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Synthesis and characterization of diquaternary di-Schiff base compounds and their potential as antimicrobial agents against different types of microorganisms

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Abstract

Three Schiff base compounds were prepared from the reaction of acetophenone and o-phenylene diamine, p-phenylene diamine and benzoimidazole designated as (AH1-AH3). The products were reacted by dimethyl sulfate to obtain three diSchiff base cationic derivatives (AH4-6). The chemical structures of the prepared diSchiff bases and their cationic derivatives were confirmed using elemental analysis, infrared spectroscopy and nuclear magnetic resonance spectra. The prepared cationic derivatives were evaluated for their antimicrobial activities against Gram-positive, Gram-negative and sulfate reducing bacteria. The evaluation was involved inhibition zone diameter and minimum inhibitory concentration measurements. The results showed that the chemical structures of the compounds play an effective role on their antimicrobial efficiency.

Keywords: Schiff base; cationic biocides; inhibition zone diameter; minimum inhibition concentration.
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1. Introduction

Schiff bases are studied widely due to their synthetic flexibility, selectivity and sensitivity towards the central metal atom, structural similarities with natural biological compounds and also due to the presence of azomethine group (-N=CH-) [1], The azomethine group is considered to be the fundamental characteristics of Schiff bases which has interesting biological significance and is found to be responsible for biological activities such as fungicidal and bactericidal [2]. Another key point to remember is that Schiff bases with oxygen, nitrogen and carbonyl group have been used as drugs and reported to possess biological activities against bacteria and fungi due to their biochemical, clinical and pharmacological properties [3].

The biological activity of Schiff bases is mainly depending on the azomethine group; hence the nitrogen atom may be involved in the formation of a hydrogen bond with the active centers of cell constituents and interferes in normal cell processes. Additionally, Schiff bases have found applications in many other fields such as agrochemical, analytical chemistry, electrical conductivity, magnetism, host guest chemistry, ion exchange, nonlinear optics and catalysis. Above all, Schiff bases have played an important role in the development of coordination chemistry and inorganic biochemistry as well [4-7]. Literature survey shows that Schiff bases show bacteriostatic and bactericidal activity [8]. Antibacterial, antifungal, antitumor, anticancer activity has been reported and they are also active against a wide range of organisms, e.g. C. albicans, E. coli, S. aureus, B. polymyxa, P. viticola, etc [9-10]. Many Schiff bases are known to be medicinally important and used to design medicinal compounds [11-13].

2. Experimental Section

Synthesis of Schiff base compounds

Schiff base compounds were synthesized according to the Schiff base reaction. In a general procedure: in one neck flask equipped by Dean-Stark connection, 0.5 mole of amine derivatives (namely: o-phenylene diamine, p-phenylene diamine and 2-amino benzimidazole) were condensed with 1 mole of acetophenone in 200 mL of xylene as a solvent and 0.1wt% of *p*-toluene sulfonic acid as a dehydrating agent. The reaction mixture was refluxed for 6 h until the water of the reaction was obtained (18 mL). Then the reaction mixture was evaporated to eliminate the solvent and the obtained matrix was washed by distilled water to remove the used catalyst and dried at 40 ^{o}C under vacuum. The obtained solid product was recrystallized from acetone and dried under vacuum at 40 ^{o}C to obtain the corresponding Schiff bases (AH1: mp. 124-126 ^{o}C, yield: 83%; AH2: mp. 135-137 ^{o}C, yield: 85%; AH3: mp. 157-159 ^{o}C, yield: 92%).

Synthesis of quaternary ammonium Schiff bases

In one neck flask, the synthesized Schiff base compounds AH1, AH2 and AH3 (0.1 mole) and 0.2 mole of dimethyl sulphate were refluxed individually for 4 h in the presence of acetone as a solvent. The reaction mixture was allowed to cool overnight and the solid products were filtered and recrystallized from acetone and dried under vacuum at 40 ^{o}C for 24 h. The synthesized cationic Schiff base surfactants were designated as: AH4: mp. 151-153

ºC, yield: 87%; AH5: mp. 158-160 ºC, yield: 86%; and AH6: mp. 174-176 ºC, yield: 82%.

Analysis

Microelemental analysis were performed using Vario Elementar Analyzer, FTIR spectra were performed using Genesis Fourier transformer FTIR™, H^1-NMR spectroscopy was obtained by Varian NMR – 300 model; Mercury 300 MHz.

Antimicrobial Evaluation

The synthesized cationic Schiff bases and their different metal complexes were screened for their antimicrobial activity against bacteria and fungi using agar well diffusion method [14-15].

Growing of microorganisms

The bacterial strains were cultured on nutrient medium, while the fungi strains were cultured on malt medium. For bacteria, the broth media were incubated for 24 h. As for fungus, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile Sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation.

Measurements of resistance and susceptibility

For preparation of discs and inoculation, 1 mL of inocula were added to 50 mL of agar media (40 ºC) and mixed. The agar was poured into 120 mm petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, holes filled up to the surface of agar with 0.1 mL of the synthesized cationic surfactants dissolved in DMF (1 mg/mL DMF). The plates were left on a leveled surface, incubated for 24 h at 37 ºC for bacteria and 48 h for fungi and then the diameters of the inhibition zones were measured.

