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SYNTHESIS AND IDENTIFICATION OF SEVERAL SCHIFF BASES DERIVATIVES AND THEIR ANTIBACTERIAL ACTIVITY ON COMMON PATHOGENIC BACTERIA

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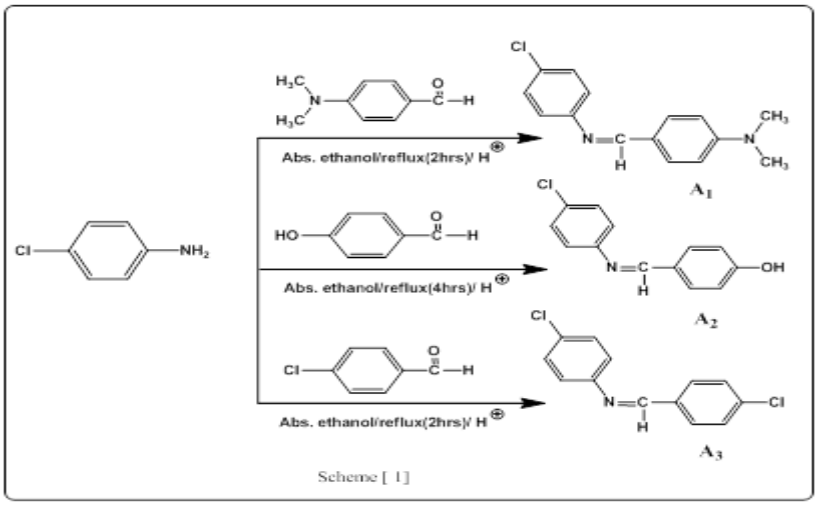
ABSTRACT

This research involves synthesis of some Schiff base compounds [A_1-A_3] were prepared from condensation of [4-(dimethylamino) benzaldehyde and 4-hydroxy benzaldehyde and 4-chloro benzaldehyde] in absolute ethanol. Each from the prepared compounds are distinguished with [FT.IR] spectroscopy, additionally to the progression of the steps of reaction through the technique of [TLC], by utilizing (ethanol-toluene,1:1) as solvent .The following scheme[1] explains the steps of reaction and the prepared compounds. Antibacterial efficiency of A1, A2 and A3 compounds were tested against eight human pathogens like two gram +ve bacteria (*Staphylococcus aureus, Streptococcus agalactiae*) as well as five gram -ve bacteria (*Escherichia coli, Enterobacter cloacae, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeroginosa* and *Salmonella typhi*) using agar well diffusion method. All compounds showed significant activity against pathogenic bacteria, but the A2 compound showed maximum inhibition zone and antibacterial activity against all pathogenic bacteria. The minimum inhibition zone was determined in A3 compound showing less antibacterial activity against the experimental bacteria.



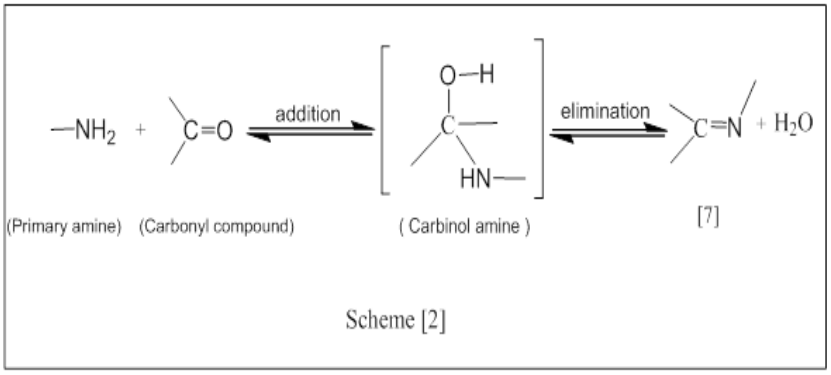
Scheme [1]

KEYWORDS: Synthesis, Identification, Schiff bases derivatives, Antimicrobial Activity.

INTRODUCTION

Para-Chloroaniline is utilized in the industry of tinctures as an intermediate like the agents of azoic coupling and pigments, as well as utilized as a mediate in the manufacture of several agricultural chemicals and the pharmaceuticals (monuron urea, herbicides, e.g.).[1,2] The noteworthy declination path for para-chloroanilne was appeared to be through The photolysis in the water of estuarine. The microbial declination of Chloroanilines was not found through short-term (over to 3 days) nurseries; while, The faster microbial declination was occurred for Chloroaniline additionally the photoproducts of aniline.[3,4,5,6] Macro porous caution exchanger including para-chloroaniline, as cautions from aqueous samples.[7] Schiff bases described the formation of N-substituted imines, These compounds typically containing Azomethine group (C=N) have been synthesized via condensation of primary amines with active carbonyls.[8,9] They are represented of the essential compounds attributable to their extensive variety of the industrial application as well as biological activities.[10] Schiff bases have establish to be in possession the pharmacological exercises like anticancer[11], antimalarial[12], antiviral[13] and antibacterial.[14] These compounds have several nomenclatures depending on the nature of the groups (R, R_1, R_2) such as azomethines, imines, benzanils, anils, aldimines and ketimines.[7] Alkyl primary amine (R-NH_2) or aryl primary amine (Ar-NH_2) add to the carbonyl group of aldehyde or ketone to initially form carbinol amines, the carbinol amine loses water molecule to form N-substituted imine as the product.[7,15] Scheme[2], The new compounds were synthesized by intensification between the carbonyl compounds aldehyde , ketones or there derivatives and primary amines in the alcohol absolute by existence of concentrated hydrochloric acid or glacial acetic acid as

