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STUDY OF NEW AZO-AZOMETHINE DERIVATIVES OF SULFANILAMIDE : SYNTHESIS, CHARACTERIZATION, SPECTROSCOPIC, ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER ACTIVITY

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ABSTRACT : A complementary for previous study, series of azo-azomethine compounds (Sb6- Sb12) have been synthesized with magnificent yield by condensation reaction of 4-((3-formyl-4-hydroxy-5-methoxyphenyl)diazenyl) benzenesulfonamide and aniline derivatives. The new azo compound was prepared from sulfanilamide by converting it to diazonium salt followed by coupling reaction with 2-hydroxy-3-methoxybenzaldehyde in alkaline medium. The structures of synthesized azo and azo-azomethine compounds have been established based on their spectral data (FT-IR,1HNMR ,13CNMR)and elemental analysis (C, H, N). The purity of compound and evaluation of R_f value were determined by TLC. The antimicrobial activity of azo-azomethine compounds have been tested *in vitro* against bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Klepsilia pneumonia*) and fungi (*Candida glabrata, Candida albicane* and *Aspergillus niger*) by agar diffusion method, to assess their inhibiting potential. Also, the antioxidant and anticancer efficiency of azo-azomethine compounds have been calculated.

Key words : Azo , azo-azo Schiff bases, azo dyes, sulfanilamide, aniline derivatives.

INTRODUCTION

Sulphonamides are the primary viable chemotherapeutic specialists utilized for bacterial disease in people. Since their disclosure, sulfonamides have been generally utilized for prophylaxis and treatment of bacterial contaminations in spite of the fact that they are bacteriostatic as opposed to bactericidal. Their worth lies in the capacity to back off or anticipate development in wounds or tainted organs without considerable harmfulness to ordinary tissues (Mansour, 2014).

Sulfanilamide and its derivatives have a wide scope of pharmacological exercises, for example, Oral hypoglycemic, antileprotic, antiepileptic, against hypertensive, antibacterial, antiprotozoal, anti-parasitic, antiretroviral, calming , utilized as diuretic. Additionally studies have demonstrated that Sulphonamides are likewise ready to obstruct cancerous cell (Sharaf El-Din, 2000; Ajeet *et al*, 2015).

Azo compounds are the biggest class of natural colors that firstly prepared in 1862 by Peter Griess. These compounds portrayed by the existence of the azo moiety (–N=N–) in their structure, conjugated with two aromatic or hetero aromatic frameworks. Due to their particular physico-chemical properties and biological activities, they have discovered flexible use in numerous practical application in pharmaceutical, cosmetic, food, coloring or material industry and analytical science. Azo compounds are notable for their therapeutic significance and perceived for their applications as antidiabetics, germicides, antifungal, calming, antineoplastics, antibacterial and antitumor (Patil and Nehete, 2015; Kareem and Salman, 2017).

Schiff base, a marvelous gathering of compounds otherwise called anils, imines or azomethines that contains azomethine group (C=N). This group in charge of the biological activity of Schiff bases since intramolecular hydrogen bonding with C=N nitrogen atoms of Schiff bases decides the properties of different molecular systems and acting a critical role in numerous biochemical systems (Pallikkavil *et al*, 2012; Sikarwar *et al*, 2016).

Schiff bases have a wide assortment of applications in various regions, for example biological chemistry, organic and inorganic science and bioinorganic science as normal non-enzymatic/enzymatic intermediates, coordination and supramolecular science as basic ligands in addition to biomedical applications and material sciences. Schiff bases have gotten noteworthiness in pharmaceutical field due to expansive scope of biological activities like antibacterial, antifungal, anti-HIV, mitigating,

pain relieving, antimicrobial, antispasmodic, tuberculosis, against malignancy, antioxidants, anthelmintic, antimalarial and against amoebic (Mumtaz *et al*, 2016; Berk *et al*, 2017).

MATERIALS AND METHODS

Materials and reagents : 4-floroaniline, 2-methyl-4-chloro aniline, 4-bromoaniline and 4-methoxyaniline was obtained from Fluka. HCl, $NaNO_2$, Sodium carbonate, 2-hydroxy 3- methoxybenzaldehyd, sulphanilamide, Sulfadiazine, sulfamerazine and sulfamethazine from sigma-Aldrich product. All solvent used of analytical grade from Fluka and used without further purification. The measurements of melting point were done on Bauchi 510.

Instrumentation : The solid state FTIR spectra of the compounds were record on shimadzu FT –IR model 8400 S Spectrophotometer using KBr pellets in the range 4000 - 400 cm^{-1}. The spectra of 1HNMR and ^{13}CNMR were done in a Brucker spectrophotometer (400 MHZ) and using DMSO –d6 as solvent and TMS as internal standard. Elemental analysis (C, H, N) were recorded by Euro vector model 3000 A (Italy).

Synthesis of 4-((3-formyl-4-hydroxy-5-methoxyphenyl)diazenyl) benzenesulfonamide (A) : The compound was prepared as described by Erdem *et al* (2009). A solution of sulfanilamide 5 mmol (0.86g) in 2N of HCl (10 mL) was stirred until a clear solution was obtained. The mixture was cooled to 0–5°C, then sodium nitrate 7.5mmol (0.5 g) in was added dropwise with stirring, maintaining the temperature below 5 °C. After the addition was completed, the solution was stirred for an additional 45 min. ortho vanillin 5 mmol (0.76g) was dissolved in 30 mL of aqueous solution containing 20 mmol of Na_2CO_3. The mixture cooled to 0–5°C in an ice bath, then gradually added to the solution of the cold diazonium salt of sulfanilamide. The resulting mixture was continually stirred at 0–5°C for 2h and keeping pH about 6–7. The originating precipitate filtered, washed several times with water and recrystallized from EtOH to give the desired azo compound. The purity azo compound was evaluated by thin layer chromatography by using ethanol / chloroform (1: 9).

Synthesis of Sulfanilamide Azo-azomethine (Sb6-Sb12) : The Schiff-base was set by the usual condensation reaction, in which equimolar 1 mmol (0.3 g) of azo compound and the aniline derivatives 1mmol were liquefied in least possible quantity of methanol ,also glacial acetic acid (2-3 drops) was added and refluxed for about 5-12hrs, the response of product was tested by TLC using acetone/chloroform (7:3), pure compounds was obtained by cooling the reaction mixture to freezing temperature. The precipitate was filtered off and washed with cold absolute methanol and dried in air (Erdem *et al*, 2009). The various synthetic preparation of azo – azomethine compounds are summarized in Scheme 1.

Antimicrobial activity : The compounds were screened in vitro for antimicrobial properties .the panel of pathogens involved *Staphylococcus aureus* as a Gram-positive bacterium, *Escherichia coli* as a Gram-negative bacterium, *Klebsiella pneumonia* and fungal species *candida glabrata*, *Candida albicane* and *Aspergillus niger*. This test was performed by agar diffusion method. 0.2 mL of fungal inocula and bacterial inocula were placed on the surface of Sabouraud dextrose agar (S.D.A) medium and Nutrient agar (N.A) medium, respectively. The tested compounds and reference drugs were dissolved in DMSO with concentration include 0.03g /mL for every compound. The synthesis compounds were placed in central pore inbacterial plates and incubated at (37 ± 2°C), while fungal plates were incubated at (25 ± 2°C) for 24 hr, the inhibition zones of each isolate were recorded in millimeter unit (NCCLS, 1998).