The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds. The mean value obtained for three individual replicates was used to calculate the zone of growth inhibition of each sample. The results were compared with a similar run of Cetyl trimethyl ammonium bromide (CTAB) [16] as an antibacterial reference and Grisofluvine as an antifungal reference. Both antimicrobial activities were calculated as a mean of three replicates.

Microorganisms

The biocidal activity of the synthesized surfactants was tested against different bacterial strains (ATCC: American Type Culture Collection) as follows: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 55422, *Desulfomonas pigra* ATCC 29098 and *Staphylococcus typhimurium* ATCC 27948.

Minimum inhibitory concentration

The biocidal activity of the synthesized surfactants against the tested strains was expressed as the minimum inhibitory concentration (MIC) values, defined as the lowest concentration of compounds inhibiting the development of visible growth after 24 h of incubation. The MIC values were determined by dilution method [15]. The compounds tested were dissolved in a mixture of distilled water/alcohol (3/1; v/v) at various concentrations and the 1 mL aliquot of the cationic surfactants solutions was added to the 14 mL agar media. The final concentrations of the tested surfactants in the medium were 300, 200, 100, 40, 20, 10 and 4 μg/mL.

3. Results and Discussion

Structure conformation of the prepared compounds

Elemental Analyses

The chemical structures of the synthesized Schiff base surfactants and their quaternary salts were confirmed using microelemental analyses, Table 1.

Table (1): Microelemental analysis of the prepared compounds

Compound	Molecular Formula	Mwt.	C%		H%		N%		S%	
			Cal.	Found	Cal.	Found	Cal.	Found	Cal.	Found
AH1	$C_{22}H_{20}N_2$	312.22	84.6	84.8	6.4	6.2	8.96	8.73	----	-----
AH2	$C_{22}H_{20}N_2$	312.22	84.6	84.2	6.4	6.1	8.96	8.72	-----	-----
AH3	$C_{15}H_{13}N_3$	235.15	76.6	76.2	5.5	5.9	17.8	18.4	-----	-----
AH4	$C_{26}H_{32}N_2S_2O_8$	564.3	55.3	55.2	5.67	5.69	4.96	4.61	11.34	11.26
AH5	$C_{26}H_{32}N_2S_2O_8$	564.3	55.3	55.6	5.67	5.42	4.96	4.66	11.34	11.29
AH6	$C_{19}H_{25}N_3S_2O_8$	487.3	46.8	46.4	5.13	4.92	8.62	8.98	13.10	13.30

FTIR and ^{1}H-NMR spectroscopy

FTIR spectra of AH1 (Figure 1) showed Peaks at 1629 indicate the presence of the azomethine group (C=N), Stretching peak appeared at 1573 cm^{-1} related to C=C ring, stretching vibration of the aromatic C-H groups give weak bands at 3007 cm^{-1}. The absorption bands in the range of 600-900 cm^{-1} are corresponded to the stretching of C-H bonds of the different phenyl groups in the chemical structures. The bending absorption band of C-H group appeared at 1363 cm^{-1}. The absorption bands in the area 1152-1108 Cm^{-1} can be attributed to C-N group.

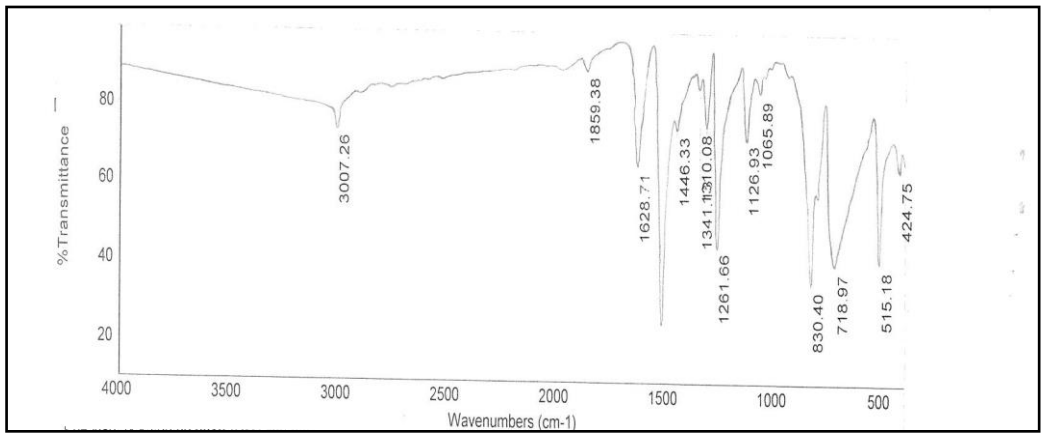


Fig (1): FTIR spectrum of compound AH1.

Its ^{1}H-NMR (DMSO-d6) showed signals at δ (ppm): 2.510 ppm (s, 3H, CH3), 2.514 (s, 3H, CH3), 6.41 ppm-6.58ppm (m, 14H, Ar-H) (Figure 2).