drops.[16]The aim of study was Synthesis of some Schiff base derivatives were prepared from condensation of some primary aromatic amine [4-chloro aniline] with different substituted benzaldehydes [N,N-dimethyl benzaldehyde, 4-chloro benzaldehyde, p-hydroxybenzaldehyde]. Every of the formation compounds were assessed for their antimicrobial efficiencies versus gram +ve and gram -ve bacteria.



Scheme [2]

Experimental Apparatus

1. Electro thermal melting point apparatus was utilizing for Melting points calculated. UK.
2. Fourier transform infrared SHIMADZU FT.IR-8400S was utilizing for FT.IR spectra calculated with utilizing infrared spectrophotometer by KBr disc, Kufa University.
3. (TLC) Thin layer chromatography was carried out by aluminum plates an plated with layer of silica gel, iodine vapor was utilizing for detecting of these compounds.

MATERIAL AND REAGENTS

Each chemicals used utilizing provided from BDH, Fluke and Merck chemicals companies.

General procedure

Synthesis of Schiff bases derivatives[A_1-A_3][17]

The 4-chloroaniline (0.01mole) was putted for the solution of the various substituted benzaldehydes (0.01mole) in absolute ethanol (40ml) as well as glacial acetic acid (two drops) were too putted to the mixing solution. This solution was refluxed for (2-4 h.) and at the end of the reaction; solvents were partially evaporated then poured into water. The precipitates were collected by filtration, washed with water.

1- 4-((4-chlorophenylimino) methyl)-N,N-dimethyl aniline[A_1]

The compound was prepared by the treatment of 4-chloroaniline (0.01mole, 0.95gm) and 4-(dimethyl amino) benzaldehyde (0.01mole, 1.49gm).

2- 4-((4-chlorophenylimino) methyl) phenol [A_2]

The compound was prepared by the reaction of 4-chloroaniline (0.01mole,0.95gm) and 4-hydroxybenzaldehyde (0.01mole,1.22gm).

3- 4-chloro-N-(4-chlorobenzylidene) aniline [A_3]

The compound was prepared by the treatment of 4-chloroaniline (0.01mole, 0.95gm) and 4-chlorobenzaldehyde (0.01mole,1.40gm).

Bacterial isolates

The following pathogenic bacterial isolates: two gram +ve bacteria (*Staphylococcus aureus, Streptococcus agalactiae*) as well as five gram -ve bacteria (*Pseudomonas aeroginosa, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis* additionally *Salmonella typhi*) were isolated from different clinical specimens like stool, urine, wound, sputum and CSF. The isolates were identified utilizing a range of biochemical as well as morphological technicalities[18], and then finally confirmed by using Vitek-2 compact system GP and GN card automated bacterial identification instrument. The stored isolates were on brain heart infusion broth including (20%) glycerol at (−20°C). The isolates were sub cultured on BHIA and incubated at 37°C for 24h. before use.

Preparation of Schiff base [A1, A2, A3] compounds concentration

0.01 g of powder each Schiff base [A1, A2, A3] compounds were dissolved in 1ml of DMSO to give concentration 10 mg/ml. These concentrations were used in the antibacterial test.

Antibacterial activity experimental

Bacterial suspensions were carried out as explained by.[19] Agar well diffusion method was used to determine the antibacterial activity of Schiff base [A1, A2, A3] compounds against bacterial isolates.[20,21]. Brain heart infusion broth (BHIB) was utilized for the elaboration of bacterial cultures. Muller Hinton agar (MHA) was used to determine the activity of the Schiff base [A1, A2, A3] compounds contra bacterial organisms, this was synthesized according to the manufacturer's instruction.

