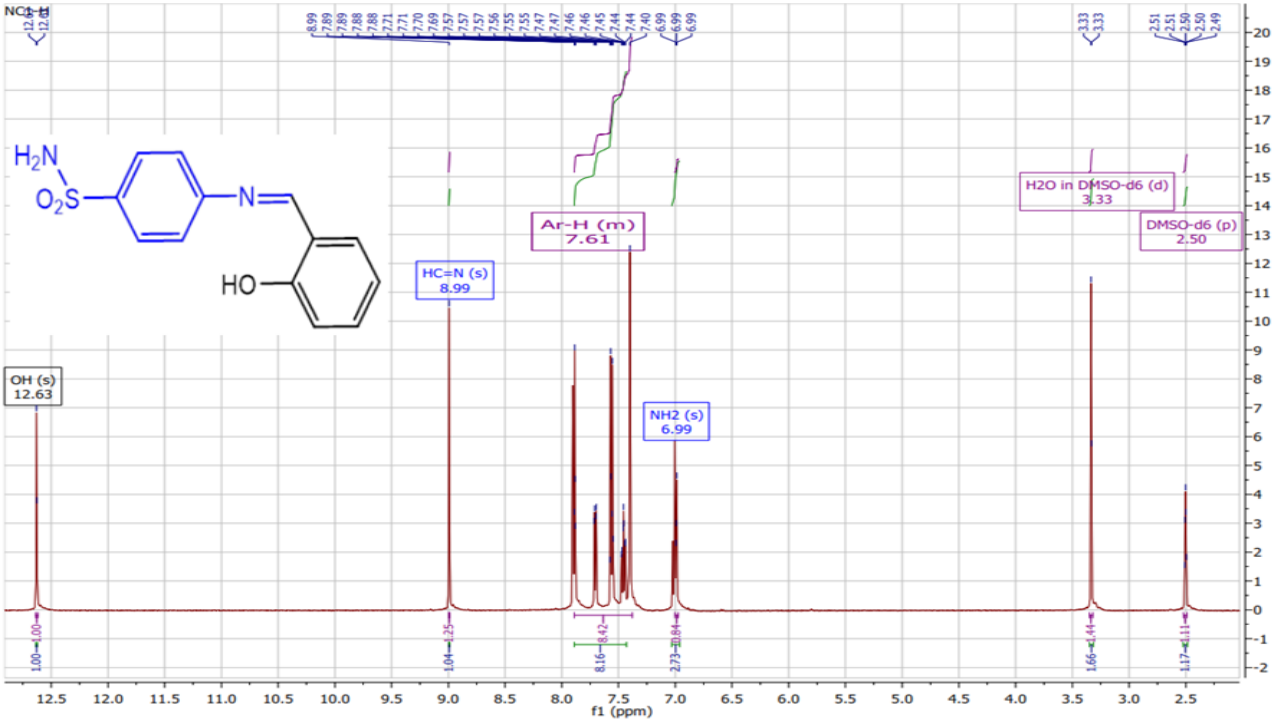


Figure 2: [1]HNMR Spectrum of the compound NC1.