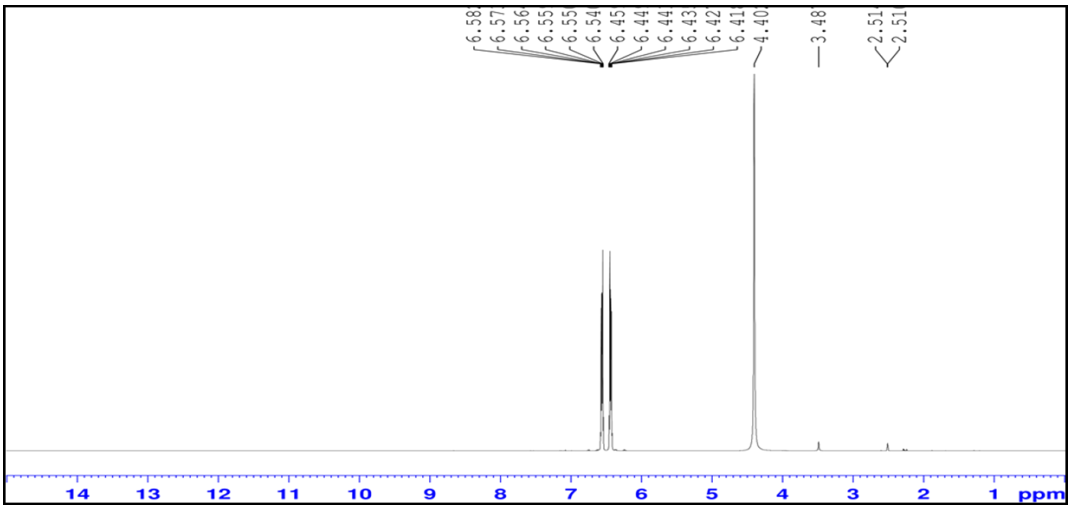


Fig (2): ^{1}H-NMR spectrum of compound AH1.

FTIR spectra (Figure 3) of AH2 showed peaks at 1627 indicate the presence of the azomethine group (C=N), stretching peak appeared at 1590 cm^{-1} related to C=C ring, The stretching vibration of the aromatic C-H groups give weak bands at 3007 cm^{-1}. The absorption bands in the range of 600-900 cm^{-1} are corresponded to the stretching of C-H bonds of the different phenyl groups in the chemical structures. The bending absorption band of C-H group appeared at 1363 cm^{-1}. The absorption bands in the area 1152-1108 Cm^{-1} can be attributed to C-N group.

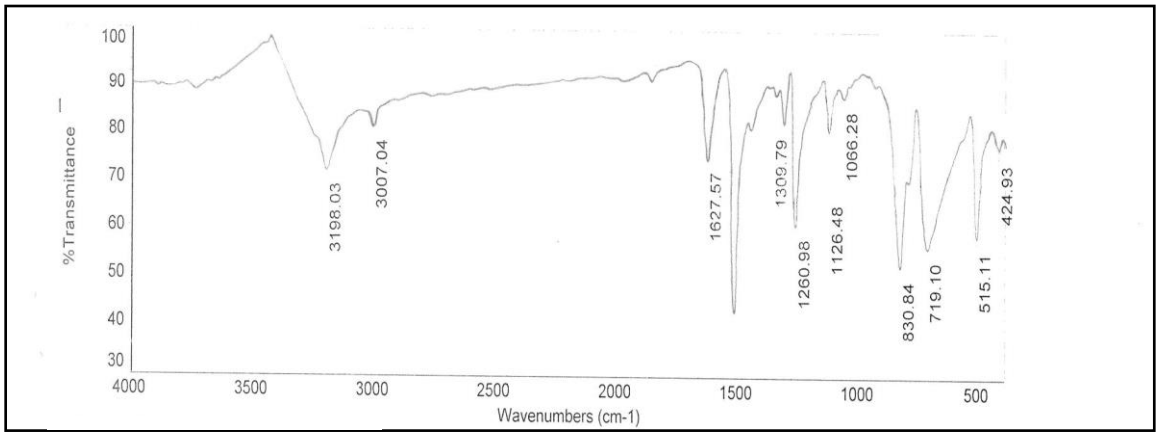


Fig (3): FTIR spectrum of compound AH2.

Its 1H-NMR (DMSO- d6) showed signals at δ (ppm) at 2.485 ppm (s, 3H, CH3), 2.481(s, 3H, CH3) 6.33 - 7.99 ppm (m, 14H, Ar-H) (Figure 4).

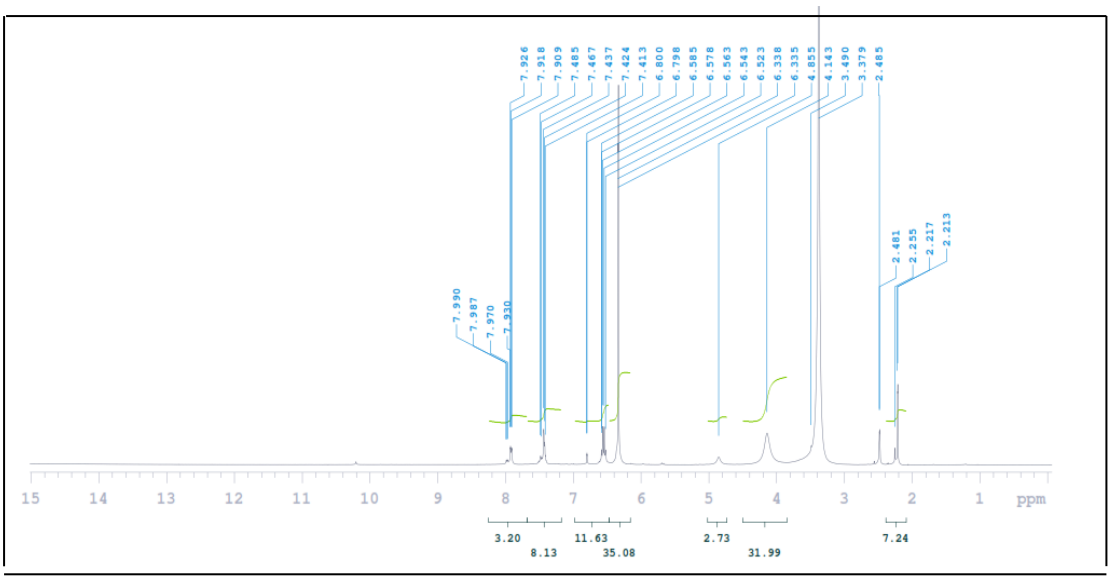


Fig (4): ^{1}H-NMR spectrum of compound AH2.