Agar well diffusion assay

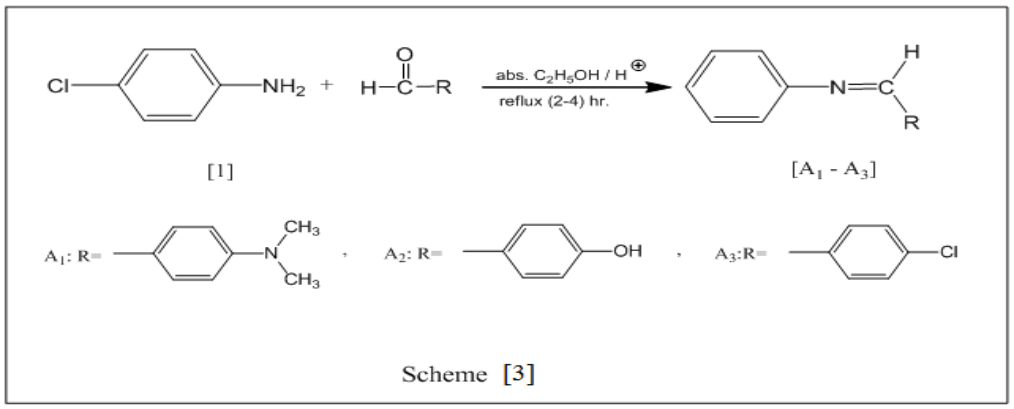
Bacterial isolates were prepared to match 0.5 McFarland standards. Utilizing the micropipette, 100 µl of organisms (BHIB) was spread over the surface of an agar plate (MHA). This approach was the like for each test bacteria. Utilizing a sterile cork borer, five

holes were punched in every of the culture plates. One of the holes was punched in the center of the plate where 100 µl of Gentamicin was added as positive control; 100µl of DMSO was added as a negative control in the other hole; 100µl of the Schiff base [A1, A2, A3] compounds were put in the remaining three holes. The culture plates were then incubated at 37°C for 24 h. The clear zone of inhibition around the Schiff base [A1, A2, A3] compound was computed in mm. The tests were done in triplicate.

RESULTS AND DISCUSSION

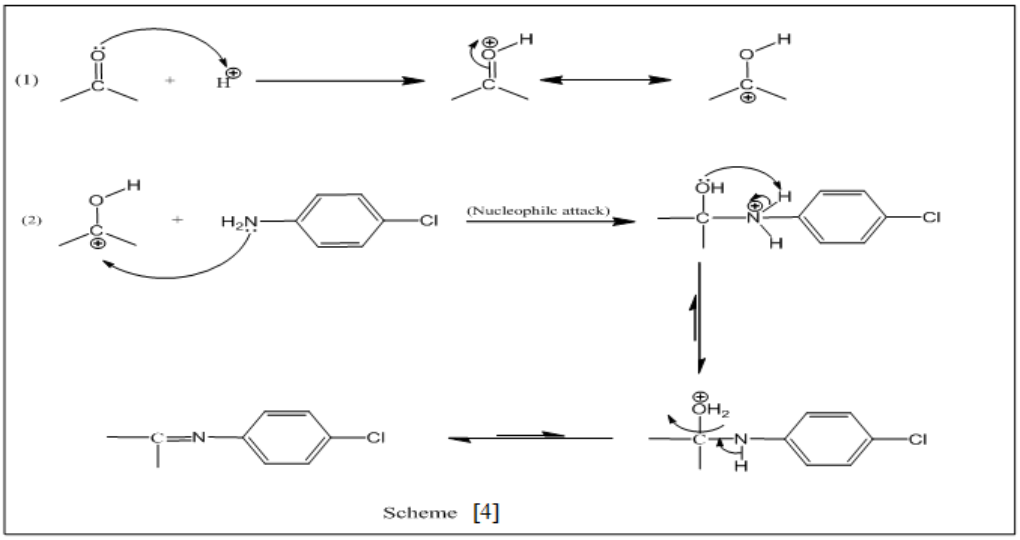
Synthesis and identification of Schiff bases derivatives [A_1-A_3]

The intermediate compounds [A_1-A_3] were prepared with the condensation of equal molar quantity of 4-chloroaniline[1] with different aromatic benzaldehydes [4-(dimethylamino) benzaldehyde,4-hydroxybenzaldehyde, 4-chlorobenzaldehyde] scheme.[3]



Scheme [3]

The proposed mechanism[15] for these reactions scheme[4]



Scheme [4]

Table [1] shows molecular formula, melting point, yield, R_f(benzene : ethanol , 3 : 1), colour and the analytic data of synthesis compounds [A_1-A_3].