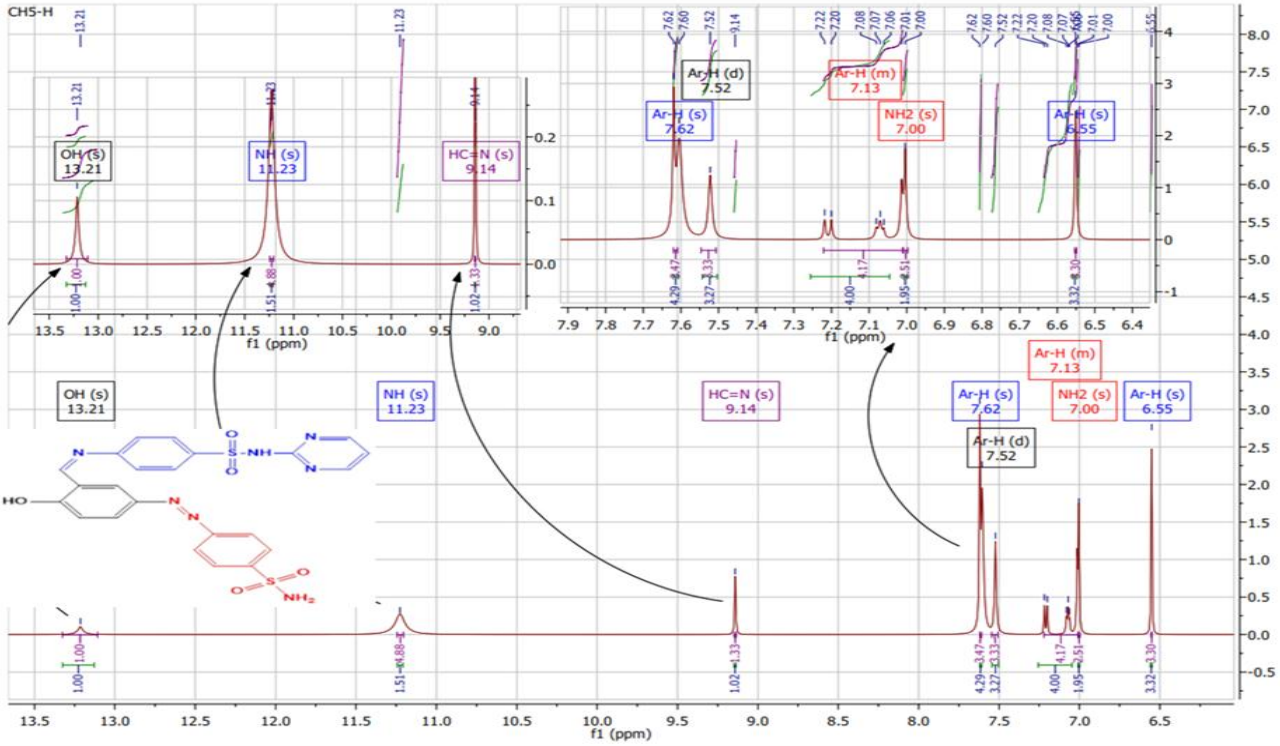


Figure 3: [1]HNMR Spectrum of the compound CH5

Antioxidant Activity

Free radicals created on account of exposure of radiation environmental pollutants and by side products of metabolized drugs . These free radicals are hostile to molecules which are naturally present as antioxidants . Damaging of the cells that caused by free radicals plays an important role in the symptoms of aging and the development of various disease .[26]

Antioxidants are widely studied for their ability for protecting the organism and cell the body from damage that is created by oxidative overtax . Many scientists in various disciplines began to research the production of important compounds as antioxidants ,

whether by producing them or obtaining them from natural sources that could supply efficient . Components to forbid or inhibit the impactof oxidative overtax on cell . [27]

The important action of antioxidants by trapping and remove the free radical. One of the most important mechanism by which these compounds donating hydrogen to free radicals and then the process of reducing these species and converting them to unreactive species , the phenolic compounds act as reducing agents by removing and trapping free radicals by donating hydrogen and remove the odd electron of oxygen . The efficiency of our synthesized compounds was estimated as antioxidants , as they contain phenolic groups and compared with standard compounds (BHT) . It was believe that the activity of compounds (CH1,CH7,NC1 and CH5) as antioxidant compounds depends on the relationship between absorbance and time , then it was compared with (BHT) and with application of the previous mathematical equations show in Table **(5)** Figures **(4,5)** . It was noted that the compound NC1 has highs efficiency , followed by the potency of active compound CH5 . Also the compounds CH7 shows moderate efficiency . Therefore , established on antioxidant activity study , the arrangement of the actively orders of these compounds as follows : NC1 > CH5 > CH7 > CH1.

Table (5) : Effectiveness results of synthesized compounds as antioxidants compared with (BHT) (solvent used DMSO) .

AA%	At*	Aj*	At	Aj	Sample
73	0.181	0.207	0.236	0.243	BHT
30	0.181	0.207	0.206	0.224	CH1
46	0.181	0.207	0.202	0.216	CH7
53	0.181	0.207	0.203	0.215	NC1
50	0.181	0.207	0.203	0.216	CH5

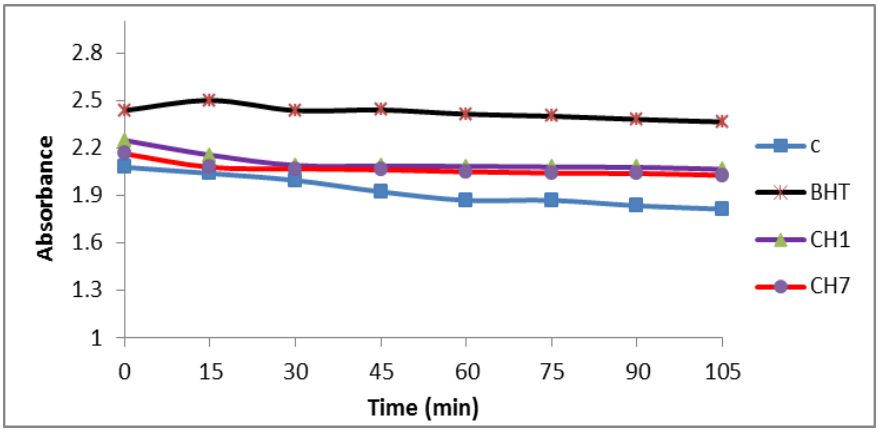


Figure -4- : antioxidant activity of (CH1 , CH7)