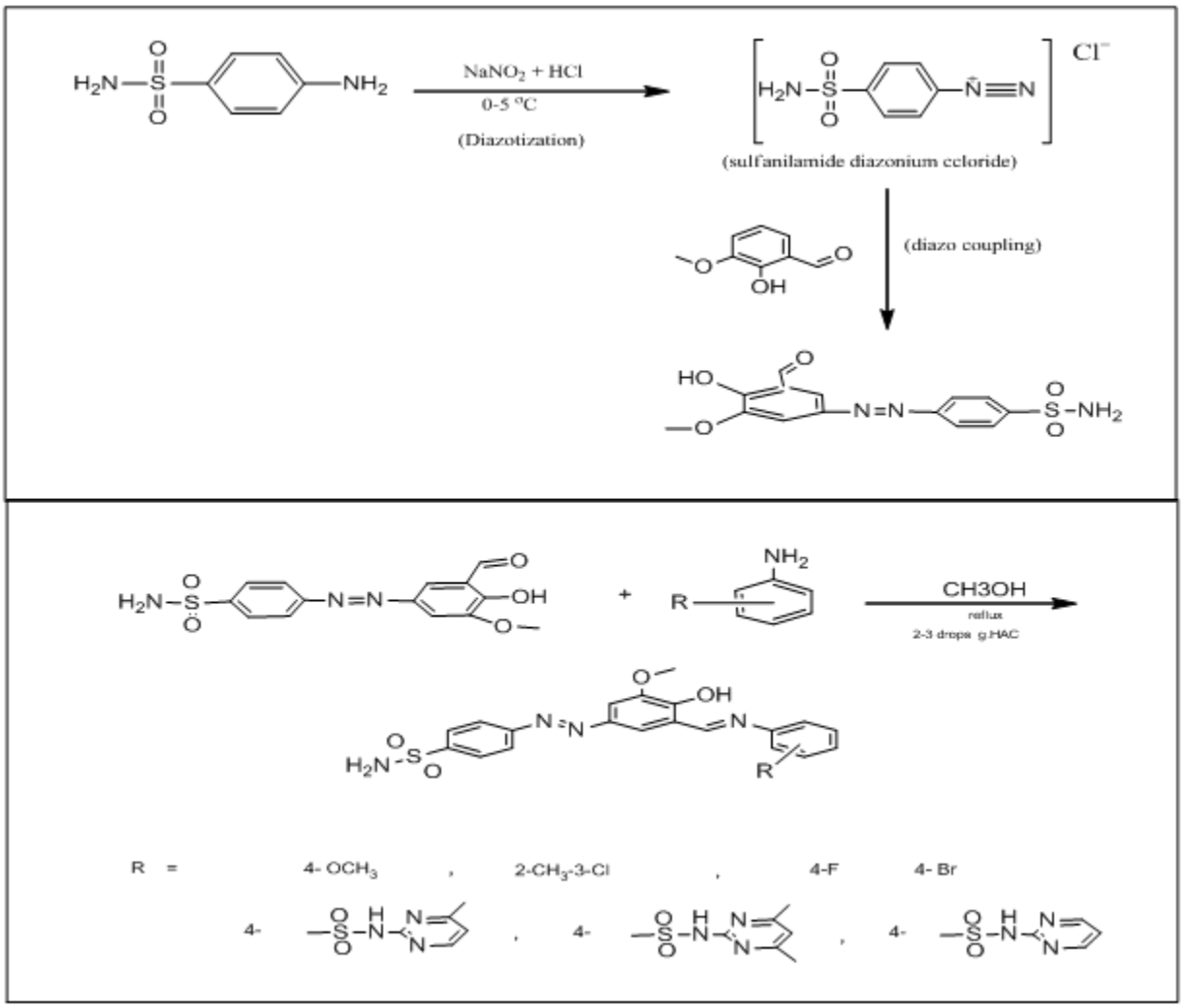
Antioxidant assay using the â-carotene bleaching method : The oxidative losses of â-carotene/ linoleic acid emulsion were used to assess the anti-oxidation ability of the synthetic organic compounds . 0.02 ml of linoleic acid and 0.2 ml of Tween 20 were placed in a round flaskand (1 ml) â-carotene (0.2 mg/ml in chloroform) was added to the flask. The mixture was evaporated and 50 ml of distilled water was added to the mixture, 3.8 ml of the mixture was then dosed with 0.2 ml of tested sample and reference (BHT) compound . The absorbance was read at 470 nm, the samples were then subjected to thermal autoxidation at 45°C in a water bath for 2 h. Absorbance was measured every 15 min (Ahmeda *et al*, 2009). Antioxidant activity (AA) was calculated using the following equation:

$$\%AA = 1 - [(Ai - At) / (*Ai - *At)] \times 100$$

Where, Ai : is the measured absorbance value of sample at zero time. At : is the measured absorbance value of sample after incubation (105)min at 45°C.

*Ai : is the measured absorbance value of control at zero time, *At : is the measured absorbance value of control after incubation (105)min at 45°C.

***In vitro* assay for cytotoxicity activity (MTT assay) :** The Cytotoxicity of samples on AMJ13 cell line and REF normal cells were determined by the MTT assay (Mosmann, 1983). Cells (1×10^5/well) were plated in 96-well plates and incubated in 37^0C with 5% CO_2 condition. The various concentrations ofthe samples were



Scheme 1 : The various synthetic preparation of azo–azomethine compounds.

added and incubated for 72 hours. After incubation, the sample wasremoved from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100ìl/well (5mg/ml) of 0.5%, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl—tetrazoliumbromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with a UV -spectrophotometer using DMSO as the blank. Measurements were performed and theconcentration required for a 50% inhibition (IC50) was determined graphically.

RESULTS AND DISCUSSION

The azo-azomethine compounds (Sb6-Sb12) were prepared via reaction of aniline derivatives with azo compound (A). The prepared compounds are solid Compound, stable in air, the elemental analysis C, H, N are in agreement with suggested formula. The physical properties, percent yield and Rf values are cited in Table 1.

Spectroscopic analysis : Their spectroscopic analysis are in agreement with the empirical structures. The infrared spectra show the position and the intensities of the peaks, which corresponds to various groups present in each compound. Moreover their elemental analyses are in conform with the theoretical expectations.

Infrared spectra : The infrared of prepared compounds (A, Sb6– Sb12) shows characteristic bonds at (1462-1485) cm^{-1}that be attributed to the stretching vibration of the azo group (-N=N-). All the infrared spectra of the compounds were characterized by a broad band at 3421–3568 cm^{-1} which corresponds to the O-H stretching vibration. IR spectra of the Schiff bases showed the absence of bands at 1647 cm^{-1}, which attribute to carbonyl υ (C=O) stretching vibrations and instead, the appearance of a strong new band at 1614–1627 cm^{-1} that assigned to the azomethine υ (C=N) linkage (Jarrahpour *et al*, 2004), which indicates the reaction between the amino and aldehyde moieties of the starting reagents no more exist and have been converted into the respective Schiff base linkages. In

Table 1 : The symbol, Synthetic formula, IUPAC name, analytical and physical data of the prepared compounds.

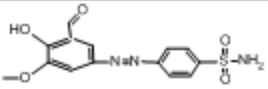
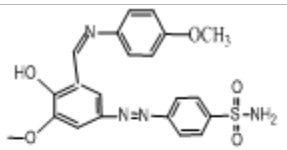
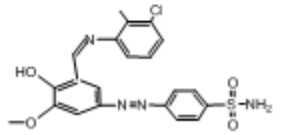
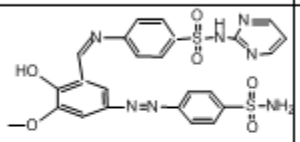
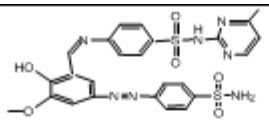
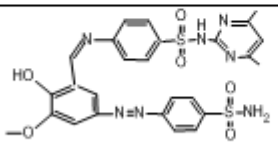
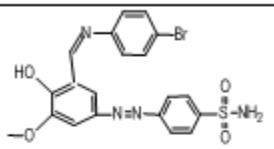
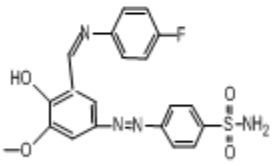
Comp. symb.	Structural formula / IUPAC name	Molecular formula / M.Wt	M.P °C	Reaction time (h)	R_f	Appearance (Yield%)	Elemental analysis CHN partical (theoretical)		
							C%	H%	N%
A	 4-((3-formyl-4-hydroxy-5methoxyphenyl) diazenyl) benzene sulfonamide	$C_{14}H_{13}O_5N_3S$ 335	200	3	0.69	Maroon powder (48)	49.81 50.14	3.61 3.91	12.23 12.53
Sb6	 4-((4-hydroxy-3-methoxy-5-(4-methoxyphenyl)imino)methyl)phenyl)diazenyl)benzenesulfonamide	$C_{21}H_{20}O_5N_4S$ 440	93	5	0.68	Light yellow /long crystals (73)	57.15 57.26	4.33 4.58	12.43 12.72
Sb7	 4-((3-(3-chloro-2-methylphenyl) imino)methyl)-4-hydroxy-5-methoxy phenyl) diazenyl)benzene sulfonamide	$C_{21}H_{19}O_4N_4SCl$ 458.6	120	5	0.77	Light yellow /long crystals (48)	54.82 54.96	3.99 4.17	12.01 12.21
Sb8	 4)-(2-hydroxy-3-methoxy-5-((4-sulfamoylphenyl)diazenyl) benzylidene)amino)-N-(pyrimidin-2-yl) benzenesulfonamide	$C_{24}H_{21}O_6N_7S_2$ 567	218	12	0.5	Maroon crystals (34)	50.48 50.79	3.59 3.73	17.16 17.27

Table 1 continued...

addition, the strong band at 1249–1265 cm^{-1} can correspond to the phenolic C-O stretching vibration.

Furthermore the SO_2 moiety appears featured bands at the range 1300-1381 cm^{-1} and in 1147-1188 cm^{-1}, which assigned to asymmetrical and symmetrical stretching vibration respectively of SO_2. In the region (1095 cm^{-1}), (972cm^{-1}), (734 cm^{-1}) strong peaks appeared related to stretching of υ (C-F), (C-Cl), (C-Br)

Table 1 continued...

Sb9	 **4-(2-hydroxy-3-methoxy-5-((4-sulfamoylphenyl)diazenyl) benzylidene)amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamid**	$C_{25}H_{23}O_6N_7S_2$ 581	305dec.	12	0.26	Yellow powder (68)	51.51 51.63	3.81 3.99	16.75 16.86
Sb10	 **N-(4,6-dimethylpyrimidin-2-yl)-4-((2-hydroxy-3-methoxy-5-((4-sulfamoylphenyl)diazenyl) benzylidene)amino)benzenesulfonamide**	$C_{26}H_{25}O_6N_7S_2$ 595	138	12	0.28	Pale brown powder (67)	52.29 52.43	4.15 4.23	16.32 16.46
Sb11	 **4-((3-(4-bromophenyl) imino)methyl)-4-hydroxy-5-methoxyphenyl)diazenyl)benzenesulfonamide**	$C_{20}H_{17}O_4N_4SBr$ 489	116-118	5	0.85	Light orange crystals (29)	48.11 49.09	3.38 3.50	11.32 11.45
Sb12	 **4-((3-((4-fluorophenyl) imino) methyl)-4-hydroxy-5-methoxyphenyl) diazenyl) benzenesulfonamide**	$C_{20}H_{17}O_4N_4SF$ 427.9	96-98	5	0.72	Pale orange /long crystal (47)	55.93 56.07	3.82 4.00	12.90 13.08

respectively for the compounds Sb12, Sb7 and Sb11. The spectral data of these synthesized compounds have been summarized in Table 2 and some spectra of compounds showed in Figs. (1-3) respectively (Modi *et al*, 2014; Al-Salami *et al*, 2014).

