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SYNTHESIS, CHARACTERIZATION AND INVITRO-ANTI-MICROBIAL ACTIVITIES OF 3-NITRO –N- (3-NITROBENZYLIDINE) ANILINE SCHIFF BASE COMPLEXES OF COBALT (II) AND CU (II)

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ABSTRACT

Schiff base derived from an equimolar amount of 3-nitrobenzaldehyde with P-nitro aniline was synthesized. The Schiff base was subsequently reacted with Cobalt (II) chloride and Copper (II) chloride to form the metal complexes. The compounds were characterized using FTIR, UV-visible, melting point/decomposition and solubility test. The solubility test of the compounds showed that they are soluble in most organic solvents and the melting point of the schiff base ligand was found to be 147.8 ^{o}C and the decomposition temperature of the Cobalt (II) and Copper (II) were found to be 158.8^{o}C and 157.9^{o}C for the Cobalt (II) and Copper (II) complexes respectively. The UV and the IR spectroscopy confirmed the formation of complex. The antimicrobial studies of the synthesized ligands and their Cobalt (II) and Copper (II) metal complexes were carried out against Aspergillus, Gram positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli. The results indicated that the metal complexes were more active than the ligand but less active compared to standard drugs (ciprofloxacin and fluconazole).

Key words: *Schiff base, 3-nitrobenzaldehyd and 3-Nitro aniline.*

INTRODUCTION

The role of antimicrobial drugs in decreasing illness and death associated with infectious diseases in animals and humans cannot be overemphasized. However, selective pressure exerted on existing antimicrobial drug has orchestrated the emergence and spread of drug-resistance traits among disease causing and commensal bacteria [1]. Of serious concern is the development of resistance by *Escherichia coli* or *E.coli* strains to the current antibiotics such as ampicillin, sulfonamide, gentamicin, streptomycin, ciprofloxacin, trimethoprim, and amoxicillin. *E. coli* is often a commensal bacterium of humans and animals but Pathogenic variants cause intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia [6]. This trend of resistance exhibited by this organism poses serious threat to human and animals health, necessitating the search for newer antibiotics.

In recent years, Schiff bases have received considerable attention because of their physiological and pharmacological activities.

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This class of organic compounds have also demonstrated significant inhibitory activity against the growth of *E. coli*, making them potential drug candidate in man's quest to curb the dangerous trend of multi-drug resistance posed by this pathogenic micro-organism [11].

Conventional drug discovery and development is characterized by trial-and-error approach. This is time consuming, costly due to the enormous expense of failures of candidate drugs late in their development and a threat to green chemistry due to enormous waste released into the environment.

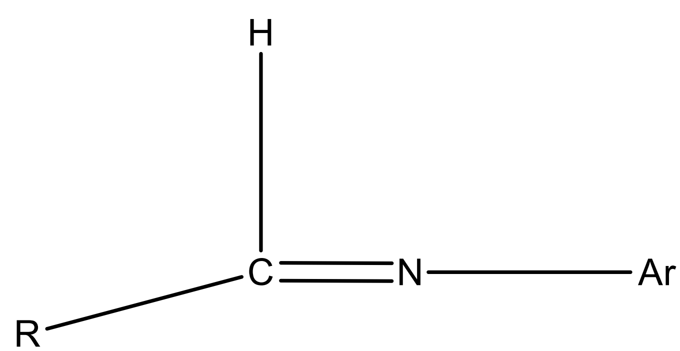
Metal complexes

Coordination complex consists of a central atom or ion, which is usually metallic and is called the coordination center and a surrounding array of bound molecules or ions that are in turn known as ligands or complexing agents. A coordination complex whose central atom is a metal is called a metal complex. The electronic configuration of transition complexes gives them some important properties such as color, magnetism and reactivity etc. Metal ions exist in solutions as coordination complexes. A huge number of metal complexes are used as catalyst in a variety of organic reactions (e.g. polymerization, hydrogenations, additions, cross coupling reaction). Several metal complexes are in clinical use for the treatment of various diseases, and many are currently being tested in potential metal-based drugs. The bonding of metals to ligands frequently results in synergistic activity. It is well known that metal ions are often crucial for the activity of drugs and are involved in their mechanisms of activity. Metal complexes are not only important as potential drug; they can also be used for analytical and diagnostic purposes in biological systems [7].