FTIR spectra (Figure 5) of AH3 showed Peak at 1631 indicate the presence of the azomethine group (C=N), stretching peak appeared at 1590 cm^{-1} related to C=C ring, The stretching vibration of the aromatic C-H groups give weak bands at 3029 cm^{-1}. The absorption bands in the range of 600-900 cm^{-1} are corresponded to the stretching of CH bonds of the different phenyl groups in the chemical structures. The bending absorption band of C-H group appeared at 1363 cm^{-1}. The absorption bands in the area 1152 cm^{-1} can be attributed to C-N group Showed absorption band at 3384 (NH).

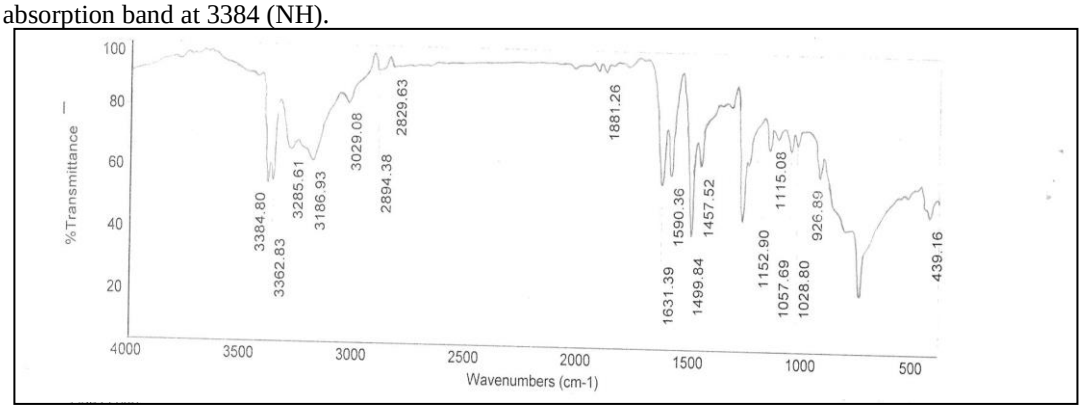


Fig (5): FTIR spectrum of compound AH3.

Its 1H-NMR (DMSO- d6) showed signals at δ (ppm) : 2. 51 ppm (s, 3H, CH3), 6.13 - 7.11 ppm (m, 9H, Ar-H), 10.69 (s, 1H, NH) .

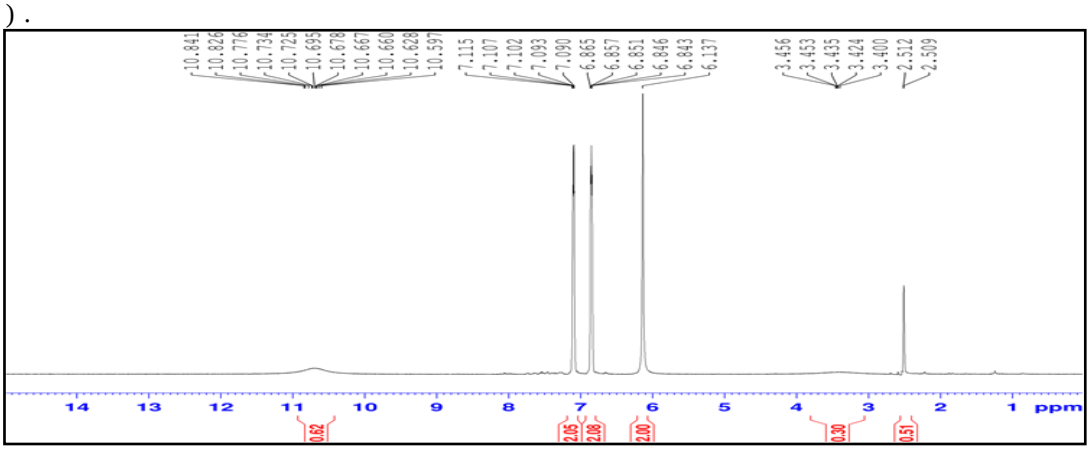


Fig (6): ^{1}H-NMR spectrum of compound AH3.

FTIR spectra of (Figure 7) AH4 showed stretching peak appeared at 1681 cm^{-1} related to C=C ring. the stretching vibration of CH_3 groups give weak bands at 2960 cm^{-1}. The absorption bands in the range of 600-900 cm^{-1} are

corresponded to the stretching of C-H bonds of the different phenyl groups in the chemical structures. The absorption bands in the area 1178 cm^{-1} can be attributed to C-N.