NMR spectra : The ^{1}HNMR and ^{13}C NMR spectral data of synthesized compounds have been listed in Tables 3 and 4. The ^{1}HNMR spectral data analysis was carried out for these compounds given results indicating the compatibility of the proposed structures. Some spectra of compounds showed in Figs. 4, 5. The ^{1}HNMR spectra of the azo compound (A) shows a single signal at δ (10.091) ppm, which attributed to the proton of azo-aldehyde group. All the compounds are characterized by showing singlet signal at δ (9.29-13.47) ppm and which can be assigned to phenolic group (OH). Also multiple signals that appear at δ 6 to 8.52 can be attribute to

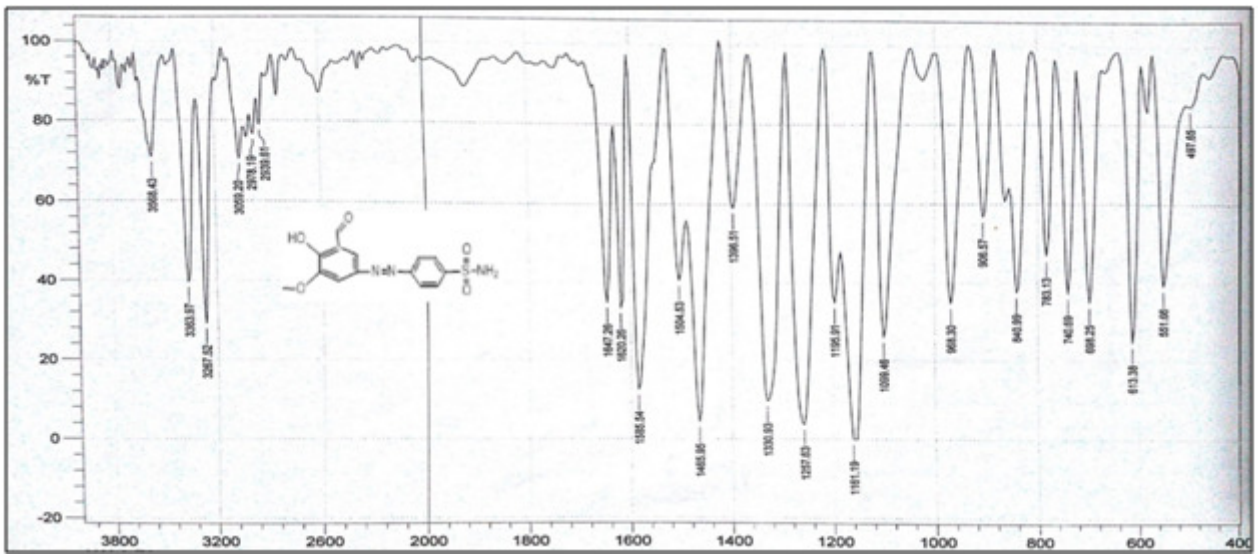


Fig. 1 : FT-IR spectrum of compound A.

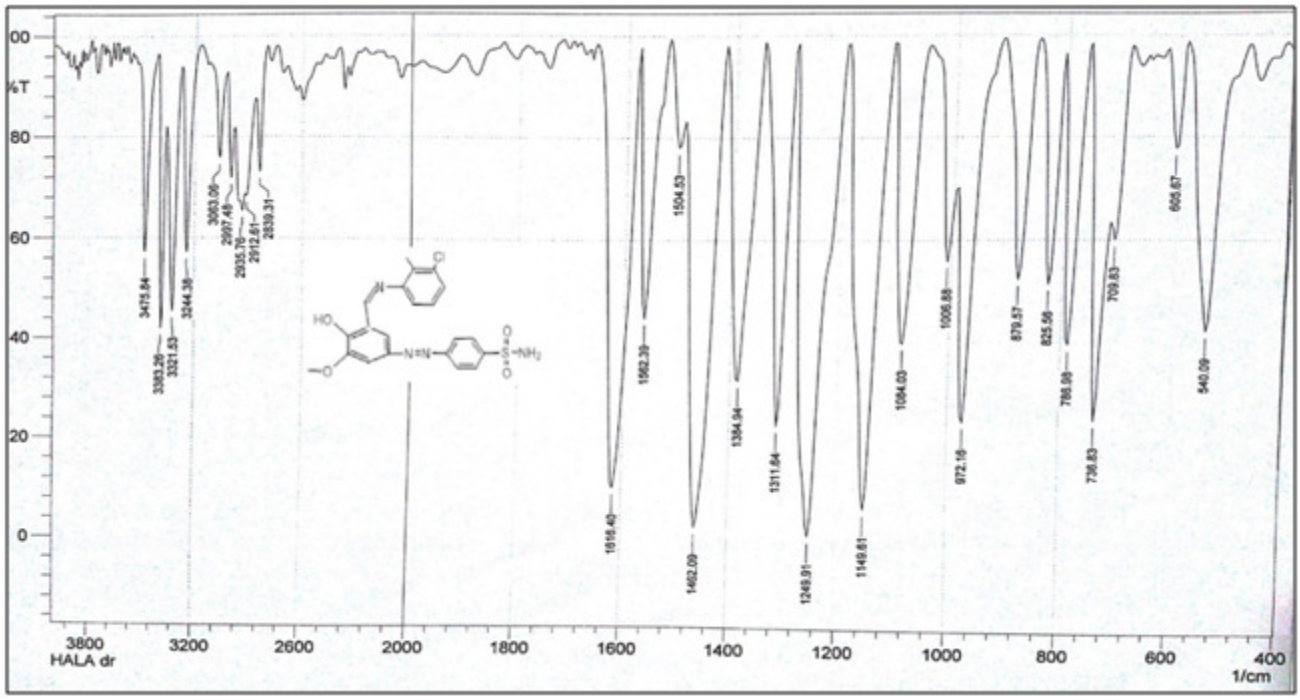


Fig. 2 : FT-IR spectrum of compound Sb7.

aromatic rings of these compounds, while we can have observed in azo-azomethine compounds (Sb6-Sb12) disappearance of the signal of azo-aldehyde group and instead of it a new single appears at δ (8.79-8.95) ppm which attributed to the proton of azomethine group (HC=N). Furthermore, the methoxy substituent of the o-vanillin was observed as a strong singlet at δ 3.79–3.84 ppm (Karabasannavar *et al*, 2017; Yang and Sun, 2006).

In addition, all azo-azomethine compounds have singlet signal at δ (6.83-6.99) ppm that due to the presence of two protons of (NH_2) group of sulfanilamide which innervate the desired results (Saeedia *et al*, 2014).

The proton of CH=N $_{ring}$ of compounds (Sb8) appear at δ (8 .47) ppm. In addition the proton of (NH) group of compounds (Sb8, Sb9 and Sb10) appear at δ (10.4 -11.17) ppm (Sharma *et al*, 2017).

Similarly, the ^{13}C NMR spectra shows signal at the range δ 161.8–165.4 ppm is due to the imine functional group (C=N), which confirms the formation of the Schiff base from new azo compound and aniline derivatives. Additionally, the signals of aromatic carbons of these synthesized compounds represented at δ 110- 162 ppm, while the signal of the methoxy carbon observed at the range δ 55.8 -56.3 ppm. The ^{13}C NMR spectral data of the azo-azo Schiff bases are in accord with suggested