Table [1] several the physical properties for new compounds [A_1-A_3]

Comp. No.	Molecular Formula	M.P. ^{O}C	Yield $^{0}/_{0}$	R_f	Colour
A_1	($C_{15}H_{15}ClN_2$)	152 -153	93.2	0.82	Yellow
A_2	($C_{13}H_{10}ClNO$)	210 - 211	82.5	0.33	Pale brown
A_3	($C_{13}H_9Cl_2N$)	122	85.7	0.53	Pale yellow

The FT .IR spectra of the compounds [A_1-A_3] appeared vanishing of the two bands of absorption at (3471 cm^{-1}) and (3379 cm^{-1}) were due to the symmetric and asymmetric respectively vibration stretching of ($-NH_2$)group of 4-chloroaniline Figure[1] and vanishing for the strong band absorption on (1680 – 1720) cm^{-1} was resulting from (C=O) for aldehyde group.[14] The FT .IR appeared showing of the stretching vibration between (1600 – 1622) cm^{-1} was resulting from the imine group (C=N).[17] Another information of the functional groups were appeared in table.[2]

Table: (2) FT.IR information of Schiff bases compounds [A_1 – A_3]

Com. No.	Fig. No.	υ(C-H)Str. Aromatic Aliphatic cm^{-1}	υ (C=N) Str. Imin cm^{-1}	υ (C-H) Ben. Aromatic cm^{-1}	Others cm^{-1}
A_1	[2]	2901 2891	1600	823	υ($N-CH_3$)Str.: 2850asym. 2808sym.
A_2	[3]	3080 2949asym. 2897sym.	1606	831	υ (Ar-OH)Str.: 3431
A_3	[4]	3020 2920asym. 2881sym.	1622	833	υ (Ar-Cl)Str.: 1083 Ben.: 1232