Schiff bases

Schiff bases are stable compounds containing the carbon-nitrogen double bond (-HC=N-), they are also called imines. Imines are present in various naturally derived compounds; ancistrocladinine and chitosan-derived Schiff base. The presence of imine group in these compounds have contributed to their activities. Generally, Schiff bases have the formula RHC=NAr (Figure 3). The chemistry of the carbon-nitrogen double bond plays a vital role in the progresses of chemical science. Schiff bases also called imines, are characterized by the presence of the azomethine functional group (-C=N-) and are usually formed by condensation of carbonyl compound (aldehyde or ketone) with a primary amine [9].

Fig 1. Schiff base functional group



R = alkyl
Ar = aryl

The presence of aryl group leads to conjugation in the system, hence increased stability of aryl Schiff bases. Most Schiff bases exhibit delocalized π-electron system which makes them

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to have large non-linear polarizabilities usually enhanced by the presence of donor or acceptor groups. Schiff bases are among the most general nitrogen coordinating ligands, because the basicity of the sp^2 hybridized nitrogen lone pair of the imine nitrogen (although lower than that of amines sp^3 hybridization) is well suited to form complexes with metal ions. Schiff bases play important roles in Coordination Chemistry as they easily form stable complexes with most transition metal ions (Atmaram and Kiran, 2011). Many of the ligand systems employed in model studies are derived from Schiff bases. Schiff bases possess structural similarities with naturally occurring biological substances. This enhances their use in elucidating the mechanism of reaction processes in biological systems such as transamination and racemination reactions. The relatively simple preparation procedures and synthetic flexibility which facilitates the introduction of tailor-made structural properties allows their use as ligands for preparation [5].

Schiff bases and their chemistry

Schiff bases are condensation products of primary amines and carbonyl compounds and they were discovered by a German chemist, Nobel Prize winner, Hugo Schiff in 1864. He discovered Schiff bases and other imines and was responsible for research into aldehydes and had the Schiff test named after him. He also worked in the field of amino acids and the Biuret reagent. Compounds containing an azomethazine group (-CH=N-) are known as Schiff bases [4].

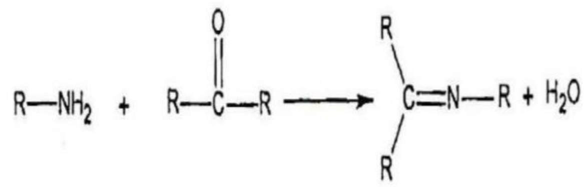

Fig 2. Formation of Schiff base by condensation reaction.

Where R may be an aliphatic or an aromatic group.

Schiff bases of aliphatic aldehydes are relatively unstable and are readily polymerizable while those of aromatic aldehydes, having an effective conjugation system, are more stable [4]. Condensation of amines with aldehydes and ketones has numerous applications which include preparative use, identification, detection and determination of aldehydes or ketones, purification of carbonyl or amino compounds or protection of these groups during complex action or sensitive reactions. An amino group found in simple amines and the Schiff bases obtained from aromatic amines are known as anilines. Schiff bases are generally bi- or tri-dentate ligands capable of forming very stable complexes with transition metals. In chemistry, Schiff bases find a versatile use; some of them are the basic units in certain dyes. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds [12].

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Biological importance of Schiff's base

Schiff bases appear to be important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most prevalent types of catalytic mechanisms in biochemical processes involves condensation of primary amine in an enzyme, usually that of a lysine residue, with a carbonyl group of the substrate to Schiff bases. Schiff Bases are characterized by the –N=CH- (imine) group which works in elucidating the mechanism of transamination reaction in biological system. Schiff bases are active against a wide range of organisms *like Candida albicans, Escherichia coli, Staphylococcus aureus, Bacillus polymxa, Trychophyton, gypseum, Mycobacteria, erysiphegraminis* and *Plasmoporaviticola* [1].