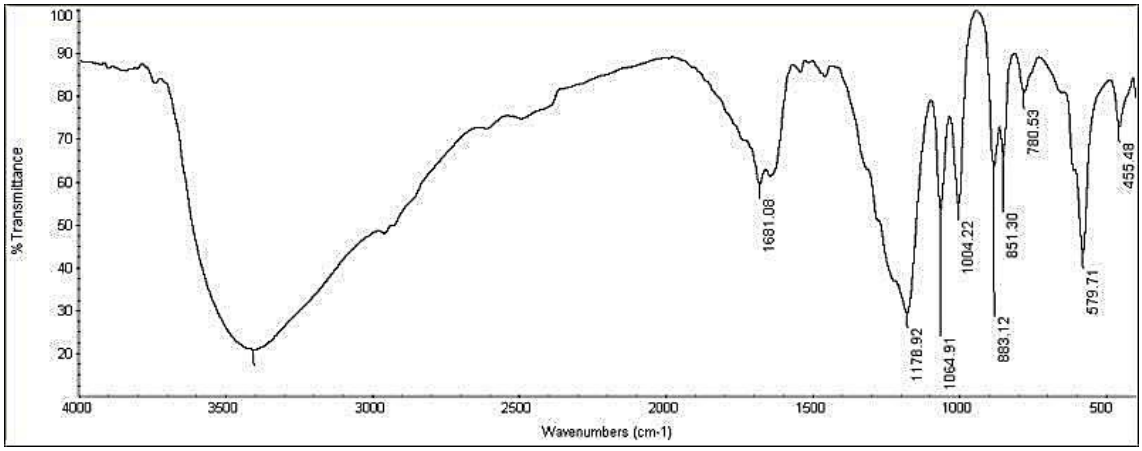


Fig (7): FTIR spectrum of compound AH4.

The ^{1}H-NMR spectroscopic analysis of AH4 (Figure 8) showed the characteristic signal shifts δ (ppm) at 3.2 ppm (s, 3H, C<u>H</u>$_3$), 6.2 ppm- 7.2 ppm corresponded to phenyl protons (m, nH, Ar-<u>H</u>).

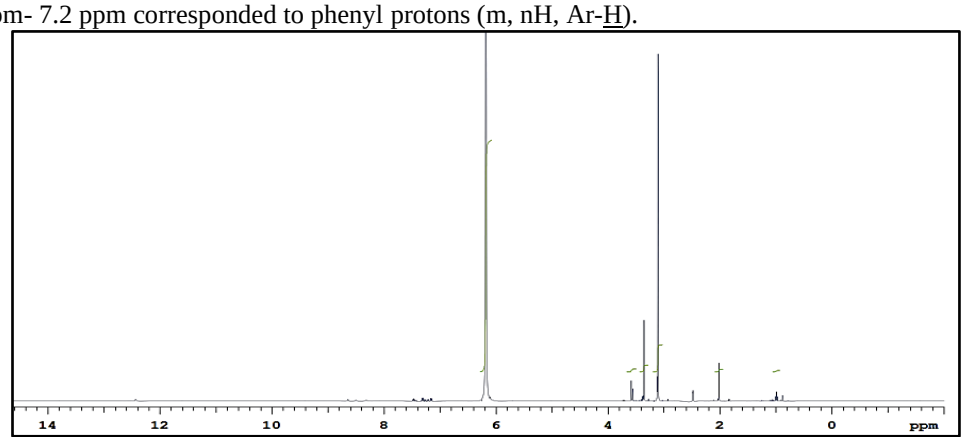


Fig (8): ^{1}H-NMR spectrum of compound AH4.

FTIR spectra (Figure 9) of AH5 showed stretching peak appeared at 1631 cm^{-1} related to C=C ring, The stretching vibration of CH_3 groups give weak bands at 2960 cm^{-1}. The absorption bands in the range of 600-900 cm^{-1} are corresponded to the stretching of C-H bonds of the different phenyl groups in the chemical structures. The absorption bands in the area 1175 cm^{-1} can be attributed to C-N.

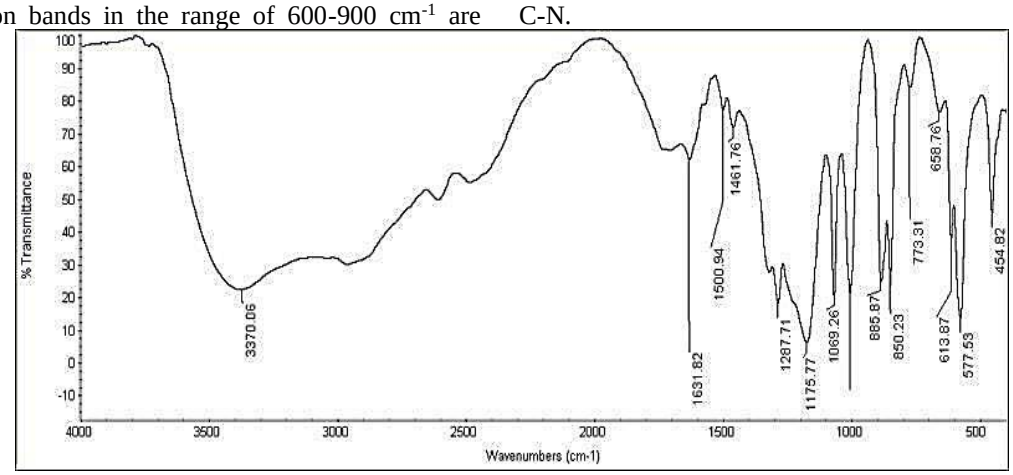


Fig (9): FTIR spectra of compound AH5.