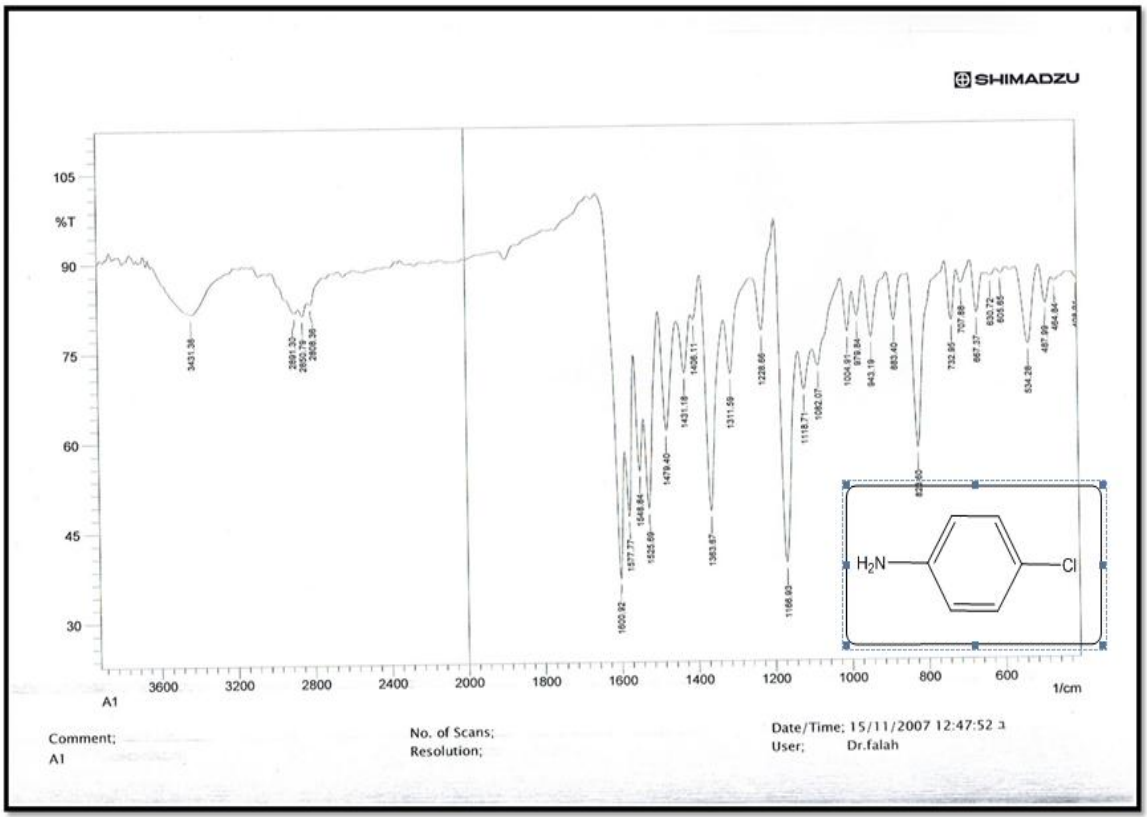


Figure (1) FT.IR spectrum of 4-chloroaniline

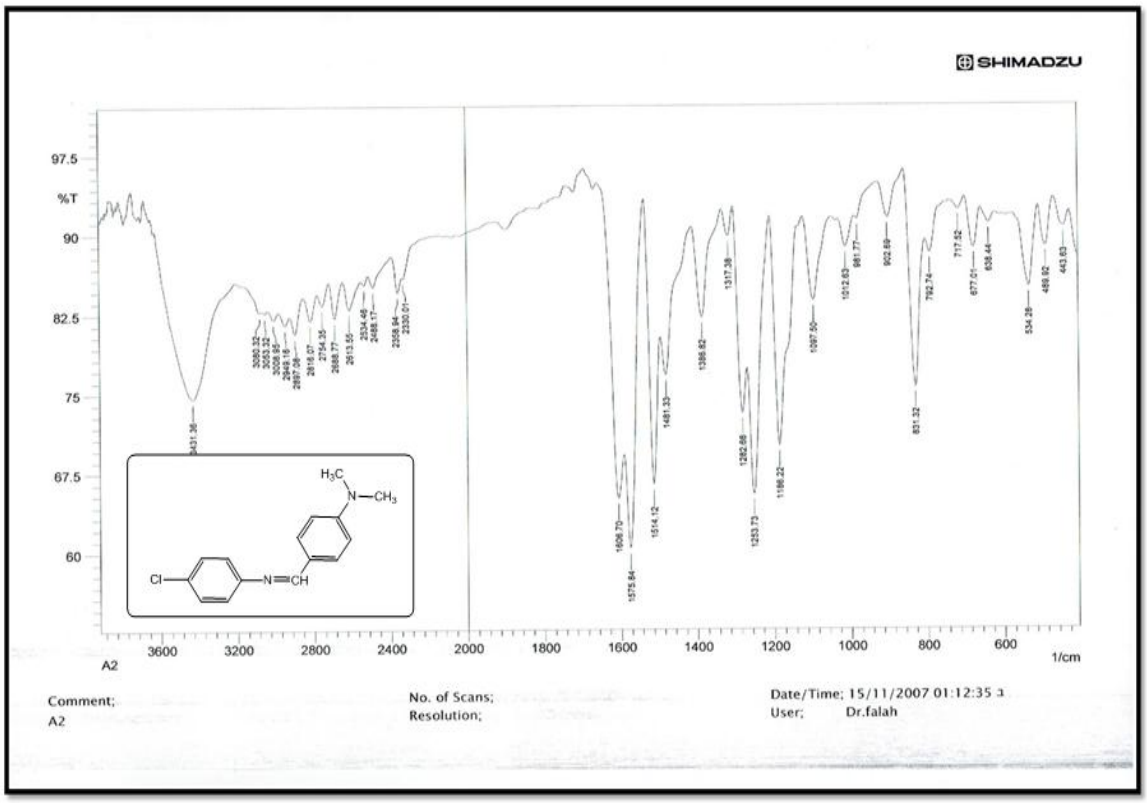


Figure (2) FT.IR spectrum of compound [A_1]

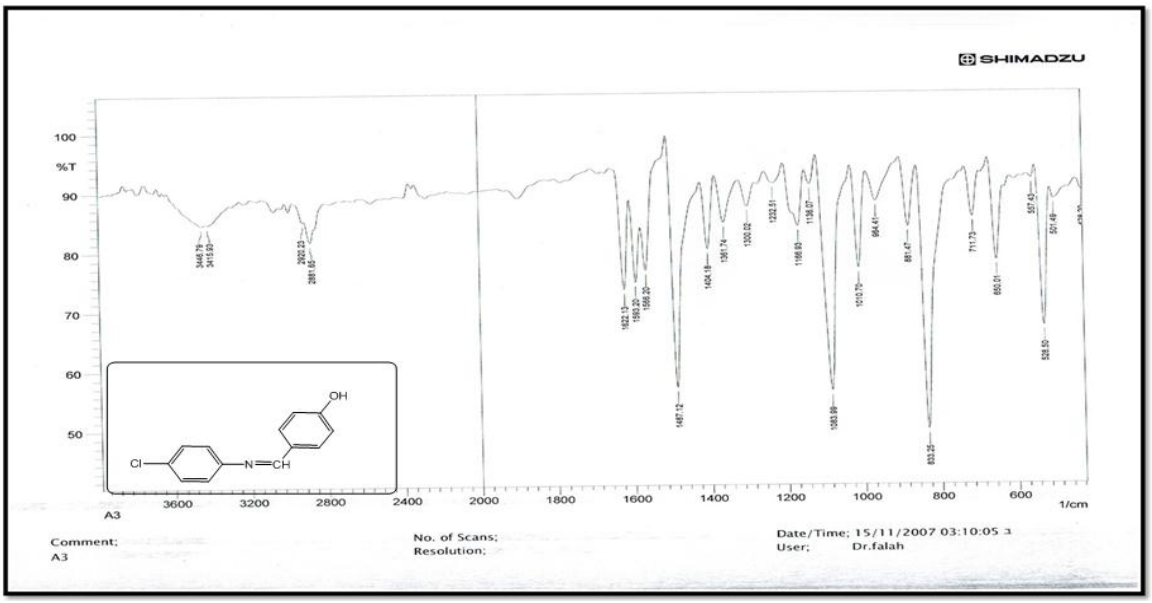


Figure (3) FT.IR spectrum of compound [A_2]