Antimicrobial activity

Antimicrobial drugs are designed to kill, or inhibit the growth of microorganisms (bacteria, fungi, virus, pirocheates and protozoa etc). Bacteria, fungi and viruses are responsible for almost all of the common infectious diseases found in Nigeria from malaria to AIDS to ulcers. The modern era of antimicrobial chemotherapy began in 1929 with Fleming's discovery of the powerful bactericidal substance penicillin and Domagk's discovery in 1935 of synthetic chemicals sulfonamides with broad antimicrobial activity [12]

The future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms especially bacteria, are becoming resistant to more and more antimicrobial agents. Currently, bacterial resistance is combated by the discovery of new drugs.

Pharmacological properties of Schiff bases

Imine complexes have a broad range of biological properties: antitumor, antiviral, antifungal, Antibacterial. They are also used in the treatment for diabetes and AIDS. As biological models, they help in understanding the structure of biomolecules and biological processes occurring in living organisms. They participate, inter alia, in photosynthesis and oxygen transport in organisms. They are involved in the treatment of cancer drug resistance, and often tested as antimalarial. It also could be used for the immobilization of enzymes [9].

MATERIALS AND METHOD

Materials and Reagents

Reagents**,** Benzaldehyde, P-nitroaniline**,** Ethanol**,** Acetic acid**,** Ammonia**,** Copper (ii) chloride**,** Cobalt (ii) chloride**,** Magnetic stirrer**,** Watch glass**,** Conical flask**,** Beaker**,** measuring cylinder Infrared spectrophotometer**,** Uvi-Visible spectrophotometer**,** Analytical balance**,** Melting point apparatus.

Synthesis of Schiff's base ligand using 3-nitrobenzaldehyde and p-nitroaniline

The schiffs base ligand was synthesized by adapting procedure used by Jadhav S. P. and Kapadnis [8]. A solution of 1.02 ml of 3-nitrobenzaldehyde [0.01m] in 10ml of ethanol,

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and 1.38gm 0f 4-nitroaniline [0.01m] in 10ml ethanol was added in a beaker. a few drops of glacial acetic acid was added to adjust the pH of the solution. The reaction mixture was stirred in 5 hours, after stirring cool water was added, the obtained precipitate was collected by filtration. It was well dried and recrystallized from ethanol and dried at room temperature, the yellow product of Schiff base ligand was obtained for re-crystalization of Schiff base ligand, the product was dissolved in same solvent used for the synthesis i.e., ethanol and was heated until the product was dissolved completely, and clear solution was obtained and filtered through cotton at hot condition. Filtrate was cooled in ice bath for overnight to get yellow crystalline solid particles of Schiff base ligand of 3-nitrobenzaldehyde and 4-nitroaniline

Synthesis of metal complex of cobalt with schiff base ligand

The complex was synthesized by adoptind the procedure by Nasira N. S. *et al*. [10]. A solution of 1.19gm of cobalt chloride [0.01m] in 10ml of ethanol, a hot solution of 2.26gm of Schiff base ligand in 10ml ethanol was added in beaker. Few drops of ammonia were added to adjust pH of solution. The reaction mixture was stirred in 2 hours. After stirring cool water was added, the obtained precipitate was collected by filtration. It was dried well and recrystallized from ethanol and dried at room temperature.

Conductivity measurement

Conductivity (or specific conductance) of an electrolyte solution is a measure of its ability to conduct electricity. The SI unit of conductivity is Siemens per meter(S/m). Conductivity measurement is used to determine the molar conductance of the complexes in any soluble solvent using conductivity meter. This measurement will assist in establishing if the new complex is an electrolyte or not, and to find out the nature of charges it contains [4].

Uv-vis spectroscopy

UV-Vis Spectroscopy is an analytical method used to measure the absorbance of ultra-violet or visible radiation through an analyte. The molecular absorption of the analyte corresponds to both excitation of valence electrons and excitation of electrons in different atomic orbitals. UV-Vis Spectroscopy is an effective technique for both qualitative and quantitative analysis of organic and inorganic compounds. UV-Vis Spectroscopy is based on the Lambert-Beer principle which states that the absorbance of a solution (A) is directly proportional to its pathlength (l) and its concentration (c) when the wavelength of the incidence light remains fixed. This is summarized as $\boldsymbol{A = \varepsilon l c}$ where ε is the molar absorptivity.