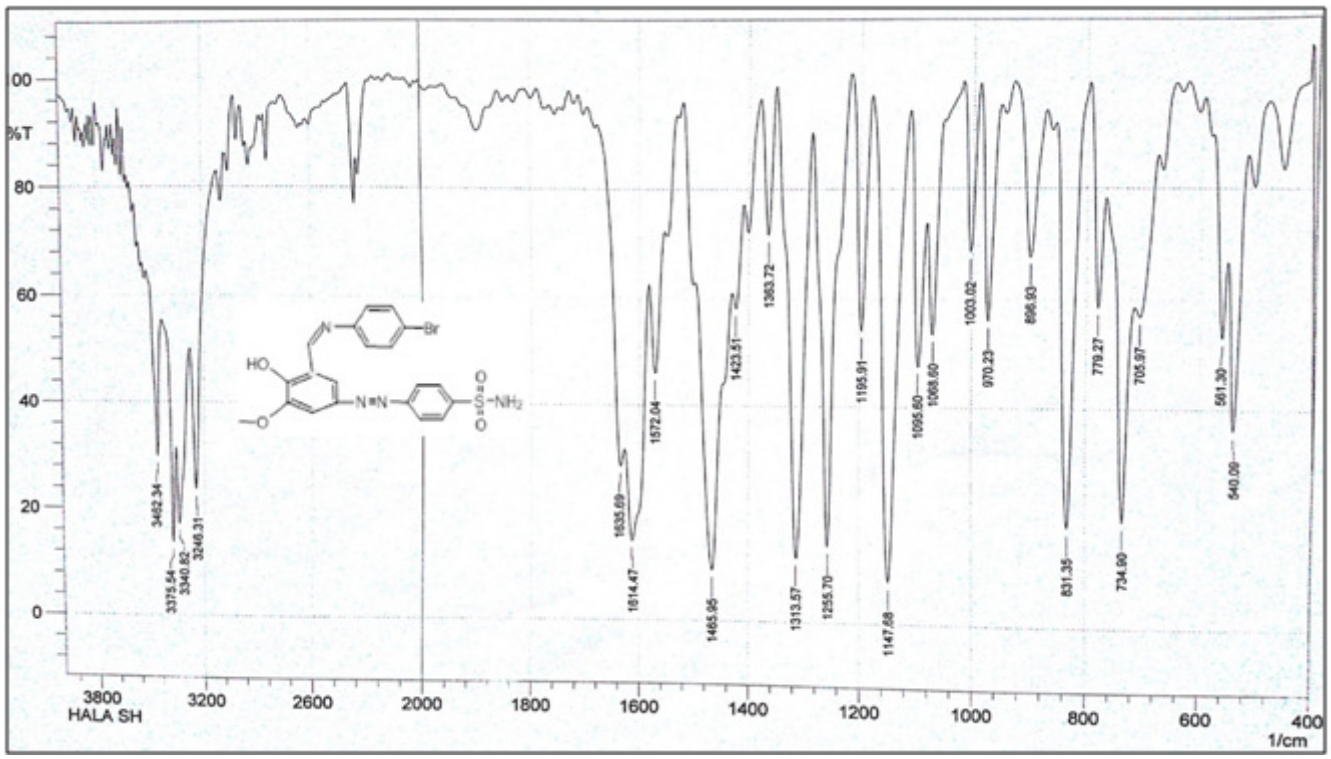


Fig. 3 : FT-IR spectrum of compound Sb11.

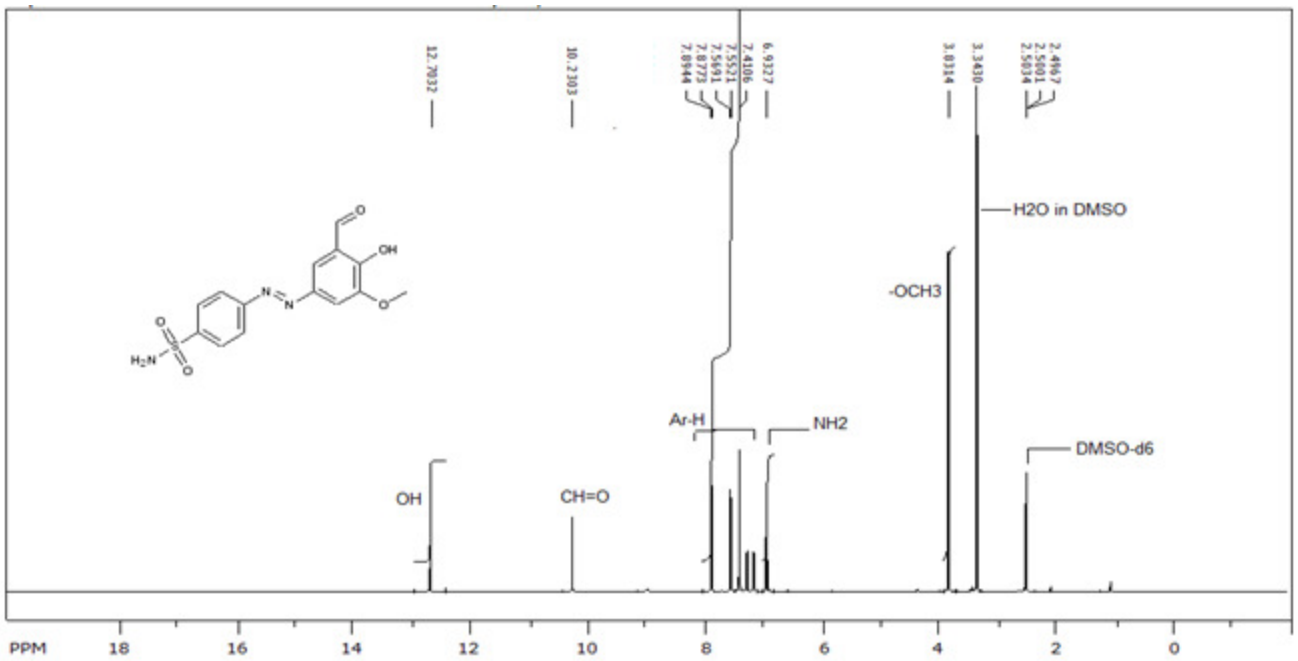


Fig. 4 : ^{1}H NMR spectrum of compound A.

structures. Some spectra of compounds showed in Figs. 6-8.

Biological activity : antifungal and antibacterial screening : *In vitro* studies of the antifungal and antibacterial activities of the investigated compounds against bacteria (*S. aureus, E.coli* and *K. pneumonia*) and fungi (*C. glabrata, C. albicane* and *A. niger*) were carried out using well diffusion method. The results have been showed in Table 5 and Figs. 9, 10. Almost all the compounds exhibited antibacterial activity against the studied microbes at concentration 30 mg/ml .

In comparison of the three kinds of studies strain we can observed that the most of prepared compounds have high to moderate antibacterial activity. It is found that compound (A, Sb10) are more effective against *Eschericahia coli* and *Staphylococcus aureus* than Sb6,

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

Com.	υ(OH) cm^{-1}	υ(NH_2) sulfa 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) cm^{-1}	Others
A	3568 m	3267-3363	1647 s	_	1465 s	1330 s 1161 s	1257 s	968 s υ(S-N)$_{str.}$ 2978 w υ$_{as}$(C-H)$_{ali.}$ 3059w υ(C-H)$_{Ar.str.}$
Sb6	3421	3390	-	1616 s	1469 s	1300 1165	1253 s	2939 w υ$_{as}$(C-H)$_{ali.}$ 3055w υ(C-H)$_{Ar.str.}$
Sb7	3475 w	3244-3321	-	1616 s	1462 s	1311 1149	1249 s	2935 w υ$_{as}$(C-H)$_{ali.}$ 3063w υ(C-H)$_{Ar.str.}$
Sb8	3475br	3379	-	1616 m	1465 m	1338 1157	1257 s	2943 wυ$_{as}$(C-H)$_{ali.}$ 1581 s υ(C=N)$_{ring.str.}$ 3043w υ(C-H)$_{Ar.str.}$
Sb9	3475br	3383	-	1627 m	1485 m	1327 1149	1265 m	2939 w υ$_{as}$(C-H)$_{ali.}$ 1589 s υ(C=N)$_{pyr.str.}$ 3032w υ(C-H)$_{Ar.str.}$
Sb10	3483 br	3221-3375	-	1627 m	1477 s	1381 1153	1261 s	2939 w υ$_{as}$(C-H)$_{ali.}$ 1589 s υ(C=N)$_{ring.str.}$ 30 70 υ(C-H)$_{Ar.str.}$
Sb11	3462 br	3246-3340	-	1614 s	1465 s	1313 1147	1255 s	2990 w υ$_{as}$(C-H)$_{ali.}$ 3049 w υ(C-H)$_{Ar.str.}$
Sb12	3448 br	3286	-	1616 s	1465 s	1361 1188	1253 s	2997 w υ$_{as}$(C-H)$_{ali.}$ 3066 w υ(C-H)$_{Ar.str.}$

Sb7, Sb8, Sb9, Sb11 and Sb12 which have moderate activity. The compound Sb7 has the higher activity against *K. pneumonia* than other compound which shows moderate activity.

Among the synthesized compounds A and Sb7 were found to be more effective against *Aspergillus niger* with an IZ 40 mm more effective than the positive control Nystatin (IZ 30 mm). Also compounds Sb9 and Sb10 showed a good activity with an IZ ranging from 20-25 mm. The rest compounds have no activity against *Aspergillus niger*.