The^{1}H-NMR spectroscopic analysis of AH5 (Figure 10) showed the characteristic signal shifts δ (ppm) at 3.5 ppm (s, 3H, 4C<u>H</u>$_3$), 6.2-7 ppm, corresponded to phenyl protons (m, nH, Ar-<u>H</u>).

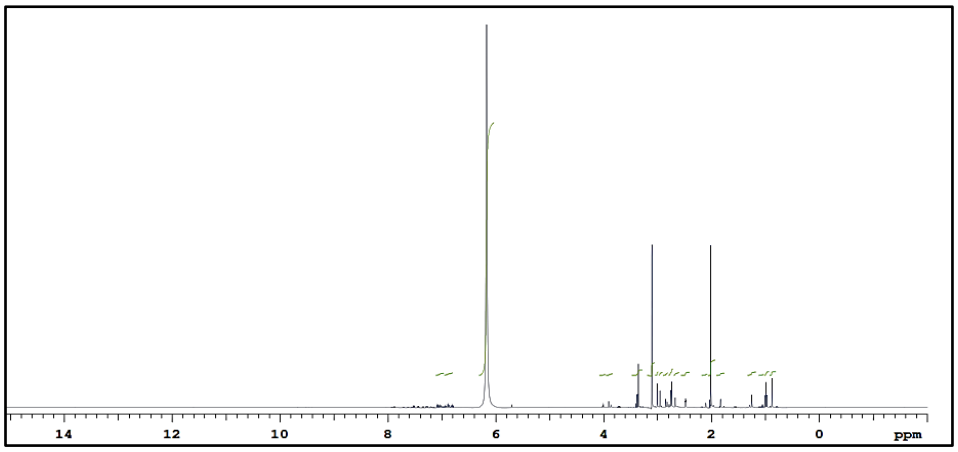


Fig (10): ^{1}H-NMR spectrum of compound AH5.

FTIR spectra (Figure 11) of AH6 showed, stretching peak appeared at 1636 cm^{-1} related to C=C ring, the stretching vibration of CH_3 groups give weak bands at 2960 cm^{-1}. The absorption bands in the range of 600-900 cm^{-1} are corresponded to the stretching of C-H bonds of the different phenyl groups in the chemical structures. The absorption bands in the area 1180 cm^{-1} can be attributed to C-N.

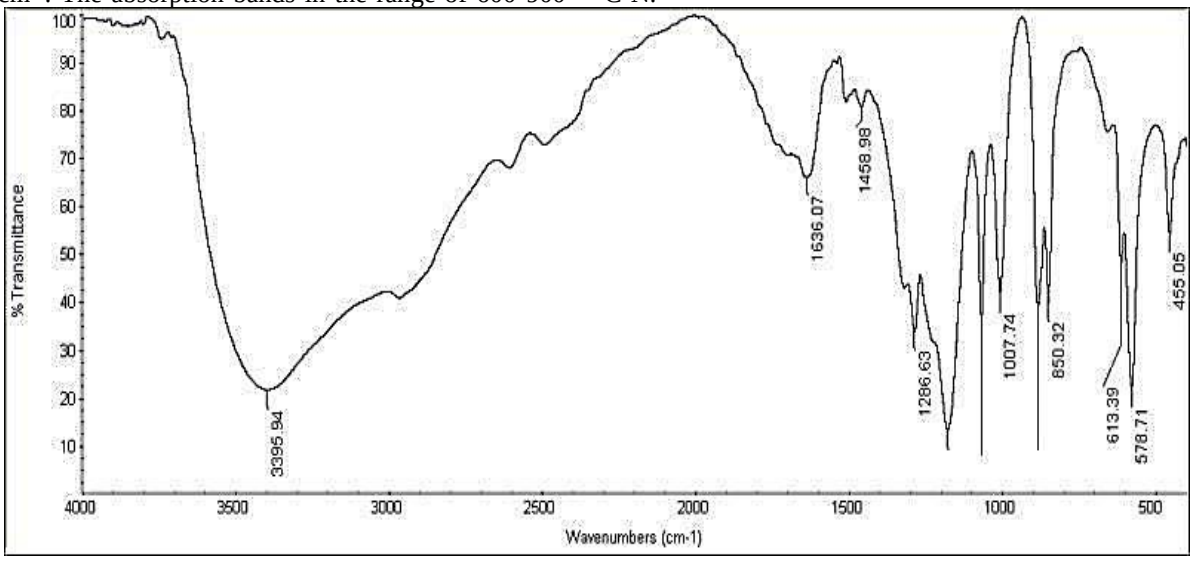


Fig (11): FTIR spectrum of compound AH6.

The ^{1}H-NMR spectroscopic analysis of AH6 (Figure 12) showed the characteristic signal shifts δ (ppm) at 3.7, 4.2 ppm (s, 3H, $C\underline{H}_3$), 7.4 ppm, 7.1 ppm corresponded to phenyl protons (m, nH, C<u>H</u>).