Melting point Determination

Melting point or decomposition temperatures of the compounds were determined with a melting point apparatus and capillary tube.

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Solubility Test

Solubility test was done using different solvents which includes ethanol, methanol, DMSO, ethyl acetate, chloroform, acetone, and water in order to determine which solvent are suitable for subsequent analysis.

Antimicrobial Activity

The test microorganisms used for this analysis were pure cultures of bacteria and fungi. The isolates were *Staphylococcus aureus*, *Escherichia coli,* and *Aspergillus*. Determination of Inhibitory Activity (Sensitivity Test) of the Synthesized Samples Using Agar Well Diffusion Method was carried out. The standardized inocula of both the bacterial and fungal isolates were streaked on sterilized Mueller hinton and potato dextrose agar plates respectively with the aid of a sterile swab sticks. Four wells were punched on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentrations of the synthesized samples which were 75 and 25mg/ml respectively. Each well was filled up with 0.2ml of the sample. The inoculated plates with the sample were allowed to stay on the bench for about an hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37°C for 24hour (plates of Mueller hinton agar) while the plates of potato dextrose agar were incubated at room temperature for about 3-5 days. At the end of the incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (Zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimeter and the results were recorded.

Infrared Spectroscopy (IR)

Infrared spectroscopy is a very powerful technique which uses electromagnetic radiation in the infrared region for the determination and identification of molecular structure as well having various quantitative applications within chemistry. The IR spectroscopy concept can generally be analyzed in three ways: by measuring reflection, emission, and absorption. The major use of infrared spectroscopy is to determine the functional groups of molecules.

Bands that are unique to each molecule, similar to a fingerprint, are found in the fingerprint region, from 1300 to 4000 cm^{-1}. These bands are only used to compare the spectra of one compound to another [2].

RESULTS AND DISCUSSION

Physical properties of the Schiff base

Some of the physical properties of the Schiff base and its Co (II) and Cu (II) metal complexes were characterized and recorded below.

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Table 1. Solubility test result

Compound	H_2O	Ethanol	Chloroform	Ethylacetate	Acetone	Ethanol
L	IS	S	SS	S	S	S
L-Cu	IS	S	IS	S	S	S
L-Co	IS	S	IS	S	S	S

Keys: S-Soluble, IS-Insoluble, SS- Slightly Soluble

Table 2. Melting Point/ Decomposition temperature

Compound	Melting Point (^{O}C)	Decomposition temperature (^{O}C)
L	147.8	-
L-Cu	_	157.9
L-Co	-_	158.8

Table 3. UV-Visible region of the synthesized complexes

Compound	L	L-Co	L-Cu
Max (nm)	414	385	352

Table 4. FT-IR Vibration frequencies of the Ligand and the complex

Compound	**N-H stretch**	**C-H bending**	**C=N stretch**	**O-H stretch**	**C=C stretch/ bending**	**N-O stretch**	**C-O stretch**	**C-N stretch**
L	3369.5	1494.7			834.9 693.3	1520.8	1107.0	1300.8
L-Co	3548.4	1498.4	1494.7	3388.2	1595.3	1297.1	1103.3	1256.1
L-Cu	3362.1	1494.7	1595.3 1494.7		1595.3 834.9	1300.8	1256.1 1107.0	

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Table 5: Zone of inhibition of 4-Nitro-N-(3-nitrobenzalidene) aniline)

Microorganism	75mg/ml	50mg/ml	25mg/ml	Control mg/ml
E. coli.	5mm	3mm	1mm	
Staph..	4mm	2mm	1mm	
ASP.	3mm	2mm	2mm	

Key: *E. coli= Escherichia coli, Staph = Staphylococcus Aureus,* ASP = *Aspergillus*

Table 6: Zone of inhibition of Cu complex of 4-Nitro-N-(3-nitrobenzalidene) aniline)

Microorganism	75mg/ml	50mg/ml	25mg/ml	Control mg/ml
E. coli.	8mm	5mm	2mm	
Staph..	7mm	5mm	2mm	
ASP.	4mm	2mm	1mm	