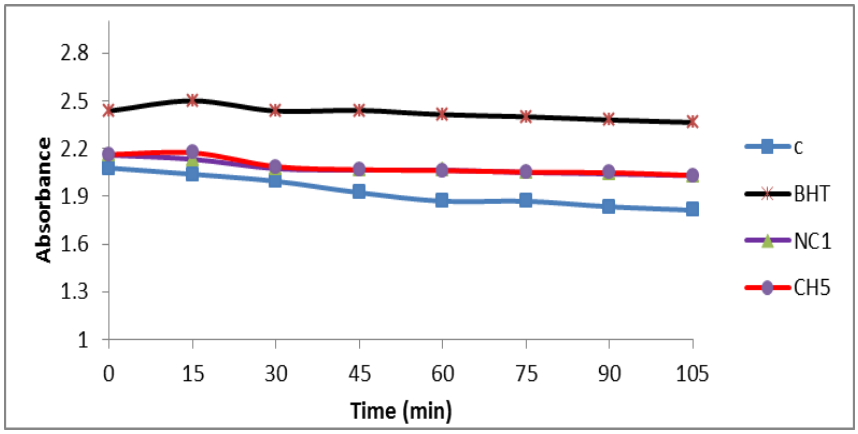


Figure -5- : antioxidant activity of (NC1 , CH5)

Biological activities : antibacterial and antifungal studying:

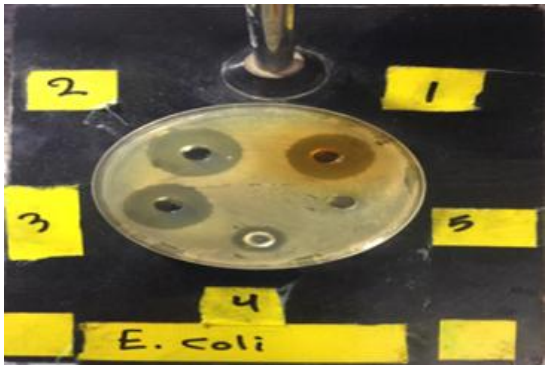
In vitro the antibacterial and antifungal activities of the synthesized compounds against two kinds of bacteria which included positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) , and the same compounds was screened for it antifungal activity against (*C.albicans*) and (*A. niger*) in DMSO by using well diffusion methods [28].

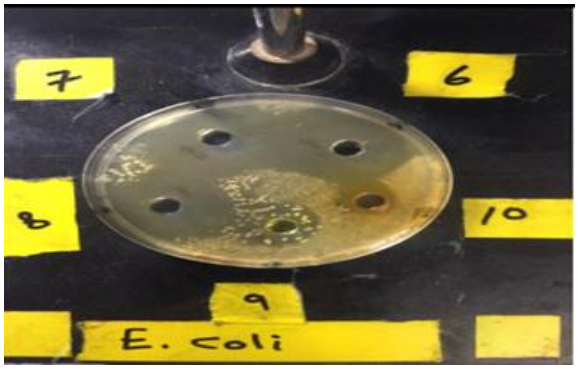
The compounds were tested at the concentration 50 mg/ml and they compared with known antibiotics and antifungal such as Amoxicillin and Nystatin respectively and pure sulfa drugs like (Sulfanilamide , Sulfaoxazol and Sulfadiazine) .The result in Table 5and Figures10 shows that the compounds (NC1, NC3 , NC4 and CH8 are more effective than other compounds against the two kinds of fungal organisms while CH4 and CH2 medium efficiency shown . Moreover compounds CH5and NC2 showed that they had no effectiveness towards the same organism . Also the synthesized compounds were evaluated for their antibacterial activity . In comparison with standard Amoxil , only compounds NC4 showed good activity against *E.coli* while compounds CH5,NC2, NC1, CH7,CH4 and CH6 were found to be active against both kind of bacteria . Remaining all the compounds were displayed moderate activity towards the gram positive except compounds CH2 and Azo3 who have demonstrated that they do not have activity against gram negative bacteria .