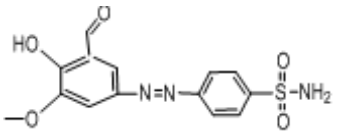
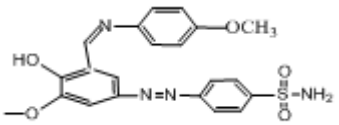
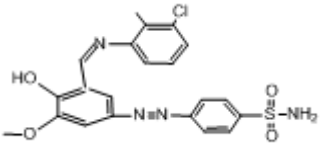
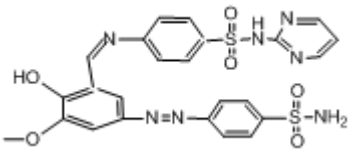
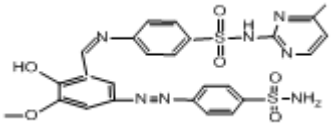
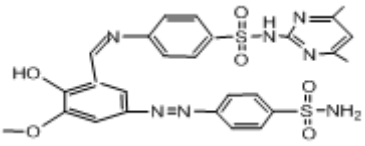
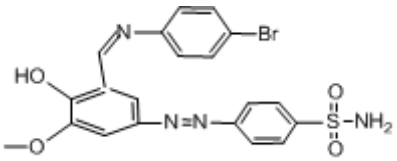
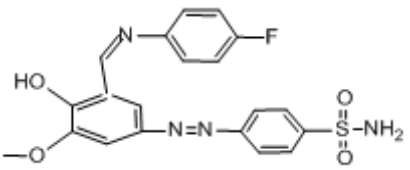
The antifungal activity against *Candida albicans* shows that Sb6 was the most active of all the compounds with an IZ = 50 mm. Also compounds A, Sb7, Sb9 showed a good activity with an IZ=40 mm. The compounds Sb10, Sb11 and Sb12 showed appreciable activity with an IZ ranging from 10-20 mm. The compoundsSb8 have no activity against *Candida albicans* . The antifungal activity against Candidaglabrata showed that Sb6 was the most active of all the compounds with an IZ = 40 mm. Also compounds Sb7, Sb8, Sb10, Sb12 showed high activity with an IZ = 30 mm. The compounds A, Sb9, Sb11 showed a good activity with an IZ ranging from 22-25 mm (Figs. 9, 10).

Schiff bases helps to clarify the mechanism of transamination and racemization reaction in biological system. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (>C=N–) with the active centers of various cellular constituents, resulting in interference with normal cellular processes (Khedr and Saad, 2015; Prakash *et al*, 2017). Also, N=N group played important role in antibacterial activity. The azoderivatives consist of -N=N- group can be protonated under acidic condition to react with the phosphate group on the polysaccharide peptidoglycan layer of bacteria, which hinder the formation of cell wall. Furthermore, compounds consist of phenyl groups and halogen atoms have also revealed that those with more lipophilic character could easily penetrates the cell wall of microorganism (Ngaini and Kui, 2017).

Antioxidant activity

The antioxidant activity of our synthesized compounds could be attributed to demesne these compounds hydroxyl group, which have ability of scavenging free radical . It was estimated the activity of compounds (A, Sb6- Sb12) as antioxidant compounds depending upon the relationship between absorbance and time as showing in Table 6 and Figs. 11, 12 and comparable those with BHT and with application of previous mathematical, the highest activity compound is Sb6 possess high activity comparable to the standard BHT. This compound have an additional methoxy group, which increase the antioxidant activity. This activity may be correlated with the introduction of electron

Table 3 : ^{1}HNMR spectral data of compounds (A, Sb6-Sb12).

Symb. of com.	Structure	Chemical δ(ppm)
A		10.23 (s, 1H, HC=O), 12.70 (s,1H,OH) 3.83(s ,3H ,OCH_3) ,7.410-7.89 (m, 6H, Ar-H) 6.93(s, 2H, NH_2)
Sb6		8.92 (s, 1H, CH=N), 13.47 (s, 1H, OH) 3.79 (s,6H, $2OCH_3$), 7.06-7.55(m, 10H, Ar-H) 6.925(s, 2H, NH_2)
Sb7		8.79 (s, 1H, CH=N), 12.93(s,1H,OH) 3.82 (s,3H,OCH_3), 6.92-7.32(m, 9H, Ar-H) 6.905(s, 2H, NH_2), 2.34 (s, 3H, CH_3)
Sb8		8.95 (s, 1H, CH=N), 12.5(s, 1H, OH) 3.84 (s, 3H, OCH_3), 6-8.52(m, 13H, Ar-H) 6.92(s, 2H, NH_2), 11.7 (br,1H, NH), 8.47(s, 1H, $CH=N_{ring}$)
Sb9		8.84 (s, 1H, CH=N), 11.12(s, 1H, OH) 3.83 (s, 3H, OCH_3), 6.5-8.3(m, 12H, Ar-H) 6.99(s, 2H, NH_2), 10.4 (br,1H,NH), 2.8 (s ,3H ,CH_3)
Sb10		8.921 (s, 1H, CH=N), 9.29(s, 1H, OH), 3.84(s, 3H, OCH_3) 6.56-8.1(m, 11H, Ar-H), 6.87(s, 2H, NH_2) 11.17(br, 1H, NH), 2.2, 2.4(s, 6H, $2CH_3$)
Sb11		8.95 (s, 1H, CH=N), 12.9 (s, 1H, OH), 3.82(s, 3H, OCH_3), 7.37-7.65 (m, 10H, Ar-H), 6.99 (s, 2H, NH_2)
Sb12		8.93 (s, 1H, CH=N), 13.06 (s, 1H, OH) 3.82 (s, 3H, OCH_3), 6.91-7.47 (m, 10H, Ar-H) 6.83 (s, 2H, NH_2)

donor substituent which stabilizes the generated radical during oxidation (Mohana and Kumar, 2013). Also the compounds A, Sb9, Sb10, Sb11 and Sb12 possess high activities comparable to the standard BHT. While the compounds Sb7 and Sb8 showed less activity. Finally, based on antioxidant activity study, the actively order of these compounds take this way; Sb6> Sb12> Sb9 = Sb10 = Sb11 >A >Sb7 >Sb8.

Commonly, the free radical scavenging compounds endue protons and convert to extra stable free radicals.

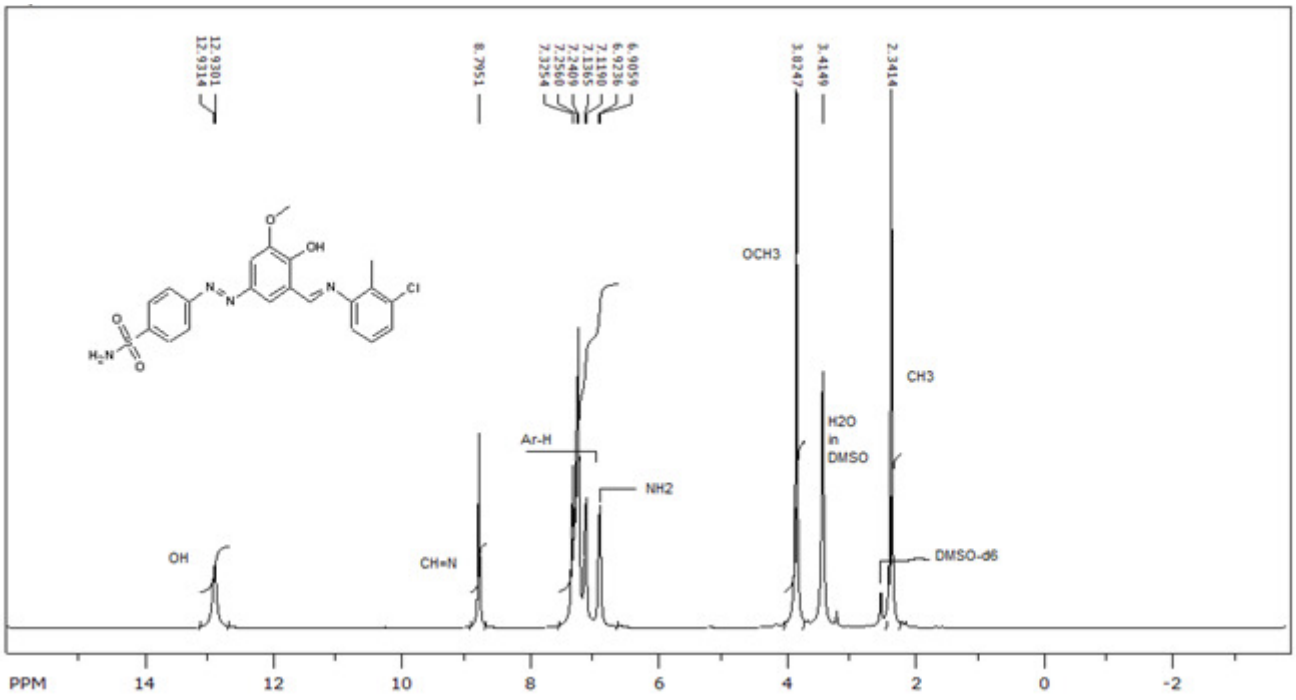


Fig. 5 : ^{1}H NMR spectrum of compound Sb7.