Key: *E. coli= Escherichia coli, Staph = Staphylococcus Aureus,* ASP = *Aspergillus*

Fig. 3: Formation of the ligand

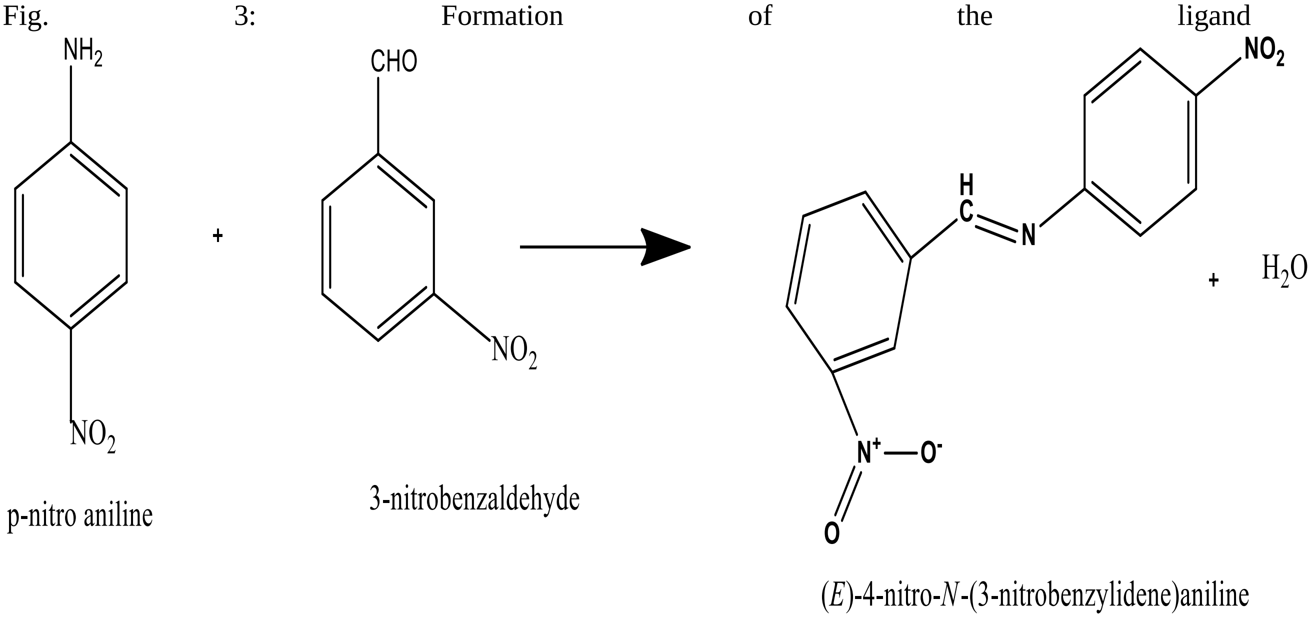

Fig. 3: Formation of the ligand

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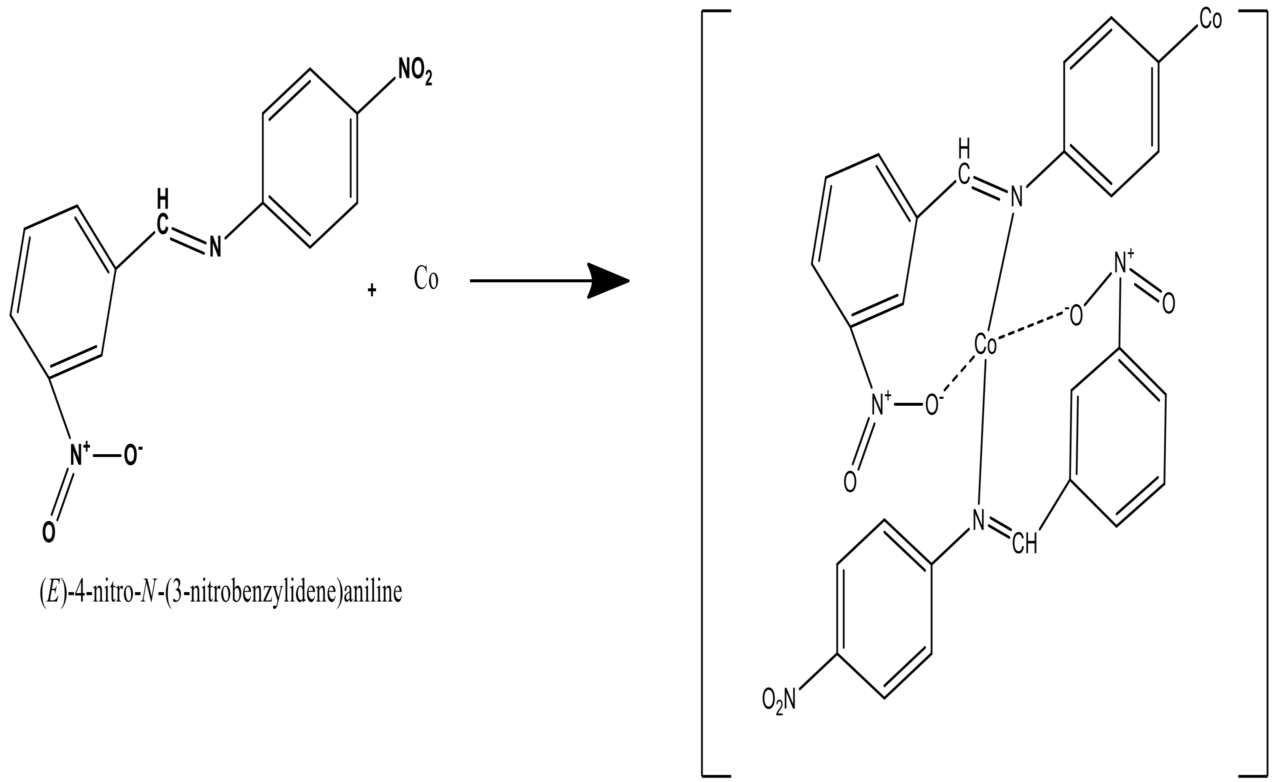

Fig. 4: Expected structure of the complex

complexes were analyzed to be soluble in most of the polar organic solvent tested. They were tested

complex formation from the ligand

Discussion

The physical properties of the Schiff base, its Cobalt (II) and Copper(II) complex were analyzed accordingly. The melting point temperature of the Schiff base was determined to be 147.8ºC and the decomposition temperature of its Co(II) and Cu(II) complex were found to be 158.8ºC and 157.8ºC respectively which indicate higher thermal stability of the compound after binding with the metal. Solubility test of the Ligand and its Cobalt(II) and Copper(II) with ethanol, methanol, ethyl acetate, water, chloroform, and acetone.

UV-Visible spectra of ligand and its Co(II) and Cu (II) metal complex were recorded in ethanol. λmax for ligand and its Cobalt (II) and Copper (II) complex are 414 nm ,385 nm, 352 nm respectively, it confirms complex formation. FT-IR spectra of the ligand shows a sharp absorption band at 1628.8 cm^{-1} corresponding to C=N frequency, and the presence of band in the region 1600- 1690 cm^{-1} supports the existence of C=N

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stretching in the metal complexes. On coordination of the azomethine nitrogen, the IR stretching frequency of C=N shows a shift and is observed in the region 1600-1690 cm^{-1}in synthesized Schiff base metal complexes. The appearance of the band around 1500-1600 cm^{-1} corresponds to N-O stretching frequency both in ligand and in metal complexes The ligand shows a sharp absorbtion at 3358.3 cm-1 which corresponds to N-H frequency, and the slight shift in the absorption band of the Co(II) and Ni(II) metal complex which were 3354.6cm-1 and 3354.8cm-1 respectively confirmed complexation. (infra-red spectroscopy absorption table)

Antimicrobial Result

The results of Schiff base and their Cu and Co complexes indicate activity against all the isolates tested even at lower concentration. The result also shows that the Schiff base has less antimicrobial activities (5mm, 4mm and 3mm at 75) than the Cu complex. The largest zones of inhibition of the Schiff base complex of Cu occurred with E. coli and Staph 8 mm and 7mm respectively at 75 mg/ml (table 6). Aspergillus shows the lowest zone of inhibition 3 mm, 2 mm and 1mm as the concentration of the compound decrease from 75 mg/ml, 50 mg/ml and 25 mg/ml.