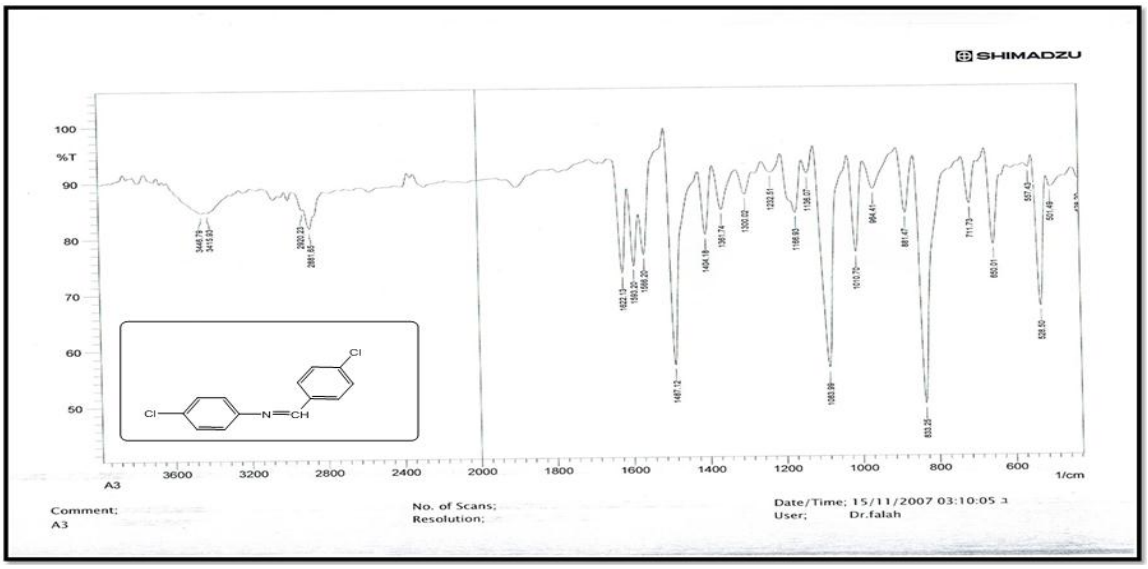


Figure (4) FT.IR spectrum of compound [A_3]

Antibacterial activity

Results of the inhibition zone values for Schiff bases derivatives [A_1-A_3] compounds against gram +ve (*S. aureus*, *Str. agalatiae*) and gram -ve bacteria (*E. coli*, *P. aeruginosa*, *S. typhi*, *Pr. merablis*, *Ent. clocae*, *K. peumoniae*) are presented in figure (5) and table (3). Both A1 and A2 compounds had better antibacterial activity against *Str. agalatiae* (the inhibition zone 30 and 34 respectively). The results also indicated that A2compound have higher antibacterial activity against all bacteria comparing to the A1 and A3compounds. The results of this test showed that A2 compound has a great effect to all bacterial isolates, A1compound has mediate effect to bacterial isolates and A3 compound give the lowest effect to bacterial

isolates. Bacterial isolates showed different susceptibility towards Schiff bases derivatives [A_1-A_3] compounds used in this study as shown in figure (5).

The highest rate of inhibition zone is seen with A2 compound (35mm), A1compound (30mm) toward *S. agalatiae* and moderately inhibition zone to A2 compound (20mm, 20mm, 16mm) toward *Pr. merablis*, *Ent. cloceae*, *S. typhi* whereas is relatively lower inhibition zone to A2 compound (15mm, 14mm, 13mm, 12mm) toward *S. aureus*, *K. pneumoniae*, *Ps. aeruginosa*, *E.coli* respectively; A1compound (13mm,10mm, 8mm, 8mm) toward *S. typhi*, *Ent. clociae*, *Pro. merablis*, *Ps. aeruginosae* respectively; A3compound (15mm,11mm,10mm) toward *Pro. merablis*, *K. pneumoniae*, *Ps. aeruginosa* respectively.