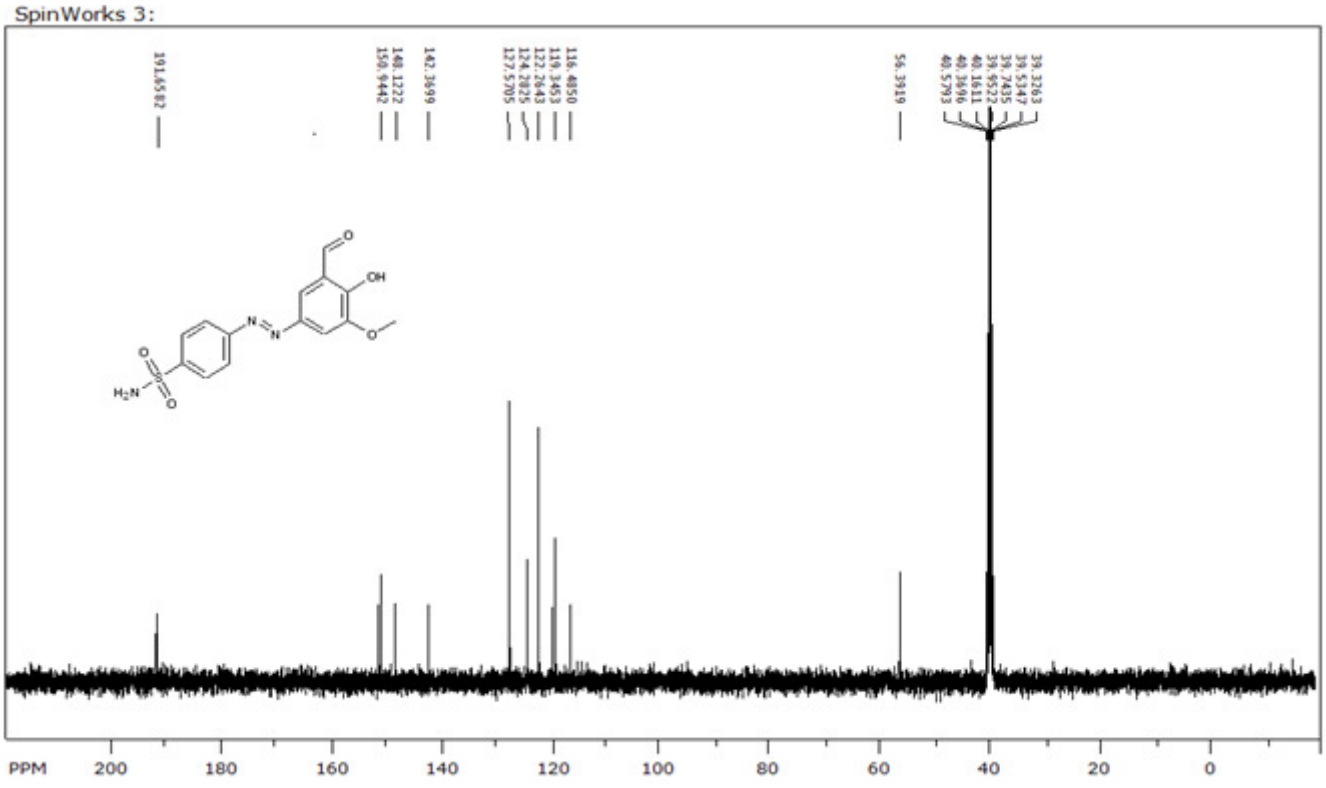


Fig. 6 : ^{13}C NMR spectrum of compound A.

This stability increases with the existence of delocalization and increases the antioxidant ability. Otherwise , presence full conjugation (ð-system) in structure and steric hindrance these features has positive effect on the antioxidant ability (Shakir *et al*, 2017). Phenolic compounds, which can represent an inhibitor of the process of oxidation, even at comparatively small concentration, usually involve an aromatic ring as part of the molecular structure, with one or more hydroxyl groups. They can act as antioxidants as their broad conjugated ð-electron systems allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals (Canadanovic-Brunet *et al*, 2005), where the phenoxide free radical ($ArO^{\cdot}$) is stabilized by resonance

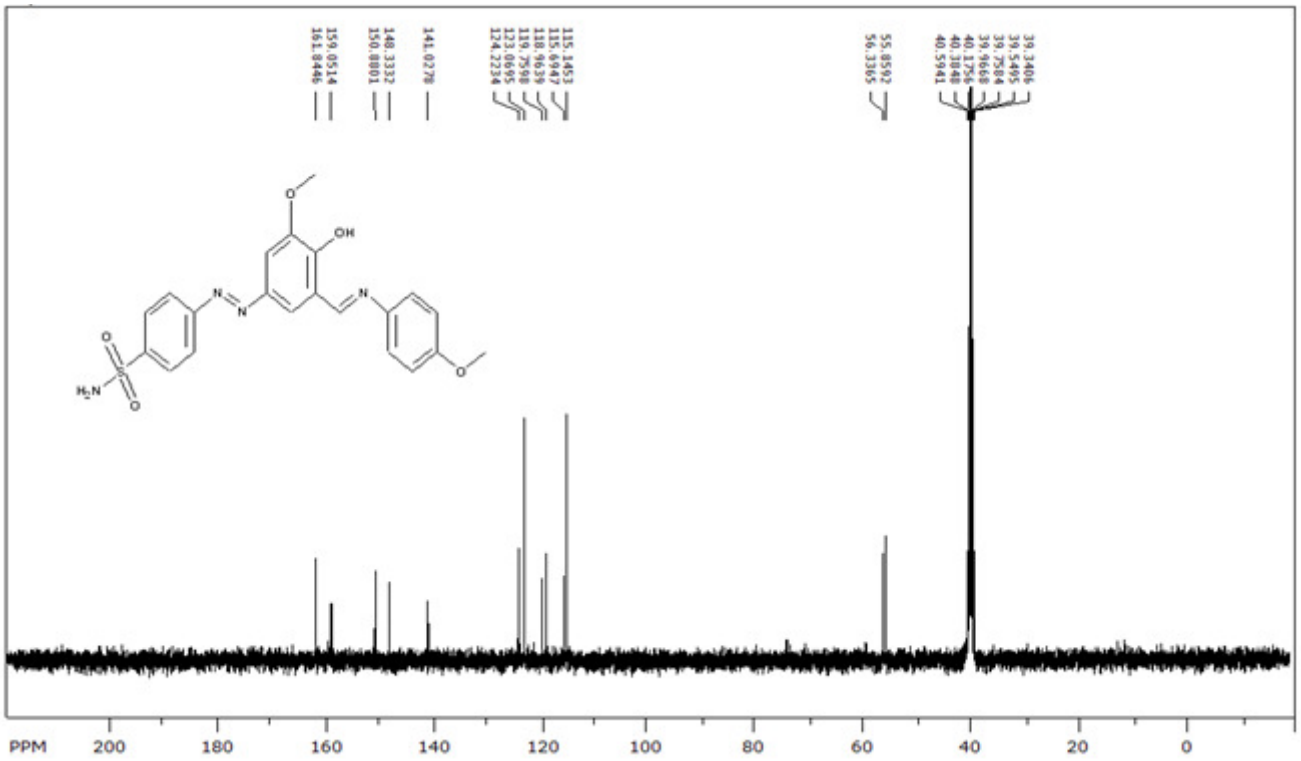


Fig. 7 : ^{13}C NMR spectrum of compound Sb6.

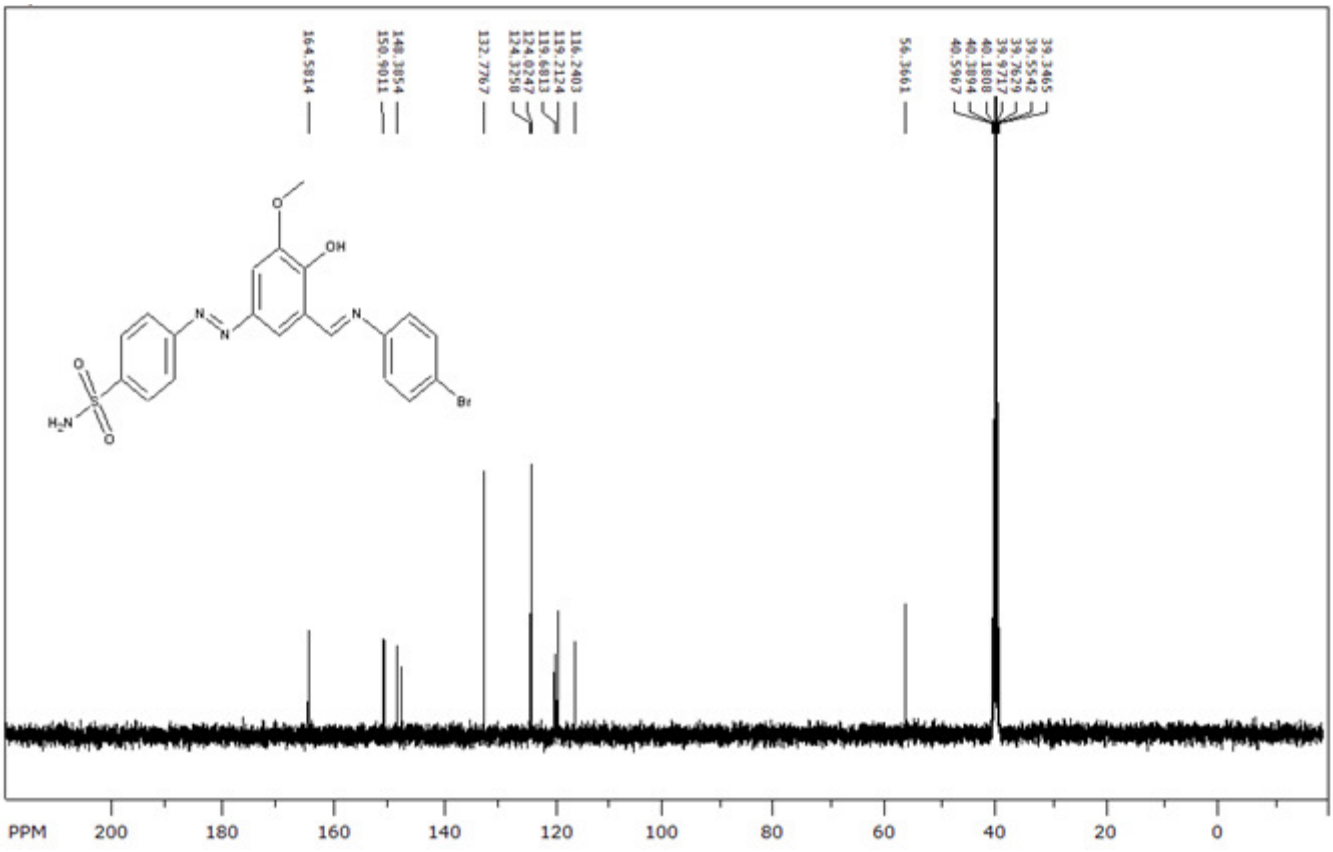


Fig. 8 : ^{13}C NMR spectrum of compound Sb11.