In general, all the compound reveals good zone of inhibition in different concentration both in fungi and bacteria isolate, this shows that the Schiff base used have anti-fungi (Aspergillus *Spp.)* and anti-bacteria component in them. Thus, the compound can also be used to treat Aspergillus *Spp.* Staphylococcus aureus and Escherichia coli.

CONCLUSION

The Benzaldehyde and p-nitroaniline Schiff base ligand and its Cobalt(II) and Copper(II) metal complexes were successfully synthesized and confirmed/characterized by melting point/decomposition temperature, solubility test, UV-visible spectroscopy and infra-red spectroscopy. The complexes and the ligand were subjected to antimicrobial activity, where they were found to be sensitive against the tested bacterial and fungal isolates studied but for L-Cu which was found to be more active against tested fungi and bacteria isolate (8mm,7mm and 5mm at 75 conc.) than the Schiff's base (5mm, 4mm and 2mm at 75conc.) The complexes exhibited better activity than Schiff base but less active compared to the standard drugs (control). Also from preliminary finding the complex is shown to possesses tetrahedral structure even though other instrumentation such as X-ray crystallography are required to confirm the structure.

REFERENCES

[1] Archana S., (2013). Synthesis and characterization of Schiff base salicyaldehyde and thiohydrazones and its metal complexes. Advances in Applied Science Research; 5(7): 708-715.

ISSN 0795 - 22066

[2] Brain E.I. and Drago R.S. (1971). Nickel (II) and nickel (IV) complexes of 2,6-diacetylpyridine dioxime. Journal of the American chemical society; 93(24): 6469-6475.

[3] Chakraborti AK, B. S. (2004). Magnesium perchlorate as an efficient catalyst for the synthesis of imines and phenylhydrazones. Tetrahedron Lett, 7641-7644.

[4] Chaudhary, N. (2013). Synthesis and medicinal use of Metal Complexes of Schiff Bases Technology and Mathematics, 75-80.

[5] Chohan ZH, A. M. (2006). Synthesis, Characterization and Antimicrobial activity of Cobalt metal complex against multi resistant bacterial and fungal pathogens. Bioinorganic Chemistry and Applications Journal, 1-13.

[6] Friedrich B. and Elaine W. (2007) @'Benzaldehyde', Ullmann's Encyclopedia of industrial chemistry (7th edition), Wiley, p 11.

[7] Gupta K, C. K. (2008). Schiff base metal complexes of Ni, Pd and Cu . Coordination Chemistry, 1420-1450.

[8] Jadhav S. P. and Kapadnis K. H. (2018). Synthesis and characterization of schiff bases of benzaldehyde with nitroanilines and their cobalt, nickel and copper metal complexes nitroanilines and their cobalt, nickel and copper metal complexes. Journal of Arts, Science & Commerce; 9: 147-152.

[9] Mohammad M. Y. K., Mohammed A. H. M., Vinod L. P., Mihir S. P., Roma K. P. and Rohit H. D. (2018). Synthesis and characterization of schiff base aniline with 5-bromo-2-hydroxyl benzaldehyde and their metal complexes. International Journal of Recent Scientific Research; 9(4) : 26026-26030

[10] Nasira N. S., Rashmi S., Kabita S., Rahul G.,Shankerdutt P., Satu T. C. and RP Chavan, (2016). Synthesis, Characterization of some Schiff's basesderived from phenylhydrazine.International Journal of Chemical Studies ; 4(1): 109-111.

[11] Pasdar H, N. F. (2018). Cobalt (II) Complexes with Schiff Base Ligands Derived from Terephthalaldehyde andortho-Substituted Anilines: Synthesis,Characterization and Antibacterial Activity. Applied Science, 50-62.

[12] Thierry Y. F., Monisola I. I., Derek T. N. and Patrick B. N., (2018) Microbial activity of some heterocyclic Schiff bases andmetal complexes. Tropical Journal of Pharmaceutical Research; 17 (12): 2507-2518.

ISSN 0795 - 22066