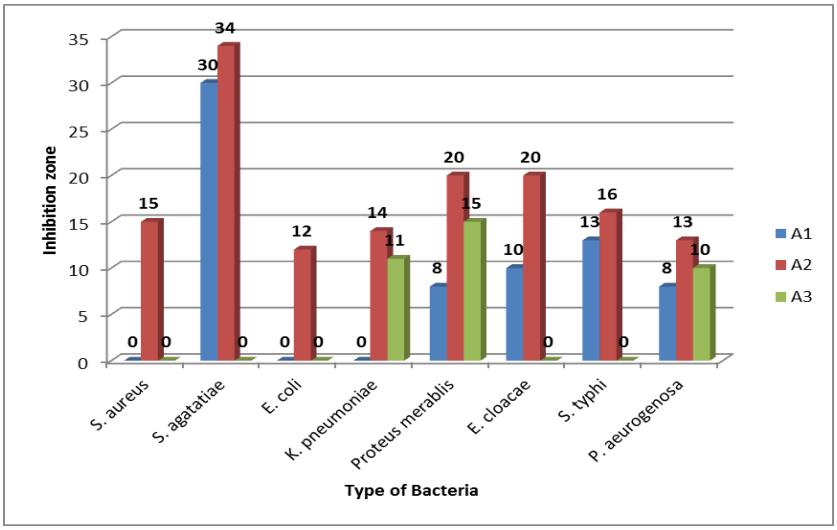
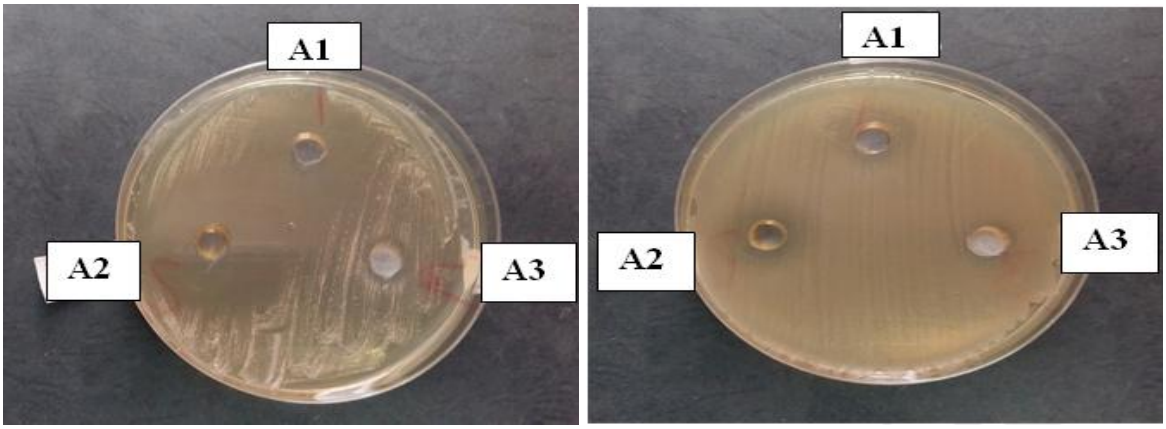


Figure (5) Comparing of antibacterial activity (inhibition zone, mm) of different Schiff base [A1, A2, A3] compounds against pathogenic bacteria.



A- **B-**

Figure (6): Antimicrobial activity of A1, A2 and A3 compounds against pathogenic bacteria. A- *St. agalatatiae* B- *Sal. typhi*.

The highest rate of inhibition zone and antibacterial activity of A2 may be belongs to hydroxyl group that existed in it. Hydroxyl radicals and Superoxide are very poisonous and make harmful influences on bacterial physiology.[22,23,24] Even under steady-state conditions. Every main types of bactericidal antibiotics (inclusive β-lactams, aminoglycosides and quinolones) enhance the formation of deadly hydroxyl radicals in both Gram-ve and Gram+ve bacteria, in spite of the completely variations in their initially drug–target overlap.[25] The Phenol chemical compounds are that be for the kind of containing a hydroxyl (OH) group linked to an aromatic phenolic group. The position(s) as well as the (OH) groups number on the phenol compound are conception to be associated to their proportional toxicity on microorganisms, by the confirmation that augmentation hydroxylation brings about augmentation toxicity.[26]

The moderate rate of antibacterial activity of A1 compound because the amine-donating group that present in this compound. The amine electron-donating group was increased the ring activity for A1 compound but there are two methyl in the amine which decreased the amine activity for donating the electron that lead to few decreased the activity of A1 compound for bacteria.[27]

The low antibacterial activity of A3 compound belongs to chloride group. Because of that the chloride group was withdraw group which that lead to make the benzene ring for the A3 compound indicative ring and which that produce the low activity for this compound for bacteria.[27]

Table (3): Antibacterial activity (inhibition zone, mm) of Schiff base [A1, A2, A3] compounds against pathogenic bacteria

Type of bacteria	A_1	A_2	A_3
Staphylococcus aureus	0	15	0
Streptococcus agalactiae	30	34	0
Escherichia coli	0	12	0
Klebsiella pneumoniae	0	14	11
Proteus mirabilis	8	20	15
Enterobacter cloacae	10	20	0
Salmonella typhi	13	16	0
Pseudomonas aeroginosa	8	13	10

CONCLUSIONS

From this study, the following conclusion could be drawn

1. The withdrawing electron groups and The donating electron groups are influenced on the assay of the reaction time. The donating electron groups raised the reaction rate, for this reason the reaction time is decreased but the withdrawing electron groups are decreased the reaction rate, that was leading to the reaction time was increased.
2. Each the prepared compounds were stabilized with resonance and these compounds were possessing rising melting points comparatively, this is other proof on the extent of the stability.
3. Evaluation of antibacterial activity of new Shiff base derivatives [A1-A3] compounds towards common multidrug resistance pathogenic bacteria.