(Apak *et al*, 2007).

Cell Cytotoxicity (anticancer) study

The most antioxidant compounds A, Sb6 and Sb12 were evaluated to their anticancer activity. The (IC50) and death cells percent of AMJ13 cancerous and REF normal cells at different concentrations ranged from 30

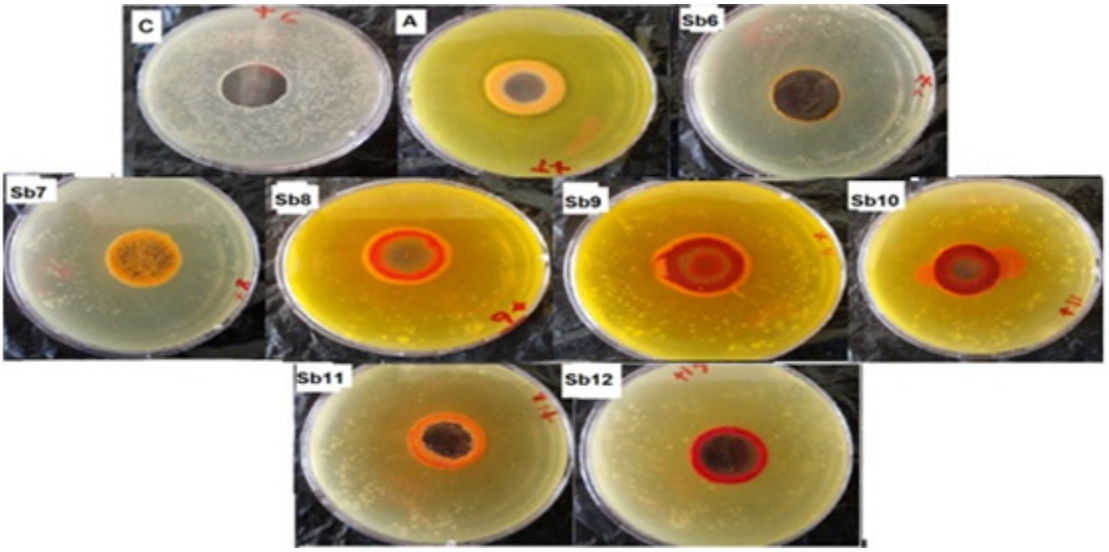


Fig. 9 : Inhibiton zone of synthesis compounds against *Staphylococcus aureus* at concentration (30mg/ml).

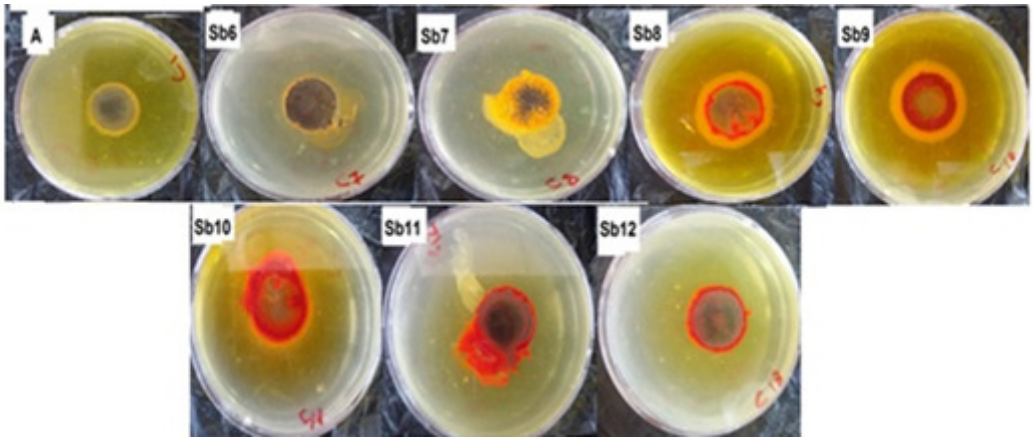


Fig. 10 : Inhibiton zone of synthesis compounds against *Candida glabrata* at concentration (30mg/ml).

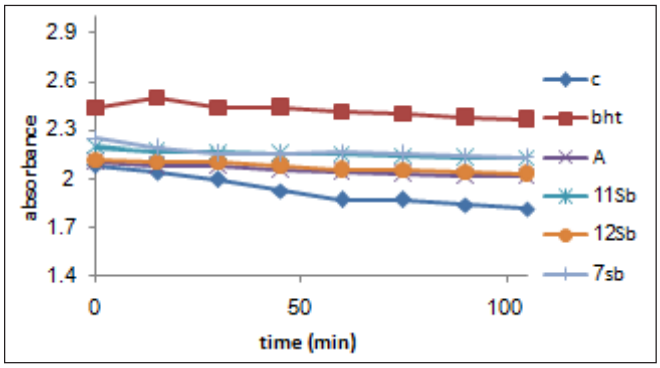


Fig. 11 : Antioxidant activity of compounds A, Sb7, Sb11 and Sb12.

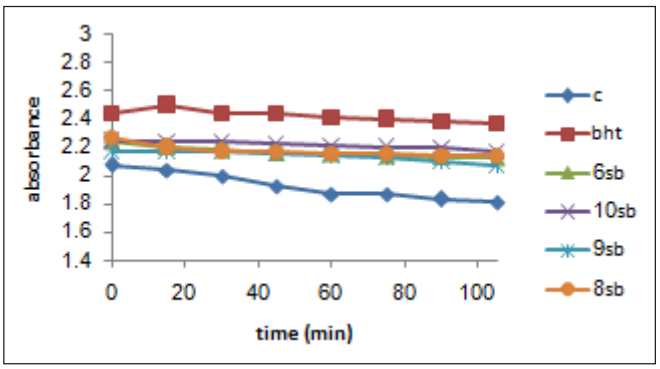


Fig. 12 : antioxidant activity of compounds Sb6, Sb8, Sb9 and Sb10.

to 500 µg/ml are given in Tables 7 and 8. It is evident that, all the tested compounds showed anticancer activity in all concentrations and the effects of these compounds were dose dependent, *i.e.* by increasing the concentration in the culture media; the percentage of death cells is increased. IC50 values ranged from 100.7 to 195.9 ìg / mL. Also, we can note that the cytotoxic activity of compounds were higher in cancerous cellswhen compared with the normal cell especially compound Sb12 its effect close to beinexistent to normal cells.

Azo compounds revealed for its pharmaceutical significance as anticancer agents. Azo molecules are known to be associated with the restraint of DNA, RNA, and protein synthesis. The presence of -N=N- in the molecular structure of azo is related to anticancer activity by the interaction with the active site of protein by means of hydrogen bonding bringing about the hindrance development of cells. The presence of halogen

Table 4 : ^{13}CNMR Spectra of prepared compounds.

Comp. symb.	Chemical shift ä (ppm)					
	C=NAzomethine	$C=N_{ring}$	Aromatic carbon	Ph-OCH_3	C=O	-CH_3
A	——	——	116-150.9	56.3	191.6	——
Sb6	161.8	——	115 - 159	56.3 , 55.8	——	——
Sb7	164.7	——	116.2 – 150.9	56.3	——	15
Sb8	165.4	158.3	112- 156.8	55.8	——	——
Sb9	163	157	110-152	55.9	——	15.05
Sb10	163	156.7	112-147	55.9	——	23.17
Sb11	164.5	——	116 - 150.9	56.3	——	——
Sb12	164	——	116.5 – 162.6	56.3	——	——

Table 5 : *In vitro* antimicrobial activity result of the synthesized compounds.

	Bacteria			Fungal		
Comp. symbol	*Klebsiella pneumonia*	*E. coli*	*Staphylocuses aureas*	*Candida glabrata*	*Candida albicane*	*Aspergillus niger*
A	20	45	49	22	40	40
Sb6	30	25	22	40	50	0
Sb7	35	21	25	30	40	40
Sb8	28	22	30	30	0	0
Sb9	23	20	20	25	40	20
Sb10	20	30	23	30	10	25
Sb11	30	22	25	25	15	0
Sb12	30	30	20	30	20	0
Sulfadiazin	0	20	18	20	0	0
Amoxicillin	40	38	40	—	—	—
Nystatin	—	—	—	0	35	30

Table 6 : Effectiveness results of prepared compounds as antioxidants compared with BHT (Solvent used DMSO).