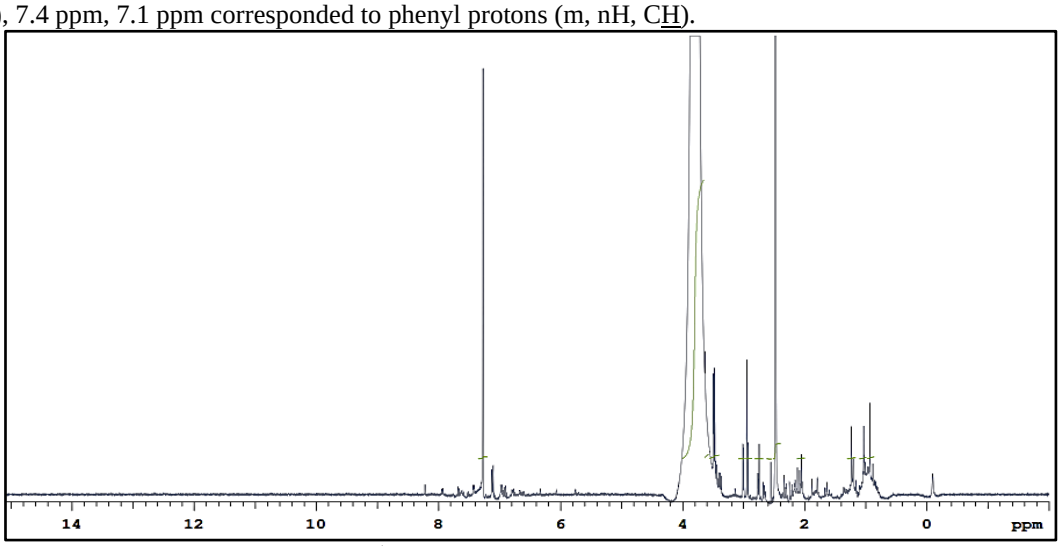
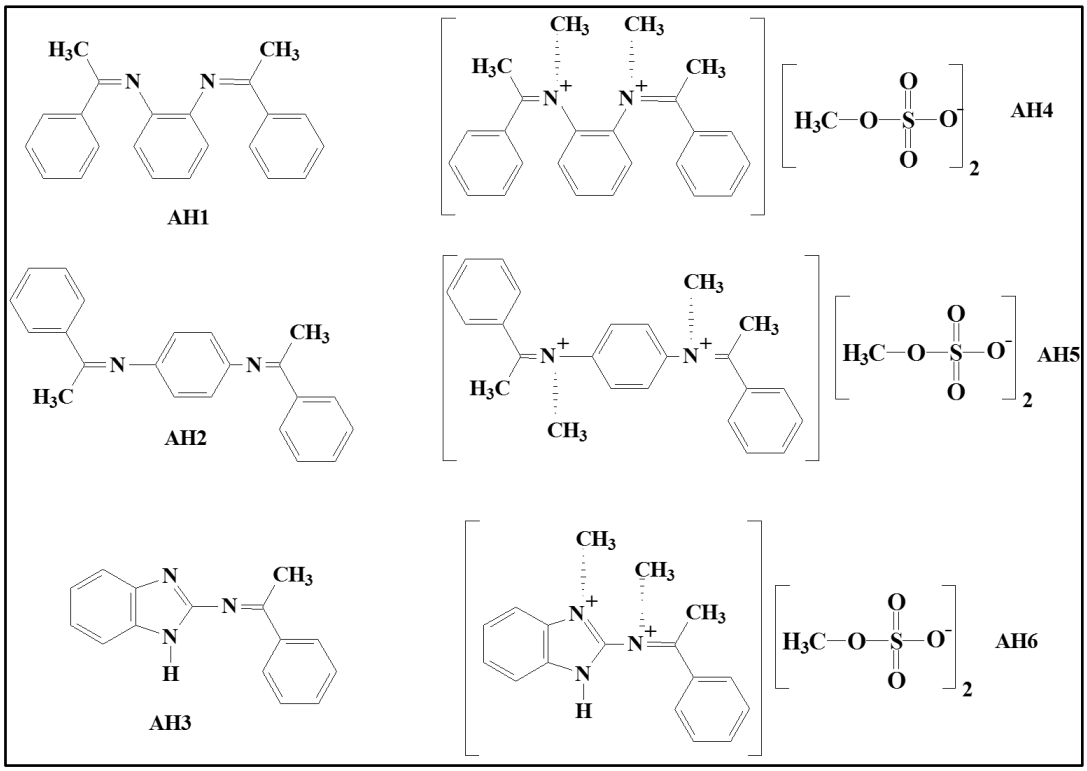


Fig (12): ^{1}H-NMR spectrum of compound AH6

The spectroscopic analysis of the prepared compounds confirmed their chemical structures as represented in Scheme 1.



Antimicrobial activity

Inhibition zone diameter of the synthesized quaternary Schiff base compounds (AH4, AH5 and AH6)

The potent action of the synthesized cationic Schiff base compounds (AH4, AH5 and AH6) was screened against Gram-positive and Gram-negative bacteria at different concentrations using the values of the inhibition zone diameter tests and the results are summarized in Table 2.

Table (2): Antimicrobial activities [a, b] in terms of inhibition zone diameter at different doses (1, 2, 5 mg/mL) of the synthesized cationic Schiff base compounds against different bacterial strains

Bacteria	*S. auerus*			*B. subtilis*			*E. coli*			*P. aeruginosa*			*Desulfomonas pigra*		
Dose, mg/mL	1	2	5	1	2	5	1	2	5	1	2	5	1	2	5
HTAB[c]	12.3	14	15	12.3	14	15	12.3	14	15	12.3	14	15	12.3	14	15
AH4	16	17	20	17	19	22	14	16	19	13	14	17	13	15	17
AH5	16	17	22	18	21	24	16	17	20	15	15	19	13	16	18
AH6	19	21	25	19	21	26	17	19	22	16	18	21	16	18	20

[a] The data is a mean of five replicates with relative error ~9%.