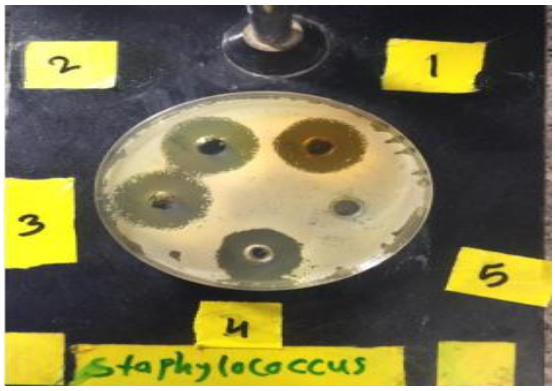
The effectiveness of these synthesized compounds is due to the fact that most of these compounds containing sulfanilamide in its composition and the action of sulfanilamide is competitively inhibit the motivation of dihydropteroate synthase and preventing the net biosynthesis of folate coenzymes , therefore its bacteriostatic . Another reason for their high efficiency can be explained by a capacity of these compounds to penetrate the layers of lipid which consists of the cell wall of the microorganism which depending on the great desire to dissolved in this lipid layer . In other hand most Schiff base have a tendency acts as sturdy bactericidal agents to kill some types of
microorganisms [29] .

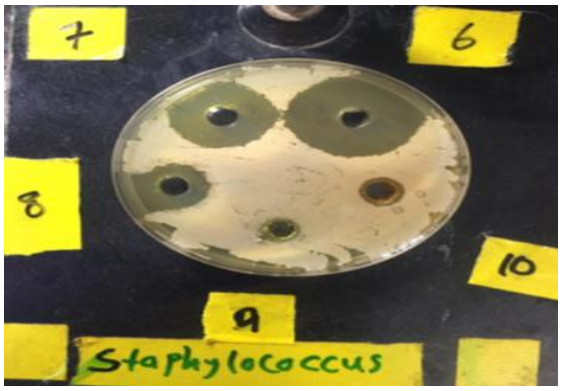
Table -6-: in vitro antimicrobial activity result of the synthesized compounds

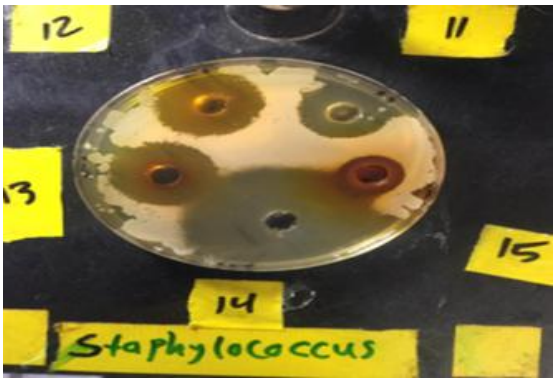
No.	Code	Conc. mg/ml	Inhibition zone(mm)		Inhibition zone(mm)	
			C. albicans	A. niger	S. aureus	E. coli
1	CH5	50	0	0	25	20
2	NC2	50	0	0	25	25
3	sulfadiazine	50	15	0	25	25
4	T.S	50	30	40	22	15
5	Nystatin	50	40	40	0	0
6	NC4	50	50	50	35	40
7	NC3	50	50	50	27	40
8	NC1	50	50	50	20	35
9	Et(NH)2	50	50	50	0	25
10	CH8	50	50	50	12	15
11	Prepoly	50	15	20	22	15
12	CH7	50	0	14	26	20
13	CH4	50	15	25	25	15
14	Amoxil	50	12	0	50	40
15	sulfaoxazol	50	0	0	20	15
16	CH1	50	15	12	25	0
17	CH6	50	15	20	25	27
18	sulfanilamide	50	15	40	26	0
19	CH2	50	13	30	25	0
20	Azo3	50	20	15	17	0

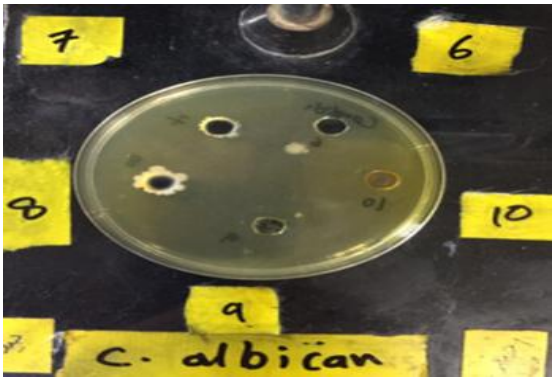












Figures -6- : shows the effect of the some compounds referred to in Table 5 on the {(*Staphylococcus aureus*), (*Escherichia coli*), (*Candida albicans*),(*Aspergillusniger*)}

Conclusion

In this study , we synthesized three kinds of compounds which derivatives from sulfa drugs . these synthesized compounds have been characterized by FT- IR , ^{1}H-NMR spectra . The analytical data confirmed the composition and structures of these compounds . The antioxidant activity and biological activity were screened by using two types of fungi and two types of bacteria and we observed that these compounds possess excellent activity as antifungal and slightly active against both kinds of bacteria .

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