Comp. symbol	Ai	At	*Ai	*At	AA%
BHT	2.436	2.364	2.078	1.813	73
A	2.108	2.014	2.078	1.813	65
Sb6	2.2	2.123	2.078	1.813	71
Sb7	2.25	2.128	2.078	1.813	54
Sb8	2.27	2.143	2.078	1.813	53
Sb9	2.157	2.070	2.078	1.813	67
Sb10	2.245	2.158	2.078	1.813	67
Sb11	2.199	2.112	2.078	1.813	67
Sb12	2.114	2.030	2.078	1.813	69

substituents in azo compound likewise assumed a significant job in the restraint of receptor enzyme. Presence of halogens in the biologically active molecules has appeared to assume a vital job in their pharmacological properties (Ho *et al*, 2017).

The active centers of cell components should get interfaced with azomethine's nitrogen molecule through framing a hydrogen bond which meddles with normal cell processes and results in the devastation of enzymatic activity of destructive cells, along these lines presents Schiff base as a potential objective to find anticancer chemotherapeutics (Parekha *et al*, 2017).

The present study concluded that the Azo-azomethine compounds derived from sulfanilamide were prepared, characterized and biological evaluated as antimicrobial, antioxidant and anticancer agents. The synthesized compounds shows a moderate antibacterial activities and moderate activities against three types of pathogenic fungi. The finest result as antimicrobial activity were

Table 7 : IC_{50} values and *in vitro* anti cancer activities of the selected compounds against AMJ13 cell line.

Comp.	% Death cells								IC_{50} µg/mL
	30 µg/mL	60 µg/mL	90 µg/mL	120 µg/mL	150 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	
A	19.3	26	23.4	26.3	23.4	55.2	37.4	36.2	100.7
Sb6	8.7	17.4	10.5	9	10	60.5	64.9	66.1	195.9
Sb12	-0.6	18.6	21.4	15.8	15.2	39.7	42	53.1	162

Table 8 : % Death cells of REF normal cells in presence of tested compounds.

Comp.	% Death cells			
	30 µg/mL	60 µg/mL	400 µg/mL	500 µg/mL
A	17.4	28	30	14
Sb6	20	18.9	14.8	17
Sb12	0	5	0	0

obtained for the compounds A and Sb6. The synthesized compounds Sb6, Sb12 showed good activities as antioxidant agents, this is due to demesne these compounds phenolic hydroxyl component which have aptitude of scavenging free radical. Compound Sb6 have greater anticancer activity and the Percentage inhibition of total cells by compound was 66.1% at concentration 500 µg/ml.

REFERENCES

Ajeet A, Mishra A K and Kumar A (2015) Recent Advances in Development of Sulfonamide Derivatives and Their Pharmacological Effects- A Review. *Amer. J. Pharm. Sci*. **3**, 1, 18-24.

Ahmeda A, Hossain M A and Ismail Z (2009) Antioxidant properties of the isolated flavonoids from the medicinal plant *Phyllanthus niruri*. *As. J. Food Ag-Ind.* **2**(03), 373-381.

Al-Salami B K, Mohammed A H and Asker K A (2014) Synthesis andcharacterization of New Schiff bases derived from 2-hydroxybenzaldehyde and Amino Acids and their vanadyl complexes. *Res. J. Pharm., Bio. Chem. Sci*. **5**, 4, 1457.

Apak R, Guclu K, Demirata B, Ozyurek M, Celik S E, Bektasoglu B, Berker K and Ozyurt D (2007) Comparative evaluation of various total antioxidant capacity assays applied to Phenolic compounds with the CUPRAC assay. *Molecules* **12**, 1496-1547.

Berk B, Ertas M and Biltekin S N (2017) Synthesis, Antimicrobial Activity Studies and Molecular Property Predictions of Schiff Bases Derived from ortho-Vanillin. *Acta Pharm. Sci.* **55** , 1, 83-96.

Canadanovic-Brunet J M , Djilas S M , Cetkovic G S and Tumbas V T (2005) Free-radical scavenging activity of wormwood (*Artemisia absinthium* L.) extracts. *J. Sci. Food Agric*. **85**, 265–272.

Erdem E, Sari E Y, Arslan R K and Kabay N (2009) Synthesis and characterization of azo-linked Schiff bases and their nickel(II), copper(II), and zinc(II) complexes. *Tran. Met Chem.* **34**, 167–174.

Ho B K, Ngaini Z, Neilsen P M, Hwang S S, Linton R E, Kong E L and Lee B K (2017) Synthesis and Anticancer Activities of 4-[(Halophenyl)diazenyl] phenol and 4-[(Halophenyl) diazenyl]phenyl Aspirinate Derivativesagainst Nasopharyngeal Cancer Cell Lines. *J. Chem.* Article ID 6760413.

Jarrahpour A A, Motamedifar M, Pakshir K, Hadi N and Zarei M (2004) Synthesis of Novel Azo Schiff Bases and Their Antibacterial and Antifungal Activities. *Molecules* **9**, 815.

Karabasannavar S, Allolli P, Shaikh I N and Kalshetty B M (2017) Synthesis, Characterization and Antimicrobial Activity of some Metal Complexes Derived from Thiazole Schiff Bases with *In-vitro* Cytotoxicity and DNA Cleavage Studies. *Ind. J. Pharm. Edu. Res*. **51**, 3.

Kareem M A and Salman H D (2017) Synthesis, Characterization and Antimicrobial Studies ofTransitionMetal Complexes with Azo Ligand derivative from 4-Aminoantipyrine. *Mesop. Environ. J*., **Special Issue**, 83-91.

Khedr A M and Saad F A (2015) Synthesis, structural characterization, and antimicrobial efficiency of sulfadiazine azo-azomethine dyes and their bi-homonuclearuranyl complexes for chemotherapeutic use. *Turk. J. Chem.* **239**, 267–280.

Mansour A M (2014) Selective coordination ability of sulfamethazine Schiff-base ligand towards copper(II): Molecular structures, spectral and SAR study . *SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy* **123**, 257–266.

Modi J A, Desai K R and Lokhandwala S R (2014) synthesis of novel 1 ,2 ,4 –triazoles and their Schiff base derivatives and evaluating their antimicrobial activity. *World J. Pharm. Pharmaceut. Sci.* **3**, 1875-1885.

Mohana K N and Pradeep Kumar C B (2013) Synthesis and Antioxidant Activity of 2-Amino-5-methylthiazol Derivatives Containing 1,3,4-Oxadiazole-2-thiol Moiety. *ISRN Organic Chemistry* Article ID 620718.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth*. **65**, 55–63.

Mumtaz A, Mahmud T, MR3 E and GW3 W (2016) Synthesis and Characterization of New Schiff Base Transition MetalComplexes Derived from Drug Together with Biological Potential Study. *J Nucl. Med. Radiat. Ther.* **7** , 7:6.

National Committee for Clinical Laboratory Standards (NCCLS) (1998) Refrence method for broth dilution antifungal susceptibility pesting of conidium forming filamentous fungi: proposed standard. M 38-P. Wayne. PA,USA.

Ngaini Z and Kui H B (2017) Synthesis and antibacterial activity of azo and aspirin – azo derivatives. *Mal. J. Ana.Sci.* **21**, 1183 – 1194.

Pallikkavil R, Ummathur M B and Krishnankutty K (2012) Schiff bases of Terephthalaldehyde with 2-aminophenol and 2-aminothiophenol and their metal complexes. *Arch. Appl. Sci. Res.* **4**, 5, 2223-2227.

Parekha, N M, Mistry B M, Pandurangan M, Shindec S K and Patel R V (2017) Investigation of anticancer potencies of newly generated Schiff base imidazolylphenylheterocyclic-2-ylmethylenethiazole-2-amines. *Chin. Chem. Let.* **28**, 602–606.

Patil C J and Nehete C A (2015) The Azo Derivatives of Salicylic Acid . *Int. J. Pharm. Sci. Rev. Res.* **33**(2), 51, 248-256.

Prakash B S, Raj I S and Raj A G (2017) Synthesis ,Characterisation And Anti Cancerus Studies of Schiff Base Complexes of Co(II),Cu(II) And Ni(II) Using DFMPM And L-Histidine. *Int. J. Eng. Res. Appl.* **7**, 7, 38-49.

Saeedia M, Golia F, Mahdavia M, Dehghanb G, Faramarzic M A, Foroumadia F and Shafee A (2014) Synthesis and Biological Investigation of some Novel Sulfonamide and Amide Derivatives Containing Coumarin Moieties. *Iran. J. Pharm. Res*. **13** (3), 881-892.

Shakir R M, Ali K F and Hussain D F (2017) Synthesis and Antioxidant Ability of Some New 6-amino-7H- [1,2,4] triazolo [3,4-b][1,3,4] thiadiazin-3-yl) Derivatives Bearing 2,6-Dimethoxy-4-(methoxymethyl) Phenol Moiety. *Orient. J. Chem.* **33**(5), 2492-2500.

Sharaf El-Din N (2000) Synthesis of some sulfonamide derivatives with potential antibacterial activity. *Chem. Het. Comp*. **36**, 4.

Sharma S, Kaur J, Kaur S and Sharma P (2017) Synethesis , antibacterial and antifungal activities of some new azoanils containing pyrazole moiety. *Ind. J. Chem.* **53B**, 227-237.

Sikarwar P, Tomar S and Singh A P (2016) Synthesis, Characterization and antimicrobial activity of transition metal (II) complexes of schiff bases derived from 2-aminophenol and vanillin. *Int. IJRTER* **02**, 02.

Yang Z and Sun P (2006) Compare of three ways of synthesis of simple Schiff base. *Molbank* M514.