[b] The antimicrobial activity expressed as the diameter of the inhibition zone formed in presence of the tested compounds.

[c] Data referred to hexadecyl trimethyl ammonium bromide (HTAB) as a blank

The obtained diameters of the inhibition zones were gradually increased by increasing the concentration of the tested quaternary Schiff base compounds, and the maximum diameters are obtained at 5 mg/mL. Moreover, the antimicrobial activities were gradually increased by increasing the unsaturation and heterogeneity of the different compounds. The benzoimidazole derivative (AH6) showed the maximum antimicrobial activities against the tested bacterial strains. General observation for data in Table 2 indicates that the Gram-negative bacteria are more resistant to the tested compounds compared with the Gram-positive bacteria. The data obtained from the inhibition zone diameter could not be considered as quantitative data. In order to evaluate the synthesized compounds quantitatively as antibacterial agents, minimum inhibitory concentrations (MIC) were measured.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) values of the synthesized cationic Schiff base compounds (AH4, AH5 and AH6) were summarized in Table 3 which also includes the MIC values corresponding to the hexadecyl trimethyl ammonium bromide [17] as a classical antimicrobial agent. Compared with the classical quaternary ammonium compound. The synthesized biocides show moderate activity level against bacteria with MIC values of 4-100 μg/mL (except *P. aeruginosa* and *S. typhimurium*: 100-300 μg/mL and > 300 μg/mL, respectively), Table 3.

Table (3): Minimum inhibitory concentration values, MIC [a] (μM) of the synthesized cationic Schiff base compounds against different bacterial strains

Bacteria	*S. auerus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*Desulfomonas pigra*
HTAB [b]	20	50	100	300	300
AH4	40	10	40	200	40
AH5	10	4	40	200	20
AH6	4	4	10	100	10

[a] The data is a mean of five replicates with relative error ~9%.

[b] HTAB hexadecyl trimethyl ammonium bromide

However, the use of quaternary ammonium compounds (QAS) in some fields is limited due to the developed microbial resistance against QAS after longer periods of applications, high acute toxicity, and low biodegradability [18-19].

The action mode of the cationic biocides is generally accepted that the cationic biocides can adsorb onto negatively charged cell membranes, which will then lead to decrease in the osmotic stability of the cell and leakage of intracellular constituents [20-21]. However, the exact mechanism of the antimicrobial action is still unknown.

Several other mechanisms that may contribute to the antimicrobial action have also been suggested, such as the formation of an impermeable coat on the bacterial surface [22], uptake of low molecular weight biocides that will interact with electronegative substances in the cell [22], and inhibition of bacterial growth through chelation of trace metals [19]. The mechanism for the interaction may be different for Gram-positive and Gram-negative bacteria.

It is clear from the MIC values of the tested cationic surfactants that (except *E. coli*) Gram-negative bacteria are resistant to these cationic Schiff base compounds in the tested concentration range, Table 3. By contrast, AH6 cationic Schiff base compound presents a similar activity against both Gram-positive and Gram-negative bacteria. That can be attributed to the presence of the heterogeneous atom of nitrogen in its chemical structure.

Gram-negative bacteria are generally more resistant to antimicrobial agents than are Gram-positive bacteria. This can be explained by the different cell membrane structure of the two bacterial types. The external layer of the outer membrane of the Gram-negative bacteria is entirely composed of lipopolysaccharides and proteins that restrict the entrance of biocides and cationic compounds [23].

An important factor influences the biocidal activity of the different biocides is the net charge on their molecules. Several studies showed that the electrostatic interactions play a key role in the action of cationic biocides, and that a decrease in the charge density of the cationic compounds results in a reduction in adsorption and biocidal efficiency [24-25].

Comparing the MIC values of the synthesized cationic Schiff base compounds with the classically antimicrobial surfactant (HTAB) showed their relatively higher biocidal activity own to their relatively lower MIC values. The relatively high biocidal activity of the synthesized cationic Schiff base compounds is accounted to the high average charge on their molecules. In case of HTAB, the head group has one positive charge locates on the nitrogen atom (N^+). On contrarily, conjugation of electrons over the phenylene diamineium nucleus sharply increases the charged centers and consequently increases the average charge of the molecules. That gradually decreases the tendency towards adsorption in addition to the biocidal activity compared by the imidazolium nucleus.

The synthesized cationic Schiff base compounds were screened for their potency against sulfate reducing bacteria (*D. pigra*), (Table 3). The data showed imperative results due to their relatively high efficiency against SRB bacteria. SRB bacteria (anaerobic bacteria) produce H_2S gas due to the reduction of sulfate compounds as soul source of energy. H_2S gas increases the acidity of the medium which causes biocorrosion of the pipelines. In addition, H_2S is responsible for the formation of sulphide salts which are highly corrosive to stainless steel than H_2S gas. To decrease the production of corrosive materials in the petroleum pipeline environments (H_2S, sulphide ions), biocides are used. The MIC values of the tested cationic surfactants showed their approved biocidal activity against SRB. Also, it is clear from the data in Table 3 that the gradual increase of the unsaturation and heterogeneity of the different compounds increases their biocidal activities against SRB.

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