REFERENCES

1. *IARC IARC Monographs on the Evaluation of Careinogenic Risk ofChemicals to Man,* Some Carbamates, Thiocarbamates and Carbazies, Lyon, 1976; 12: 167-17.
2. *US National Library of Medicine Hazardous Substances Data Bank (HSDB No, 207), Bethesda, MD,* 1992.
3. Schaefer e.H., Colwell A.E. and Dupras E. E, Jr. The occurrence of p-chloroanilne and p-chlorophenyl urea from the degradation of diflubenzuron in water and fish. *ln: Grant, e.D.* & Washino R. K., eds. *Proceedings and Papers of the 48th Annual Conference of the Califomia,* Mosquito and Véctor, *Control Association (CMVCA), Inc.,Visalia, CA, CMVCA Press,* 1980; 8489.
4. Sakagami Y., Yokoyama H. and Ose Y., Degradation of disinfectantsby Pseudomonaaeruginosaisolated from activated sludge- identification of degradation products.*1 Hyg. Chem.,* 1986; 32: 427-432.
5. Huang H. M., Hodson R. E., and Lee R. F.,: Degradation of anilne andchloroanilnes by sunlight and microbes in estuarine water. *Water Res.,* 1987; 21: 309316.
6. Mutanen R. M., Siltanen H.T., Kuukka Y. P., Annila E. A. and VaramaM. M. O., Residues of diflubenzuron and two of its metabolites in a forestecosystem aftercontrol of the pi moth, *Bupaluspiniarius L. Pestic. Sei.,* 1988; 23: 131-140.
7. Kaczvinsky J. R., Jr, Saitoh K. and Fritz J. S., Cation-exchange concentration of basic organic compounds from aqueous solution. *An l. Chem.,* 1983; 55: 1210-1215.
8. H. Schiff, *Justus Liebigs Ann. Chem*., 1864; 131: 118-119.
9. Mladenove R. *et al. ED. Eur. Polym. J.,* 2002; 38: 989–999.

10. Wang L., Feng Y., Xue J. and Li Y., *J. Serb. Chem. Soc.*, 2008; 73: 1-6.
11. Li Y. *et al., Bioorg. and Med. Chem.*, 2003; 11: 4363-4368.
12. Villar R. et al. *Bioorg. and Med. Chem.*, 2004; 12: 963-968.
13. Venugopal K.N. and Jayashree B.S.; *Indian J. Pharm. Sci.*, 2008; 70: 88-91.
14. Karthikeyan M.S. *et al., Bioorg. and Med. Chem.*, 2006; 14: 7482-7489.
15. F. M. Menger, D. J. Goldsmith and L. Manden; "Organic Chemistry", 2nd, Ed., 1975; 318.
16. Milan Cacic, Maja Molnar, BojanSarkanj, Elizabeta Has-Schon and Valentina Rajkovic, *Molecules*, 2010; 15: 6795-6809.
17. Abd El-Fatah M. O.uf, Mayada S. Ali, Mamdouh S. Solaiman, Ahmed M. El-Defrawyand Sahar I. Mostafa,*Journal of the Korean Chemical Society*, 2010; 54: 4.
18. Macfaddin, J.F. Biochemical tests for identification of medical Bacteria. 3rd-ed, williium and Wilkins, U. S. A., 2009.
19. Ramalivhana JN, Obi CL, Samie A, Iweriebor BC, Uaboi-Egbenni P, Idiaghe J.E. and Momba M. N. B. Antibacterial activity of honey and medicinal plant extracts against Gram negative microorganisms. African Journal of Biotechnology, 2014; 13(4): 616-625.
20. Murray, PR. ; Baron EJ , Pfaller MA, Tenover FC and Yolken HR. Manual of clinical microbiology, 6th Ed. ASM press , Washington DC, 1995; 15-18.
21. Olurinola, P.F. A laboratory manual of pharmaceutical microbiology. Idu, Abuja, Nigeria, 1996; 69-105.
22. Imlay, J. A. Pathways of oxidative damage. Annu. Rev. Microbiol, 2003; 57: 395–418.
23. Imlay, J. A. How oxygen damages microbes: oxygen tolerance and obligate anaerobiosis. Adv. Microb. Physiol, 2002; 46: 111–153.
24. Davies, B. W. et al. Hydroxyurea induces hydroxyl radical-mediated cell death in Escherichia coli. Mol. Cell, 2009; 36: 845–860.
25. Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A. and Collins, J.J. A common mechanism of cellular death induced by bactericidal antibiotics. Cell, 2007; 130: 797–810.
26. Cowan, M.M. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews, 1999; 12(4): 564-582, ISSN 0893-8512.
27. Brown, W.H. ; Foote, C.S. and Iverson, B.L. Organic chemistry. 5th Ed. 2009.